第十二届"挑战杯"中国大学生创业计划竞赛

获奖证书

敖若寅 王坤 李凌宏 龚禹瑞 陈本佳 刘金涛 何迷 黄静 胡庆毅同学

你(们)的项目《 多年生稻科技小院—深入云南边境的乡村振兴稻路 》

在第十二届"挑战杯"中国大学生创业计划竞赛中荣获

铜奖

指导老师：胡凤益 秦世雯 黄光福

特颁此证，以资鼓励。

主办单位：共青团中央 教育部 中国科协 全国学联 黑龙江省人民政府

承办单位：东北林业大学 共青团黑龙江省委

第十二届"挑战杯"中国大学生创业计划竞赛组织委员会

二〇二〇年十二月

组织委员会

23010201014444

获奖证书

Certificate of Award

李凌宏、李军、唐筱韵、贺汝来、李昆翰、李禹甫、普新援、黄广一、凌霄

你们的作品《多年生稻——中国粮食安全“新防线”》，在第七届中国国际“互联网+”大学生创新创业大赛中荣获**金奖**

指导老师：胡凤益、黄光福

特发此证，以资鼓励。

主办单位：教育部、中央统战部、中央网络安全和信息化委员会办公室、国家发展和改革委员会、工业和信息化部、人力资源和社会保障部、农业农村部、中国科学院、中国工程院、国家知识产权局、国家乡村振兴局、共青团中央、江西省人民政府

承办单位：南昌大学、南昌市人民政府

中国国际“互联网+”大学生创新创业大赛组织委员会

二〇二一年十月

“互联网+”大学生创新创业大赛 组织委员会

编号：2021200051

荣誉证书

CERTIFICATE OF HONOR

王慈嘉、谢雨琪、张超超、李缘、张利娜、王天喜、吴嘉丽、王冰蓉 同学：

你们的作品《魔宴——中国西部的致富盛宴》，在“俊发杯”第七届云南省“互联网+”大学生创新创业大赛中荣获：

金 奖

指导老师：吴学尉、张盛林

特发此证，以兹鼓励！

主 办：云南省教育厅
承 办：云南大学
协 办：俊发集团、云大启迪K栈众创空间、
云南省高等学校创新创业教育教学指导委员会

云南省教育厅
二〇二一年十一月

荣誉证书

CERTIFICATE OF HONOR

黄广一、张时扬、张树忻、喻好好、薛笑然、陈佳佩、王少阳、
贺鸿、蒋文龙、李凌宏、潘志立、卢玠、李贝、张佳欣、陈锦荟 同学：

你们的作品《滇咖余香——全国咖啡公益科技服务引领者》，在“俊发杯”第七届云南省“互联网+”大学生创新创业大赛中荣获：

银奖

指导老师：何飞飞

特发此证，以兹鼓励！

主 办：云南省教育厅
承 办：云南大学
协 办：俊发集团、云大启迪K栈众创空间、
云南省高等学校创新创业教育教学指导委员会

云南省教育厅
二〇二一年十一月

荣誉证书

CERTIFICATE OF HONOR

张佳欣、汪铭、张粱、徐玉龙、韩泽邦、孙帅、
赵峰、周光信、孙岳、黄广一、诸圣航、周高锐 同学：

你们的作品《“咖妃”的表与里——咖啡果皮深加工技术推动咖啡产业可持续发展》，在“俊发杯”第七届云南省“互联网+”大学生创新创业大赛中荣获：

银奖

指导老师：何飞飞

特发此证，以兹鼓励！

主 办：云南省教育厅
承 办：云南大学
协 办：俊发集团、云大启迪K栈众创空间、
云南省高等学校创新创业教育教学指导委员会

云南省教育厅
二〇二一年十一月

荣誉证书

CERTIFICATE OF HONOR

田青霖、姬梦琳、薛治峰、张树竹、薛笑然、龚禹瑞、胡斌、
姚凡、焦淑亮、朱蓉静、张晶、潘志立、陈慧媛、杨骏安 同学：

你们的作品《香蕉生防“军”——保卫云南香蕉绿色食品牌》，在“俊发杯”第七届云南省“互联网+”大学生创新创业大赛中荣获：

银奖

指导老师：秦世雯、郑泗军

特发此证，以兹鼓励！

主 办：云南省教育厅
承 办：云南大学
协 办：俊发集团、云大启迪K栈众创空间、
云南省高等学校创新创业教育教学指导委员会

云南省教育厅
二〇二一年十一月

云南省第八届“互联网+”大学生创新创业大赛获奖名单 （省级）

序号	项目名称	负责人	赛道	组别	团队成员	指导老师	奖项
1	云沐科技：智慧温室一站式方案提供商	张宇	“青年红色筑梦之旅”赛道	创意组	王燚、张俊杰、郝康、丁予晴、万星雨、刘鸿瑜、李诗阳、郑汉卿、齐振坤、史心竹、胡钦行、辜欣曼、董昊哲、张铭信	李振东、李娅、马子红、朱要龙	金奖
2	律动——民族音乐从传承创新到共享共筑	侯诗睿	“青年红色筑梦之旅”赛道	创意组	杜匡辰，卢俊宇，杨文静，谭亚轩，吕春保，李婷，程迪，王兴猛，沙文海，李文哲，方一佳，陈虹，彭涛	李振东，周观琪，陈雪，李豪杰，汪洋	金奖
3	“一个都不能少”我国跨境居住少数民族数字影像化传承保护与发展项目	李思媛	“青年红色筑梦之旅”赛道	公益组	王文俊，宦宏明，丁晓文，龙睿超，薛帆，石晶雯，唐培耕，朱令昌	木永跃，付秋静	金奖
4	金“芋”良品——助力边疆民族地区乡村振兴的“金疙瘩”	王蕊嘉	“青年红色筑梦之旅”赛道	公益组	赵青青，张利娜，李文涵，张惠敏，胡斌，王煜涵，韩茂，王天喜，谢雨琪，魏怡，杨冬，张超超，李缘，李炎婷	吴学尉，杨舒然，张盛林	金奖
5	数字医疗村村通——打通边疆民族地区乡村医疗健康“末梢”	李鑫鑫	“青年红色筑梦之旅”赛道	公益组	李兴兴，胡文杰，段荣坦，李顺彩，王宏硕，穆婧秋，曾丽涛，赵鋆，曾正媛，王云霞，王斌，王铁林	郑家亮，姚建文，杨舒然，赵淞，韦嘉	金奖
6	共赴“心”生——智慧医疗助力偏远山区先心病全面救助	成焱雄	“青年红色筑梦之旅”赛道	公益组	张晓庆，夏军，汪琴，樊庆玲，田英杰，马鹏钥，杨轩宇，周姣，晏紫荆，肖桂章，刘源志，王越玥，王楠	池宗琳，余鹏飞，普园媛	金奖
7	品味“真”薯——非常规原生态干马铃薯片产业化推广	王馥瑛	“青年红色筑梦之旅”赛道	创意组	李鑫鑫，朱协，刘泯辰，王斌，何月，李晓启，张凌杉，余建华，刘彦明，何欣然，王诗莹，孟曦农	赵德森，邓伟升，李豪杰，姚建文，陈楠	金奖
8	济群科技——中国智慧城市地下管网守护者	杨键	主赛道	本科生初创组	吕志钦，李佳凝，蒋群利，周文瑾，王艺璇，刘子愉，姜刚涛，许心怡，白雨珊，陶宇彬，郑舒心，吕佳钰，金文汉	刘晓倩，袁娥，马凡松，姚建文，梁虹	金奖
9	“孕乐宝”早产自检试剂盒——国内居家早产检测新方案	赵颖雯	主赛道	本科生创意组	肖承容，吴少骞，张子寒，刘文彦，陈雨菲，张小姣	郭春明，邓双胜	金奖
10	绿砼——多领域火山石生态混凝土行业引领者	梁亚鹏	主赛道	本科生创意组	陈秋雨，孔颜，张俊康，田京鑫，徐航，黎佳文，谢艺宁，林杰，方文裕，李云昊，刘欢，陈亭璇	雷真，何曼榕	金奖
11	精准视疝——智能可视化轻量型补片领航者	朱楠	主赛道	本科生创意组	温君博，李耀伟，李欣洁，罗帅，张艺旖，李瑾怡，张鹤舰，张天翊，耿皓天，禹玺，舒雨诚，曾丰	吴俊，杨舒然，孙亮，普圆媛，李洁超，高松，朱科铭	金奖
12	汇光增能——实现全光谱太阳能发电的转光玻璃	李天宇	主赛道	本科生创意组	周杨名，姚志兰，李润超，莫宇峰，阮敬娥，文湘，蓝鸿，靳嘉琪，田东林	熊飞，马玉超	金奖
13	饮水洁源——新型铁碳微电解地下水净化系统	胡明达	主赛道	本科生创意组	金奕扬，孙婷，卢洪波，罗梦齐，花悦珊，袁绪卓，李加涵，何亚琳，罗芸，唐彬，姜科，张非凡，刘西悦	常军军	金奖
14	南亚东南亚企业顾问——您的全过程涉外法律管家	杨杨	主赛道	本科生创意组	方圆，彭心怡，丑盈盈，刘芳缘，张奕珣，石景瑞，李春辉，李云昊，秦瑜洁，刘语童，王华欣，胡诗雨	唐国淞，李晶磊，李一非	金奖
15	一灯——目前世界领先的自动追光灯系统	高竟涛	主赛道	本科生创意组	张锦超，陈铭，崔晓，高瑜蔓，李恒佳艺，张瑾，王行知，杨文静，张怡嘉，戴兴竹	李振东，周开邻，马子红	金奖
16	“越鸟南栖”——基于数据库技术的少数民族文化灵感供应商	朱子莹	主赛道	本科生创意组	周嘉雨，穆婧秋，苏城，乔星嘉，张哲瑞，柏涵解，何巧，钟木腾，陈芊希，宋颖，奉敏燕，孙明欣	唐国淞	金奖
17	绳农之策——国内首创绳驱并联机器人助力表型测定智能化	施文博	主赛道	本科生创意组	田辉，赵亚倩，王海凡，邹星宇，袁辰，范宸佑，王楚沅，唐逸飞，段宏斐，苏浩明，杨启元，吴洪波，吴婷玉，李张瑶	刘金卓，李娅佳，汤开磊	金奖

云南省第八届“互联网+”大学生创新创业大赛获奖名单 （省 级）

序号	项目名称	负责人	赛道	组别	团队成员	指导老师	奖项
18	精诚治疝---多模态可视化补片固定系统提供商	张兆民	主赛道	研究生创意组	夏芪芩，王强，周易，蔡铨纬，田娟，王蓉，田冀雯，陈佳男，姚波，张菁菁，陈妍君，周景鸿，刘文彦，赵彬霞	吴俊，普园媛，孙亮，李洁超，高松，颜光前	金奖
19	光驰科技——超高时空分辨多功能光学仪器	高健文	主赛道	研究生创意组	张林林，张闰华，李云海，石福燕，李俊邑，肖娟，侯凯夫，车俊叶，杨成宇	任杨，孟伟东	金奖
20	智能+柔性电极——柔性传感器综合解决方案	宋振林	产业命题赛道	国赛命题组	段良飞，吕天平，王晓朦，赵建红，卢清杰，字包叶，陈明鹏，荣茜，Wei，高成凤，陈丽敏	柳清菊	金奖
21	基于昇思MindSpore的智慧医疗创新解决方案	王佳舜	产业命题赛道	国赛命题组	阳芷，汪瑞斌，宋明远，麦嘉成，常相超，郭竞，张佳佳，华鹏举，王晶晶，王耀威，刘博，杨鹏，肖亮	杨云	金奖
22	甜蜜事业---传播养蜂技术，爱心助农致富	李一帆	“青年红色筑梦之旅”赛道	创意组	邝钦峄，谢致黎，赵宗秋，陈建勇，陈雨菲，王诗莹，任秉灵，张硕，顾耘华，叶蔻	朱科铭，邓伟升，姚建文，龚志文，何乐生，张冬冬	银奖
23	阿细记忆--彝族阿细人非物质文化遗产数据库	张馨予	“青年红色筑梦之旅”赛道	创意组	刘航麟，牛丹，赵楠，李加涵，李雨洋，艾合买提尼牙孜·阿不力米提，吴顺，蒋晓梅，赵宣宏	祝湘辉，廖雷昭，李伟华	银奖
24	“护农知”——打造一体多元农业知识产权服务平台	王蕊	“青年红色筑梦之旅”赛道	公益组	李承洲，彭卓异，吴桂红，陈鹏宇，胡亚情，马畅，何海龙，陈青，杨凤，宋仁杰，段然，刘鑫	谭立力，于福科，赵春玉	银奖
25	数字灵眸打造沉浸式VR“四史”学习教育新方案	潘映伶	“青年红色筑梦之旅”赛道	公益组	廖贵彩，张秋月，刘逍	马志霞	银奖
26	"啡”同凡响——咖啡产业助力云南边疆乡村振兴	张树竹	“青年红色筑梦之旅”赛道	公益组	薛笑然，肖作敏，邵忠思，何琦，周露，谭丹，柏惠子，孔德婷，刘爽，唐锐，薛治峰，姬梦琳，田青霖	何飞飞，秦世雯，杨志玲，杨孟禹，董相书，高晶	银奖
27	云农智链——全产业链AI农业科技解决方案	史芳菲	主赛道	本科生初创组	赖丽珍，张唯一，刘茜，张紫雯，黄新渝，朱雅丽，张一三，袁宇航，濮虹文，吴钟岱	何鸣皋，普园媛，邵志会，景贵飞	银奖
28	张杨的多肉世界——边疆地区乡村振兴的金钥匙	张杨	主赛道	本科生初创组	吴佩桦，唐吉星，李晓启，王绍围，曹明涵，巴赫，王苡先，唐萌煜，周静怡，鲁丽萍，李璐璐， 罗梦佳	杨舒然，沈聪燕，吴学尉，姚建文，吴白雨，吴玉美	银奖
29	“灯峰照极”多功能路灯智慧控制系统	栗瑶瑶	主赛道	本科生创意组	董彦均，李晓静，李雪艳，马溶，王思予，郑方艳，丁卓鹰，李雪静，彭婷，刘晴晴	杨艳华，杨舒然	银奖
30	及时雨--陆天一体化火灾监测预警灭火系统	龚伟	主赛道	本科生创意组	赵泽，李思源，陈梦鑫，任秉灵，马宏伟，黄静，高瑜蔓，徐倩雪，王俊杰，赖丽珍，王俊兵，肖力铭，胡仕杰，匡正之	丁洪伟，杨俊东	银奖
31	书画文物“寻医问诊”：基于目标检测技术的书画文物病害识别系统	李子璇	主赛道	本科生创意组	刘芮，黄俞婷，蔡羲辰，王佳怡，萧纯一，张文清，康钰莹，赵海铭，张莹，蓝鸿，李柏萱，金樱，唐榕蔚，黄飞杨	张云春，赵明雄	银奖
32	"芸"香雅韵——打造民族生态芳香品牌	高瑜蔓	主赛道	本科生创意组	周琼，朱楠，于沁，张非凡，王欣彤，吉小萌，赵苗臣，李继飞，胥堡惠，张艺旖，杨雯，李天宇，朱薇焱，李志鹏	朱科铭，张亮，李晶，王进进，胡凌	银奖
33	UAV安防——基于图像识别的无人机检测系统	唐榕蔚	主赛道	本科生创意组	金樱，杨镘闻，周锐，黄俞婷，岳廷沁，孟云浩，于昕阳，李东骏，邓佳宁，师振昆，郭正阳，李雪静	江倩	银奖

云南省第八届“互联网+”大学生创新创业大赛获奖名单（省级）

序号	项目名称	负责人	赛道	组别	团队成员	指导老师	奖项
34	“弃而不舍，变废为宝“——尾菜资源化处理系统	刘馨	主赛道	本科生创意组	王馨若，邓洁，郑申奥，冯远衡，杨旭，娄晏如，翟羽佳，高瑜蔓，何亚琳	朱科铭，孟伟东	银奖
35	日和安-定制中国节日饰品的原创工作室	王欣彤	主赛道	本科生创意组	王姝懿，王楚沅，黄欣欣，滕彩凤，王鹏绮，王娅婷，朱子晴，秦诗雯，马晓婧，程国涵，马晓蓓，王识羽，范家豪，陆飞	黄天慧，蒋颖荣，凌霄	银奖
36	尚品食品——高端健康发酵肉行业引领者	张悦	主赛道	本科生创意组	支亚若，王馨柔，罗海燕，王丽媛，赵芯蕊，朱晨婧，胡秀春，喻薇，曾内勇	王兴红，杨凡	银奖
37	萍花物语——利用新型浮岛技术推动云上高原湖泊生态修复与经济发展双轨共进	刘西悦	主赛道	本科生创意组	孙婷，李云昊，刘芳缘，侯悦，李克雪，张羿菲，周杰，廖美欣，姬梅梓，孙佳楠，李洁	常学秀，潘晓斌，徐润冰	银奖
38	心有“灵析”——便携式心脏病监测系统	郑慧洁	主赛道	本科生创意组	王颖，付屹雯，孔基帆，殷龙祥，唐庆勇，刘佳，魏梅琼，宋奇龙，郭琪，刘欢，秦耕，宋佳轩，刘子怡	何敏，王威廉，邓伟升	银奖
39	"绿源之滇"——云南碳达峰碳中和实施者项目组	郑智远	主赛道	本科生创意组	祝延浩，郭宇婷，赵梓言，李会改，齐悦颐，周一诺，李星泽，汪旭，魏进兰，谢瑞瑞，高雨阳	张禹，朱娟萍，李振东	银奖
40	伤口自愈——多领域微生物自修复混凝土行业引领者	濮成侣	主赛道	本科生创意组	李海霞，罗丽姣，罗瑞，聂子雯，王逸凡，张孜妍，王林峰，赵兴璐，靳晗，陈俏伊，钟莹银，韦洁婷	雷真，李宗菊	银奖
41	“乳”此爱妳--乳腺癌早筛智能掌上超声领航者	熊丽	主赛道	研究生创意组	黄凯，王致诚，姚瑞晗，朱婧颖，谭浩，雷建，潘灵蕊，唐红，梁洲源，姚鹏，刘旭珩，潘威霖，袁梦，杨健会	张榆锋，何冰冰	银奖
42	"从肠计议"——UC智能评估专家	刘燕红	主赛道	研究生创意组	罗旭东，李博，王先旺，周奇浩，杨蕊绮，邹芷欣，李诗楠，张鑫，王肖，陈萍，白雪松，康倩，叶艳，赵阳	张俊华	银奖
43	AI安全帐篷-中国户外露营安全引领者	刘博	主赛道	研究生创意组	潘佳艺，彭思薇，郭竞，杨成荣，熊维东，南峰涛，吕梦凡，黎世锋，杜飞，王佳舜	杨云	银奖
44	南云科技：打造辐射南亚东南亚的AI视觉算法开放平台	张榕倩	主赛道	研究生创意组	肖一鸣，王子睿，潘佳艺，邓彪，包聆言，杨昭杰，罗佳，张贤琪，包慧琪，鲁丽萍，张菊，张集力，赵世豪，张琴	赵明雄	银奖
45	仁迹文化——校园双创综合服务平台先驱者	李春仁	高教主赛道	本科生成长组	韦鳗珂，彭艺娇，王姝霏，张庆辉，姚雪珂，王彩兰，胡文杰，陈洁筠，周洁，周依典，洪朗，杜匡辰，张瑞	马春，杨云川，张玉	银奖
46	易检——基于视觉识别的全自动抗原检测系统	杨谨瑞	高教主赛道	本科生创意组	张丹阳，黄传翔，郝梓帆，王曦，周谱，王思扬，张敏萱，赵紫薇，宋明远，顾耘华，马兴摇，敖志浩	谢和均，许若群，张鹏杨	银奖
47	小德—AI新冠医疗废弃物分类机器人	黄春华	高教主赛道	本科生创意组	龚伟，何杰奥，刘恒贝，郭正阳，廖美欣，黄纯熙，闫浩，侯凯夫，马宏伟，林国修，崔子韫，沈跃	丁洪伟，何鸣皋，黎玉洁	银奖
48	蚁穴系统——依托无人机的道路交通安全隐患识别系统	葛雪晴	高教主赛道	本科生创意组	黄俊铭，周帅宇，刘云鹏，林文浩，常启航，傅佳怡，鲁洪，何海欣，王佳怡，韩伊敏，禹玺，刘珊，许祝愿，蒋瀚谊	赵明雄，陈清毅，张云春	银奖
49	云裳之湄	刀欣	高教主赛道	本科生创意组	何腊仙，宋晨熙，张万海，武倩，祁王斌，蒋玛阳，周映辰，朱湘江，李俊筱	庞俊彩	银奖
50	移动心医——先心病相关肺高压辅助诊断开拓者	杨炫锴	高教主赛道	研究生创意组	王原林，沈伊，张伟，沈城标，王昕，王春宇，彭鸿坤，谭浩，李瑾怡，张艺旖，周姣，李国正	孙静，池宗琳，普园媛，姚建文，谢汝生	银奖
51	葡愈—葡萄病害有机菌剂研发者	桂仟	“青年红色筑梦之旅”赛道	创意组	储梦娴，徐安琪，韦亚含，蔡少鸿，杨文恬，郭雨蒙，王煜涵，代涛，李欣洁，杨金燕，李若婧，何昱，罗婧	杨明挚，周观琪	银奖
52	云岭天蕉——香蕉枯萎病综合防控技术提供者	周光东	“青年红色筑梦之旅”赛道	创意组	邵凯，王欣彤，蔡婉华，于沁，关晓菲，王茂森，朱酉正捷，周昀璠，高瑜蔓，田利波，姜润震，韦仁星，王培辉，杨鼎	郑泗军，黄斌全，李迅东	银奖

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序号	项目名称	负责人	赛道	组别	团队成员	指导老师	奖项
53	防诈于未然	杨京虎	“青年红色筑梦之旅”赛道	创意组	彭思薇，陈兴通，游苏娟，邹星竹，丁雨洁，陈首冠，高阳，徐建华，周韬，韩炳萱，王利峰，杜俊笙，何明龙，李忠斌	岳昆，段亮	银奖
54	红韵兰乡——打造附生兰产业致富新模式	周梦娇	“青年红色筑梦之旅”赛道	创意组	王睿彬，周谱，陈珍涓，李倩倩，张宝月，梁应冲，李泽宇，钱清清，赵茹卿，吴志伟，杨强雪，崔昊，陆文秀，董净玉	沈聪燕，王娟，赵大克，牟宗敏	银奖
55	一杯佳酿，会泽百家——盐水石榴酒振兴钱王之乡	陈东琴	“青年红色筑梦之旅”赛道	创意组	范瑞雯，杨康，桂贤定，李晓芳，陈双艳，陈坤，谢瑞瑞，邢晋嘉	王文兵，李秀，杨明挚	银奖
56	Mocap动作管家——基于动作捕获技术的民族舞蹈资源数字化整合平台	柳雁安	“青年红色筑梦之旅”赛道	创意组	杨英琪，杨涵，韩啸，尹文霞，张佳琪，秦茂桐，刘晓阳，袁业瑾，宋倩云，罗月英，孙守政，熊婴宇，徐启豪，张浩	徐丹，贺康建，锁箭	银奖
57	农废不再愁——基于农废再利用的太阳能净水发电构建精准培育智慧大棚	赵嵘	“青年红色筑梦之旅”赛道	创意组	赵春波，王秀君，张景浩，金央央，武明薇，骆江俞，耿学敏，王春阳，牟泓雨，李文君，佟金丽，袁长鑫	万艳芬，张艮林，李豪杰，杨鹏	银奖
58	守护稻源——云南野生稻资源保护平台构建	王淑雅	“青年红色筑梦之旅”赛道	公益组	薛珊，杜雨濛，熊子璇，王洪敏，管娟，雷凌云，杨得正，欧雯	程在全，刘丽，秦世雯	银奖
59	防微杜疫——基层智慧防疫的引领者	吴鑫然	“青年红色筑梦之旅”赛道	公益组	逯桐，祝徐，王利峰，陈祥，杨思怡，陈首冠，李精明，姚健，徐官杰，李圆媛，刘华帅，李忠斌，姜博厚，江嘉伟	岳昆，祁志卫，段亮	银奖
60	污水处理-资源回收利用解决方案	华文婷	产业命题赛道	国赛命题组	张睿朴，陈雨菲，武文斌，吴昊天，吴紫微，张峻菲，曾皓，鲁婷，刘天立，周子钦，李若然，韦修炜	汤立红，宁致远	银奖
61	新智慧城市内涝预警系统	杨键	产业命题赛道	国赛命题组	吕志钦，李佳凝，刘子愉，姜刚涛，蒋群利，周文瑾，王艺璇，陶宇彬，许心怡，白雨珊，吕佳钰，郑舒心	袁娥，姚建文，罗美娟，梁红，刘晓倩，马凡松，武鹏	银奖
62	基于华为云IoT开发创新应用	黄春华	产业命题赛道	国赛命题组	何杰奥，马宏伟，马思琦	何鸣皋，黎玉洁	银奖
63	云养宠--治愈时代的爱宠陪伴新模式	蔡玮	高教主赛道	本科生创意组	文湘，兰天，李晓芳，冯小操，罗淋，范伊兰，张艳玲，李欣芮	余虹，孔莉	铜奖
64	共工云控——让您放心的AI消防安全管家	李楚怿祯	高教主赛道	本科生创意组	肖志杰，林焕咏，赵梦婷，李梦瑶，王雨曦，叶婉莹，陈敏，蒋钰婷，朱文华，耿皓天，黄贺，张艳玲，曾智	何鸣皋，聂仁灿	铜奖
65	矢量职业——一款真实体验职业的模拟游戏	何昀潞	高教主赛道	本科生创意组	韦洁婷，杨逸飞，田真，袁绪卓，依沙叫，罗钦月，庞鑫慧，郭正阳，王华欣，李志伟，孟涛，李杨，陈芳	王旭，王亚丽	铜奖
66	凤翎栖——专注于云南珍稀鸟类文创设计	朱湘江	高教主赛道	本科生创意组	刘芳缘，曹玉薇，喻潇，范黎萱，匡季莹，李杭峰，李忠华，王巡，王俊兵，刀欣，黄钰雯	邓兵	铜奖
67	地涌金莲——地莲花种植技术推广	李婧娴	高教主赛道	本科生创意组	胡英伟，李思棋，郭宥麟，宋晓茜，张兴缘，常金昊，要春晖，李函羲，牛晓琳	谢志昆	铜奖
68	潮流不含“毒”——云南野生菌相关潮玩手办的开发	莫星宏	高教主赛道	本科生创意组	施雨涵，张文，王雪，吴宝萍，谢玉香，王逸凡，胡湘袖，肖伦菲，赵秋宇，李俊筱，张文欣，白夏楠	盛春宇，黄光能，李堂英	铜奖
69	颐家智助——世界首款居家新冠抗原检测和照护机器人	李明康	高教主赛道	本科生创意组	刘社仪，杨达坤，曾文，杨莉，杨皓，林伟田，吴心月，白沂鑫，黄煦，吴熙，朱朝冉，韩菲儿	何鸣皋，丁海燕	铜奖
70	傣相陶——非遗视角下傣陶传承发展与设计创新	涂旭	高教主赛道	本科生创意组	冯佳琪，渠雪鑫，赵洁，莫星宏，费菲，饶婉钦，郭金，张博傑，王婉格，吕春保，傅树铭，何柱成，杜兵，赵茹卿	盛春宇，史陇鹏	铜奖

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序号	项目名称	负责人	赛道	组别	团队成员	指导老师	奖项
71	构库Web3D—改变传统工科教育的三维模型Web引擎	戴凡皓	高教主赛道	本科生创意组	李昊霞，代瑞，王颖，宋敏行，刘文翠，吴婷玉，刘鹏辉，林豪健，李函羲，杨雨荷	刘杨	铜奖
72	云游石林——三色可餐的乐享生活	刘欢	高教主赛道	本科生创意组	郑慧洁，张睿薪，孙卓航，孙萌谦，宋雨乔，蔡双琳，韩菲儿，王巡，周雅文琪，蔡挺，付树豪，张瑾，普怡瑶	王克岭，倪向丽，杨梦诗，吴奇志	铜奖
73	基于绿化废弃物与生活污泥共堆肥零碳技术推广	唐彬	高教主赛道	本科生创意组	花悦珊，黄文涛，张非凡，李安宁，关晓菲，成立雯，高瑜蔓，胡诚皓，马斯腾，胡明达，赵淑婕，徐安昆	李世玉，王新菊，段连丽	铜奖
74	洞穴精灵——云南特有金线鲃的人工扩繁与高端鱼业的提供者	喻厚泽	高教主赛道	本科生创意组	杨思丹，郁辰潇，刘雨薇，王欣怡，李增智，柏金彪，张晓辉	杨舒然，李春青	铜奖
75	光舞时空--突破飞秒时域技术封锁打造三场调控集成仪器	张皓霖	高教主赛道	本科生创意组	张逸龙，迟思棋，顾茂绮，金博文，李欣悦，杨耘赫，张国奎，张路思，蒋志曜	任杨，孟伟东	铜奖
76	云醉南谣——利用区块链NFT技术保护云南少数民族非物质文化遗产	纳唯源	高教主赛道	本科生创意组	陈俊蓉，陈佳林，成运，罗富琳，罗晓周，李海梅，战雅静，徐婧柯，肖伦菲，张晓薇，杨亚茹，李昂，龚泰霖，徐惠玲	李娟，赵征鹏	铜奖
77	允臻智能——国内首家打造“无废城市”的餐厨垃圾处理器	李佳凝	高教主赛道	本科生创意组	戴牧言，白雨珊，许心怡，李欣璇，李芳霞，刘西悦，康政，黄炳豪，史芸欣，芦宇，刘恒贝，王丽媛	何曼榕，张春红	铜奖
78	慧眼-基于深度学习的人脸真伪识别系统	王千年	高教主赛道	本科生创意组	李丽娜，刘昕蕊，付昌陇，杨镘闻，吴涛，吴娅苏，唐榕蔚，凌洁，陈梦芸，车钰英，周映辰，吕伟杰，黄俞婷，金樱	江倩	铜奖
79	森林奇境—基于COP15宣传“共建地球生命共同体”理念的系列盲盒	袁辰	高教主赛道	本科生创意组	米婷婷，刘若瑜，李泽慧，王雪，杨欣睿，李汶倩，王清莹，黎启美，何福伟，池华怡，周星可，岭思琪，王霄云，李恒佳艺	蒋颖荣，刘玉鹏，杨惟钦	铜奖
80	蘑菇力量——基于生物质碳催化剂的超高能量密度金属空气电池	李忠华	高教主赛道	本科生创意组	高展鹏，赵新雨，李杰，严江莲，曹峻铭，黄才权，穆童，陈梦鑫，李奥，孙铭泽，雷鑫林，毕皓怡	张龙舟，赵云刚	铜奖
81	云墨-国内首款骨胶无苯酚环保健康墨	武文斌	高教主赛道	研究生初创组	马溶，张峻菲，亢文彤，胡琛，墨帅聪，曹喆奕，成立雯，艾贤思琪，陈静蕾，华文婷，时欣，王悦，辛恺，邓仙红	何曼榕，汤立红	铜奖
82	InGaAs/InP单光子探测器	刘辰	高教主赛道	研究生创意组	赵伟林，李云雪，马旭，叶海峰，刘泽林，李再波，杜夏露	史衍丽，孟伟东	铜奖
83	“湖泊革命”——全流域长效化治理开拓者	罗薇	高教主赛道	研究生创意组	徐文杰，徐瑞骋，康小霖，袁嘉辉，贺星耀，吴凯香，赵奕，徐官杰，金学林，武佩瑶	梁虹	铜奖
84	欧诺德——癌症精准治疗药物筛选专家	吴春玥	高教主赛道	研究生创意组	李璐，王霄文，张树地，胡丁言，皮洋，张强荐，孙晓彤，蒙雅淼，马司颖，魏妮斯，何峻伟，杜夏露	郭春明，陈茂荣，余星云，羊晓东，余鹏飞	铜奖
85	能源管理云平台助力双碳行动的智慧能源管理者	崔秀秀	高教主赛道	研究生创意组	姚波，冉言，张璐，李思璋，王致远	张俊，王毓德，钱伟	铜奖
86	“云检”试纸—绿色食品与环境安全的守护者	赖亭润	高教主赛道	研究生创意组	任杰，姚波，舒慧，金标剑，赵荣俊，黄艳梅，施航，张煜佩，李文君	王毓德，陈善元	铜奖

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87	焕肤消灼灵——烧伤烫伤了无痕，重塑自我焕新生	姚琳琳	高教主赛道	研究生创意组	刘佳琦，杨锐铛，杨于军，梁晶晶	蔡乐	铜奖
88	i克松—干细胞&外泌体生产转化工厂	余俊旭	高教主赛道	研究生创意组	杨丽黎，李珊珊，胡海燕，杨璐涵，严亚萍，何用娟，余瑞	郑冰蓉，侯宗柳，孟明耀	铜奖
89	气体安全云卫士——基于气敏传感材料的安全健康监测平台	宋振林	高教主赛道	研究生创意组	吕天平，赵建红，段良飞，卢清杰，字包叶，WeiGuan，曾吉阳，陈丽敏，徐东，陈丰颖，高成凤，王晓朦	柳清菊	铜奖
90	刻骨铭心——AI人体骨骼三维重建开拓者	李博	高教主赛道	研究生创意组	赵阳、杨蕊绮、李诗楠、普钟、罗旭东、刘燕红、王骞、凌威、罗晋、王思威、王晓燕	张俊华	铜奖
91	"鉴骨识疾"——脊柱侧凸智能辅助诊断行业领跑者	赵阳	高教主赛道	研究生创意组	李博，杨蕊绮，张鑫，黄昆，周奇浩，罗旭东，李诗楠，禄瑶，罗晋，普钟，谭露露，肖培，刘燕红，凌威	张俊华	铜奖
92	蛙图研学机构	魏环	高教主赛道	研究生创意组	王蒙，冯帆，王一涵，莫绮婷，王晖杨，李权，黄龙城	赵凌云，盛春宇	铜奖
93	织兰予树——真菌技术打造兰科花园	马婉秋	高教主赛道	研究生创意组	丁允宇，黄丹，孙云宏，袁子涵，沈跃，周克，任淑君，肖桔炜，许萱，武雅晶，张星驰，王一菡	赵大克，牟宗敏，沈聪燕，王娟	铜奖
94	红”篇“剧”制——实景高沉浸式剧本杀助力红色旅游发展	邓焱	“青年红色筑梦之旅”赛道	创意组	王紫茹，孙宁远，淦睿杰，张豫，刘龙泉，李艾仪，陈雪，张俊康，杨月婧，那琳，荀福玉，胡少伟	杨莉红，徐琰超，光映炯，朱丹	铜奖
95	优助——云帮扶平台（以会泽新城为试点）	黄钰雯	“青年红色筑梦之旅”赛道	创意组	范家豪，郭宇婷，赵子怡，赵倩，杨光祥，廖欣悦，马思佳，佘国杰，王胜钒，朱湘江，吴顺	赵毅达，光映炯，朱艳萍	铜奖
96	芳年华月——滇越铁路上的网“红”乡村缔造者	和心怡	“青年红色筑梦之旅”赛道	创意组	张雅雯，陈钰，谭露露，王诗莹，郭宇桐，李嘉奇，耿子怡，林豪健，杨芮姗	撒莹，王玲，马春	铜奖
97	云染绣——留住人·传下去，让非遗研学助力乡村振兴	张颖	“青年红色筑梦之旅”赛道	创意组	周梦娇，刘宸玮，邵红粉，李祖辉，邓蠢，杨婷，陈新德，杜兵，何月，韩菲儿，周晨雨，潘子珩	邓永进，王筱春，沈聪燕	铜奖
98	菌鉴——基于AI的野生菌识别先行者	彭婷	“青年红色筑梦之旅”赛道	创意组	张曼琪，王艺璇，邓亚萍，于也，马荣璘，艾贤思琪，李佳凝，贾芸菁，刘敏，栗瑶瑶，车钰英，张闻迪	余鹏飞，邓崧，普园媛	铜奖
99	艺久弥新——数字智能文物修复与展示的倡导者	熊婴宇	“青年红色筑梦之旅”赛道	创意组	宋倩云，杨涵，宋墨累，李毅，柳雁安，韩啸，李承洲，秦茂桐，秦家成，王翊豪，赵丽媛，张浩，陈牧秋，周义巧	徐丹，锁箭，贺康建	铜奖
100	智仓优选——打造云南孟连牛油果供应链新模式	李函羲	“青年红色筑梦之旅”赛道	创意组	王一茳，董苡菡，李婧娴，谭天好，谭淳丰，栗瑶瑶，要春晖，戴凡皓	宋乐	铜奖
101	盘溪早桔：云南高原湖盆地区乡村振兴路上最红的果	周露	“青年红色筑梦之旅”赛道	公益组	张树竹，王彬彬，段圆媛，邓紫煜，马敏，林虎，陈首冠，周烨，杨迪闲，张琪，刀晨峰	杨志玲，孔莉，胡凤益，姚绍文，欧晓昆	铜奖
102	区块链技术应用	王基书	产业命题赛道	国赛命题组	苗晨，吕声隆，娄建辉，熊维东，黄河祥，张玉朱，石琴琴，韩瑞文	张璇，朱锐，王旭	铜奖
103	数字经济下民间非遗传统文化传承和典藏数字营销模式设计	蒋艳蓉	产业命题赛道	国赛命题组	彭璐璐，赵裕民，韩静，张雨，宋海贤，Shanshan He	马翀炜	铜奖
104	AI+智慧养老——人工智能赋能养老服务的场景应用创新	邱笛	产业命题赛道	国赛命题组	赵仁瑨，李润思，马乐融，李洁，白林鹭	李娅，马子红	铜奖
105	渐进式可视化编程	李竿影	产业命题赛道	国赛命题组	郑舒予，艾祖康，谢珮舒	杨孟禹	铜奖

证 书 号 第 16635673 号

实用新型专利证书

实用新型名称：一种用于液氮磨样的可拆解防冻手套

发　　明　　人：王蕊嘉;李缘;吴学尉;王天喜;张利娜;谢雨琪;张超超

专　　利　　号：ZL 2022 2 0160551.4

专利申请日：2022 年 01 月 21 日

专 利 权 人：云南大学

地　　址：650500 云南省昆明市呈贡区大学城东外环南路

授权公告日：2022 年 05 月 31 日　　授权公告号：CN 216627588 U

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发明人：

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证 书 号 第 14994545 号

实用新型专利证书

实用新型名称：一种适宜魔芋种芋催芽的装置

发　　明　　人：王蕊嘉;吴学尉;张利娜;王天喜;吴昊林;刘海英;李永萍
吴治成;蒋春竹

专　　利　　号：ZL 2021 2 1338251.2

专利申请日：2021 年 06 月 16 日

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王蕊嘉;吴学尉;张利娜;王天喜;吴昊林;刘海英;李永萍;吴治成;蒋春竹

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PCT

国际申请号和国际申请日通知书

(PCT 细则 20.2(c))

发文日（日/月/年）
17.11 月 2020(17.11.2020)

申请人或代理人档案号	重 要 通 知
FYPCT2020101	

国际申请号	国际申请日（日/月/年）	优先权日（日/月/年）
PCT/CN2020/123602	26.10 月 2020(26.10.2020)	04.1 月 2020(04.01.2020)

申请人

云南大学 等

发明名称

一种生态智能室内立体植物种植墙及其培育方法

1. 通知申请人，对国际申请给出上述国际申请号和国际申请日。
2. 通知申请人，国际申请的登记本：

☒ 已于 17.11 月 2020(17.11.2020) 传送给国际局。

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PCT 申请确认表(需委托方盖章确认)

案号：FYPCT2020101　　顾问：高 双

委托项目	■ PCT 申请（国际阶段） □ PCT 申请（国家阶段）（如为该项，请在下面填写国家/地区名称） 国家/地区：_____
专利类型	☑ 发明 □ 实用新型
申请人	名称/姓名（中英）： 云南大学、昆明绿岛园艺有限公司 Yunnan University、Kunming lvdao gardening co., ltd
	组织机构代码证号或统一社会信用代码或身份证号： 125300004312032649、915301123230545729
	地址（中英）：云南省昆明市呈贡新区呈贡大学城云南大学 Yunnan university, chenggong university city, chenggong new district, kunming, yunnan province 邮编：650000
发明人	姓名（中英文对照）吴学尉、王丽花、刘海英、李永萍、何飞飞、刘丹丹、张达、李莲莲、葛继东 Wu xuewei, Wang lihua, Liu haiying, Li yongping, He feifei, Liu dandan, Zhang da, Li lianlian, Ge jidong 地址（中英文对照）云南省昆明市呈贡新区呈贡大学城云南大学 Yunnan university, chenggong university city, chenggong new district, kunming, yunnan province 邮编：650000
	第一发明人身份证号：440106197709121879
发明名称	**一种生态智能室内立体植物种植墙及其培育方法**
PCT 信息	**国际申请日：** **国际申请号：** **国际公布号：** **国际公布语言：** **国际公布日：**
特殊要求	□ 同时提实质审查请求 □ 提前公开 □ 费用减缓 □ 不丧失新颖性公开 □其他 （请注明） □同时提 PPH
优先权	优先权号： 2020100070207 优先权日：2020-01-04 原受理机构： 云南凌云律师事务所 绝限期：2021-01-04
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记笔记

一种菌株及其在枯萎病防治中的应用

专利类型： 发明公开

申请(专利)号： CN201911168789.0 **申请日：** 2019-11-25

申请公布号： CN111172056A **公开公告日：** 2020-05-19

申请人： 云南大学

地址： 650000 云南省昆明市翠湖北路2号

发明人： 秦世雯; 胡凤益; 黄立钰; 王振中; 王松

分类号： C12N1/20;A01N63/00;A01N63/22;A01P3/00;C12R1/125

主分类号： C12N1/20 **国省代码：** 53

页数： 15

代理机构： 成都市鼎宏恒业知识产权代理事务所(特殊普通合伙) **代理人：** 谢敏

主权项：

1.一种菌株,其特征在于,具有如下所示的核苷酸序列中任意一项：Ⅰ、具有SEQ ID NO.1所示的核苷酸序列；Ⅱ、具有SEQ ID NO.1所示的核苷酸序列经修饰、取代、缺失或添加一个或多个碱基获得的核苷酸序列。

摘要：

本发明公开了一种菌株及其在枯萎病防治中的应用,该菌株具有如下所示的核苷酸序列中任意一项：Ⅰ、具有SEQ ID NO.1所示的核苷酸序列；Ⅱ、具有SEQ ID NO.1所示的核苷酸序列经修饰、取代、缺失或添加一个或多个碱基获得的核苷酸序列。该菌株对香蕉、瓜类和茄科植物枯萎病菌均具有拮抗效果。

查看法律状态

法律状态公告日	法律状态	法律状态信息
2020-05-19	公开	公开
2020-06-12	实质审查的生效	实质审查的生效 IPC(主分类):C12N1/20

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相似专利

[1] 一种拮抗枯萎病的芽孢杆菌及其应用[P]. 朱育菁,郑梅霞,陈峥,刘波,许炼,潘志针,肖荣凤,邓文琼,李慧敏. 中国专利:CN110964654A, 2020-04-07

[2] 一种抑制枯萎病的香蕉茎叶残体腐熟菌剂及其制备方法[P]. 蒋常德,胡艳晖. 中国专利:CN106244491A, 2016-12-21

[3] 一株马来西亚链霉菌及其应用[P]. 邓晓,李勤奋,武春媛,李怡,刘景坤. 中国专利:CN105349455A, 2016-02-24

本领域科技成果与标准

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[1] 黄瓜枯萎病拮抗菌的筛选鉴定及抗菌物质研究[Z]. 张雪辉,唐蕊,孟春燕,胡永利,牛伟涛,石晓云,王僧虎. 国家科技成果.

[2] 植物青枯病与枯萎病分子协同控制技术[Z]. 国家科技成果.

研究与应用

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实用新型名称：普洱茶快速陈化仓储室

发　　明　　人：刘丹丹;时鸿迪

专　　利　　号：ZL 2019 2 1444990.2

专利申请日：2019 年 09 月 02 日

专 利 权 人：云南大学

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实用新型专利证书

实用新型名称：普洱茶快速发酵设备

发　明　人：刘丹丹;时鸿迪

专　利　号：ZL 2019 2 1445006.4

专利申请日：2019年09月02日

专利权人：云南大学

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Plant Signaling & Behavior

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/kpsb20

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To cite this article: Hong-Di Shi, Wan-Qiao Zhang, Hong-Ye Lu, Wen-Qian Zhang, Hui Ye & Dan-Dan Liu (2020) Functional characterization of a starch synthesis-related gene *AmAGP* in *Amorphophallus muelleri*, Plant Signaling & Behavior, 15:11, 1805903, DOI: 10.1080/15592324.2020.1805903

To link to this article: https://doi.org/10.1080/15592324.2020.1805903

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PLANT SIGNALING & BEHAVIOR
2020, VOL. 15, NO. 11, e1805903 (8 pages)
https://doi.org/10.1080/15592324.2020.1805903

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RESEARCH PAPER

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Functional characterization of a starch synthesis-related gene *AmAGP* in *Amorphophallus muelleri*

Hong-Di Shi[a*], Wan-Qiao Zhang[a*], Hong-Ye Lu[b], Wen-Qian Zhang[c], Hui Ye[a], and Dan-Dan Liu[a]

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ABSTRACT

Amorphophallus has attracted tremendous interest because of its high contents of glucomannan and starch. Very few genes regulating glucomannan and starch were reported in *Amorphophallus*. In this study, an ADP-glucose pyrophosphorylase (*AGP*) gene that plays a significant role in plant starch synthesis was cloned from *Amorphophallus muelleri*. It was shown that it encoded a predicted protein containing a conserved plant ADP-Glucose-PP repeat domain and seven potential ligand-binding sites. The real-time quantitative PCR showed that *AmAGP* was most abundant in tubers, and it was positively correlated with starch content. Additionally, its influencers about temperature and exogenous plant hormone were also discussed, showing that *AmAGP* expressed highly in tubers under treatments using 25°C and IAA. Furthermore, starch content was closely related to *AmAGP* expression level, suggesting that *AmAGP* was involved in the regulation of starch synthesis in *A. muelleri*. Therefore, identifying the sequence of *AmAGP* and its expression pattern during tuber enlarging and the changes of its transcript levels in response to temperature and plant hormones would contribute to a better understanding of starch synthesis, and also providing a reference information for future preferable breeding for obtaining more starch or more glucomannan in *Amorphophallus*.

ARTICLE HISTORY
Received 11 June 2020
Revised 23 July 2020
Accepted 26 July 2020

KEYWORDS
Amorphophallus muelleri; starch; ADP-glucose pyrophosphorylase; temperature; plant hormone

1. Introduction

Amorphophallus is a perennial, herbaceous monocot that is mainly distributed in countries of tropical and subtropical Asia, including China, Japan, Myanmar, Vietnam, and Indonesia. At present, more than 170 species of *Amorphophallus* have been identified, and 21 species have been discovered and named in China, and nine of these are endemic to China.[1] *Amorphophallus* tuber is edible, which is rich in konjac glucomannan (KGM) and starch. KGM is a kind of dietary fiber, which has a beneficial effect on human health. Because of its non-toxicity, biocompatibility, biodegradability, and hydrophilicity, KGM has attracted people's attention.[2] At present, *Amorphophallus* is the only plant that can produce large amounts of KGM. In addition to KGM, starch is seemed as the major caloric source in a variety of human diets and is also a major nutrient in *Amorphophallus*.[3] *A. muelleri* is a special *Amorphophallus* species, its leaves displayed living bulbils on the proximal petiole. Because of its high disease resistance and its apomictic characterization, it has a strong competitive advantage and a very high economic value.[2] Studies have shown that *A. muelleri* starch can moderate the rate of digestion of foods, and can also reduce glycemic response and insulin resistance in patients with diabetes via influencing the controlled glucose release profiles.[4]

Starch, an important storage substance produced by photosynthesis in most higher plants, is synthesized inside the plastid stroma within plant cells.[5] There have been many studies on *Amorphophallus* glucomannan at home and abroad, but fewer have focused specifically on *Amorphophallus* starch,[6] especially in its biosynthesis. Biosynthesis of starch is at least regulated by four classes of enzymes, including ADP-glucose pyrophosphorylase (AGPase, EC 2.7.7.27), Starch Synthase (SS, EC 2.4.1.21), Starch branching Enzyme (BE, EC 2.4.1.18), and Starch Debranching Enzyme (DBE, EC3.2.1.68).[7,8] Each of them plays a different role in starch synthesis. AGPase is the first key regulatory and rate-limiting enzyme for starch biosynthesis and has a high degree of control over starch biosynthesis.[9,10] In plants, it is a heterotetramer that composed of two pairs of large and small subunits.[11,12] The small subunit has both catalytic and regulatory functions, it was found in the leaf and contributes to increase plant biomass,[13,14] and the large subunit has largely a regulatory function,[15] it was found in the seed endosperm plays an important role in determining grain yield in cereals.[13,16,17] The small subunit includes the cytosolic small subunit (SSUI) and the plastidial small subunit (SSUII), the large subunit includes the cytosolic large subunit (LSUI) and the plastidial large subunit (LSUII).[18] Genes encoding SSUII, SSUII, LSUI, and LSUII have been characterized at the genome level across many plant species such as *Arabidopsis*,[19] rice,[20,21] sweet potato,[22] banana,[23] potato,[24] wheat,[25] maize,[26] and cassava.[27] AGPase genes are closely associated with starch synthesis in plant tissues. For example, in the *OsAPL3*-defective mutants of rice, starch content was

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reduced with the decreased AGPase activity in leaves.[28] Additionally, it was also regulated by environmental factors. In potato, the expression of AGPase genes (*StAGP*) was regulated by low temperature;[29] the expression of tomato *AGPL1* and *AGPS1* was up-regulated in salt-stressed developing fruits; the expression of two banana AGPase genes was induced by cold, salt, and drought;[23] starch accumulation in developing rice grains was positively correlated with an increase in the AGPase and SS activity under drought.[30] Additionally, phosphorylation is also found to regulate starch synthesis by controlling carbon flux into starch while simultaneously modulating expression of genes involved in starch synthesis. Over the past century, starch phosphorylation has aroused increasing interest as the only naturally occurring covalent modification in starch.[31] It occurs during starch synthesis, although the rates of phosphorylation are likely to be different.[32] The starch levels are in a continual state of flux during plant development; thus, starch accumulation is determined by the regulation of both synthetic and degradative enzymes.[33] Research in the past decade has uncovered new and surprising information about the pathways of starch synthesis and degradation. Starch degradation in chloroplasts requires β-amylase (BAM) activity, but in *Arabidopsis*, there are nine BAM proteins, five of which are thought to be catalytic.[34] These discoveries also uncover complexities of starch synthesis mechanism: results from model plants (such as *Arabidopsis*) are not necessarily applicable to other plant species or to growth in natural.

The genome size of *Amorphophallus* is quite large, and it is, therefore, challenging to sequence the entire genome of *Amorphophallus* species. So far, due to limitations in genomic data, fewer studies have screened for suitable functional genes in *Amorphophallus*. Recently, transcriptome data were obtained from *A. muelleri*, and most of the significant genes in the sphingolipid metabolic pathway were identified.[35] In the present study, *AmAGP* gene was isolated because of its important role in starch synthesis, and its expression patterns were explored during tuber expansion in *A. muelleri*. Additionally, the function of *AmAGP* was further characterized by examining its response to temperatures and hormones that are favorable for starch accumulation. Taken collectively, considering the relationship between starch and KGM, the assessment of *AmAGP* function provides a theoretical basis for the identification of starch- and glucomannan-related regulatory networks, and also a support for directional breeding in agricultural production.

2. Materials and methods

2.1. Plant materials and growth conditions

The test materials were *A. muelleri* which were grown in orchard in Yunnan University, Yunnan Province, China. *A. muelleri* is a special *Amorphophallus* species and widely planted in Yunnan and Sichuan Province, China.

2.2. Asexual seed evaluation of A. muelleri

To examine the formation of asexual seeds, the spathe was cut off before flower buds had opened to expose pistils to the air for pollination. The stigma was removed with a sharp blade and covered with a paper bag. Compared with the total number of decapitated flowers, the number of parthenogenetic fruits that produced seeds in the absence of fertilization is calculated as a percentage of asexual seeds.

2.3. Cloning and homology analysis of the AmAGP gene

For cloning *AmAGP* gene in *A. muelleri*, total RNA of tubers was isolated using Trizol (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. First-strand cDNA was synthesized using the M-MLV system and used as a template for RT-PCR. Referring to the conserved sequences of ADP-glucose pyrophosphorylase genes from *Amorphophallus konjac* and *Amorphophallus albus*, the forward primer 5′-ATGGATGCCAGCAGTATGATGGT-3′ and reverse primer 5′-GTATGATGGTCACAAGCTTCGGT-3′ were designed and synthesized by the Beijing Genomics Institute (BGI), the sequences were download from NCBI and Gene number were JF727266.1 and AF316326.1, respectively. PCR conditions were as follows: 3 min at 94°C (one cycle), followed by 30 s at 94°C, 30 s at 55.5°C, and 45 s at 72°C (30 cycles) for 25 μL reaction buffer. The PCR products were run on a 1.0% (m/v) agarose gel and detected using an image analyzer (white/UV transilluminator), and the desired band was recovered and ligated to the pMD18-T cloning vector for sequencing.

For homology analysis of *AmAGP* gene. The sequence of the amplified *AmAGP* gene was used to identify its ORF by Edit sequence software, and then the nucleotide sequence was translated into an amino acid sequence by DNAMAN software. Conserved domain analysis was also used DNAMAN software. Nucleotide and amino acid sequence similarity alignments were performed by Blast in NCBI (http://blast.ncbi.nlm.nih.gov/). The ProtParam protein analysis tool (http://web.expasy.org/protparam/) was used to analyze the molecular weight, theoretical isoelectric point, and other protein properties.

2.4. Phylogenetic tree construction

Firstly, BLAST was performed on the AmAGP protein sequence, and then 17 AGP protein sequences were downloaded from NCBI (http://ncbi.nlm.nih.gov). A phylogenetic tree was constructed in the MEGA6 program using the neighbor-joining method with the 'pairwise deletion' option, the 'Poisson correction' model, and 1000 bootstrap replicates.[36]

2.5. Real-time quantitative PCR (qRT-PCR) and semi-quantitative PCR (sqRT-PCR) analysis

Both total RNA and first-strand cDNA of *A. muelleri* from tubers of different developing stages, root, petiole, petiolar base, and leaf were isolated according to 2.2 methods. For qRT-PCR, two pairs of specific primers were designed based on the *AmAGP* gene sequence: *AmAGP-F*, 5′-CGGCGGCATCATAGGAGGGAG-3′ and *AmAGP-R*, 5′-GACCGCAGCCACGTTCTTC-3′. qRT-PCR assays were performed using the Power SYBR Green PCR Master Mix (Applied Biosystems, Foster, USA). The amplification reaction was conducted in a 20 μL reaction volume containing 10 μL

SYBR Green using a BIO-RAD iQ5 instrument (USA). Thermocycling parameters were 2 min at 56°C, 10 min at 95°C, and 50 cycles of 15 s at 95°C, and 60 s at 60°C. Three technical replicates were performed for each sample. The ΔCt was calculated using the *A. muelleri 18S* gene (forward primer 5′-GTATGATGGTCACAAGCTTCGG-3′ and reverse primer 5′-CCTCAACAAATCCATCCCCAAA-3′) as an endogenous reference gene.[37] Relative quantitation values were calculated using the $2^{-\Delta\Delta Ct}$ method.[38] The specificity of the amplification was determined by performing a dissociation curve analysis. For sqRT-PCR, PCR conditions were 3 min at 94°C, 30 s at 94°C; 30 s at 54°C and 45 s at 72°C (32 cycles) for 25 μl reaction buffer. The PCR products were separated on a 1.0% (m/v) agarose gel and detected using an image analyzer (white/UV transilluminator).

2.5. Treatment of tubers with different temperatures and hormones in A. muelleri

A. muelleri bulbils of consistent size were used as seeds and grown under greenhouse conditions at Yunnan University (Kunming, Yunnan). Each treatment was performed using 30 number of tubers at 60 d after planting (DAP), 90 DAP, 120 DAP, and 150 DAP, respectively.

For temperature treatments, according to the preferable temperature for the development of *A. muelleri*, the temperature treatments were chosen from 20°C to 35°C. *A. muelleri* was grown at temperatures of 18°C for 8 h in the dark and 20°C, 25°C, 30°C, and 35°C for 16 h in the light. Tubers were harvested at 90 DAP, immediately frozen in liquid nitrogen, and stored at −80°C.

For hormone treatments, 1.0 mg L^{-1} IAA, 0.5 mg L^{-1} TDZ, or 25 mg L^{-1} SA were sprayed on the leaves of *A. muelleri* every 7 d, and tubers sprayed with water were used as controls. After the treatments had been repeated five times using the same hormone concentrations, 90 DAP tubers were harvested, immediately frozen in liquid nitrogen, and stored at −80°C.

2.6. Measurement of starch and glucomannan

Starch measurements were performed using an iodine-starch colorimetry assay with a minor revision.[39] Firstly, three 90 DAF tubers were chopped and mixed together, and 0.5 g tubers were added into 15 ml 80% calcium nitrate solution. Secondly, 20 mL ddH_2O was added to the boiling mixture, and then the mixture was centrifuged for 5 min at 5000 rpm. After removal of the supernatant, the subsidence was mixed with 50 mL ddH_2O, and the solution was measured. Finally, 8 ml of the starch extract was added to a centrifuge tube containing 2 ml of 0.5% iodine solution, mixed, allowed to stand for 15 minutes, and centrifuged for 5 min at 5000 rpm. The supernatant was then discarded to make a working solution. After the color was developed, the absorbance was measured with an ultraviolet (UV) spectrophotometer at a wavelength of 590 nm. Three technical replicates were performed for each sample. Contents of starch in tubers were calculated as follows:

$$\text{Starch Content}(\%) = \frac{100 \times C \times V0}{V1 \times 1000 \times m}$$

where C is the starch content obtained from the standard curve, V_0 is the total volume of the starch extraction solution, V_1 is the volume of the measured solution, and m is the weight of measured tubers.

Content of glucomannan was determined by Huang's method.[40] The absorbance of working solutions of glucomannan was measured at 550 nm with a UV spectrophotometer, and the glucomannan content was calculated as follows:

$$\text{Glucomannan Content}(\%) = \frac{\varepsilon T \times 100}{M} \times 100$$

where ε = 0.9, T is the starch content obtained from standard curves, and M is the weight of purified powder (mg).

3. Results

3.1. Molecular cloning and phylogenetic analysis of AmAGP

A. muelleri is a special *Amorphophallus* species, its leaves displayed living bulbils on the proximal petiole (Figure S1A). Leaf bulbils were easily separated from the leaf after it was withered. Besides, *A. muelleri* displayed a special growth type in which one to three seedlings all supported the same underground tuber development, thereby making full use of photosynthesis to promote tuber expansion. The inflorescence might eventually reach up to 1 m or more in height, and it was composed of a colorful outer spathe and an inner spadix with stamen and pistil born all-around upper and down, respectively (Figure S1C, D). Interestingly, fruit could develop spontaneously after removing its stigma, and a mature fruit contains one or two seeds at least (Figure S1E–G), suggesting that *A. muelleri* displays apomictic seed formation (Figure S1H). Because of its high disease resistance and its apomictic characterization, it has a strong competitive advantage and a very high economic value.

To identify the function of AGPase genes of *A. muelleri*, an *AmAGP* gene was identified according to sequence similarity with AGPase genes from *Oryza sativa* (AAA33891.1), *Triticum aestivum* (CAA46879), *Hordeum vulgare* (ABX72229.1), and *A. konjac* (AEH27531.1). AmAGP had a long open reading frame that encoded a predicted protein containing 552 amino acid residues, with a calculated molecular mass of 61,091 Da, and an isoelectric point of 8.63. The predicted AmAGP protein contained a conserved plant ADP-Glucose-PP repeat domain (CD02508) and seven potential ligand-binding sites including the 'LGGG' motif (Figure 1), which were similar to those of AGPase proteins from other plant species, suggesting that it was conserved among different plants.

To further clarify the evolutionary relationships of AmAGP in plants, phylogenetic analysis of AGPase proteins was performed using MEGA6.0. Eighteen AGPase proteins were obtained from different plant species. The results showed that AGPase proteins were classified into two groups, group I and group II, respectively. Interestingly, all of the monocots were belonged to group I and most of the dicots were fell into group II with the exception of *Zea mays* (Figure 2).

3.2. AmAGP was expressed highly in tubers

To characterize the potential function of *AmAGP* in different *A. muelleri* tissues, the temporal and spatial expression patterns

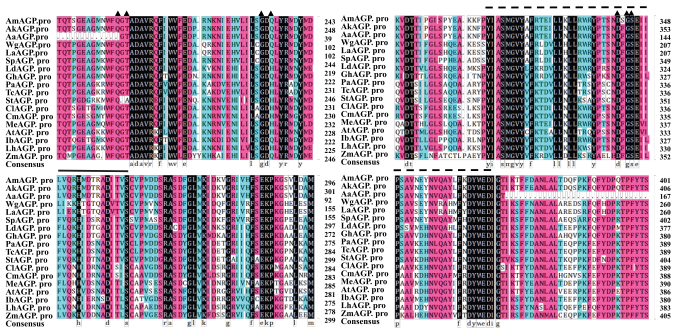


Figure 1. Sequence analysis of the predicted AmAGP protein and comparison of its sequence with other AGP protein sequences. *Ak, Amorphophallus konjac* (JF727266.1); *Aa, Amorphophallus albus* (AF316326.1); *Wg, Wolffia globosa* (KR263899.1); *La, Lemna aequinoctialis* (KR263896.1); *Sp, Spirodela polyrhiza* (JN180633.1); *Ld, Lilium davidii var. unicolor* (AJG44462.1); *Gh, Gladiolus hybrid cultivar* (AIO11223.1); *Pa, Populus alba* (TKS10148.1); *Tc, Theobroma cacao* (EOY14815.1); *St, Solanum tuberosum* (CAA52917.1); *Cl, Citrullus lanatus* (AF032472.1); *Cm, Cucumis melo* (NM_001297522.2); *Me, Manihot esculenta* (KU243122.1); *At, Arabidopsis thaliana* (AY096657.1); *Ib, Ipomoea batatas* (JQ797692.1); *Lh, Lycopersicon hirsutum* (AF184345.1); and *Zm, Zea mays* (CAA 86227.1). Two ADP-Glucose-PP repeat domains are highlighted with solid and dashed lines, respectively. The potential ligand-binding sites are highlighted with triangle.

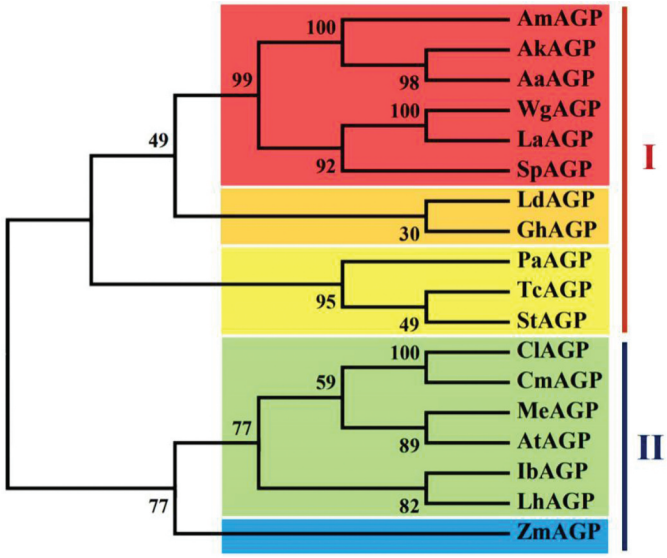


Figure 2. Phylogenetic analysis of the AmAGP protein and other homologous proteins, including *Ak, Amorphophallus konjac* (JF727266.1); *Aa, Amorphophallus albus* (AF316326.1); *Wg, Wolffia globosa* (KR263899.1); *La, Lemna aequinoctialis* (KR263896.1); *Sp, Spirodela polyrhiza* (JN180633.1); *Ld, Lilium davidii var. unicolor* (AJG44462.1); *Gh, Gladiolus hybrid cultivar* (AIO11223.1); *Pa, Populus alba* (TKS10148.1); *Tc, Theobroma cacao* (EOY14815.1); *St, Solanum tuberosum* (CAA52917.1); *Cl, Citrullus lanatus* (AF032472.1); *Cm, Cucumis melo* (NM_001297522.2); *Me, Manihot esculenta* (KU243122.1); *At, Arabidopsis thaliana* (AY096657.1); *Ib, Ipomoea batatas* (JQ797692.1); *Lh, Lycopersicon hirsutum* (AF184345.1); and *Zm, Zea mays* (CAA 86227.1). Multiple sequence alignment of AGP proteins was performed using ClustalX2 with default parameters. The unrooted phylogenetic tree was constructed in MEGA 6 with the neighbor-joining (NJ) method and 1000 bootstrap replicates.

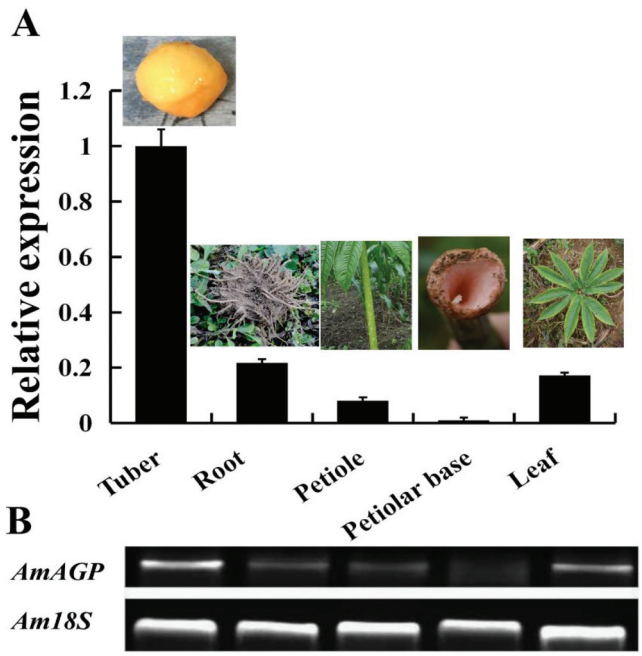


Figure 3. qRT-PCR (a) and sqRT-PCR (b) analysis of *AmAGP* in different tissues. *Am18S* was used as a loading reference.

were analyzed using qRT-PCR assays (Figure 3(a)) and sqRT-PCR (Figure 3(b)). The results showed that *AmAGP* was expressed not only in reproductive organs but also in vegetative ones. It was expressed constitutively in all tissues tested, although its expression levels varied among tissues. Its transcript level was the lowest in the base of petiole and the highest in the tuber, respectively. The tuber is the economically valuable part of the *Amorphophallus* plant and which produces abundant starch, and its high level of *AmAGP* expression suggested that it might be probably important for the synthesis of starch in tuber.

3.3. AmAGP was induced in tubers under 25°C treatment and was positively correlated with starch synthesis in A. muelleri

As commercial products, *A. muelleri* tubers can be divided into two types, the starch type and the glucomannan type,

respectively. To document the preferred characteristics and the relationship between starch and glucomannan in tubers, both of them were assessed during tuber elongation. Tubers were collected at 60, 90, 120, and 150 DAP for measurement of starch and glucomannan content. The results showed that the starch content increased significantly over time and reached a maximum at 120 DAP, it then decreased at 150 DAP, indicating that the starch had transformed into other monosaccharides gradually. In contrast, glucomannan accumulation increased throughout the development period (Figure 4(a)). Our results showed that both starch and glucomannan were significantly increased at 90 DAP, indicating that 90 DAP was the essential period for tuber elongation. Additionally, to better identify the relationship of transcript level of *AmAGP* and starch synthesis, the expression level of *AmAGP* was detected in 60, 90, 120, and 150 DAP tubers using qRT-PCR. The results showed that *AmAGP* expressed highly in 120 DAP tuber, correspondingly, starch content increased significantly over time and reached a maximum at 120 DAP, it then decreased at 150 DAP (Figure 4(b)), indicating that the starch had transformed into other monosaccharides gradually.

To assess the effect of temperature on starch and glucomannan synthesis, 90 DAP tubers were treated using different temperatures of 20°C, 25°C, 30°C, and 35°C, respectively. As treating temperature increased, the starch content reached 13.64% at 30°C, which was not significantly different from the 25°C treatment. However, when the treatment temperature was 35°C, the starch content decreased significantly to 8.85%. The glucomannan content was increased gradually with temperature changes from 20°C to 35°C, and reached a maximum of 65.98% at 35°C in tubers (Figure 5(a)). The differences in starch and glucomannan content using different temperature treatments were significant, and the optimal temperatures for starch and glucomannan accumulation were 25°C and 35°C, respectively. The results showed that a temperature of 25°C was most conducive to the accumulation of starch in *A. muelleri*.

To further confirm the regulation of *AmAGP* in starch synthesis in *A. muelleri*, its expression levels were measured in 90 DAP tubers using different temperature treatments. The results showed that 90 DAP tubers which grown at 25°C produced higher *AmAGP* transcript levels than any other treatment groups (Figure 5(b)), indicating that *AmAGP* expression level was positively correlated with starch content in *A. muelleri*.

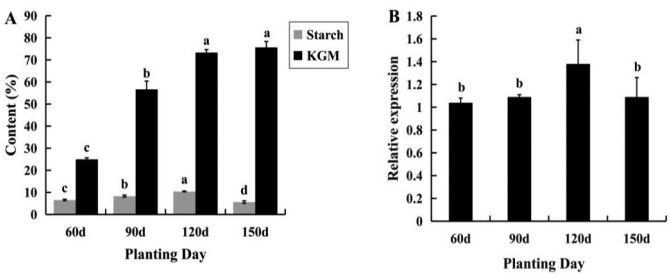


Figure 4. Starch and glucomannan content (a) and *AmAGP* transcript level (b) during tuber expansion in *A. muelleri*. For a given substance, values that share a letter do not differ significantly ($P < .05$, $N = 3$ biological replicates). Error bars represent standard errors.

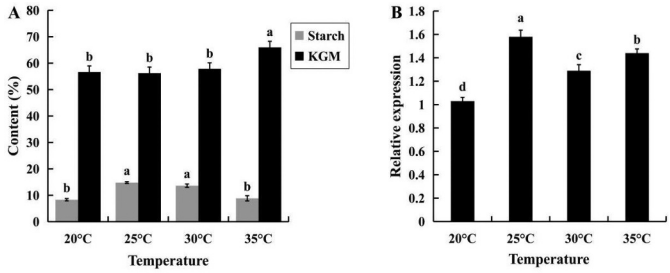


Figure 5. Analysis of starch and glucomannan content (a) and *AmAGP* transcript levels (b) in 90 DAP tubers under different temperature treatments. Values that share a letter do not differ significantly ($P < .05$, $N = 3$ biological replicates). Error bars represent standard errors.

3.4. *Both starch synthesis and* AmAGP *expression were induced by IAA*

Starch could be induced by hormones, to assess which hormones were involved in starch synthesis, three different hormones (IAA, TDZ and SA) were used to spray the leaves of *A. muelleri*. Compared with control tubers, all the tubers treated using 1.0 mg L^{-1} Indole-3-acetic acid (IAA), 0.5 mg L^{-1} Thidiazuron (TDZ), and 25 mg L^{-1} Salicylic acid (SA) displayed higher contents of both starch and glucomannan. Additionally, they were significantly higher (8.23% and 68.2%, respectively) in response to IAA treatment than those in response to other treatments (Figure 6(a)). Therefore, both starch and glucomannan were promoted by IAA in *A. muelleri*.

In order to further identify the involvement of *AmAGP* into starch synthesis, the abundance of *AmAGP* was detected in 90 DAP tubers that treated using different hormones as above. It was showed that *AmAGP* expression was lowest in the control tubers and highest in the tubers treated with 1.0 mg L^{-1} IAA, followed by 25 mg L^{-1} SA and 0.5 mg L^{-1} TDZ (Figure 6(b)). These findings further confirmed that *AmAGP* was induced by IAA and acted as a positive regulator for starch synthesis.

4. Discussion

A. muelleri is an *Amorphophallus* species with a high competitive ability and economic value. Both the bulbs and tubers of *Amorphophallus* are rich in the biopolymers starch and glucomannan, at levels that differ among species. In recent years, these biomaterials have received considerable attention as potential components of drug delivery systems for the controlled release of drugs at specific targeted delivery sites.[41] Among the natural polymers, cassava starch KGM is regarded as a potential carrier for a site-specific bioactive protein drug delivery system.[42] In view of this potential function, an increased understanding of starch and glucomannan biosynthesis and metabolism may help to increase the abundance of this relatively easily digestible and fermentable polymer.

In plants, both starch and KGM are derived from carbon products accumulated during photosynthesis in leaves.[43] In their synthesis pathway, the first key enzyme is AGP, which catalyzes the production of ADP-glucose from glucose-1-phosphate. At the same time, glucose-1-phosphate is acted upon by GGP to produce GDP-glucose (or to further produce GDP-mannose), and glucomannan is synthesized under the action of

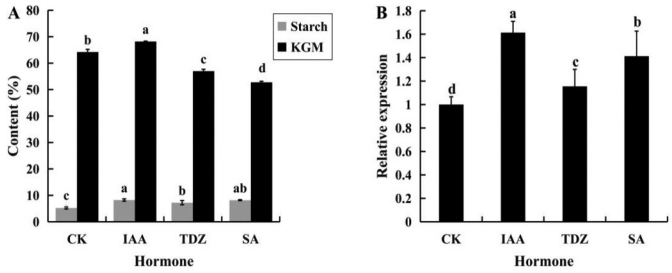


Figure 6. Analysis of starch and glucomannan content (a) and *AmAGP* transcript levels (b) in 90 DAP tubers under different hormone treatments. Tubers sprayed with water were used as controls. Values that share a letter do not differ significantly ($P < .05$, $N = 3$ biological replicates). Error bars represent standard errors.

CSLA3. AGP and GGP catalyze the production of ADP-glucose and GDP-glucose from glucose-1-phosphate,[43] and these sugar phosphates then participate in starch or glucomannan synthesis, respectively. Therefore, the starch content would affect the glucomannan content in *Amorphophallus*. High *AmAGP* expression in tubers might lead to high contents of starch, which providing genetic improvement and potential utilization in oriented breeding of *A. muelleri*. To date, the traditional view of starch metabolism has focused on the multiplicity of enzymes and enzyme isoforms contributing to the production of the constituent polymers.[44] Among these enzyme complexes, AGPase is the well studied.[45] Regulation of the AGPase gene has been shown to change starch content and to accelerate or reduce the synthesis of reducing sugars. In potato, antisense-*AGP* transgenic plants exhibited significantly reduced starch, whereas sucrose and glucose were increased in the tubers.[46] AGP was not only involved in the regulation of starch production, but it also indirectly regulated sugar formation. There are seven *MeAGPs* in cassava, which are the same as rice AGPase genes,[20,21] but it obviously differs in number from AGPase genes of *Arabidopsis*,[19] potato,[24] maize,[26] banana,[23] and sweet potato.[22] There might be some AGPase genes in *A. muelleri*. In this study, a starch synthesis-related gene *AmAGP* was isolated from *A. muelleri*, and its predicted protein was shown to have the highest homology with AkAGP and AaAGP. There were seven binding sites of substrates in AmAGP, which showed some differences from other AGPs, i.g. the binding site of 116 amino acid showed that it was 'Q' in most of monocots but was 'H' in most of dicots, suggesting that AmAGP might have some differences from other AGPs proteins in both substrate binding and enzyme activity regulation. According to protein homology, AGPs proteins could be divided into dicot and monocot types. Interestingly, maize was differed from other monocots, it might be due to its high contents of starch, suggesting that AGPs genes showed a species specific evolution among monocots and dicots.

AGP is differentially expressed not only among different plant organs but also within the same organ at different developmental stages, as shown in wheat,[47] barley,[48] maize,[49] etc. As for wheat, the cytosolic *AGP* gene is expressed in the late stages of endosperm development, while the plastidial *AGP* gene is expressed in young endosperm and leaves.[47] In hull-less barley, *AGP* gene had higher expression in grain than in leaves and stems.[48] In *A. muelleri, AmAGP* was highly expressed in tubers with high starch content and low expressed in petioles and base of petioles with low starch content. Furthermore, the expression level of *AmAGP* was consistent with the change of starch content during tuber enlarging, suggesting that *AmAGP* expression was positively correlated with starch content. Interestingly, the expression level of *AmAGP* was decreased in 150 DAP tuber, it might be induced by the feedback regulatory mechanism of *AmAGP* in *A.muelleri*. Plants such as potato, rice, and tomato also have shown tissue-specific expression patterns similar to those documented here in *A. muelleri*. Additionally, *AGP* transcript levels changed in different tissues during different growth stages of barley.[48] By contrast, maize has three genes that encode *AGP* large subunits, and one does not show obvious tissue-specific expression.[49] The tissue-specific expression of the AGP large subunit, therefore, appears to vary among species.

Additionally, the expression of the AGPs genes was greatly affected by the environmental factors,[23,29,50] suggesting that environmental conditions have an impact on AGPase activity,[27] such as hormone, temperature, and other conditions. Specifically, IAA, SA, TDZ affect the bulbous expansion and the accumulation of inclusions, and appropriate exogenous hormone treatments can increase starch content. Both endogenous and exogenous hormones such as IAA, SA, and TDZ influence metabolites and tuber expansion in plants.[51–53] In *A. muelleri*, the accumulation of starch was increased significantly at 90 DAP, and *AmAGP* transcript levels were highest at 25°C and in response to IAA treatment. It has been suggested that *AmAGP* is involved in the regulation of starch formation in *A. muelleri*. Regulation of AGP during starch synthesis is complex, and our study contributes to a better genetic improvement of *A. muelleri*.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

Funding

This work was supported by the National Natural Science Foundation of China under Grant (No. 31660566).

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Genome-Wide Analysis of Purple Acid Phosphatase Genes in *Brassica rapa* and Their Association with Pollen Development and Phosphorus Deprivation Stress

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Abstract: PAPs (purple acid phosphatases) belong to the metallo-phosphoesterase superfamily and play important roles in developmental processes, phosphorus foraging, and recycling. However, the specific functions of BrPAPs in *Brassica rapa* are poorly understood. In this study, 39 *BrPAPs* were identified and divided into three major clades and nine subgroups. In 8 of the 39 BrPAPs, some invariant amino acid residues were lost or shifted. Based on an expression profiling analysis, *BrPAP11*, *14*, *20*, *24*, *29*, and *34* were specifically expressed in fertile floral buds, indicating their critical roles during pollen development. A total of 21 *BrPAPs* responded to Pi deprivation in either shoots or roots. Of these, *BrPAP4*, *5*, *19*, and *21* were upregulated in roots under Pi depravation conditions, while *BrPAP12* was upregulated in the roots in normal conditions. *BrPAP28* was upregulated in shoots under Pi depravation conditions, indicating its function shifted compared with its *Arabidopsis* homolog, *AtPAP26*. The present work contributes to further investigation of *BrPAPs* as candidate genes for genetic improvement studies of low phosphorus tolerance as well as for creating male sterile lines based on gene editing methods in *Brassica rapa*.

Keywords: *Brassica rapa*; purple acid phosphatases; *BrPAP*; Pi-deprivation; pollen development

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Citation: Cai, Y.; Qi, J.; Li, C.; Miao, K.; Jiang, B.; Yang, X.; Han, W.; Wang, Y.; Gao, J.; Dong, X. Genome-Wide Analysis of Purple Acid Phosphatase Genes in *Brassica rapa* and Their Association with Pollen Development and Phosphorus Deprivation Stress. *Horticulturae* **2021**, *7*, 363. https://doi.org/10.3390/horticulturae7100363

Academic Editors: Xiaowu Wang, Jian Wu and Xu Cai

Received: 31 August 2021
Accepted: 30 September 2021
Published: 5 October 2021

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1. Introduction

Purple acid phosphatases (PAPs) belong to the metallo-phosphoesterase superfamily and are a type of acid phosphatase (APase) comprised of a binuclear metal center binding Fe (III)-M (II) complex (where M = Fe, Zn, or Mn) at the active site [1,2]. Due to the existence of a tyrosine residue ligated to a ferric iron, this group of acid phosphatases has a purple color [3]. They have been isolated from most eukaryotic organisms and some bacteria [3,4]. APases can hydrolyze a wide range of anhydrides and phosphate esters [5]. Therefore, improvement of APase activity is an important avenue for improving the efficiency of phosphorus utilization of plants in a low Pi (inorganic phosphate) environment. This enables plants to utilize extracellular and intracellular organic phosphorus (Po) [5–7].

With the aid of five conserved motif blocks (**D**XG/G**D**XX**Y**/G**N**H(D/E)/VXX**H**/G**H**X**H**, where bold and underlined letters indicate metal ligating residues that are required for metal coordination) and the availability of plant genome sequences, many *PAPs* have been identified and annotated from plant species at the genomic level [2,8,9]. For example, there are 29 *PAPs* in *Arabidopsis thaliana*, 38 in *Glycine max*, 33 in *Zea mays* ssp. *mays* var. B73, 19 in *Camellia sinensis*, 25 in *Jatropha curcas*, 26 in *Oryza sativa*, and 25 in *Cicer arietinum* [2,8–15]. Based on protein mass and structure, the PAPs isolated from plants can be divided into two forms: low molecular-mass monomeric PAPs (LMMs, with a mass of approximately 35 kDa) and high molecular-mass oligomeric PAPs (HMMs, with a mass around 55–60 kDa) [2,8,15].

LMM PAPs usually contain only one catalytic domain, and HMM PAPs are homodimeric or heterodimeric proteins, where each subunit has a C-terminal domain with an active site and an N-terminal domain of unknown function [16].

Due to the APase activity of PAPs, phosphorus acquisition and metabolism are the focus of most studies relating to PAP function. In *Arabidopsis*, *AtPAP10* was induced by Pi starvation and was shown to play a role in plant tolerance to Pi limitation [17]. *AtPAP15* was related to the mobilization of phosphorus reserves during seed and pollen germination [18]. *AtPAP12* and *AtPAP26* appear to be the predominant PAP isozymes responsive to Pi starvation and they can utilize Po [19,20]. In rice, APase activity and Po degradation are significantly increased by the overexpression of *OsPAP10a*, *OsPAP10c*, and *OsPAP21b*, and the expression levels of these can be induced by Pi starvation [21–24]. In soybean, *GmPAP14* was induced in response to Po and utilized external Po to promote plant growth and development [7].

In addition to phosphorus acquisition and metabolism, the biological functions of *PAP* genes include other biological processes. Heterologous expression of *AtPAP2* in potato can lead to a higher photosynthesis rate, faster growth, higher tuber number, and tuber starch content [25]. In tobacco (*Nicotiana tabacum*), beta-glucan synthesis and cellulose deposition were increased in *NtPAP12* overexpressing cells during cell wall biosynthesis [26]. In soybean, *GmPAP3* is related to the adaptation of soybean to NaCl stress through the involvement of ROS (reactive oxygen species) metabolism processes [27]. In *Arabidopsis*, seven *AtPAPs* (*AtPAP6*, *11*, *14*, *19*, *23*, *24*, and *25*) are predominantly expressed in flowers [4], and *AtPAP11* and *AtPAP5* are involved in the biological process of pollen tube growth [28]. This implies their potential functions during flower, pollen, and pollen tube development processes. Taken together, evidence suggests that *PAPs* have diverse functions during plant growth and development under normal or environmental stress conditions.

Brassica rapa is an important vegetable crop in China, Korea, and other Asian countries. PAP genes in *B. rapa* have been isolated due to the importance of *PAPs* for plant growth and development [9]. The detailed characteristics and features of *B. rapa* purple acid phosphatase genes (*BrPAPs*) are limited. In this study, *BrPAPs* were systematically identified and characterized. In silico and semi-RT-PCR analyses indicated that *BrPAP11*, *14*, *20*, *24*, *29*, and *34* might be related to pollen development, and their potential functions were highlighted by co-expression analyses. The responses of *BrPAPs* to Pi deprivation conditions were also investigated. The results from this study provide a basis for further understanding of the functions of *BrPAPs* during pollen development and *B. rapa* responses to Pi deprivation.

2. Materials and Methods

2.1. Plant Growth and Pi Deprivation Treatment

Brassica rapa L. *ssp. pekinensis* 'Chiifu' seeds were germinated in Petri dishes at 23 ± 1 °C in darkness. The germinated seeds were sown into plastic pots (7 cm × 7 cm) filled with vermiculite and grown for 20 d at 23 ± 2 °C with a light intensity of 6000–7000 Lux on a 16:8 h (L:D) photoperiod. During this growth phase, the plants were watered every two days with Hoagland solution (in mmol/L: KNO_3 6, $Ca(NO)_3 \bullet 4H_2O$ 4, KH_2PO_4 1, $MgSO_4 \bullet 7H_2O$ 1, H_3BO_3 0.046, $MnSO_4 \bullet H_2O$ 0.014, $ZnSO_4 \bullet 7H_2O$ 1.36×10^{-3}, $CuSO_4 \bullet 5H_2O$ 4.8×10^{-4}, $(NH_4)_6MoO_{24} \bullet 4H_2O$ 1.62×10^{-5}, $FeSO_4 \bullet 7H_2O$ 2×10^{-5}, and $C_{10}H_{14}N_2Na_2O_8$ 2×10^{-5}) [29]. The pH of the solution was adjusted to 6.0 using HCl and NaOH. For Pi deprivation treatment, the KH_2PO_4 in the Hoagland solution was replaced with KCl. After 20 d of cultivation, 15 to 20 plants were selected randomly. Five plants were used for root length, root weight, fresh weight, leaf area, and phosphorus content measurements. The roots and shoots of the remaining plants were separated and sampled. After sampling, the roots and shoots were frozen immediately in liquid nitrogen and stored at −80 °C until use. All Pi deprivation treatments were carried out with three independent biological replicates.

For the fertile and sterile plants of *B. rapa*, seeds were germinated in Petri dishes at 23 ± 1 °C in darkness, then the germinated seeds were transferred to a 4 °C growth

chamber in darkness for 30 d to induce vernalization. After vernalization, the seeds were sown into pots (15 × 15 × 18 cm) containing potting soil and grown in a greenhouse at 23 ± 1 °C with a light intensity of 6000–7000 Lux and a 16:8 (L:D) photoperiod. The floral buds were then collected from five plants using three biological replicates, as previously described [30]. Root and shoot tissues were collected from three-week-old seedlings without vernalization. Stems and stem leaf tissues were sampled from these plants seven days after bolting. All tissues were stored at −80 °C until use.

2.2. Leaf Area and Phosphorus Content

For leaf area determination, the outermost leaf of Chiifu seedlings was dissected, and the leaf area was determined with a Yaxin-1241 leaf meter (Beijing Yaxinliyi, China) following manufacturer's instructions. Total phosphorus concentration was determined as described previously [31]. In brief, 0.2 g of leaves was ground and extracted using a laboratory-scale high-performance microwave digestion system (Milestone Ethos UP, Italy, Sorisole) with 8 mL HNO_3-H_2O_2 (3:1) reagent. Afterward, the mixtures were reacted with molybdenum blue reagent and measured 20 min later at 700 nm by using a spectrophotometer (UV-1200, Instrument Co., Ltd., Shanghai, China).

2.3. Identification of BrPAP Genes in Brassica rapa

To identify the PAP genes from *B. rapa*, all putative protein sequences of *B. rapa* (version 3.0) were downloaded from BRAD (http://brassicadb.agridata.cn/brad/ accessed on 28 January 2021) [32]. All of the protein sequences of the 29 *AtPAPs* (*A. thaliana purple acid phosphatase*) were retrieved from TAIR (https://www.arabidopsis.org/index.jsp; Araport11 accessed on 28 January 2021) based on a previous publication [2] and used as queries to search against all putative protein sequences of *B. rapa* using BLASTP. A total of 43 putative BrPAP proteins were obtained based on two criteria: an E-value below 10^{-5} and sequence identity above 20%. Next, the amino acid sequences of the 43 putative BrPAP proteins were analyzed to determine the presence of conserved sequence motifs (**D**XG/G**D**XX**Y**/G**N**H(E/D)/VX2**H**/G**H**X**H**) defined previously for BrPAPs [2], and BraA02g012470.3C, BraA04g002700.3C, BraA06g026740.3C, and BraA10g003360.3C were removed. As a result, 39 proteins containing these conserved sequence motifs were identified. To find all potential PAPs in *B. rapa*, each of the 39 BrPAPs were used as queries for BLAST searching at NCBI and Phytozome 13 (https://phytozome-next.jgi.doe.gov/phytomine/begin.do accessed on 28 January 2021). However, no additional predicted BrPAPs were found. All BrPAPs were named BrPAP1 to BrPAP39 based on their genomic locations.

2.4. Phylogenetic Tree and Bioinformatics Analysis of BrPAPs

To carry out phylogenetic tree analysis, the protein sequences of PAPs from *Arabidopsis* [2] and *B. rapa* were aligned using MUSCLE with default parameters [33]. An unrooted phylogenetic tree was constructed using MEGA6 with the Neighbor-joining method and parameters set as follows: Jones–Taylor–Thornton (JTT) model, pairwise deletion, and a bootstrap test of 1000 replications [34]. The position of each BrPAP on the *B. rapa* chromosomes was isolated from BRAD (http://brassicadb.agridata.cn/brad/ accessed on 28 January 2021) and visualized with a custom Python script. The isoelectric point (PI) and molecular weight (M.W.) of BrPAPs were determined using the ProtParam tool (https://web.expasy.org/protparam/ accessed on 28 January 2021) [35]. Conserved motifs in BrPAPs were identified using the MEME suite 5.1.1 (http://meme-suite.org/ accessed on 28 January 2021) [36]. The predictions of signal peptides and N-glycosylation sites were carried out using SignalP-5.0 (http://www.cbs.dtu.dk/services/SignalP/ accessed on 28 January 2021) [37] and NetNGlyc 1.0 (http://www.cbs.dtu.dk/services/NetNGlyc/ accessed on 28 January 2021) with default settings, respectively. Gene structures of *BrPAPs* were drawn using the Gene Structure Display Server (GSDS, version 2.0, http://gsds.cbi.pku.edu.cn/ accessed on 28 January 2021) [38].

2.5. Expression and Co-Expression Analysis of BrPAPs

All RNA-seq reads from shoots (vernalized and non-vernalized), leaves, stems, roots, flowers, differential developmental seeds, and male sterile lines of *B. rapa* [39–44] were downloaded from the SRA (sequence read archive) on NCBI (https://www.ncbi.nlm.nih.gov/sra accessed on 28 January 2021) from accession IDs PRJNA339187, PRJNA185152, PRJNA310313, and PRJNA579431. The quality of these RNA-seq reads was analyzed using fastQC (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/ accessed on 28 January 2021), and the adapter sequences and low-quality reads were removed using the FASTX-Toolkit (Version 0.0.13) [45]. For gene expression, the groomed reads were mapped to genes using Bowtie2 [46], and fragments per kilobases of exons per million mapped reads (FPKM) values for transcripts were calculated using Cufflinks [47]. Heatmaps were generated using Multiple Experiment Viewer (T-MEV) with log transformed FPKM values [48].

For co-expression analysis, RNA-seq data from male sterility lines of *B. rapa* were re-assembled [40,41,43,44], and the genes with no differences between fertile and sterile floral buds were removed. Pearson's correlation coefficient (PPC) values were calculated in Excel using *BrPAP* candidates as queries. The threshold values for PPCs were set between 0.60 and −0.60. GO (Gene Ontology) enrichment analysis was carried out using agriGO (http://systemsbiology.cau.edu.cn/agriGOv2/ accessed on 28 January 2021) [49] and the clusterProfiler package in R [50].

2.6. RNA Extraction, Semi-RT-PCR, and qRT-PCR

Total RNA was isolated using the RNAiso Plus Reagent (Takara Biomedical Technology Co., Ltd., Beijing, China), based on manufacturer protocols. The PrimeScript™ RT reagent Kit with gDNA Eraser (Takara Biomedical Technology Co., Ltd., Beijing, China) was used to synthesize 1st strand cDNA with 1 µg of total RNA. After synthesis, this cDNA was diluted to 10 ng/µL for PCR analysis. Semi-RT-PCR was carried out using 20 ng/µL cDNA with the following program: denaturation at 94 °C for 5 min, followed by 28 cycles of 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 60 s. For qRT-PCR analysis, 10 ng/µL of cDNA was used and performed with a 30 s pre-denaturation at 95 °C, followed by 40 cycles of 95 °C for 5 s, 55 °C for 30 s, and 72 °C for 60 s. All of the primers used are listed in Table S1. The *B. rapa* actin gene was used as an internal control. The products of semi-RT-PCR were separated on 1.5% agarose gels and stained with GeneGreen (Tiangen Technology Co., Ltd., Beijing, China). The qRT-PCR results were analyzed using the $2^{-\Delta Ct}$ method with three biological replicates. After removing the genes with Ct value above 35 and less than two-fold change, the results are represented by a heatmap using R scripts with row normalization.

3. Results

3.1. Brassica rapa PAP Genes

After BLASTP search and conserved motif determination, 39 *PAP* genes were identified from the *B. rapa* genome and designated as *BrPAP1* to *BrPAP39* according to their positions on chromosomes (Figure 1). Among them, 31 BrPAP proteins contained the seven conserved metal-binding residues (D, D, Y, N, H, H, and H) in five consensus blocks (Table 1). Another three BrPAPs (BrPAP14, BrPAP17, and BrPAP21) lacked the first conserved block. The fourth block was lost in BrPAP9. For BrPAP7, one conserved residue in the fourth block was changed from H to S and the fifth block was missing. In the proteins from BrPAP22, BrPAP32, and BrPAP39, one conserved residue in the second block changed from Y to F, which has been reported in *Arabidopsis* [2], soybean [51], rice [11], maize [12], chickpea [13], and tea [15].

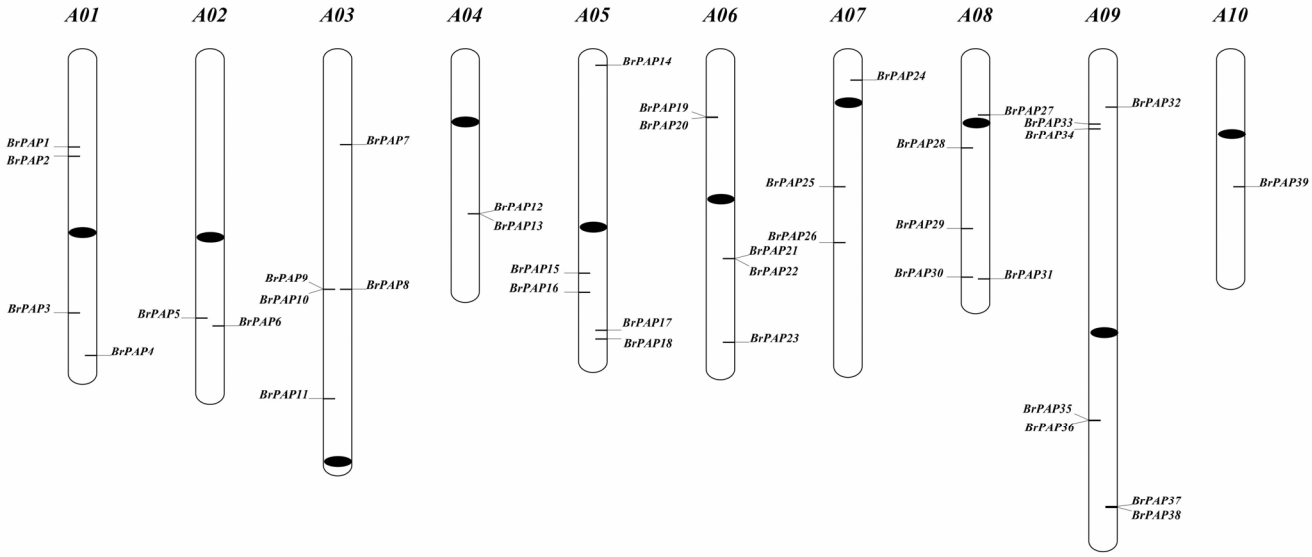


Figure 1. Chromosomal locations of the 39 *BrPAP* genes identified in this study. Chromosome number is indicated above each chromosome. Black ovals on each chromosome represent centromeric regions.

Table 1. Conserved metal ligating amino acid motifs present in BrPAP proteins. Conserved residues are underlined.

Gene ID	Gene Name	PAP Conserved Motifs				
		GDXG	GDXXY	GNH(D/E)	VXXH	GHXH
BraA01g015600.3C	*BrPAP1*	GDMG	GDITY	GNHE	FIAH	GHVH
BraA01g017190.3C	*BrPAP2*	GDLG	GDLSY	GNHE	VLNH	GHVH
BraA01g034870.3C	*BrPAP3*	GDWG	GDNFY	GNHD	VVGH	GHDH
BraA01g041630.3C	*BrPAP4*	GDTG	GDVSY	GNHE	VTWH	GHVH
BraA02g035210.3C	*BrPAP5*	GDWG	GDNFY	GNHD	VVGH	GHDH
BraA02g036110.3C	*BrPAP6*	GDMG	GDISY	GNHE	VQGH	GHVH
BraA03g017330.3C	*BrPAP7*	GDLG	CDSCY	GEHE	ATWS	
BraA03g043110.3C	*BrPAP8*	GDLG	GDFSY	GNHE	VLMH	GHVH
BraA03g043120.3C	*BrPAP9*	GDLE	YDTKY	GNCD		
BraA03g043130.3C	*BrPAP10*	GDLG	GDLSY	GNHE	VLNH	GHVH
BraA03g059950.3C	*BrPAP11*	GDLG	GDLSY	GNHE	VIVH	GHVH
BraA04g019300.3C	*BrPAP12*	GDLG	GDLSY	GNHE	VLVH	GHVH
BraA04g019310.3C	*BrPAP13*	GDLG	GDLSY	GNHE	VLVH	GHVH
BraA05g001000.3C	*BrPAP14*		SDMHY	GNHD	VYVH	GHDH
BraA05g026730.3C	*BrPAP15*	GDLG	GDLSY	GNHE	ALFH	GHVH
BraA05g029790.3C	*BrPAP16*	GDWG	GDNFY	GNHD	VVGH	GHDH
BraA05g036550.3C	*BrPAP17*		GDVVT	GNHD	IFWH	GHNH
BraA05g038510.3C	*BrPAP18*	GDLG	GDVSY	GNHE	VSWH	GHVH
BraA06g009980.3C	*BrPAP19*	GDMG	GDICY	GNHE	FLAH	GHAH
BraA06g010040.3C	*BrPAP20*	GDMG	GDISY	GNHE	VQGH	GHVH
BraA06g026750.3C	*BrPAP21*		GDNIF	GNHD	AYFH	GHDH
BraA06g026760.3C	*BrPAP22*	ADMH	GDNIF	GNHD	AYFH	GHDH
BraA06g040030.3C	*BrPAP23*	GDWG	GDNFY	GNHD	VVGH	GHDH
BraA07g002610.3C	*BrPAP24*	GDLG	GDLSY	GNHE	VLVH	GHVH
BraA07g012620.3C	*BrPAP25*	GDWG	GDNIY	GNHD	VVGH	GHDH
BraA07g020920.3C	*BrPAP26*	GDLG	GDLSY	GNHE	AVMH	GHIH
BraA08g006360.3C	*BrPAP27*	GDLG	GDFTY	GNHE	ATMH	GHVH
BraA08g009450.3C	*BrPAP28*	GDLG	GDLSY	GNHE	VLMH	GHVH
BraA08g021060.3C	*BrPAP29*	GDLG	GDLAY	GNHE	VMVH	GHVH
BraA08g030080.3C	*BrPAP30*	GDWG	GDNFY	GNHD	VVGH	GHDH

Table 1. *Cont.*

Gene ID	Gene Name	PAP Conserved Motifs				
		GDXG	GDXXY	GNH(D/E)	VXXH	GHXH
BraA08g030430.3C	*BrPAP31*	GDMG	GDISY	GNHE	VQGH	GHVH
BraA09g007730.3C	*BrPAP32*	ADMH	GDNIF	GNHD	AYFH	GHDH
BraA09g010460.3C	*BrPAP33*	GDLG	GDFSY	GNHE	VLMH	GHVH
BraA09g011240.3C	*BrPAP34*	GDLG	GDLSY	GNHE	VLVH	GHVH
BraA09g043870.3C	*BrPAP35*	GDLG	GDLSY	GNHE	AVIH	GHVH
BraA09g043900.3C	*BrPAP36*	GDLG	GDLSY	GNHE	VLLH	GHVH
BraA09g059830.3C	*BrPAP37*	GDMG	GDISY	GNHE	VQGH	GHVH
BraA09g059970.3C	*BrPAP38*	GDMG	GDICY	GNHE	FLAH	GHAH
BraA10g015040.3C	*BrPAP39*	ADMH	GDNIF	GNHD	AFFH	GHDH

General information for *BrPAPs*, including gene size, molecular weight, *Arabidopsis* homologs, and potential N-linked glycosylation sites, were also analyzed (Table 2). The sizes of the genomic DNA sequences for *BrPAPs* varied from 1196 bp (*BrPAP16*) to 6766 bp (*BrPAP23*), with an average length of 2443 bp. Molecular weight of identified BrPAPs ranged from 34.8 kDa (BrPAP21) to 75.79 kDa (BrPAP20). Based on predictions from SignalP, signal peptides were found in 34 BrPAPs except for BrPAP2, BrPAP9, BrPAP21, BrPAP25, and BrPAP39. N-glycosylation site prediction analysis indicated that BrPAPs exhibited different numbers of glycosylation sites, from 0 to 6 (Table 2).

3.2. Phylogenetic and Gene Structure Analyses of BrPAPs

A phylogenetic analysis was carried out using amino acid sequences from BrPAPs and AtPAPs with the neighbor-joining method. All PAP proteins were classified into three major clades (I to III), and a further classification of these three major clades produced nine subgroups (Figure 2). This analysis was similar to the findings of a previous study in *Arabidopsis* [2], with one exception. AtPAP10, 12, and 26 were classified into the same group in *Arabidopsis* [2], whereas AtPAP26 was separated from AtPAP10 and 12 and classified into a new subgroup (I a-3) in this study. Previously, a single gene was identified as a potential ortholog of AtPAP26 in maize and rice, indicating its conserved functions in the Pi deprivation response, which was also observed in *B. rapa* and was grouped into I a-3 (Figure 2). Based on our phylogenetic analysis, one AtPAP was related to one to three BrPAP homologs, which may have been due to whole-genome polyploidization and a gene loss event of *B. rapa* after separation from *Arabidopsis*, although this was not the case for AtPAP10 (Table 2 and Figure 2). For AtPAP10, five homologs (BrPAP2, BrPAP8, BrPAP9, BrPAP10, and BrPAP33) were identified in *B. rapa*, which indicated the expansion of AtPAP10 in *B. rapa* over evolutionary time.

During the evolution of a multigene family, gene structure usually diversifies. We therefore wanted to study *BrPAPs* related to their functional diversification and evolution. The intron size and number of *BrPAPs* were highly variable, showing 11 distinct exon–intron organization patterns. Seven exons separated by six introns was the most common organizational pattern and was present in 10 *BrPAPs* (Table 2 and Figure 3). Except for *BrPAP6*, most *BrPAPs* contained more than one intron, indicating the possible existence of alternative splicing during gene expression. For Ia-1, IIb, and IIIb, genes from the same subgroups showed similar gene structures, indicating that they might have conserved functions. The diversity of gene structures of genes from IIIa implied their diverse functions (Figure 3).

Table 2. General information for BrPAP genes and their deduced proteins. M.W., molecular weight; PI, isoelectric point.

Gene Name	Chromosome Location	No. of Exons	Gene Length(bp)	M.W. (kDa)	Protein Length (a.a.)	*PI*	Signal Peptide (Length, Cleave Site)	Number of N-Glycosylation Sites	Best Hit to *Arabidopsis*		
									***At*_ID**	***At*_Name**	***E*-Value**
BrPAP1	A01	10	2778	72.67	642	6.09	24, GHA-SN	1	*AT4G24890.1*	*AtPAP24*	0
BrPAP2	A01	8	2942	45.42	393	5.81	—	4	*AT2G16430.1*	*AtPAP10*	4.05×10^{-171}
BrPAP3	A01	3	1240	37.80	334	5.28	26, TNG-EL	1	*AT3G17790.1*	*AtPAP17*	0
BrPAP4	A01	6	2607	60.35	541	5.23	32, SDA-IP	6	*AT3G07130.1*	*AtPAP15*	0
BrPAP5	A02	7	4923	37.60	331	6.3	24, SFS-KL	2	*AT2G01880.1*	*AtPAP7*	0
BrPAP6	A02	1	1929	72.89	643	6.07	18, VHS-TP	4	*AT2G03450.1*	*AtPAP9*	0
BrPAP7	A03	6	2705	60.27	536	4.97	21, VDA-FP	6	*AT2G32770.3*	*AtPAP13*	0
BrPAP8	A03	7	2372	56.76	489	7.16	24, CHG-GT	1	*AT2G16430.2*	*AtPAP10*	0
BrPAP9	A03	4	1726	34.34	309	9.37	—	1	*AT2G16430.2*	*AtPAP10*	2.48×10^{-51}
BrPAP10	A03	7	2070	53.73	467	7.76	25, CNG-GI	5	*AT2G16430.2*	*AtPAP10*	0
BrPAP11	A03	7	1894	52.19	461	6.22	22, ING-GM	3	*AT1G56360.1*	*AtPAP6*	0
BrPAP12	A04	8	2354	55.49	482	6.16	28, CDG-GI	4	*AT2G27190.1*	*AtPAP12*	0
BrPAP13	A04	7	2057	54.08	470	6.25	28, CDG-GI	3	*AT2G27190.1*	*AtPAP12*	0
BrPAP14	A05	5	1682	44.56	394	8.47	24, VDA-YG	1	*AT2G46880.1*	*AtPAP14*	0
BrPAP15	A05	4	1947	47.48	414	5.68	19, VAA-DD	0	*AT3G20500.1*	*AtPAP18*	0
BrPAP16	A05	3	1196	38.33	338	5.57	—	1	*AT3G17790.1*	*AtPAP17*	0
BrPAP17	A05	4	1399	42.91	385	5.86	23, AVG-WE	1	*AT3G10150.2*	*AtPAP16*	0
BrPAP18	A05	5	2307	61.43	541	5.64	26, SSA-DY	4	*AT3G07130.1*	*AtPAP15*	0
BrPAP19	A06	9	2755	69.52	621	7.9	22, GGA-IQ	2	*AT1G13750.1*	*AtPAP1*	0
BrPAP20	A06	2	2599	75.79	680	5.6	22, ANA-EA	3	*AT1G13900.1*	*AtPAP2*	0
BrPAP21	A06	3	1378	34.08	308	6.24	—	0	*AT5G63140.1*	*AtPAP29*	0
BrPAP22	A06	3	1460	42.19	383	9.01	31, ASA-QG	1	*AT5G63140.1*	*AtPAP29*	0
BrPAP23	A06	14	6766	69.10	607	5.51	29, SRA-EL	3	*AT2G01890.1*	*AtPAP8*	0
BrPAP24	A07	7	2127	52.10	460	5.86	24, SHA-GV	4	*AT4G36350.1*	*AtPAP25*	0
BrPAP25	A07	8	2551	52.34	468	7.17	—	2	*AT1G25230.3*	*AtPAP4*	0
BrPAP26	A07	6	4130	50.56	437	8.25	22, SQA-YN	1	*AT3G52810.1*	*AtPAP21*	0
BrPAP27	A08	7	2273	61.20	548	5.02	31, AGG-ES	4	*AT4G13700.1*	*AtPAP23*	0
BrPAP28	A08	9	2313	55.40	481	7.35	27, GEG-GI	1	*AT5G34850.1*	*AtPAP26*	0
BrPAP29	A08	7	1887	52.42	460	6.6	19, ING-GI	1	*AT1G56360.1*	*AtPAP6*	0
BrPAP30	A08	7	2595	38.03	335	5.81	29, STA-EL	1	*AT1G14700.1*	*AtPAP3*	0
BrPAP31	A08	2	2540	73.13	652	5.95	2, ANA-KA	6	*AT1G13900.1*	*AtPAP2*	0
BrPAP32	A09	3	1746	42.92	386	9.2	32, TSS-HR	2	*AT5G63140.1*	*AtPAP29*	0
BrPAP33	A09	8	2490	54.12	469	6.77	29, CHG-GR	3	*AT2G16430.2*	*AtPAP10*	0
BrPAP34	A09	7	1977	52.82	468	5.49	23, SHA-GV	3	*AT2G18130.1*	*AtPAP11*	0
BrPAP35	A09	6	2974	46.99	415	6.01	21, VSS-YD	1	*AT3G52780.1*	*AtPAP20*	0
BrPAP36	A09	6	3163	51.08	448	5.79	35, SQA-DV	2	*AT3G52820.1*	*AtPAP22*	0
BrPAP37	A09	2	2436	73.36	653	6.18	21, ANA-KA	4	*AT1G13900.1*	*AtPAP2*	0
BrPAP38	A09	9	3035	69.22	622	6.16	24, ALG-GR	4	*AT1G13750.1*	*AtPAP1*	0
BrPAP39	A10	4	1966	45.34	404	6.83	—	3	*AT5G57140.1*	*AtPAP28*	0

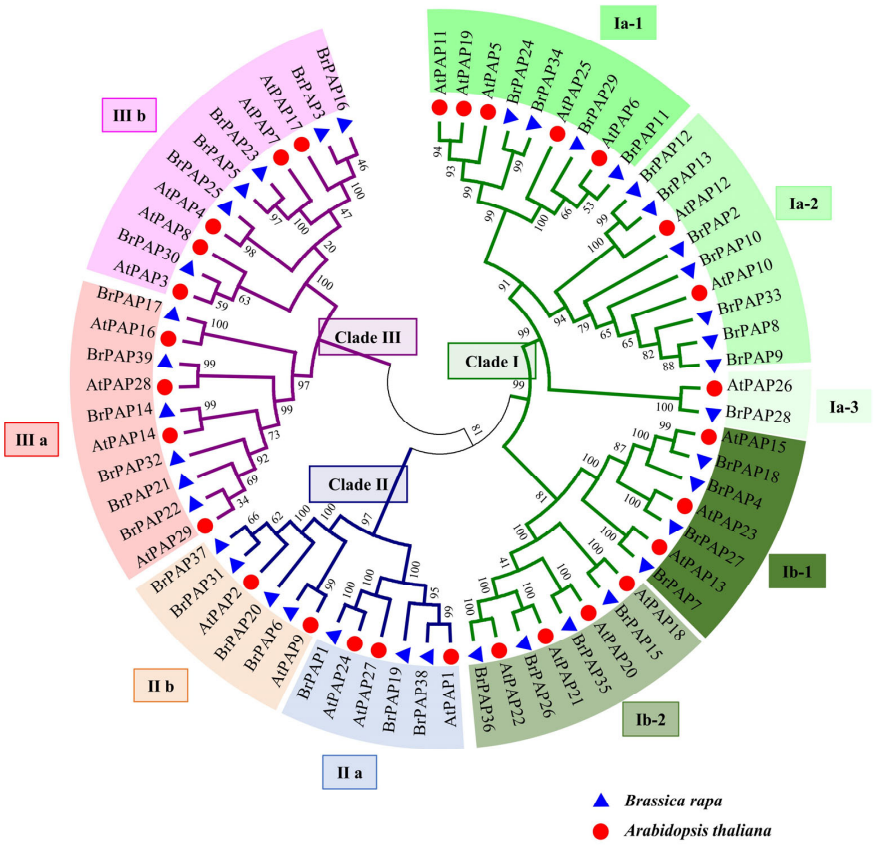


Figure 2. Phylogenetic relationships of BrPAP and AtPAP genes. Multiple protein sequence alignment was performed using ClustalW implemented in MEGA6. A neighbor-joining (NJ) tree was constructed in MEGA6 using the Jones–Taylor–Thornton (JTT) model with the following options: homogeneous pattern, pairwise deletion, and 1000-replicate bootstrap. Different clades and branches (subclades) are indicated by the colors of the branched lines and background, respectively.

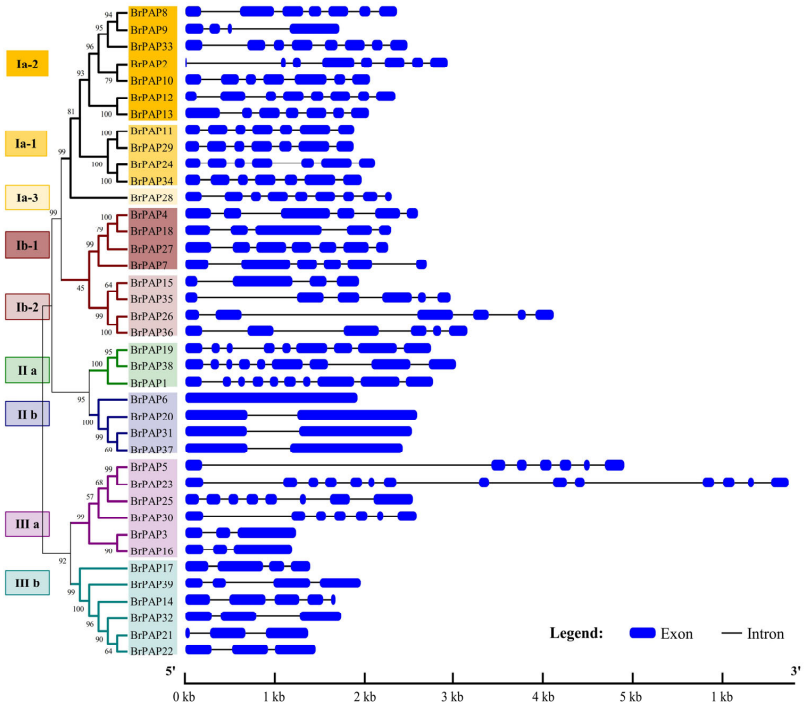


Figure 3. Gene structures of *BrPAP* genes.

3.3. Potential Functions of BrPAPs during Pollen Development

To study the functional divergence of *BrPAPs*, the spatial and temporal expression patterns of *BrPAPs* in *B. rapa* were analyzed. RNA-seq data derived from shoots (vernalized and non-vernalized), leaves, stems, roots, flowers, differential developmental stages of seeds, and male sterility lines were explored [22,39,41–44]. Based on this RNA-seq data, *BrPAP* transcripts accumulated in all tissues examined (Figure 4), as previously reported for *AtPAPs* under standard growth conditions [4]. This indicated that *BrPAPs* might play diversified functions during development. Interestingly, *BrPAP11*, *24*, *29*, and *34* from subgroup Ia-1 showed specific expression in fertile buds in *B. rapa* (Figure 4). In addition to these four genes, *BrPAP14* and *20* also showed high expression in fertile buds in *B. rapa*. This indicated that these genes specifically expressed in fertile buds might be related to pollen development. To confirm the expression patterns of these six genes, different developmental stages of floral buds from genetic male sterility (GMS) lines were collected based on a previous study (Figure 5A) [30]. According to our semi-RT-PCR results, *BrPAP11* and *24* were first detected in F2 floral buds (with tetrad pollen), and these *BrPAPs* were highly expressed in F3 floral buds (with pollen grain after tetrad formation but before maturation) (Figure 5B). *BrPAP14* and *29* were highly expressed in both F2 and F3 floral buds. *BrPAP20* and *BrPAP34* were only expressed in F4 (with pollen grain after tetrad formation but before maturation) and F3 (with mature pollen grains) floral buds, respectively (Figure 5B). To narrow down and predict the functions of *BrPAP11*, *14*, *20*, *24*, *29*, and *34* during pollen development, we carried out co-expression analysis using previously published male-sterility RNA-seq data in *B. rapa* [22,41,43,44]. When the threshold value for PPC (Pearson's correlation coefficient) was set between 0.60 and −0.60, 1511, 979, 2355, 1593, 1886, and 1667 genes were found to be co-expressed with *BrPAP11*, *14*, *20*, *24*, *29*, and *34*, respectively (Table S2 and Figure 6A). GO enrichment analysis was carried out to highlight the biological processes influenced by targeted *BrPAPs* (Figure 6B). The pollen development process was overrepresented by the co-expressed genes of *BrPAP11*, *20*, *24*, and *34*. The pollination process was only highlighted by the co-expressed genes of *BrPAP20*, *24*, *29*, and *34*. Lipid metabolic and fatty acid biosynthetic processes were enriched by co-expressed genes of *BrPAP14*. Carbohydrate transport, pollen sperm cell differentiation, pectin catabolic, polysaccharide catabolic, cell wall organization, pollen germination, and carbohydrate metabolic processes were only overrepresented by the co-expressed genes of *BrPAP20*.

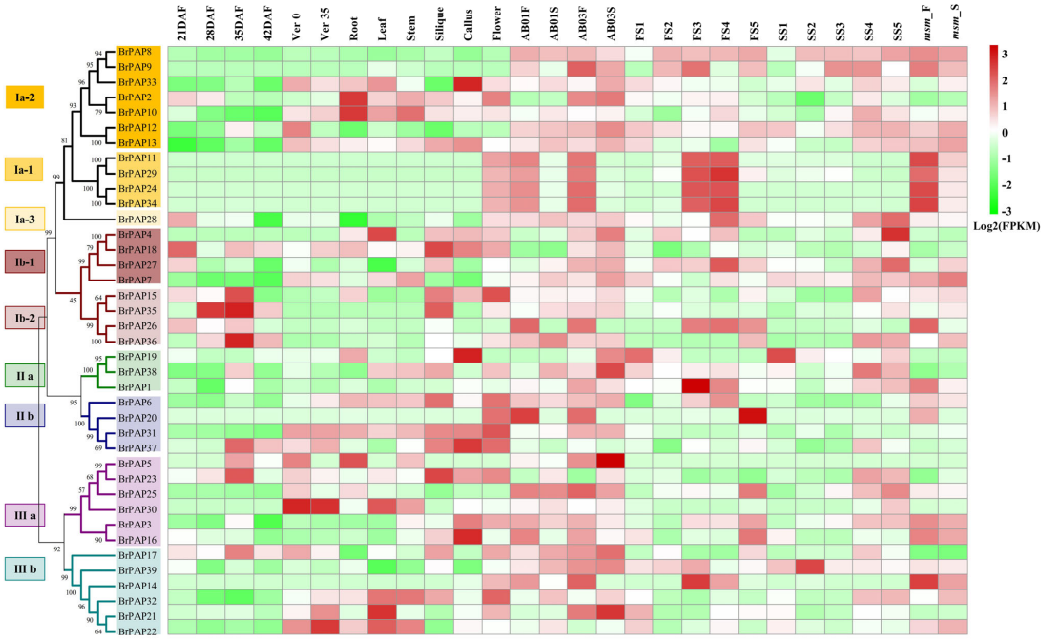


Figure 4. Heatmap representation of expression patterns for *BrPAPs* with various tissues and male sterility lines. DAF,

days after flowering; Ver0, 0 day after vernalization; Ver35, 35 days after vernalization; AB01_F and AB01_S, indicate fertile sterile floral buds of GMS Chinese cabbage line 'AB01', respectively; AB03_F and AB03_S represent fertile sterile floral buds of GMS Chinese cabbage line 'AB03', respectively; FS1–FS5, fertile flower buds stage 1 to 5 of GMS Chinese cabbage 'Bcajh97-01A/B'; SS1-SS5, sterile flower buds stage 1 to 5 of GMS Chinese cabbage 'Bcajh97-01A/B; *msm*_F and *msm*_S, floral buds from GMS Chinese cabbage line '*msm*'.

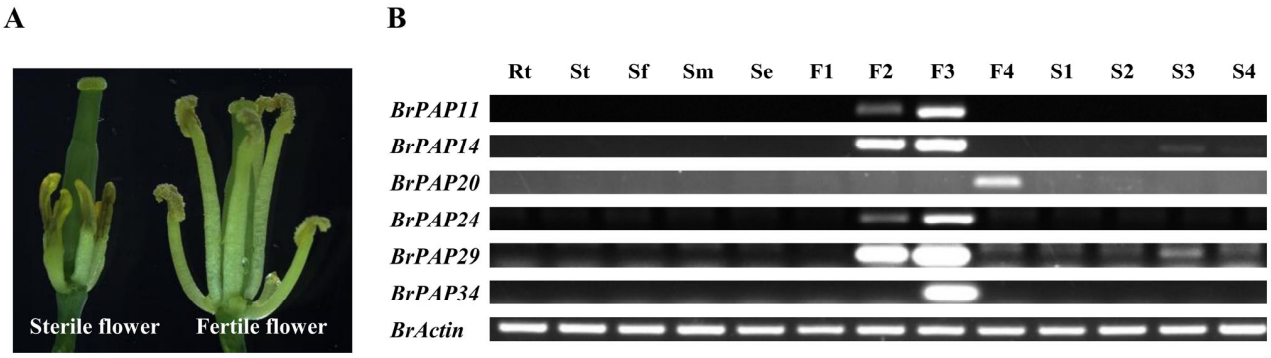


Figure 5. Confirmation of the expression patterns of *BrPAPs* in fertile buds using semi-RT-PCR. (**A**) Flower structure of fertile and sterile lines of *Brassica rapa*. In order to focus on the stamen, the petals and sepals were removed. (**B**) Analysis of the expression patterns of fertile bud specifically expressed genes using semi-RT-PCR. Rt, root; St, shoot; Sf, stem leaf; Sm, stem; Se, silique; F1–F4 indicated the floral buds from fertile plants. F1, before the tetrad stage. F2, at the tetrad stage. F3, after the tetrad stage but before containing mature pollen. F4, containing mature pollen. S1–S4, floral buds from sterile plants. S1, before the tetrad stage. S2, at the tetrad stage. S3 and S4, after the tetrad stage with aborted pollen grains.

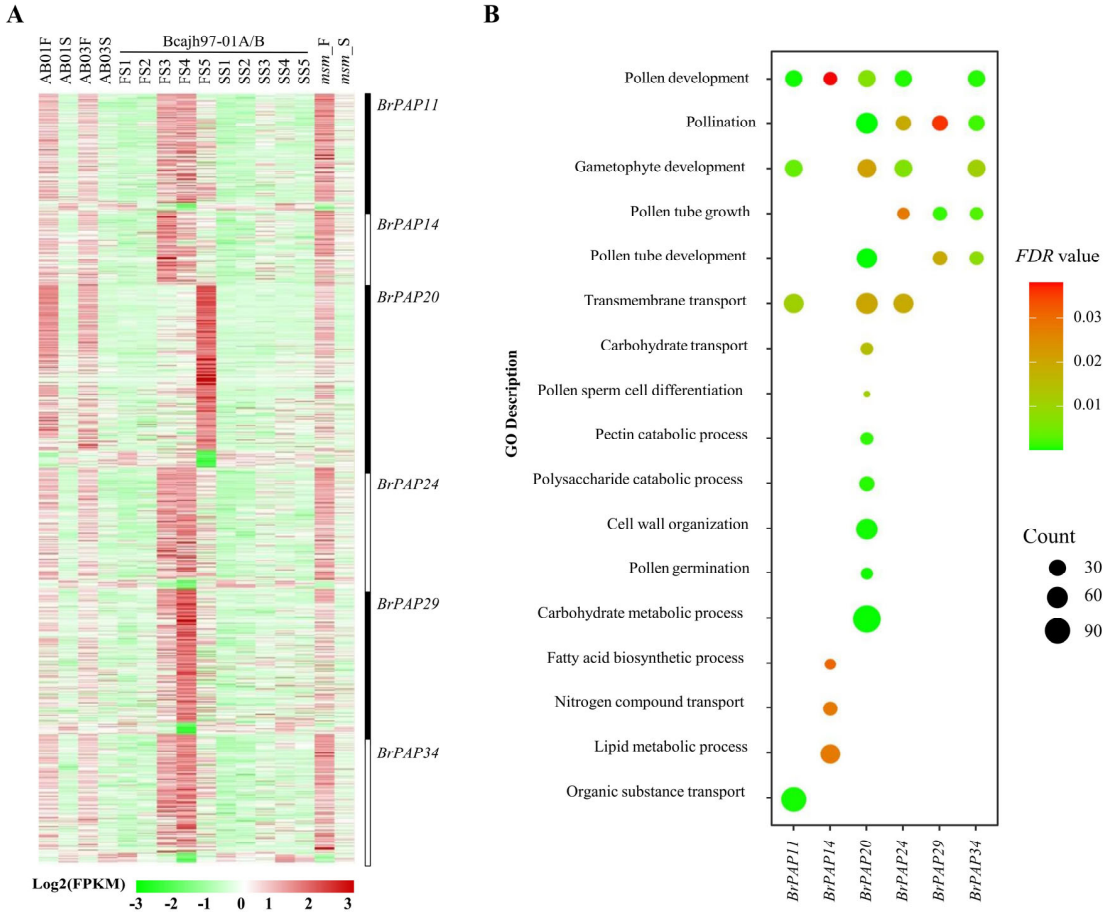


Figure 6. Analysis of co-expression genes of *BrPAP11*, *14*, *20*, *24*, *29*, and *34*. (**A**), Heatmap representation for expression patterns of co-expressed genes of *BrPAP11*, *14*, *20*, *24*, *29*, and *34*. (**B**), GO enrichment analysis of co-expression genes of *BrPAP11*, *14*, *20*, *24*, *29*, and *34*.

3.4. Expression of BrPAP Genes in Response to Pi Deprivation in Brassica rapa

PAPs are considered to play an essential role in the processes of phosphorus foraging and recycling [5,14]. To identify the potential functions of *BrPAPs* related to phosphorus acquisition and utilization, germinated *B. rapa* seeds were sown in pots containing vermiculite and watered with Hoagland solution [28]. For Pi deprivation treatments, the KH_2PO_4 in Hoagland solution was replaced with KCl. After treatments, the expression patterns of *BrPAPs* and physiological characteristics were measured. With Pi deprivation treatment, the plant height, fresh weight, leaf area, and Pi-content were all significantly decreased, while the root–shoot ratio of biomass increased compared with the normal growth condition (Figure 7). The shoot and root parts of seedlings were sampled separately for qRT-PCR analysis. Using a Ct value below 35 and fold change above two as thresholds, 21 *BrPAPs* responded to Pi deprivation either in shoots or roots (Figure 8). Four genes were mainly upregulated in roots under Pi deprivation (Figure 8A). Three genes were upregulated both in shoots and roots under Pi deprivation (Figure 8B). One gene, *BrPAP12*, was preferentially upregulated by Pi deprivation (Figure 8C). Ten genes were induced by Pi deprivation in shoots (Figure 8D). Three genes were consistently upregulated in shoots (Figure 8E).

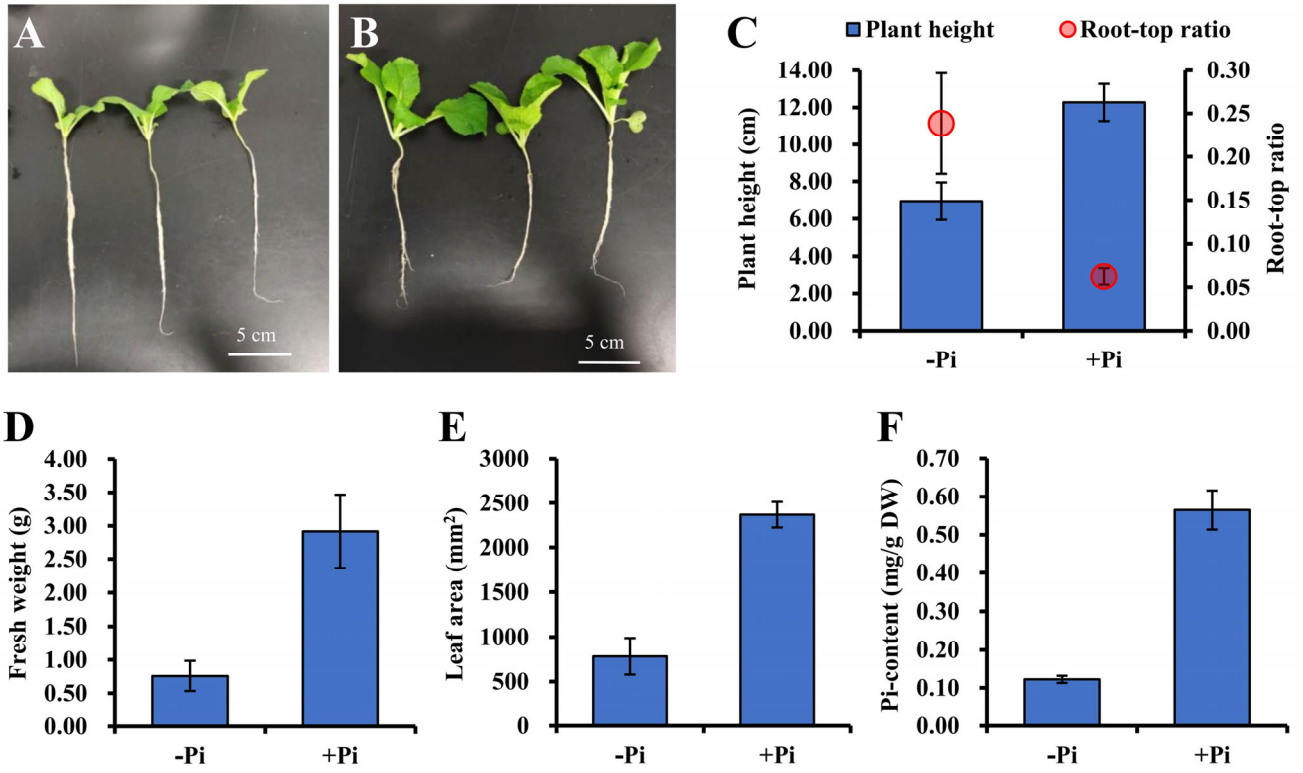


Figure 7. Phenotypical characteristics of Chiifu seedlings after 20 days of Pi deprivation treatment. (**A**,**B**) Picture representing Chiifu seedlings under –Pi or +Pi growth conditions. (**C**–**F**) Plant height, root–shoot ratio of biomass, fresh weight, leaf area, and Pi-content differences of Chiifu seedlings in –Pi or +Pi growth conditions.

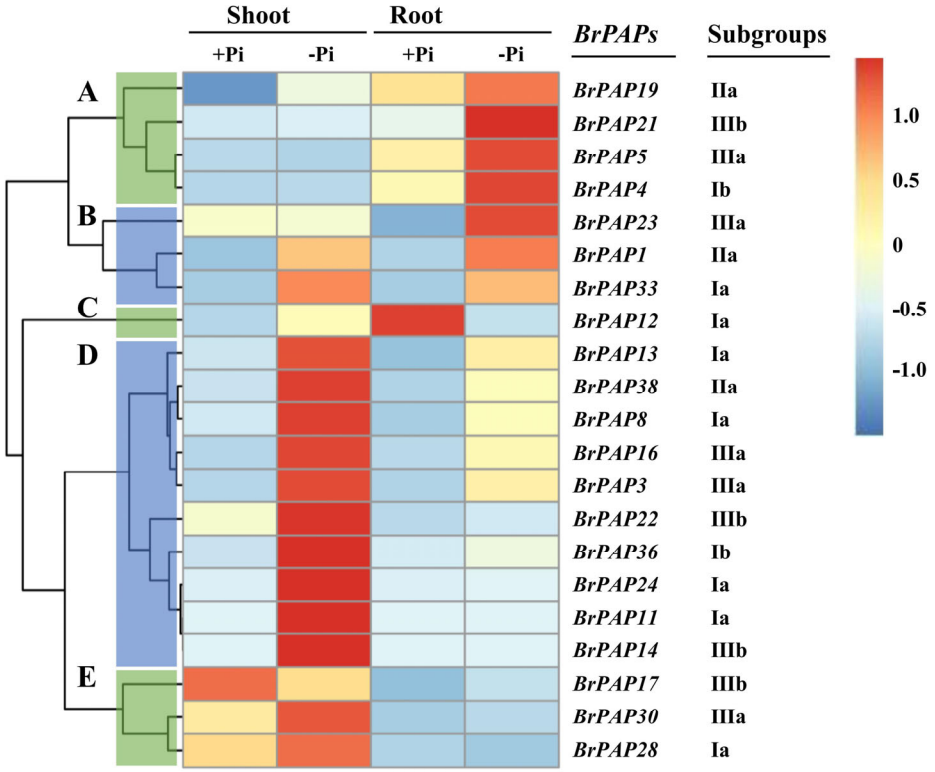


Figure 8. qRT-PCR analysis of expression patterns of *BrPAP* genes in response to Pi deprivation. (**A**) Genes upregulated in roots under Pi deprivation. (**B**) Genes upregulated both in shoots and roots in response to Pi deprivation. (**C**) Genes upregulated in roots under normal growth conditions. (**D**) Genes upregulated in shoots under Pi deprivation. (**E**) Genes upregulated in shoots both under normal and Pi deprivation conditions. The expression of *BrPAPs* was calculated using the $2^{-\Delta Ct}$ method and plotted using R with row normalization.

4. Discussion

4.1. Identification and Analysis of BrPAPs

Based on conserved sequences and motifs, genome-wide analysis of *PAPs* has been performed on many plants, including *Arabidopsis* [2], soybean [51], maize [12], tea [15], rice [11], *Brassica napus* [10], and ten vegetable species [9]. In this study, 39 BrPAPs were identified and classified into three different clades with nine subgroups based on a phylogenetic analysis using PAPs from *Arabidopsis* and *B. rapa* (Figure 2). Previously, 37 *BrPAPs* were identified in *B. rapa* [9], which could be explained by the fact that a higher genome version was used [32], leading to significantly higher accuracy of identification of *BrPAPs* [9]. The classification results in our study are mostly consistent with previous results from *Arabidopsis* and rice [2,11]. AtPAP26 from Ia-2 clustered into Ia-3 with BrPAP28 (Figure 2) [2]. Due to the whole-genome duplication events and gene loss events that have occurred in *B. rapa* over evolutionary time, one *Arabidopsis* gene usually has one to three *B. rapa* homologs [32], and the results from this study also demonstrated this observation except for AtPAP10. Based on our phylogenetic analysis, AtPAP10 corresponded to five BrPAPs (BrPAP2, BrPAP8, BrPAP9, BrPAP10, and BrPAP33), indicating the functional expansion of AtPAP10 (Figure 2). Consistent with studies in maize and rice, only one single gene, BrPAP28, was identified to be the ortholog of AtPAP26 in subgroup Ia-3, indicating its highly conserved function during evolution (Figure 2) [12].

Based on the results from *Arabidopsis*, rice, maize and other plants, some PAPs lack the invariant amino acid residues involved in the coordination of the di-metal nuclear center of known PAPs [2,11,12]. In *B. rapa*, 31 of 39 BrPAPs contained seven invariant amino acid residues (Table 1). The first block was absent in BrPAP14, BrPAP17, and BrPAP21. BrPAP9 did not possess the fourth and fifth blocks, and the fifth block was also lost in BrPAP7.

The third invariant amino acid residue Y shifted to T or F in BrPAP17, BrPAP21, BrPAP22, BrPAP32, and BrPAP39. In BrPAP7, the fourth and fifth conserved residues N and H were replaced by E and S, respectively. Compared with *Arabidopsis*, all BrPAPs had *Arabidopsis* homologs except for BrPAP9, and there was no conserved block absence in *Arabidopsis* (Tables 1 and 2) [2]. This indicates that these atypical BrPAPs might not be biochemically active PAPs and their true functions need further study.

4.2. BrPAPs and Pollen Development

Previous studies have suggested that *PAPs* play roles in the processes of carbon metabolism [25], cell wall synthesis [26], ROS metabolism, and response to salt stress [27]. Little is known about their functions related to pollen development, even for *AtPAP6*, *11*, *14*, *19*, *23*, *24*, and *25*, which are predominantly expressed in flowers [4]. In this study, *BrPAP11*, *14*, *20*, *24*, *29*, and *34* were specifically expressed in fertile floral buds in *B. rapa* (Figures 4 and 5). Combining the differences between fertile and sterile floral buds in the current and previous studies (Figure 5A) [30], in addition to our semi-RT-PCR results, we concluded that these *BrPAPs* might function and play different roles during the process of pollen development (Figure 5B). In support of this hypothesis, *BrPAP11* was initially expressed in F2 floral buds and highly expressed in F3 floral buds, and its co-expressed genes were involved in the biological processes of organic substance transport, transmembrane transport, gametophyte development, and pollen development. This suggested that *BrPAP11* might be associated with nutrition transport during pollen development (Figure 5B). *BrPAP14* was specifically expressed in F2 and F3 floral buds, and the biological processes of lipid metabolic, nitrogen compound transport, fatty acid biosynthetic, and pollen development were enriched in its co-expressed genes (Figures 5B and 6B). This implies that *BrPAP14* might be involved in secondary metabolism during pollen development. The expression levels of *BrPAP20* were high in F4 floral buds (containing mature pollen grains), and carbohydrate metabolic, polysaccharide catabolic, and pectin catabolic were enriched in its co-expressed genes (Figures 5B and 6B), suggesting that this gene might have functions during pollen maturation. The normal processing of cell wall degradation and reorganization are required for the development and functions of pollen grains [30,41]. In tobacco, *NtPAP12* were found binding to the cell wall and enhanced the activities of cellulose and callose synthases [26]. The highlighted cell wall organization process by *BrPAP20* and its co-expressed genes indicate that the functions of *BrPAP20* might have similar functions during pollen development to *NtPAP12* (Figure 6B). Even *BrPAP24* had similar expression patterns to those of *BrPAP11*, and pollination and pollen tube growth were only enriched by *BrPAP24* and its co-expressed genes. The processes of transmembrane transport, gametophyte development, and pollen development were enriched by the co-expressed genes of *BrPAP11* and *BrPAP24*. This observation suggests that the functions of *BrAPA11* and *BrPAP24* partially overlap. A similar phenomenon was observed for *BrPAP14* and *BrPAP29*. For *BrPAP34*, the biological processes of pollen development, pollination, pollen tube growth, and pollen tube development were revealed by its co-expressed genes based on GO enrichment analysis, highlighting its possible roles during pollen germination and pollen development. Previously, *AtPAP5* and *11* were highly expressed in anthers and involved in pollen tube growth processes [28]. In this study, *BrPAP11*, *BrPAP24*, *BrPAP34*, *AtPAP11*, and *AtPAP5* were classified into subgroup Ia-1, indicating the importance of subgroup Ia-1 during pollen tube growth. Phytic acid is one of the most important components of pollen, *AtPAP15* and *AtPAP23* were expressed in pollen or floral buds with phytase activity in *Arabidopsis* [4,18], indicating the specifically expressed *BrPAP11*, *14*, *20*, *24*, *29*, and *34* in fertile floral buds might have similar functions. Collectively, these analyses suggest that *BrPAP11*, *14*, *20*, *24*, *29*, and *34* have multiple functions during pollen development with some overlap. These findings will assist future molecular breeding for GMS in *B. rapa*.

4.3. Responses of Brassica rapa and BrPAPs under Pi Deprivation Conditions

Phosphorus is one of most important essential nutrients for plant growth and is involved in many metabolic processes such as respiration and photosynthesis [12]. However, the content of inorganic phosphorus (Pi) is lower than that required for plant growth in most soils, and most phosphorus is present in the Po (organic phosphorus) form or is fixed by iron, calcium, and other elements. This means that plants cannot directly utilize it [52]. APases can enable plants to use intracellular and extracellular organic phosphorus to survive in low Pi conditions [5]. Among the plant APases, PAPs are one of the most important classes and they can be induced by low Pi conditions [53]. In *Arabidopsis*, several *AtPAPs* can be induced by Pi deprivation such as *AtPAP10*, *11*, *12*, *17*, and *26* [2,17,54]. In rice, the overexpression of *OsPAP10a* and *OsPAP10c* can improve the utilization of external organic phosphorus, and both of these can be induced by Pi starvation [21,22,24]. In the current study, 21 of the 39 *BrPAPs* were found to be responsive to Pi deprivation and these can be divided into five groups (Figure 8). The first group showed a high expression in roots under Pi deprivation, including *BrPAP4*, *5*, *19*, and *21* (Figure 8A). *BrPAP1*, *23*, and *33* were clustered into a second group and their expression patterns were higher in both shoots and roots under Pi deprivation compared with normal growth conditions (Figure 8B). The third group contained only one gene, *BrPAP12*, which was upregulated in roots in normal conditions and shoots with Pi deprivation (Figure 8C). Most of the Pi deprivation-responsive genes were clustered into the fourth group, which included ten *BrPAPs* (*BrPAP3*, *8*, *11*, *13*, *14*, *16*, *22*, *24*, *36*, and *38*); these genes were predominantly upregulated in shoots under Pi deprivation (Figure 8D). *BrPAP17*, *28* and *30* were classified into a fifth group and were upregulated in shoots in both normal and Pi deprivation conditions (Figure 8D). In previous studies, *AtPAP10* was specifically induced by Pi limitation on the root surface and was considered to be one of the major Pi starvation-induced APases in *Arabidopsis* [17,55]. Among *BrPAPs*, five homologs of *BrPAP10* were found and only two of them, *BrPAP8* and *BrPAP33*, were Pi deprivation-responsive genes (Figures 2 and 8), indicating the functional expansion of the *AtPAP10* group during evolution. The expression levels of *AtPAP12* increased in low phosphate conditions in *Arabidopsis*, and its homologs, *BrPAP12* and *BrPAP13*, were also induced in shoots under Pi-deprivation (Figures 2 and 8) [2]. *AtPAP26* functions as a dual-targeted PAP and is the predominant intracellular APase as well as a major secreted APase in Pi limited conditions [19]. *BrPAP28*, the homolog of *AtPAP26*, was only upregulated in shoots in Pi deprivation, indicating that its function had shifted compared with *AtPAP26* (Figures 2 and 8E). Future studies exploring BrPAP responses to Pi deprivation will provide insights into the functions of different *BrPAPs* and novel gene sources for engineering crops with increased tolerance to Pi deprivation.

5. Conclusions

We systematically identified and analyzed the *BrPAP* genes from the *B. rapa* genome at the genome-wide level. A total of 39 *BrPAPs* were identified and divided into three major clades with nine subgroups. Expression profiles revealed that all *BrPAPs* were expressed in all examined tissues, thus indicating their various functions under standard conditions. Interestingly, *BrPAP11*, *14*, *20*, *24*, *29*, and *34* were specifically expressed in fertile floral buds, indicating their critical roles during pollen development. Their potential functions were further highlighted by co-expression analysis. Twenty-one *BrPAPs* responded to Pi deprivation in either shoots or roots. Further studies are required to determine the functions of *BrPAPs*. The information presented here provides a foundation for future investigations to gain a deeper understanding of the roles of each *BrPAP* as well as the molecular mechanisms underlying pollen development and low Pi tolerance.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/horticulturae7100363/s1, Table S1: List of primers used in this study, Table S2: List of genes co-expressed with *BrPAP11*, *14*, *20*, *24*, *29*, and *34*, with a Pearson's correlation coefficient (PPC) threshold value set between 0.60 and −0.60.

Author Contributions: Conceptualization, Y.C.; data curation, Y.W., J.G. and X.D.; formal analysis, Y.C.; funding acquisition, X.D.; project administration, X.D.; supervision, X.D.; validation, J.Q., C.L., K.M., B.J., X.Y. and W.H.; writing—original draft, Y.C.; writing—review and editing, J.G. and X.D. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the National Science Foundation of China (31601771), the Applied Basic Research Project of Yunnan (202001BB050017 and 202101AT070016), and the Yunnan University Innovation Fund for Graduate Students (2019175 and 2020324).

Acknowledgments: We thank LetPub (www.letpub.com accessed on 2 July 2021) for the linguistic assistance and pre-submission expert review.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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Characterisation and evaluation of interspecific cross-incompatibility in two *Delphinium* species

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ABSTRACT

Delphinium yunnanense is an endemic species in the Yunnan Province of China, while *Delphinium elatum* 'Guardian Blue' is widespread as cut flowers in the world. They all bloom attractive blue flowers, which are a crucial ornamental trait for floriculture. In this experiment, the cross-compatibility between *D. yunnanense* and *D. elatum* was characterised and evaluated. The results showed that a strong cross-incompatibility was observed in *D. yunnanense* × *D. elatum*, resulting in hybrid sterility. The F_1 hybrid generation produced neither fruits nor seeds. The cross-incompatibility of the hybrids resulted from the disturbed structure of the pollen tubes penetrating pistil style and callose deposits on stigma and in style. Moreover, the germination rate of *D. elatum* × *D. yunnanense* F_1 seed was lower than their own allogamic crosses, although there was no difference in fruit set rate. The results confirmed the cross-incompatibility between *D. yunnanense* and *D. elatum*, suggesting that *D. elatum* is more suitable as a maternal parent for hybridisation breeding. Our study characterised and evaluated the interspecific cross-compatibility through the pollen–stigma interaction and the reproductive system in *Delphinium*, which could provide the basis for the development and utilisation of potential ornamental traits in *Delphinium*.

Keywords: allogamic cross, compatibility, *Delphinium*, pollen-stigma interaction, reciprocal cross, reproduction

INTRODUCTION

The genus *Delphinium*, a member of the Ranunculaceae family, is widely distributed in the temperate regions of the Northern Hemisphere (Malyutin, 2001). *Delphinium* comprises about 350 species of perennial flowering plants, of which 173 species have been described in China (Wang and Warnock, 2001). In particular, most of the species are concentrated in Southwest China, which is one of the most important distribution centres and covers most of the morphological primitive representatives (Yuan and Yang, 2008). Yunnan Province, known as the 'Kingdom of Plant', is a centre of species diversity and endemism of *Delphinium*, consisting of 49 species and 13 varieties (Yuan and Yang, 2019). According to reports, there are 24 species with a high ornamental value that has potential for development and utilisation, such as *D. yunnanense* and *D. grandiflorum* (Guan and Li, 2002). Moreover, *Delphinium* is widely cultivated in the world for a long time, that is, *Delphinium elatius flore plena* was cultured by J. Parkinson in Western Europe as early as 1640 (Legro, 1961). In floriculture, the most important hybrid species is *D. elatum*, which developed as a series of 4,000 named cultivars from hundreds of different breeders and growers in the past few decades (Royal Horticultural Society, 1949). Besides, through hybridisation, mutagenesis, chemical and radiation treatment of different species within the

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genus *Delphinium*, a wide range of flower colours from white, yellow, red, violet to blue were introduced into the new cultivars as well as improved quality of cut flower, mildew resistance and toughness of the stems (Mehlquist et al., 1943; Wegulo and Vilchez, 2008; Chen et al., 2011; Lin et al., 2014). In addition to the ornamental purposes of showy flowers, the plants of *Delphinium* contain various alkaloids with spectacular medicinal values, such as diterpenoid alkaloids, trichodelphinines and flavonoids, which increase the utilisation and economic value of *Delphinium* (Chen et al., 2011; Agnihotri et al., 2014; Lin et al., 2014).

Delphinium yunnanense (*D. yunnanense*) is an endemic species in the Yunnan Province of China with blue flowers, which is considered a precious ornamental germplasm to be developed. However, *Delphinium elatum* (*D. elatum*) 'Guardian Blue' is one of the most popular flowers in the world as an attractive high-grade cut flower. Both species are the potential candidates for developing new *Delphinium* varieties with blue flowers through hybridisation. The chromosome number and the ploidy level of many species and varieties in the genus *Delphinium* have been investigated (Legro, 1961; Yuan and Yang, 2008). Most of the wild species, including *D. yunnanense*, are diploids with 16 chromosomes ($2n = 2x = 16$), whereas *D. elatum* was tetraploid ($2n = 4x = 32$). An intraspecific difference in chromosome number was detected neither in diploid nor in tetraploid species (Legro, 1961; Yuan and Yang, 2008). Nevertheless, the breeders have had success in interploidy crosses using different *Delphinium* species. For example, the frequently used tetraploid cultivars of *D. elatum* were widely crossed with diploid species to form sterile triploid hybrids, which, after the colchicine treatment, became fertile, produced seeds and viable seedlings (Lewis et al., 1951; Samuelson, 1957; Tjebbes, 2010). Moreover, the reproductive system with pollen–stigma interactions has been previously investigated in *D. grandiflorum*, *D. cardinale*, *D. nudicaule*, *D. barbeyi* and *D. nuttallianum* and their interspecific hybrids (Honda et al., 1999; Williams, 2007; Elliott and Irwin, 2009; Briggs et al., 2016). Some studies have shown that the breeding system of *Delphinium* is facultative xenogamy or obligate outcrossing, accompanying with partly self-compatibility requiring pollinators (Price and Waser, 1979; Williams et al., 2001; Zhang et al., 2014). Self-incompatibility (0–50%) and autogamy (1–90%) in different degrees among different species in *Delphinium* have been reported (Macior, 1975; Varney, 1979; Powell and Jones, 1983; Bosch et al., 2001). To our knowledge, both of the studied species are selfing; however, no previous study concerned the cross-compatibility in the species of *D. yunnanense* and *D. elatum*.

In this study, we explored and investigated the interspecific cross-compatibility of *D. yunnanense* and *D. elatum* 'Guardian Blue' to establish the mating ability of these two *Delphinium* species, which could be helpful in developing new varieties of these ornamental plants.

MATERIALS AND METHODS

Plant material and growth conditions

D. yunnanense and a commercial cultivar of *D. elatum* 'Guardian Blue' were used in this study. The seeds of *D. yunnanense* used in this study were randomly collected in natural habitats (N25°7′33″, E102°45′31″) in Kunming, Yunnan Province at an altitude of 2,139 m. The seeds of *D. elatum* 'Guardian Blue' were obtained from the local flower market with 500 seeds in the same bag. A total of 100 seeds of each species were first germinated in the petri dish covering with a moist filter paper. Then, 50 seedlings of each species were separately transferred and planted in 100-well plant trays. After the root system filled the holes, the plants were separately planted in pots (diameter = 8 cm, height = 8 cm) filled with substrate (red soil:peat = 1:1) and grown in the solar greenhouse (without heating) at the experimental farm of Yunnan Agricultural University. The flowering dynamics were continuously observed when the plants begin to bloom.

Pollen viability and stigma receptivity

The examination of pollen viability was conducted with three plants of each species during anthesis based on *in vivo* germination method (He et al., 2017). In a full flowering stage, fresh pollen was collected every 2 h from three flowers per plant and was incubated for 4 h at 25°C in the liquid medium (5% sucrose, 150 mg · L^{-1} boric acid and 20 mg · L^{-1} $CaCl_2$) from 9:30 to 15:30. The total number of pollen grains and the number of germinating pollen grains were counted in three separate fields of view per slide under the light microscope. The percentage of germinating pollen was estimated by germinating pollen number/total pollen number × 100. The active pollen was identified by the length of the pollen tube longer than the diameter of pollen grain, otherwise recorded as inactive pollen (Rodriguez-Riano and Dafni, 2000).

Stigma receptivity was examined by the benzidine–hydrogen peroxide method (Li et al., 2018). During anthesis (from day 1 to day 8), three flowers at the full-open stage of each species were collected every day and were soaked in the benzidine–hydrogen peroxide solution (1% benzidine: 3% hydrogen peroxide: water = 4:11:22, v/v, Cato Research Chemicals, Inc.). The receptive stigma shows peroxidase activity and turns blue with a mass of bubbles surrounding it. The depth of the blue color and number of bubbles indicate the intensity of stigma receptivity.

Observation of pollen–stigma interaction

To observe the pollen–stigma interaction in interspecific hybridisation, the reciprocal crosses between *D. yunnanense* and *D. elatum* were conducted as well as intraspecific allogamic crosses. The *D. elatum* × *D. yunnanense* was treated as an orthogonal cross, and the *D. yunnanense* × *D. elatum* referred to reciprocal cross. To avoid self-pollination, the flowers were

emasculated and isolated by plastic bags until the anther dehiscence. This treatment was applied in each cross combination with a random selection of 10 plants (three flowers per plant). Before pollination, the stigma was examined by a magnifier for a secretion check. The pollen sac was then gently dipped onto the stigma with secretions using tweezers. Each stigma was pollinated several times to ensure a sufficient amount of pollen and was covered by bags after pollination.

The pistils of each cross were collected at 2, 4, 6, 24, 72 and 120 h after pollination and fixed in Carnoy's solution (ethanol: glacial acetic acid = 3:1, v/v, Shanghai Fusheng Industrial Co., Ltd.) for 24 h at 4°C and then stored in 70% of ethanol. The samples were then treated with 6 mol · L^{-1} NaOH for 12 h. After repeated rinsing with distilled water, the pistils were immersed in a 0.1% water-soluble aniline blue solution (dissolved in 0.1 mol · L^{-1} K_3PO_4, ScyTek Laboratories) for 24 h. The stained ovaries were then observed with Leika fluorescent microscope under ultraviolet light to investigate pollen adherence, pollen germination, callose formation and pollen tube growth. For each treatment, six pistils in three replications were used to calculate the average number of pollen grains.

Calculation of fruit setting and germination rate

Thirty flowers were used to evaluate the fruit setting for each cross. The number of mature fruits and seeds per fruit were counted, and the thousand kernel weight (TKW) was measured. The fruit-setting rate was calculated by the formula: the number of mature fruits/ the number of pollinated flowers × 100.

The F_1 seeds of each cross were germinated in a pre-sterilised culture dish (Guangzhou Jet Bio-Filtration Co., Ltd., TCD000060) on wet filter papers. The seeds were then cultivated in environmentally controlled growth chambers (LRH-400-GSI, 400 L; Zhujiang, China) under the condition of 12 h photoperiod at 25°C. The experiment was repeated in triplicate (100 seeds per repeat). The germination rate was calculated as germinated seeds/total seeds × 100, in which germinated seeds refer to the sprout length equal to or longer than the seed length.

Statistical analysis

Data analysis and statistics were performed using Microsoft Excel 2016 and Data Processing System (Tang and Zhang, 2013). One-way and two-way ANOVA with Tukey's HSD post-hoc test was used to compare multiple samples at the 5% significance level.

RESULTS

Stages of flower development

The inflorescence of *D. yunnanense* and *D. elatum* is racemose, in which the development of individual flowers is from bottom to top. Eight flowering stages could be identified in the two species throughout the entire flower developmental period, including flower bud stage, stem elongation stage, bud expansion stage, sepal open stage, petal open stage, flower semi-open stage, flower full-open stage, and anther cracked stage (Figure 1). The flower of *D. yunnanense* contains five large and bright bluish violet sepals, and the real petals are very small and hidden between the staminode and the upper sepal. Besides, there are two blue staminodes with yellow barbs on the ventral surface. For *D. elatum*, there are two layers of hyacinthine ovate sepals, and petals and staminodes are white. The flowering period of *D. yunnanense* was about 10 days, whereas the flowers of *D. elatum* can maintain a longer period of 15 days. This may be affected by the number of petals since *D. elatum* has double petal flowers.

Pollen viability and stigma receptivity

The pollen viability, estimated in pollen germination in vitro test, of *D. elatum* was significantly weaker than that of *D. yunnanense* during the day, and the diurnal variation displayed a trend of increase in the morning but decrease in the afternoon (Figure 2). Moreover, the pollen viability of *D. yunnanense* and *D. elatum* showed a peak at noon (11:30 AM) with germination ratio at 40.89% ± 15.65% and 18.78% ± 5.18%, respectively. This suggested that *Delphinium* is better to pollinated around noon, at least in these two tested species.

The observation of stigma receptivity revealed that the stigma of *D. yunnanense* was not receivable at the beginning of the flowering period, but displayed a positive reaction with pollen from 6th day to 8th day (Table 1). This positive reaction was strengthened at the 7th day of flowering and then declined until the end of the measurement. In contrast, the stigma receptivity of *D. elatum* presented one day earlier than that of *D. yunnanense* and had a strong receptivity the next day. However, the stigma receptivity of *D. elatum* had a shorter duration than that of *D. yunnanense*, since it became only partial receivable at the 7th day of flowering.

Pollen–stigma interaction

The number of pollen grains adhering to the surface of stigma and the germination rate was measured to reflect and evaluate the cross-compatibility of each cross combination.

The results showed that pollination time (F = 73.3260, p = 0.0000) and cross combination (F = 110.5710, p = 0.0000) had significant effects on the number of attached pollen grains in *Delphinium*, and their interaction was significant as well (F = 18.9170, p = 0.0000) (Table 2). The number of attached pollen grains significantly increased at 24 h after pollination in four crosses. The number of attached pollen grains of *D. yunnanense* was significantly higher than that of *D. elatum* after pollination on any type of stigma (Figure 3). This indicated that the pollen survival rate of *D. yunnanense* was higher than that of *D. elatum*, which was in line with the result of pollen viability. However, there was no statistically significant difference in the

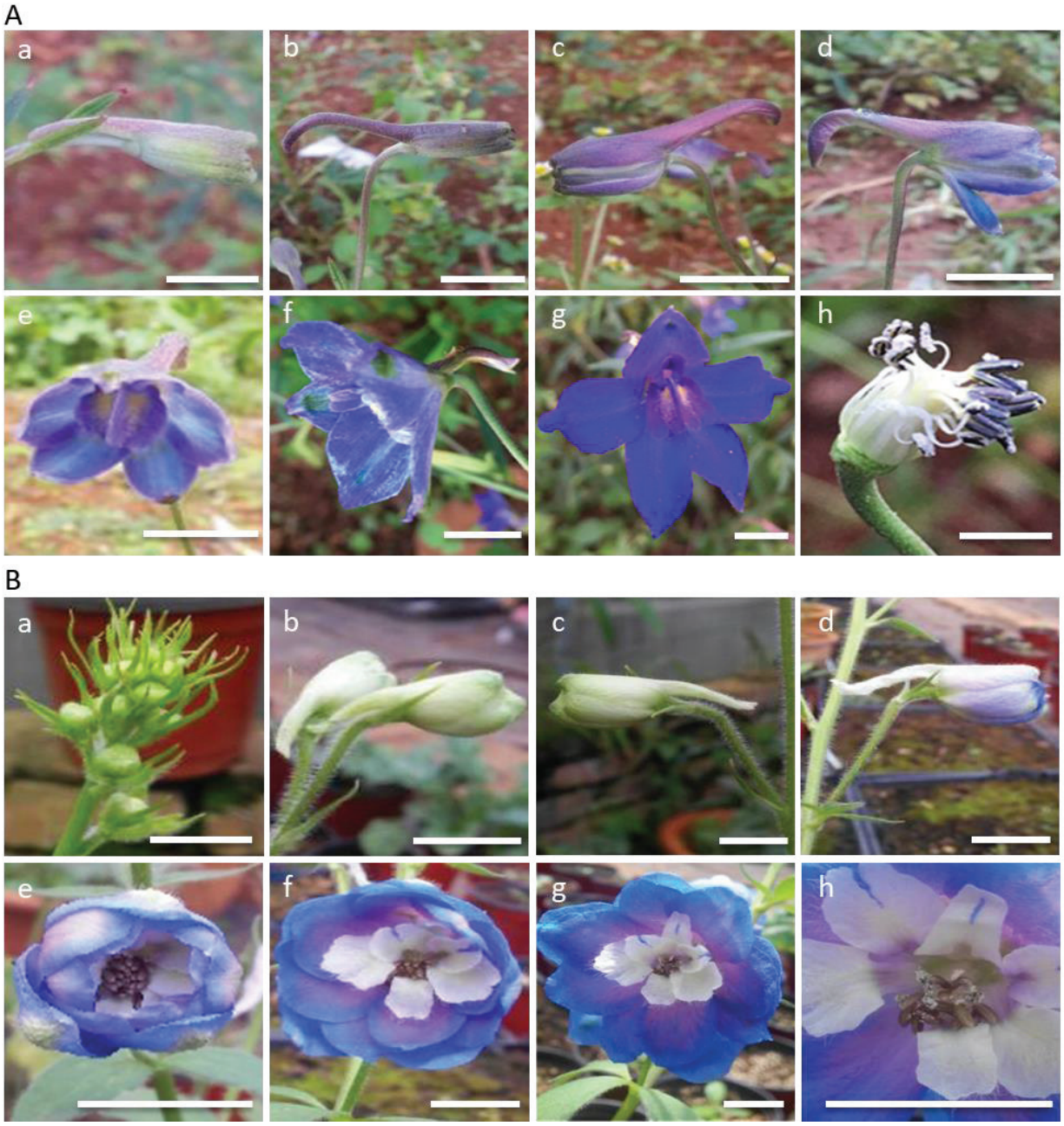


Figure 1. Stages of flower development of *D. yunnanense* (A) and *D. elatum* 'Guardian Blue' (B). Bud stage (a), stem elongation stage (b), bud expansion stage (c), sepal open stage (d), petal open stage (e), flower semi-open stage (f), flower full-open stage (g) and anther cracked stage (h). Bar = 1 cm.

number of pollen grains attached to the stigma between interspecies crosses of *D. elatum* × *D. yunannense* and intraspecific crosses of *D. yunanense*, similarly as in the cross of *D. yunannense* × *D. elatum* and intraspecific crosses of *D. elatum*.

From the overview of the dynamics of attached pollen grains in four crosses, some pollen grains had been attached to the stigma after 2 h of pollination (Figure 3). The number of attached pollens in the orthogonal cross (*D. elatum* × *D. yunnanense*) was higher than that of other three crosses, which were at the same low level in the early period after pollination. This trend continued until 6 h after pollination, in which the attached pollen grains of *D. yunnanense* allogamic cross (*D. yunnanense* × *D. yunnanense*) dramatically increased over ten-fold (from 10.67 ± 1.53 to 118.67 ± 11.02, $n = 3$). The same result as multiple comparisons, the attached pollens reached the peak after 24 h of pollination with a significant difference in each cross combination. Then, the attached pollens gradually decreased, except for that of *D. yunnanense* allogamic cross showing a sharp decrease.

The frequency of germinated pollen grains in different crosses after pollination displayed the same

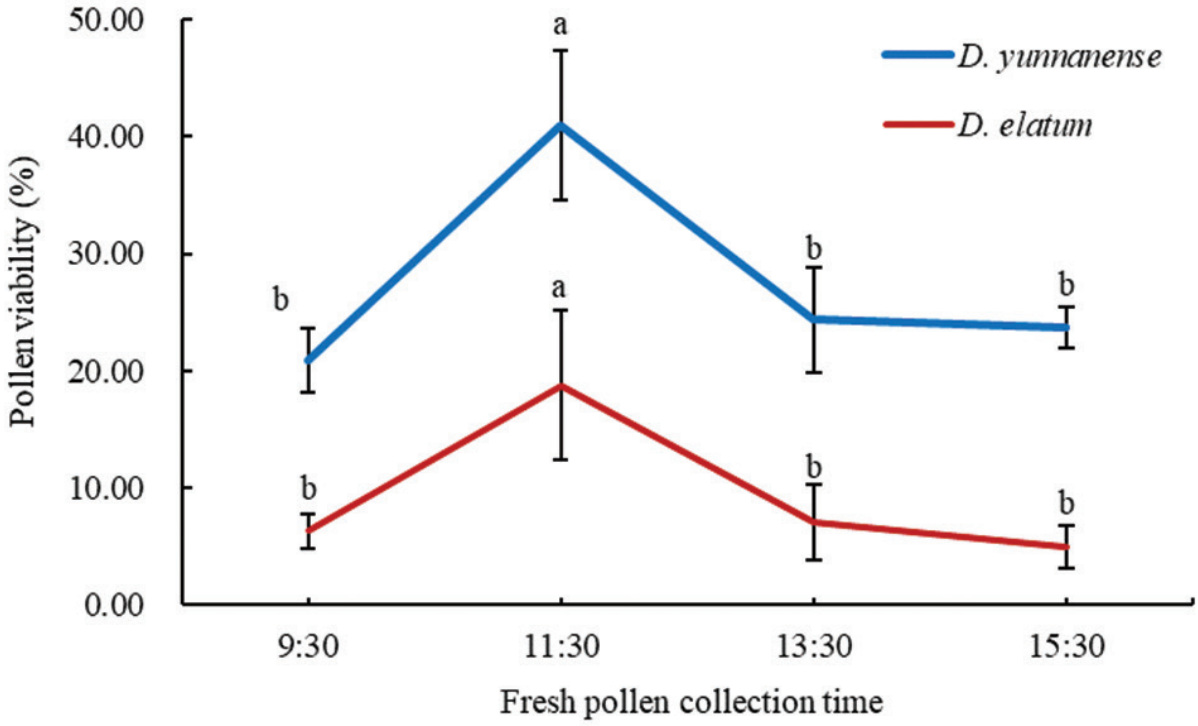


Figure 2. The diurnal variation of pollen viability in *D. yunnanense* and *D. elatum*. One-way ANOVA was used to assess statistical significance, and *p* values were calculated with Tukey's HSD test ($\alpha = 0.05$). Multiple comparisons indicated by small letters were performed in different collection time within the same species. The data represent the mean value ± standard deviation from three replicates.

Table 1. Stigma receptivity of the full-open stage during the flower opening process in *D. yunnanense* and *D. elatum*. '−', stigma not receivable; '±', partial positive reaction; '+', positive reaction; '++' strong positive reaction.

Species	1st day	2nd day	3rd day	4th day	5th day	6th day	7th day	8th day
D. yunnanense	−	−	−	−	−	+	++	+
D. elatum	−	−	−	−	+	++	±	−

The data were obtained from 10 flowers.

Table 2. Results of statistical analysis about pollination time and cross combination on the number of attached pollen grains in *Delphinium*.

Source of variation	Sum of square	Degree of freedom	Mean square	*F* value	*p* value
Pollination time	27,746.7778	5	5549.3556	73.3260	0.0000
Cross combination	25,104.2778	3	8368.0926	110.5710	0.0000
Time × Combination	21,474.2222	15	1431.6148	18.9170	0.0000
error	3632.6667	48	75.6806		
Total variations	77,957.9444	71			

trend as the attached pollen grains in the corresponding cross combination (Figure 4). There was also a significant interaction of pollination time and cross combination on the germinated pollen number revealed by the multiple comparisons ($F = 6.0460$, $p = 0.0000$, Table 3). Moreover, the number of germinated pollen grains reached its peak 24 h after pollination. The frequency of germinated pollen of *D. yunnanense* was significantly higher than that of *D. elatum* in four crosses. This was in line with the variation of attached pollens, indicating that the amount of germinated pollen grains was positively correlated with the amount of attached pollens in the species of *Delphinium*.

Dynamics of pollen tube growth in different cross combinations

In the orthogonal cross (*D. elatum* × *D. yunnanense*), most of the pollen grains germinated on stigma 2 h

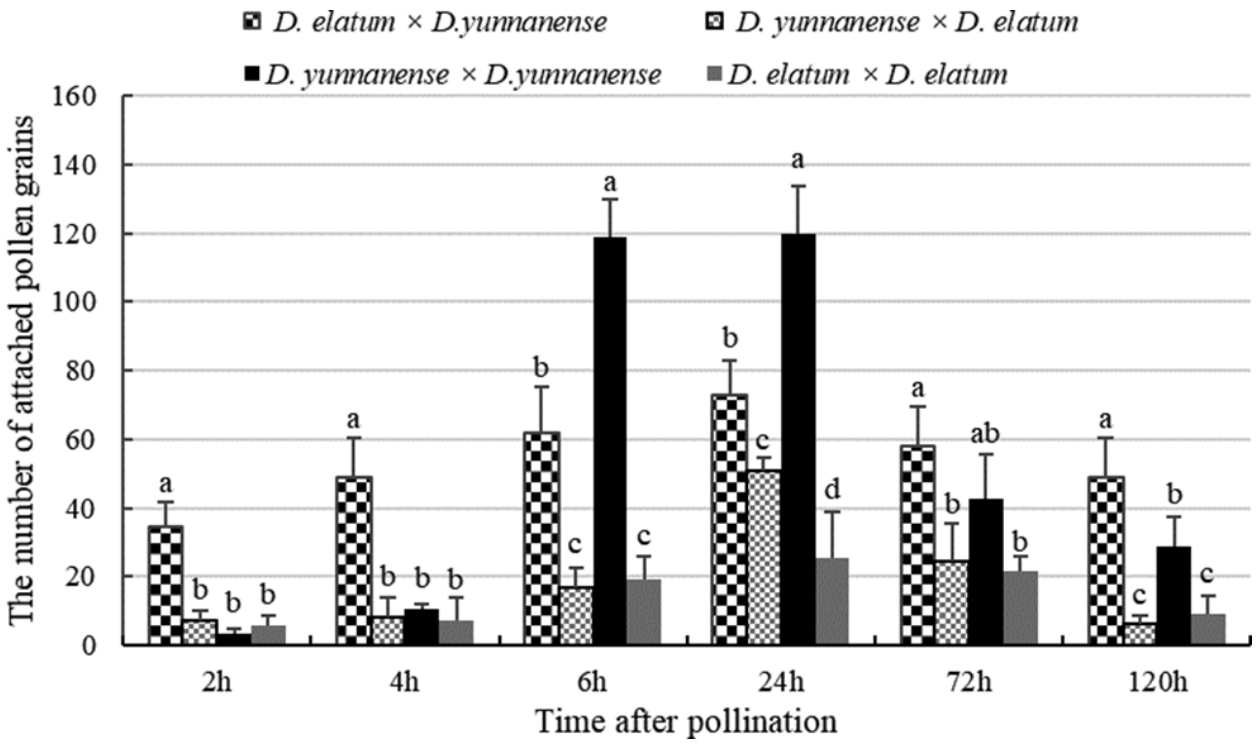


Figure 3. The number of attached pollen grains of each cross combination after different pollination time. One-way ANOVA was used to assess statistical significance, and *p* values were calculated with Tukey's HSD test ($\alpha = 0.05$). Multiple comparisons indicated by small letters were compared in different cross combinations within the same time after pollination. The data represent the mean value ± standard deviation from three replicates.

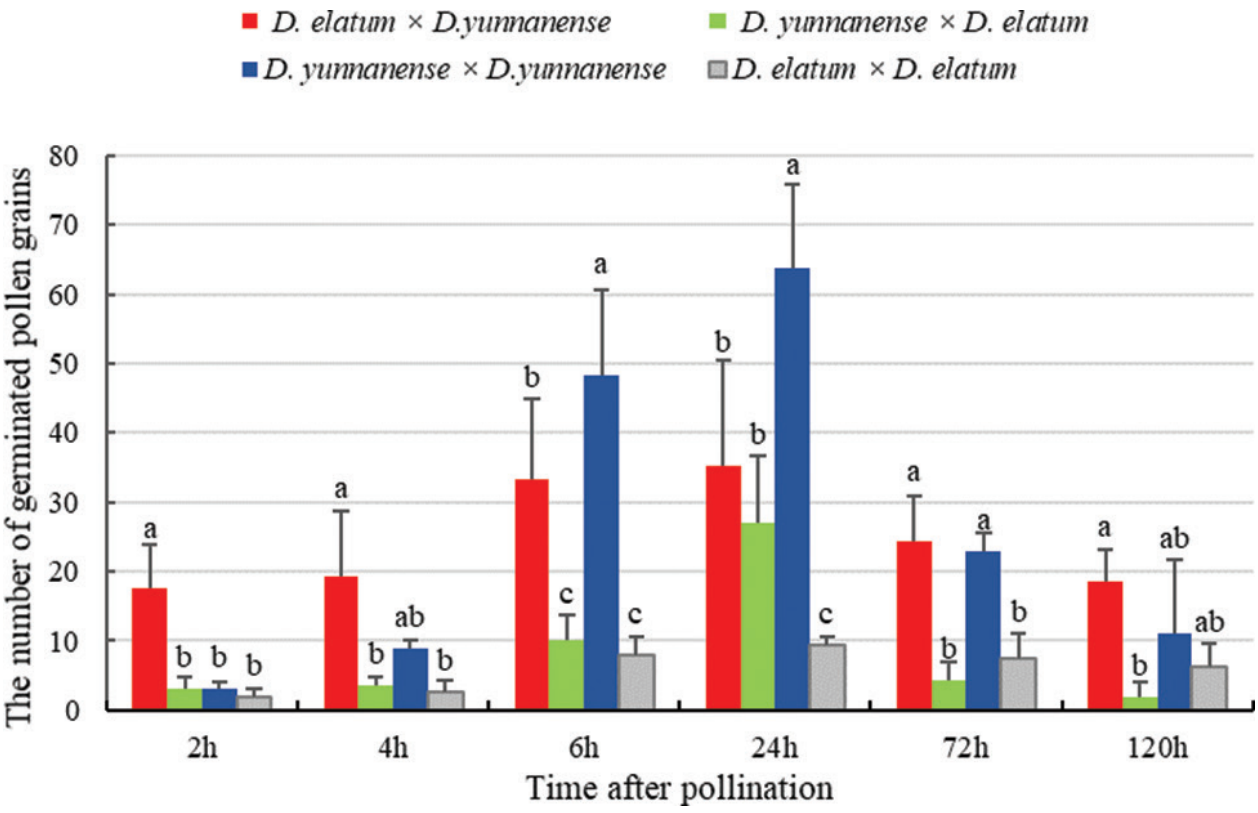


Figure 4. The number of germinated pollen grains on each cross combination after different pollination time. One-way ANOVA was used to assess statistical significance, and *p* values were calculated with Tukey's HSD test ($\alpha = 0.05$). Multiple comparisons indicated by small letters were compared in different cross combinations within the same time after pollination. The data represent the mean value ± standard deviation from three replicates.

Table 3. Results of statistical analysis about pollination time and cross combination on the number of germinated pollen grains in *Delphinium*.

Source of variation	Sum of square	Degree of freedom	Mean square	*F* value	*p*-value
Pollination time	7034.9028	5	1406.9806	29.7860	0.0000
Cross combination	6178.2639	3	2059.4213	43.5980	0.0000
Time × Combination	4283.8194	15	285.5880	6.0460	0.0000
error	2267.3333	48	47.2361		
Total variations	19,764.3194	71			

after pollination, showing pollen tubes entered the style and few callose deposited in the style (Figure 5A). After 4 h, callose depositions appeared in large number in the style, some pollen tubes had entered the ovary at this stage. Six hours after pollination, pollen tubes arrived near the ovule and started to bend and twine. Finally, pollen tubes entered the ovule 24 h after pollination. In the reciprocal cross, pollen tube growth differs that in the orthogonal cross (Figure 5B). Only a small amount of pollen grains germinated on the stigma, whereas a large amount of callose deposition was visible in the style 2 h after pollination. Four hours after pollination, pollen tubes twined, knotted and spiraled, accompanied by callose formation. As a result, only a small number of pollen tubes reached the ovule 24 h after pollination, but none of them entered the ovule as observed.

In allogamic crosses of *D. yunnanense*, pollen tubes entered the ovule 24 h after pollination (Figure 5C). However, in the allogamic cross of *D. elatum*, a small amount of pollen germinated and callose deposition appeared 2 h after pollination. Six hours after pollination, the pollen tubes were visible in the style and reached the ovary 24 h after pollination (Figure 5D). Three days after pollination, the pollen tube entered the ovule. The pollen of *D. yunnanense* displayed a significantly strong interaction with stigmas of *D. yunnanense* and *D. elatum*, indicating that the pollen activity of *D. yunnanense* was stronger than that of *D. elatum*, which was in line with the results of pollen viability. It also suggested that *D. elatum* is more suitable as a maternal parent for hybridisation breeding.

Fruit and seed setting of F1 hybrids

After crossing, the fruit set rate was evaluated in each cross according to the between the fruit number and the number of previously pollinated flowers. The results showed differences in fruit set rate between the orthogonal and reciprocal cross of *D. yunnanense* and *D. elatum* (Table 4). In the orthogonal cross (*D. elatum* × *D. yunnanense*), the fruit set rate was the highest (90.0%) and generated 17.1 seeds per fruit. In contrast, there was no seed obtained in the reciprocal cross. This was consistent with the observation of microscopic dynamics that no pollen tube was found entering the ovule of *D. yunnanense* × *D. elatum*. On the other hand, there was no significant difference in fruit set rate between the allogamic crosses of *D. yunnanense* and that of *D. elatum*, showing the rate of 86.7% and 83.3%, respectively. Moreover, the seed number per fruit of *D. elatum* in allogamic cross was the highest, suggesting that there were no disturbances in the sexual reproductive system of *D. elatum* since both male and female lines were fertile. Therefore, the cross-incompatibility probably caused the sterility of *D. yunnanense* × *D. elatum* F_1 hybrids. Furthermore, the seed germination rate of *D. elatum* allogamic cross was the highest, while seeds derived from the cross of *D. elatum* × *D. yunnanense* displayed the lowest germination rate of 16.7% as well as the lowest thousand kernel weight. This may relate to the formation of abnormal seeds detected in the orthogonal cross of *D. elatum* × *D. yunnanense*.

DISCUSSION

The genus of *Delphinium* contains several species with a high ornamental value, which is crucial for the development of new floricultural varieties. In this study, we investigated the cross-compatibility of two *Delphinium* species, *D. yunnanense*, a wild endemic species of Yunnan province and a commercial variety *D. elatum* 'Guardian Blue'. Strong cross-incompatibility was observed in the cross of *D. yunnanense* × *D. elatum*, whereas less cross-incompatibility was detected in the cross of *D. elatum* × *D. yunnanense*, which provided a better understanding of the hybridisation affinity in *Delphinium*.

The measurement of pollen viability has an important theoretical and practical significance in hybrid breeding. The level of pollen viability affects the success and efficiency of hybrid breeding. Therefore, it is essential to measure the pollen viability before hybridisation, which could provide a useful reference for the formulation of hybrid combinations. Our results showed that the diurnal variation of pollen viability displayed a trend of increased in the morning but decreased in the afternoon in both *D. yunnanense* and *D. elatum*. This was consistent with previous independent observations of *D. grandiflorum*, in which the pollen viability increased at the early stage and decrease later in daily variation (from 9:30 to 15:30) with a peak at 11:30 AM (Zhang et al., 2014). Moreover, in *Lilium davidii* var. unicolor,

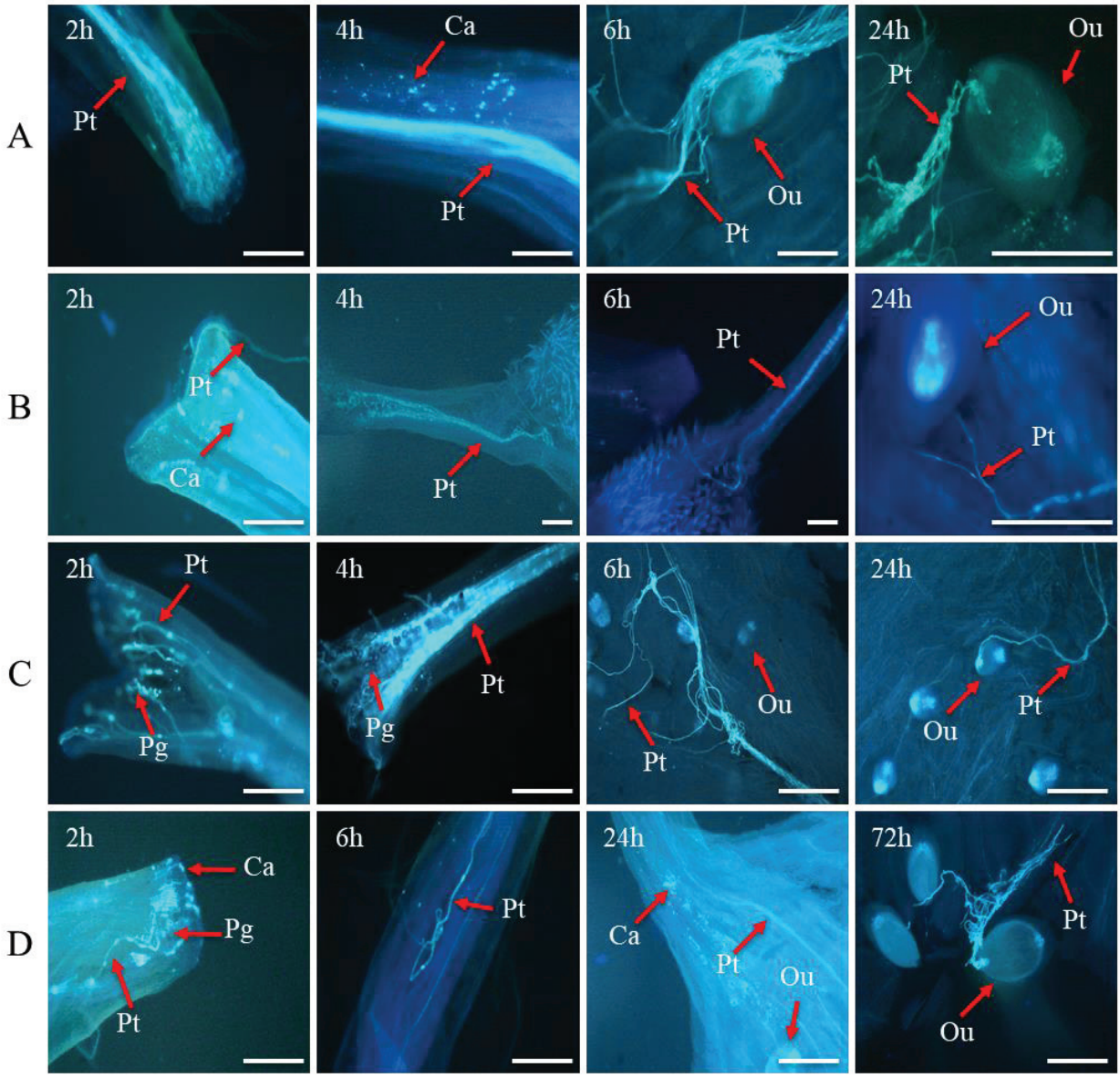


Figure 5. Dynamics of pollen tube growth of the interspecific and allogamic crosses between *D. yunnanense* and *D. elatum*. **A:** *D. elatum* × *D. yunnanense*. A-2h: A bundle of pollen tubes (Pt) in the style of pistil, pollen grains (Pg) visible on stigma. A-4h: Large number of callose (Ca) depositions appeared in the style. A-6h: Pollen tubes arrived near the ovule (Ou) and started to bend and twine. A-24h: Pollen tubes entered the ovule. **B:** *D. yunnanense* × *D. elatum*. B-2h: Few pollen grains germinated on the stigma with a large amount of callose depositions visible in the style. B-4h: The twined, knotted and spiraled pollen tubes with callose. B-6h: Six hours after pollination, the state of pollen tube remains the same as 4 h after pollination. B-24h: Few pollen tubes were close to the ovule. **C:** *D. yunnanense* × *D. yunnanens*. C-2h: A number of pollen tubes in style of pistil, pollen grains also visible on stigma. C-4h: A bundle of pollen tubes grow along the style. C-6h: Pollen tubes arrived near the ovule. C-24h: Pollen tubes entered the ovule. **D:** *D. elatum* × *D. elatum*. D-2h: A small amount of pollen germinated and callose deposition appeared. D-6h: Pollen tubes were visible in the style. The pollen tubes were twined and knotted in the style. D-24h: Pollen tubes reached the ovary with visible callose deposition. D-72: Pollen tube entered the ovule. 'Pg' refers to pollen grain, 'Pt' means pollen tube, 'Ca' is the abbreviation of callose and 'Ou' represents ovule. Bar = 200 μm.

the highest pollen vigor appeared at 08:00–14:00 with the highest germination rate appeared at 10:00 AM (Sun et al., 2019). The pollen vigor of *D. yunnanense* was significantly higher than that of *D. elatum*. This is probably because the pollen vigor of *D. elatum* has decreased after numerous artificial selections during the process of breeding.

Furthermore, we investigated the pollen–stigma interaction between *D. yunnanense* and *D. elatum*. The results showed that the number of pollen grains attached or germinated of *D. yunnanense* were relatively higher in the reciprocal or allogamic crosses. This was in line with the large pollen quantity and high pollen vitality of *D. yunnanense*. Moreover, it was detected that no pollen

Table 4. Characteristics of F_1 seeds in the interspecific and allogamic cross of *D. yunnanense* and *D. elatum*.

Cross combination	Pollinated flowers number	Fruit number	Fruit set rate	Seed number	Seed number per fruit	Thousand kernel weight (g)	F_1 seed germination rate (%)
D. elatum × *D. yunnanense*	30	27	90.0%	463	17.1	1.3	16.7 ± 2.08 a
D. yunnanense × *D. elatum*	30	0	0.0%	/	/	/	/
D. yunnanense × *D. yunnanense*	30	26	86.7%	421	16.2	1.7	24.7 ± 2.52 b
D. elatum × *D. elatum*	30	25	83.3%	527	21.1	2.0	80.0 ± 4.00 c

One-way ANOVA was used to assess statistical significance, and *p* values were calculated with Tukey's HSD test ($\alpha = 0.05$).

tube entered the ovule in the reciprocal cross, suggesting that there was a strong cross-incompatibility in the reciprocal cross of *D. yunnanense* × *D. elatum*. This was related to the cell recognition of the pollen–stigma interaction, which could prevent pollen with genetic variation from germinating on stigma and entering into ovule (Bedinger et al., 2017). It was reported that pre-fertilisation obstacles of interspecific hybridisation in plants were mainly caused by the abnormal growth of pollen tubes, the callose formed on stigma surface and excessive growth of pollen tubes (Heslop-Harrison, 1982; Dumas and Knox, 1983). In our study, the abnormal growth of pollen tubes was observed in the cross of *D. yunnanense* × *D. elatum*, such as winded, knotted and spiraled pollen tubes. The formation of callose in stigma mastoid cells was also observed after pollination, which prevented the pollen tube from entering the ovule and consequently resulted in the lack of fertilisation and seed production. Moreover, barriers to reproduction are the most important factor for cross-incompatibility, which can be effective before (prezygotic barriers) and after (postzygotic barriers) the formation of a zygote (Pease et al., 2016). Prezygotic barriers are arguably the most important and effective barriers that contribute to reproductive isolation acting before hybrids formation (Henrich and Kalbe, 2016). In the cross of *D. yunnanense* × *D. elatum*, although some pollen grains germinated, the pollen tubes were arrested in the pistils and were unable to reach the ovules. Thus, the pre-fertilisation reproductive barriers are the main factor affecting the cross of *D. yunnanense* × *D. elatum*. This phenomenon was also observed in the crosses of different petal phenotypes in *Jasminum sambac* Aiton (Deng et al., 2017).

Although the fruit set ratio of *D. elatum* × *D. yunnanense* was the highest and plenty of F_1 seeds were generated, yet the germination ratio of F_1 seeds was really low. In addition, we did not harvest any seed or fruit from the cross of *D. yunnanense* × *D. elatum*. These results demonstrated that the incompatibility indeed existed between *D. yunnanense* and *D. elatum*. One possible theory is that the development of embryo was disturbed and generated immature seed with normal appearance but containing aborted embryos, which reduced the seed vitality (Zeng et al., 2010; Chai et al., 2011; Dai et al., 2014; Chong et al., 2018). This also indicated by the thousand kernel weight of F_1 seeds in *D. elatum* × *D. yunnanense*, which displayed the lowest thousand kernel weight. Moreover, the germination traits study of *D. chefoense* showed that the seed germination rate was about 38.0% and 7.3% under 20°C and 25°C, respectively (Qian, 2012). In our study, the seed germination rate of *D. yunnanense* was about 24.7% at 25°C. The similar low seed germination rate of the wild type in *Delphinium* species indicated that reproductive strategy of this genus was relatively conserved, which probably provides high recruitment rates for the existing populations with numerous, but the genetic variation and regeneration of natural population may not be able to maintain in the long term (Orellana et al., 2009). Thus, it is necessary to protect the species in *Delphinium*, since it was also reported that the species number of *Delphinium* decreased in China (Yuan, 2006). In conclusion, our study provided a fundamental information for the cross-incompatibility in *D. yunnanense* and *D. elatum* related to hybridisation. These findings may help ornamental breeders overcome reproductive barriers to develop new cultivars in the species.

FUNDING

This work was funded by the Major Science and Technology Project of Yunnan Provincial Science and Technology Department (2019ZG006), the Special Funding for High-end Talents Cultivation of Floriculture Research Institute from Yunnan Provincial Department of Finance (530000210000000024520), the Innovative Talents Training Base for High-level Universities in Yunnan Province-Horticulture Innovation and Entrepreneurship Training Program (2016CX001), and the Construction of Modern Agricultural Industry System in Floriculture and Seedling in Yunnan Province (2017KJTX0010). We thank Dr. Chunlian Jin (Ghent University) for assistance with the English language grammar and syntax of this manuscript.

AUTHOR CONTRIBUTIONS

Y.C. and W.G. conceived and designed the experiments. Y.C. completed the experiments. Y.C. and F.L. conducted the data analysis and drafted the manuscript. F.L. critically revised the manuscript. W.G. and F.L. jointly provided supervision. All authors reviewed and approved the manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Received: July 15, 2020; accepted: October 21, 2020.

View publication stats

Gene 796-797 (2021) 145791

Contents lists available at ScienceDirect

Gene

journal homepage: www.elsevier.com/locate/gene

Research paper

Evaluation of genetic diversity and population structure of *Fragaria nilgerrensis* using EST-SSR markers

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ARTICLE INFO

Keywords:
Fragaria nilgerrensis
EST-SSR marker
Genetic diversity
Population structure

ABSTRACT

Fragaria nilgerrensis is a diploid wild strawberry widely distributed in Southwest China. Its white color and "peach-like" fragrance of fruits are valuable characters for the genetic improvement of cultivated strawberry plants. Its strong biotic and abiotic resistance and tolerance also enable it to survive in different habitats in the field. In this study, we evaluated the level of genetic variation within and between 16 populations with 169 individuals of *F. nilgerrensis* using 16 newly developed EST-SSR (expressed sequence tag-simple sequence repeats) markers. The results show that the genetic diversity of this species was high, based on Nei's genetic diversity (0.26) and polymorphic loci (0.41), although it is self-compatible and has clonal propagation. Significant genetic differentiation among populations was also detected by AMOVA analysis (Fst = 0.34), which could be indicative of little gene flow (Nm = 0.43) in *F. nilgerrensis*. The phylogenetic tree indicates that most of individuals from the same population have clustered together. These populations were not grouped based on the geographical distance, consistent with the Mantel test result ($R^2 = 0.0063$, $P > 0.05$). All the populations were assigned into two ancestral groups, with some individuals admixed, suggesting ancestral gene flow had occurred between these two groups. Our developed EST-SSR markers as well as the genetic diversity and population structure analysis of *F. nilgerrensis* are important for genetic improvement in the breeding process. Moreover, the populations that contain high genetic diversity would be a priority for collection and conservation.

1. Introduction

The strawberry is a very popular small berry. Diploid wild strawberries are important breeding materials due to their various fruit colors and special fragrance. *Fragaria nilgerrensis* is a self-compatible diploid wild strawberry with clonal reproduction, and it is widely distributed in Southwest China (Guo et al., 2018). *F. nilgerrensis* is robust and has long, erect and brownish-yellow hairs on stolons, petioles, peduncle and inflorescences. These characters give it strong biotic and abiotic stress resistance and the plant can survive in different habitats in the field, including understories, valley forests, roadsides, dry limestone and meadows on mountain slopes. Our previous dated phylogeny indicated that *F. nilgerrensis* is grouped with an East Asian clade with origin time about 6.78 Mya (Qiao et al., 2016). Considering its major distribution is around the Qinghai-Tibet Plateau (QTP), different populations of *F. nilgerrensis* may have undergone significant genetic changes to adapt to stress factors following the uplift of the QTP. Furthermore, the fruits of *F. nilgerrensis* are white to cream with a unique peach aroma. The

Abbreviations: EST-SSR, expressed sequence tag - simple sequence repeats; QTP, Qinghai-Tibet Plateau; SSR, Simple Sequences Repeat; RAPD, Random Amplified Polymorphic DNA; AFLP, Amplified Fragment Length Polymorphism; ISSR, Inter-Simple Sequence Repeat; Na, observed number of alleles; Ne, the effective number of alleles; Ho, observed heterozygosity; He, expected heterozygosity; PIC, polymorphic information content, H, Nei's genetic diversity index; I, Shannon diversity index; PCoA, Principle Coordinate Analysis; AMOVA, analysis of molecular variance; NJ, Neighbor-Joining.

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https://doi.org/10.1016/j.gene.2021.145791
Received 22 December 2020; Received in revised form 18 May 2021; Accepted 22 June 2021
Available online 25 June 2021
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unique aroma and white color from *F. nilgerrensis* were introduced into a derivative of cultivated strawberry (*F. xananassa*) by interspecific crossing to form a decaploid white strawberry variety 'Tokun' (Noguchi, 2011). Key information on genetic diversity and population structure of *F. nilgerrensis* is lacking, preventing the evaluation of current status of this species and its application in molecular breeding.

Molecular markers, like SSR (Simple Sequences Repeat), RAPD (Random Amplified Polymorphic DNA), AFLP (Amplified Fragment Length Polymorphism), and ISSR (Inter-Simple Sequence Repeat) have extensively been used in conservation genetics. SSR markers have become a particularly important tool because of their co-dominant, polymorphic and cross-transferable nature. Compared with SSR, EST-SSR (Expressed Sequence Tag - Simple Sequences Repeat) has the advantage of transferability among plant species, and is widely used in plant genetic mapping (Dong et al., 2019; Yang et al., 2019; Zheng et al., 2019), genetic diversity evaluation (Ge et al., 2019; Ohbayashi et al., 2019; Sharma et al., 2020), and species identification (Li et al., 2014). Its location in the transcriptional region suggests polymorphism may be directly related to some functional genes (Zhang et al., 2015). Increased knowledge of the genetic diversity and population structure of

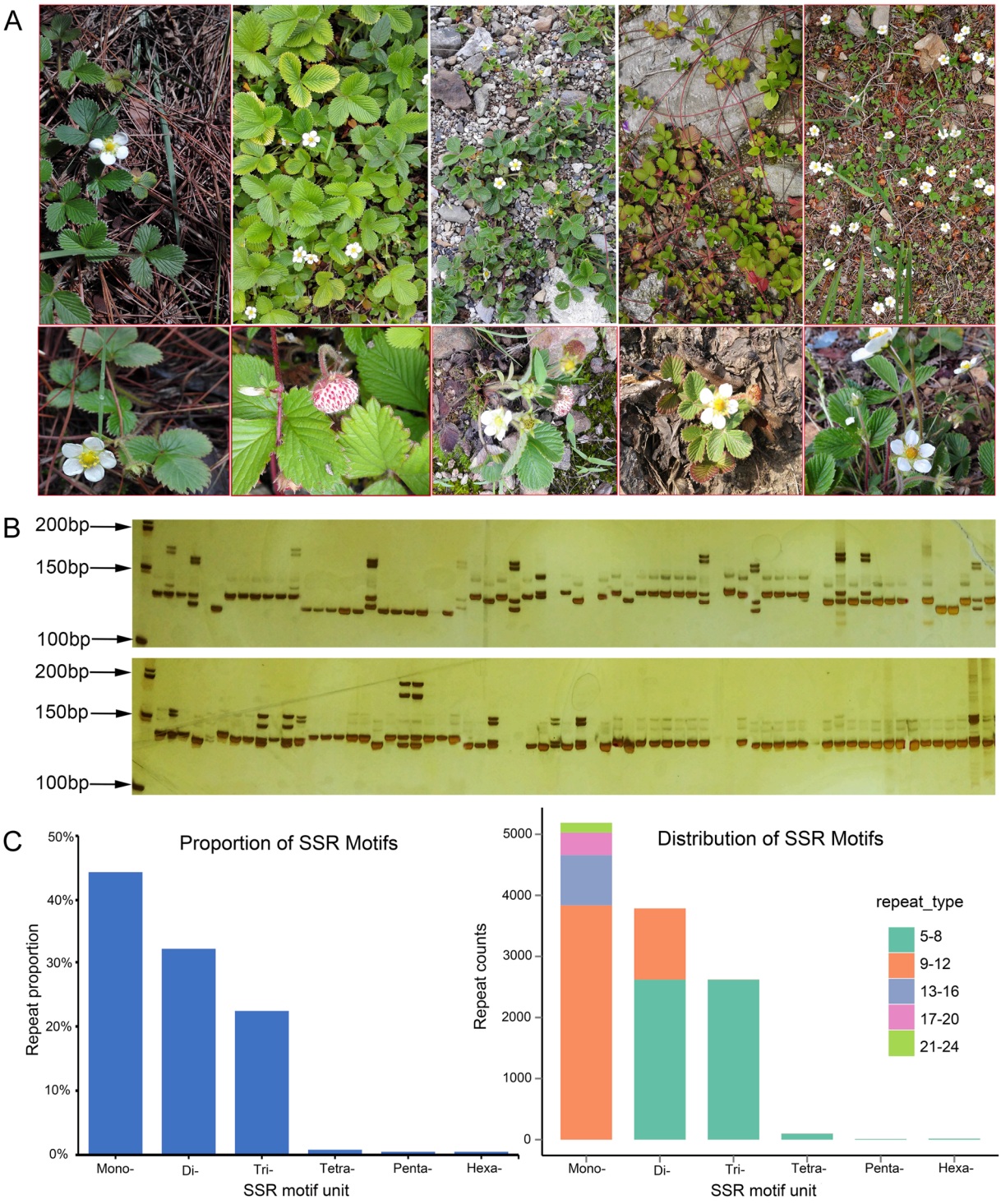


Fig. 1. (A) Habitat and morphology of *Fragaria nilgerrensis* in the wild. (B) Primer polymorphism detection results. (C) Proportion and Distribution characteristics of different EST-SSR motifs in *F. nilgerrensis*. Left: proportion of SSR motifs, Right: distribution of SSR motifs.

germplasm collections is an important foundation for crop improvement. In *Fragaria*, several researchers have developed high cross-species transferable EST-SSRs markers in *F. vesca* (Bassil et al., 2010), and performed genetic diversity and population structure analyses in *F. nubicola* (Meng et al., 2015) and multiple accessions of cultivated strawberry using SSR markers (Yoon et al., 2012).

In the present study, we detected EST-SSRs in the assembled unigenes from the transcriptome of *F. nilgerrensis*. Then we developed reproducible and polymorphic EST-SSR primers to assess the level of genetic diversity and genetic structure of *F. nilgerrensis*. Our study will provide novel insights for the utilization of *F. nilgerrensis* core germplasm resources.

2. Material and methods

2.1. Plant material and DNA extraction

In this study, a total of 169 individuals from 16 populations of *F. nilgerrensis* were collected, which covered Yunnan, Guizhou and Sichuan provinces in Southwest China (Fig. 1A, 2A, Table 1). Some of the individuals were transplanted in the strawberry germplasm resources nursery at Yunnan University. The collected healthy young leaves were stored in allochronic silica gel. Genomic DNA was extracted from 30 mg dried leaves of each individual using a DNA secure Plant kit (DP320, Tiangen Biotech Co., Beijing, China). The DNA quality and concentration were evaluated through a NanoDrop 2000 spectrophotometer (Thermo Scientific, Wilmington, USA). All DNA samples were diluted to 20 ng/μl and stored at −20 °C for further experiments.

2.2. EST-SSR mining from RNA-Seq and primer design

In our previous research, the RNA-Seq of pooled samples including leaves, stems, flowers, and fruits of *F. nilgerrensis* was conducted using an Illumina Hiseq 2500 platform and paired-end reads were generated and submitted to NCBI with accession number SRR4030219 (Qiao et al., 2016). EST-SSRs were detected in the assembled unigenes using the MIcroSAtellite (MISA) identification tool (http://pgrc.ipk-gatersleben.de/misa/misa/. Html, Beier et al., 2017). The SSRs were considered to contain mono-, di-, tri-, tetra-, penta- and hexa-nucleotides with minimum repeat numbers of 10, 6, 5, 5, 5 and 5, respectively. Unigenes with a sequence of more than 150 bp before and after the SSR region were used for primer design by Primer v3.0 (Rozen and Skaletsky, 2000). Oligonucleotides were synthesized at Shanghai Sangon Biological Engineering Technology (Shanghai, China).

Table 1
Population information of *F. nilgerrensis* examined in EST-SSR analyses.

Population code	Location	Numbers	Longitude	Latitude	Altitude (m)
QXH	Sichuan	12	29.630	102.610	1800
BMH	Yunnan	12	28.355	99.033	3940
LS	Sichuan	12	28.662	102.233	1900
LCH	Sichuan	11	31.060	103.560	2480
PS	Sichuan	13	27.869	102.420	2074
HSH	Yunnan	8	27.517	99.892	3380
LZP	Sichuan	9	28.943	102.318	1872
WT	Sichuan	7	28.805	102.281	2301
LP	Yunnan	12	25.976	98.681	3500
KM	Yunnan	18	24.771	102.909	2480
DLJ	Yunnan	4	27.839	98.481	3220
JLT	Yunnan	14	26.654	99.783	2780
JCP	Guizhou	9	25.919	104.699	2820
GS	Yunnan	15	25.589	104.249	2253
ZDH	Yunnan	6	27.902	99.649	3682
ZL	Yunnan	7	27.604	99.786	3190

2.3. Practicability test of the designed EST-SSR primers

All designed primer pairs were initially tested by amplification using 10 individuals, which were randomly selected from 10 populations of *F. nilgerrensis*. The volume of the PCR reaction system was 15 μL, including 1.5 μL of 10 × Taq Buffer, 1.2 μL of dNTP, each of forward and reverse primers for 0.5 μL, 1.2 μL of Taq DNA polymerase, 2 μL of template of DNA, and 9.1 μL of ddH_2O. The PCR reaction and amplification conditions were performed as follows: initial denaturation at 95 °C for 7 min followed by 35 cycles of 95 °C for 30 s, the annealing temperature (depending on EST-SSR primer) for 30 s, extension at 72 °C for 30 s. The final extension was performed at 72 °C for 7 min. Then, the amplified PCR products were detected by 8% non-denaturing polyacrylamide gels and stained by nucleic acid dye (Fig. 1B). Finally, the primer pairs that yielded clear, reproducible and polymorphic bands with expected size were selected for allele identification and genetic diversity analysis.

2.4. Data analysis

SSR bands generated near the expected product size were scored visually for all the genotypes and this SSR genotype data was analyzed for genetic diversity and population structure. The band size of amplified products was determined by comparing with a 50 bp plus DNA ladder. The SSR bands scored in *F. nilgerrensis* genotypes were subjected to statistical analysis.

The observed number of alleles (Na), the effective number of alleles (Ne), observed heterozygosity (Ho) and expected heterozygosity (He), polymorphic information content (PIC), Nei's genetic diversity index (H) and Shannon diversity index (I) were calculated using POPGENE 2.0 (Takezaki et al., 2010) and Power Marker 3.25 (Liu and Muse, 2005). Principle Coordinate Analysis (PCoA) and the Mantel test, which evaluates the correlation between geographical and genetic distance were conducted using GenALEx V6.5 (Peakall and Smouse, 2012). An Unweighted Neighbor-Joining tree based on dissimilarity was constructed using DARWin 6.0 (http://darwin.cirad.fr/Darwin/Html, Perrier and Jacquemoud-Collet, 2006). Population structure was estimated by the software STRUCTURE 2.3.4 (Pritchard et al., 2000). The admixture model was used with a burn-in of 50,000 and 100,000 iterations for 1 to 10 K populations with ten independent runs each. The LnPD derived for each K was then plotted to find the plateau of the ΔK values (Evanno et al., 2005). Genetic differentiation of populations was estimated by the analysis of molecular variance (AMOVA) implemented in Arlequin 3.0 (Excoffier et al., 2005) and Power Marker 3.25 (Liu and Muse, 2005).

3. Results

3.1. EST-SSR markers primer development

In total, 11,722 EST-SSR loci were detected from 82,537 unigenes, distributed in 9,785 sequences, with an average length of 5,526 bp per EST-SSR loci. The mono-nucleotide (5,188, 44.20%) had the highest frequencies, followed by di-nucleotide (3,786, 32.22%) and tri-nucleotide (2,623, 22.38%) (Table 2, Fig. 1C). The total of tetra-, penta- and *hexa*-nucleotides only accounted for less than 1% (Table 2). Among these, the A/T repeat motif type was the most abundant (5,111, 43.6%) followed by AG/CT (2,791, 23.8%) and AAG/CTT (918, 7.8%) (Supplementary Table S1). Further analysis indicated that the copy number of repeat motifs varied from 5 to 24 and was unevenly distributed in different unit types (from mono- to hexa- nucleotide repeats). The most frequent copy numbers in mono-nucleotide repeats were 9 to 12, while 5 to 8 were the most frequent copy numbers in di- and tri-nucleotide repeats respectively (Fig. 1C). Out of 125 EST-SSR primer pairs which were selected for validation, 38 (30.4%) pairs of primers could amplify unambiguous bands. Among the primers that amplified successfully, 16 pairs of primers (12.8%) obtained well-sized products

Table 2
Proportion of different repeat unit motifs in EST-SSR of *F. nilgerrensis*.

Repeat type	Repeat motifs	Total number of repeat units	Percentage (%)
Mononucleotide	A/T	5111	43.6
	C/G	77	0.6
Dinucleotide	AC/GT	532	4.5
	AG/CT	2791	23.8
	AT/AT	448	3.8
	CG/CG	15	0.12
Trinucleotide	AAC/GTT	178	1.5
	AAG/CTT	918	7.8
	AAT/ATT	87	0.74
	ACC/GGT	243	2.1
	ACG/CGT	91	0.77
	ACT/AGT	55	0.47
	AGC/CTG	205	1.7
	AGG/CCT	382	3.3
	ATC/ATG	347	3
	CCG/CGG	117	1
Tetranucleotide	AAAG/CTTT	31	0.26
	AAAT/ATTT	21	0.17
	AACC/GGTT	5	0.04
	ACTC/AGTG	7	0.06

Total number of repeat units: the sum of the frequency of different types of repeats (distributed from 5 to 24 repeats).

with polymorphic and reproducing bands (Table 3, Supplementary Table S2).

3.2. Polymorphism of EST-SSR markers

The 16 EST-SSR primer pairs were used for genetic diversity study of *F. nilgerrensis*. A total of 71 alleles were detected with an average of 4.44 alleles per locus. As shown in Table 4, the number of alleles (Na) per locus ranged from 2 (primer pair FNG3) to 8 (primer pair FNG10); and the number of effective alleles (Ne) varied between 1.09 (FNG5) and 3.84 (FNG44), with a mean value of 1.89. The observed heterozygosity (Ho) ranged from 0.01 (FNG72) to 0.44 (FNG18), with a mean value of 0.19. The genetic diversity (expected heterozygosity, He) of the 16 loci ranged from 0.08 (FNG72) to 0.74 (FNG44), with an average of 0.40. The Shannon information index (I) and Nei's genetic diversity index (H) both showed that primer pairs FNG44 and FNG72 was of highest and lowest polymorphic site respectively. The average value of polymorphism information content (PIC) in 16 loci was 0.41 and ranged between 0.08 (FNG5) and 0.70 (FNG44 and FNG91). All the above results suggested that the screening of 16 SSR loci genetic diversity was more abundant. The Hardy-Weinberg equilibrium test (HW-test) showed that most of the loci deviated significantly from equilibrium, except for primer pair FNG10 (Table 4).

3.3. Genetic diversity of F. nilgerrensis populations

As shown in Table 5, the genetic diversity parameters of populations were estimated as follows: the number of alleles (Na) ranged from 1.44 (populations DLJ, JCP) to 2.56 (LZP, WT and ZDH), with the average number of 1.98; the effective alleles (Ne) varied from 1.19 (GS) to 2.11 (ZDH), with a mean value of 1.52. Ho and He were 0.28 and 0.21 in 16 populations, respectively. The I and H indices both showed that ZDH had the highest genetic diversity, while JCP had the lowest genetic diversity. Across the 16 populations, the average of polymorphic loci was 10.31, which accounted for 64.45% of all the detected loci. Furthermore, the average of I was 0.42 and mean H was 0.26. The results also indicated that the populations ZDH, WT, LZP, ZL had higher genetic diversity, while the GS, JCP, KM, BMH populations exhibited relatively lower genetic diversity.

3.4. Genetic differentiation between populations

The analysis of different scale genetic differentiation and inbreeding coefficients of F-statistics (Fis, Fit and Fst) estimates for 16 loci were 0.22, 0.51 and 0.40 respectively (Table 4). Among the 16 loci of *F. nilgerrensis*, three loci (FNG10, FNG18, and FNG34) showed excess

Table 3
List of 16 pairs of primers.

Primers No.	Sequence (5′-3′)	Sequence SSR	Expect size (bp)	Allele size (bp)	TM (°C)
*FNG*3	F:AGTCCCTATGCAACTGTGGC R:CCCGGTTTTTCTGCTGCAAA	(AGG)7	115	115\118	57
*FNG*18	F:CCATCTGATCTGCAGCGCTA R:GAGAGCTCAGCGTATCCACC	(ACG)6	162	150\162\163\165\168	55
*FNG*5	F:AGCCACGGAATGTCCAAACT R: TTCGCTTTCTTCTCCGCCTT	(CGG)5(CAG)5	135	129\132\135	57
*FNG*91	F:GCCCCAACAAACAAAACCCA R:CCGCCTCCATTGCATTTACG	(CCT)5	145	141\142\145\148\151\152\154	57
*FNG*97	F:ACATGCCAGATCCAAAGCCA R:GAGGTTCAGGAAGGGCATGA	(CCT)5	127	124\127\130\136	57
*FNG*71	F:CAGCTGGTGCATGATTACGC R:CCGGTGAAAACACAACAGCC	(GCG)6	124	121\124\127\136	57
*FNG*100	F:ACCACAGCATTCCGGTCAAT R:GTGACGCTAACTCCCAGGTC	(GA)9	166	166\176\188\190\204	57
*FNG*103	F:CCCACGAAGAAAGCTCCCAT R:CCAGAGGAGACGAGTCATGC	(CCG)5	161	158\161\167	58
*FNG*112	F:ACGAGGAGAATGCCAAGGTG R:CACAGAAGAGGCCAGTGGAG	(GGA)7	156	144\147\156\159	58
*FNG*34	F:AGTCCCTATGCAACTGTGGC R:CCCGGTTTTTCTGCTGCAAA	(AGC)5	126	120\126\129	57
*FNG*41	F:CCATCTGATCTGCAGCGCTA R:GAGAGCTCAGCGTATCCACC	(CGA)5	131	131\132\134\137\152\155	58
*FNG*44	F:AGCCACGGAATGTCCAAACT R: TTCGCTTTCTTCTCCGCCTT	(CTC)5	135	119\125\130\132\135\138	57
*FNG*51	F:TCTTTCAACTCTCTGCCCGG R:ACCAATGTGCCACAGACACT	(GAC)6	123	112\123\126\132	59
*FNG*53	F:GCCCCAACAAACAAAACCCA R:CCGCCTCCATTGCATTTACG	(GAG)5	153	147\150\153\156	57
*FNG*72	F:ACATGCCAGATCCAAAGCCA R:GAGGTTCAGGAAGGGCATGA	(ACC)6	138	135\138\141	58
*FNG*10	F:CAGCTGGTGCATGATTACGC R:CCGGTGAAAACACAACAGCC	(TC)9	113	113\115\117\119\126\130\132\134	57

Table 4
Analysis of EST-SSR Locus Diversity and F-Statistical Test of EST-SSR Locus in in *F. nilgerrensis* population.

Locus	Number of Genotypes	Na	Ne	Ho	He	I	H	PIC	HW-Test	Fis	Fit	Fst	Nm
FNG3	3	2	1.27	0.08	0.22	0.37	0.22	0.19	**	0.30	0.65	0.49	0.26
FNG5	5	3	1.09	0.05	0.09	0.21	0.09	0.08	**	0.05	0.30	0.27	0.68
FNG10	8	8	1.57	0.4	0.36	0.84	0.36	0.35		−0.49	−0.10	0.26	0.71
FNG18	7	5	1.95	0.44	0.49	0.87	0.49	0.43	*	−0.22	0.09	0.25	0.75
FNG34	3	3	1.5	0.27	0.34	0.62	0.34	0.31	**	−0.09	0.29	0.35	0.47
FNG41	10	6	2.06	0.21	0.52	1.04	0.51	0.48	**	0.21	0.56	0.44	0.32
FNG44	15	6	3.84	0.22	0.74	1.5	0.74	0.7	**	0.39	0.68	0.48	0.27
FNG51	7	4	1.65	0.09	0.4	0.78	0.4	0.37	**	0.70	0.80	0.33	0.50
FNG53	7	4	2.09	0.14	0.52	0.95	0.52	0.46	**	0.52	0.71	0.41	0.36
FNG71	8	4	1.62	0.26	0.38	0.76	0.38	0.36	**	0.14	0.32	0.21	0.93
FNG72	4	3	1.09	0.01	0.08	0.19	0.08	0.08	**	0.66	0.85	0.58	0.18
FNG91	15	7	3.03	0.27	0.67	1.35	0.67	0.7	**	0.31	0.57	0.37	0.42
FNG97	6	4	1.1	0.02	0.09	0.23	0.09	0.38	**	0.25	0.66	0.55	0.20
FNG100	10	5	2.67	0.24	0.63	1.27	0.63	0.69	**	0.39	0.65	0.43	0.33
FNG103	5	3	1.57	0.12	0.36	0.63	0.36	0.49	**	0.29	0.65	0.51	0.24
FNG112	9	4	2.19	0.22	0.55	1.04	0.54	0.54	**	0.15	0.55	0.48	0.28
Mean	7.81	4.44	1.89	0.19	0.4	0.79	0.4	0.41		0.22	0.52	0.40	0.43

Note: Na: Number of alleles; Ne: Number of effective alleles; Ho: Observed heterozygosity; He: Expected heterozygosity; I: Shannon Information index; H: Nei's genetic diversity index; PIC: Polymorphic information; HW-Test: Hardy-Weinberg equilibrium test. (Only *FNG10* followed Hardy-Weinberg's equilibrium and *FNG18* (*) showed significant Deviation). Fis: Intraspecific inbreeding coefficient; Fit: Population inbreeding coefficient; Fst: Fixed coefficient; Nm: Gene flow.

Table 5
Genetic Diversity Analysis of 16 populations of *F. nilgerrensis*.

Population	Polymor phic site	Polymor phic locus ratio (%)	Na	Ne	He	Ho	I	H	Fst
QXH	12	75.00	2.00	1.50	0.17	0.28	0.43	0.27	0.32
BMH	8	50.00	1.63	1.24	0.10	0.16	0.25	0.15	0.36
LS	10	62.50	2.00	1.48	0.17	0.25	0.40	0.24	0.34
LCH	11	68.75	1.81	1.42	0.21	0.26	0.39	0.25	0.34
PS	11	68.75	2.25	1.53	0.31	0.27	0.44	0.26	0.33
HSH	10	62.50	2.06	1.65	0.26	0.30	0.47	0.28	0.34
LZP	14	87.50	2.56	1.76	0.26	0.39	0.63	0.37	0.33
WT	15	93.75	2.56	1.96	0.33	0.47	0.71	0.43	0.35
LP	10	62.50	1.88	1.40	0.07	0.24	0.36	0.23	0.34
KM	8	50.00	1.69	1.29	0.12	0.18	0.27	0.17	0.35
DLJ	6	37.50	1.44	1.27	0.11	0.17	0.23	0.15	0.35
JLT	11	68.75	2.06	1.71	0.18	0.33	0.50	0.32	0.33
JCP	5	31.25	1.44	1.24	0.14	0.13	0.20	0.12	0.35
GS	9	56.25	1.69	1.19	0.11	0.14	0.23	0.13	0.35
ZDH	14	87.50	2.56	2.11	0.51	0.52	0.76	0.47	0.32
ZL	11	68.75	2.00	1.62	0.25	0.32	0.48	0.30	0.34
Mean	10.31	64.45	1.98	1.52	0.21	0.28	0.42	0.26	0.34

Note: Na: Number of alleles; Ne: Number of effective alleles; He: Expected heterozygosity; Ho: Observed heterozygosity; H: Nei's genetic diversity index; I: Shannon information index; Fst: Genetic differentiation index.

heterozygosity with negative Fis values (−0.49, −0.22, −0.09), while the other 13 sites showed insufficient heterozygosity with positive Fis values. The global inbreeding coefficient Fit ranged from −0.10 (FNG10) to 0.85 (FNG72), with an average of 0.51. Across the 16 loci, Fst ranged from 0.21 (FNG71) to 0.58 (FNG72), with an average value of 0.40. Similarly, among the 16 populations, the Fst values varied from 0.32 (QXH) to 0.36 (BMH), with an average of 0.34 (Table 5). This result was consistent with the analysis of AMOVA, which showed that genetic variation among populations of *F. nilgerrensis* accounted for 34.04% of the total variation, while the genetic variation within populations accounted for 65.96% (Table 6). These results indicate that significant genetic differentiation had occurred among the populations of *F. nilgerrensis*. Correspondingly, the average value of gene flow (Nm) was only 0.43 (Table 4).

Table 6
AMOVA analysis of genetic variances within and among populations.

Source of variation	df	Sum of squares	Variance components	Percentage of variation (%)	Fst
Among populations	15	106.45	0.31Va	34.04	0.34
Within populations	322	193.32	0.60Vb	65.96	–

3.5. Clustering analysis of F. nilgerrensis populations

To detect the population structure of *F. nilgerrensis*, we performed the STRUCTURE program for Bayesian clustering analysis. The results showed that when K = 2, the ΔK reached the maximum value, indicating that the 16 populations mainly came from two ancestral groups. Of them, one group was composed with admixed populations, while another group exhibited relatively pure composition (Fig. 2B). In addition, the un-weighted Neighbor-Joining (NJ) tree derived through DARwin 6.0.1 software revealed that all the individuals clustered into three groups (Fig. 2D). Although most of individuals from same population clustered together in the NJ tree, JLT, LP and ZL exhibited scattered distribution. We also matched the individuals in the NJ tree to the two groups that STRUCTURE assigned and the results showed that the individuals with same ancestral genetic origin usually clustered together except for some intermixed individuals (Fig. 2D).

The PCoA results showed that the first and second principal components accounted for 28.97% and 21.19% of the total genetic variation, respectively (Fig. 2C). All 169 individuals could be separated into two clusters on the second principal coordinate, which was in agreement

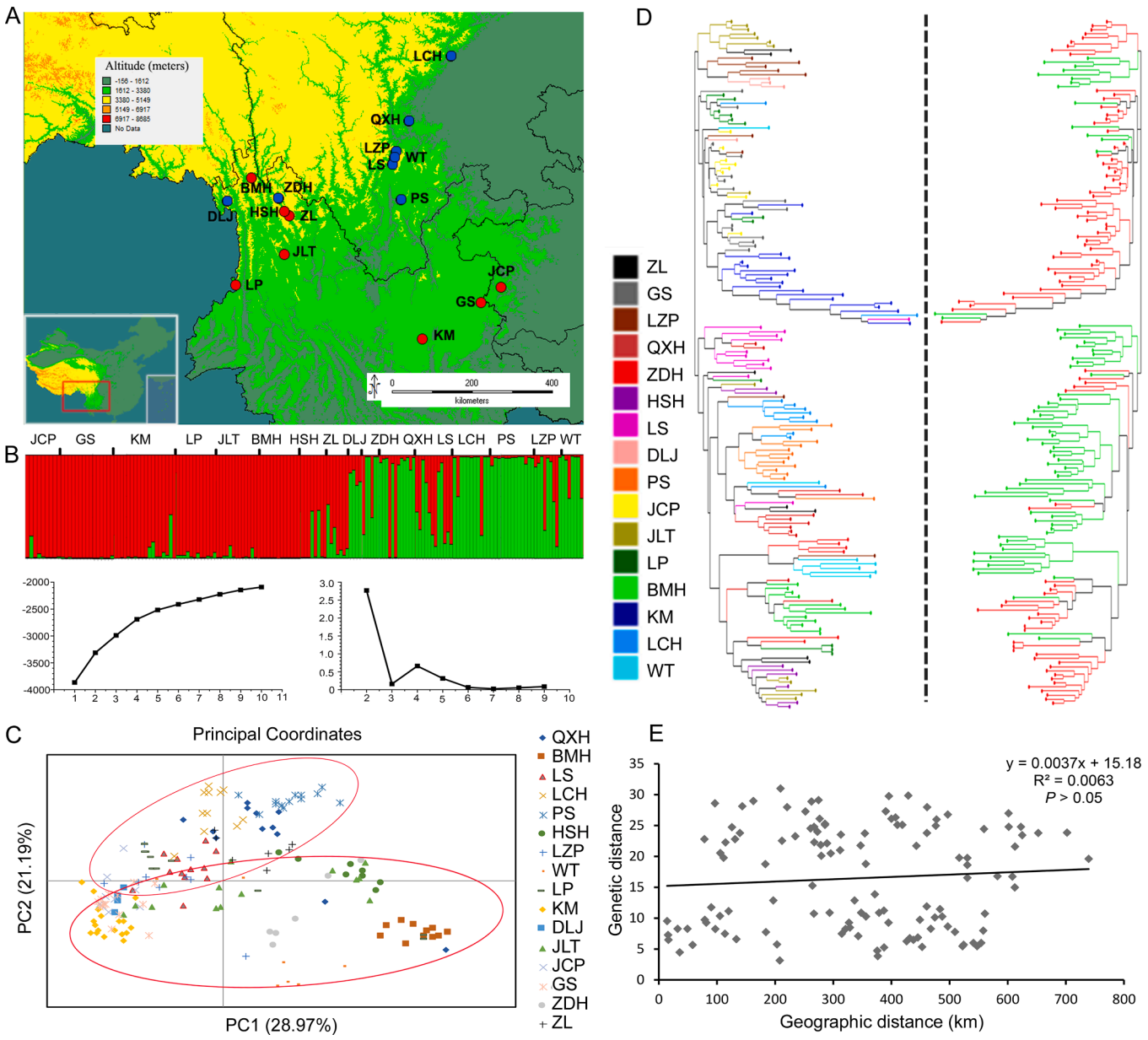


Fig. 2. Genetic structure of *F. nilgerrensis*. (A) The collection sites of experimental materials. (B) Structure of *F. nilgerrensis* inferred by Bayesian clustering of SSR data. Upper: Assignment of individuals into K = 2 genetically distinguishable groups. Below: Log probability of data L(K) as a function of K for 10 STRUCTURE runs at K = 1–10; C. Rate of change in the probability between successive runs, ΔK, as a function of K. (C) PCA of 169 individuals from *F. nilgerrensis*. (D) Unrooted Neighbor-joining tree of 169 individuals from *F. nilgerrensis*. Left: Individuals' tree with different colors for different populations; right: individuals' tree colored as two groups that Structure assigned. (E) Relationship between pairwise estimates of genetic distance and the corresponding geographic distance among 16 populations of *F. nilgerrensis* ($R^2 = 0.0063$, $P > 0.05$).

with the Structure result. Most individuals were clustered according to corresponding populations. However, the populations with higher genetic diversity index (WT, QXH, JLT, LP, LZP) showed several individuals admixing with other populations. Furthermore, both STRUCTURE and the NJ dendrogram showed that populations did not cluster based on the geographical distance. This observation was also supported by the Mantel test, and no significant correlation was detected between genetic distance and the geographic distance ($R^2 = 0.0063$, $P > 0.05$) (Fig. 2E).

4. Discussion

This study is the first attempt to develop and use EST-SSR markers to detect genetic diversity of *F. nilgerrensis*. The dominant EST-SSR repeat motifs were mono-, di- and tri-nucleotide repeats of A/T, AG/CT and AAG/CTT in *F. nilgerrensis*, that were similar to cultivated strawberry, in which A/T, AG/CT, AAG/CTT and GAA/TTC commonly occurred (Zou et al., 2020). According to our results, the tri-nucleotide repeats exhibited more polymorphisms than other types.

The level of genetic diversity can reflect the strength of a population's ability to adapt to the environment and reveal the utilization potential of the species, providing a basis for resource conservation and selection of breeding materials (Nachimuthu et al., 2015). Generally, outcrossing species tend to harbor more genetic variation than self-pollinating species. As a self-compatible species, the genetic diversity in *F. nilgerrensis* (0.26) is higher than that reported for out-crossing species *F. nubicola* (0.212), another diploid wild strawberry distributed in Southwest China (Meng et al., 2015). It was also higher than average

values obtained for mainly selfing species (Hamrick and Godt, 1996). The relatively high level of genetic diversity of *F. nilgerrensis* seemed to contradict with its clonal propagation and self-compatibility. In fact, the level of genetic diversity of clonal plants is not necessarily very low, and previous studies show that some clonal plant populations can maintain a large amount of genetic diversity (Kirsten et al., 1998). Clonal plants can compensate for the loss of genetic variation through facultative sexual reproduction, to increase the genetic variation within populations and decrease the level of genetic variation among populations (Menken et al., 1995). In addition, spontaneous somatic mutations can also be used to maintain genetic variation in clonal plants, such as dandelion (Eckert et al., 2003) and cactus (Nason et al., 2002). As we observed, the seedlings of *F. nilgerrensis* growing in the field frequently undergo somatic mutation (the number of petals increases, the leaves deeply split, the petals turn red, etc.). Because the ancestors of the cloned population may come from multiple genotype individuals, and different populations under heterogeneous environments may fix different loci by natural selection, they were able to maintain high genetic diversity. Although the breeding system of *F. nilgerrensis* is mainly selfing, long distance of pollen and seed dispersal would occur occasionally by flower visitors and human distribution. We speculate that the above factors may be the cause of its relatively high genetic diversity.

The mean Fis > 0 and the lower observed heterozygosity than the expected heterozygosity at species level indicates lack of heterozygosity in the populations of *F. nilgerrensis*. It was reported that lack of heterozygotes among the related individuals is due to either random mating with a restricted paternal pollen pool, or non-random mating (Zhang et al., 2020). Both a self-compatible breeding system and clonal propagation in *F. nilgerrensis* could cause the low outcrossing rates and heterozygosity. In fact, the results of the HW-test of 16 loci also suggest non-random mating in *F. nilgerrensis*, which showed that 14 loci deviated from the Hardy-Weinberg equilibrium.

The Fst values of 16 populations obtained from Popgene software were consistent with the results of AMOVA analysis, indicating that there was significant genetic differentiation among populations. The genetic variation was maintained by both trade-off between factors increasing (mutation and balance selection) and decreasing (natural selection and genetic drift) genetic variations. Generally, it is believed that when $Nm > 1$, the gene flow is enough to inhibit genetic drift and prevent genetic differentiation among populations, while when $Nm < 1$, the gene flow is insufficient to offset the effects of genetic drift, the main factor leading to the genetic differentiation among populations (Slarkin, 1985; Schnabel et al., 1998). In *F. nilgerrensis*, Nm was only 0.43, suggesting that low levels of gene exchange among populations could not weaken the divergence among populations caused by natural selection and genetic drift. Therefore, we can speculate that the low gene flow could be an important reason for genetic differentiation of *F. nilgerrensis*, caused by asexual reproduction and a self-compatibility breeding system. Moreover, *F. nilgerrensis* is distributed from moderate to high elevations (up to 4000 m) in a number of different environments, as we have observed in the field. The high elevation mountains usually generate heterogeneous habitats, where reproductive isolation in spatially discrete populations likely occurs (Qian et al., 2014). The unique microhabitats for plants also could provide structural heterogeneity that enhances genetic diversity (Camp and Knight, 1997). Therefore, the heterogenous habitats are likely to favor the maintenance of high levels of genetic variation and cause significant divergence of populations in *F. nilgerrensis*. Consistent with our results, the high genetic diversity of other clonal plants was also detected, and some of them have more genetic variation among populations than that within populations due to the barrier of gene flow (Ellstrand and Roose, 1987).

According to the clustering analysis and Mental test, populations of *F. nilgerrensis* were not clustered based on the geographical distances. This means that geographic isolation is not a major factor leading to high genetic differentiation of *F. nilgerrensis*. Considering that bumblebees and butterflies are the main flower visitors of *F. nilgerrensis* in the field, long distance of pollen dispersal would only occur occasionally.

The results of STRUCTURE assigned all the individuals into two ancestral groups. Several individuals displayed an intermixed composition, which could be attributed to a historical gene flow between them. In fact, the populations with admixed individuals also exhibited higher genetic diversity and scattered distribution in the NJ tree PCoA according to our results. It suggests that these populations (e.g. ZDH, WT, LZP) contain more genetic variation of *F. nilgerrensis* and have priority to be conserved and utilized in the future.

It is worth noting that in our study, the sample number of each population was uneven, for example, DLJ population had only four samples, while KM population had 18 samples. Although the sample size was small in DLJ, it exhibited the same level of genetic diversity as KM did according to Nei's genetic diversity. This may due to the different sampling strategies in the two populations. Individuals of DLJ came from four subpopulations because *F. nilgerrensis* has scattered distribution at high-altitude levels. In contrast, the samples of KM population were collected about 20 m away between each other in a big population. This suggests that for genetic diversity study, greater distance is needed for clonal plants which live in homogeneous environments.

5. Conclusions

F. nilgerrensis is a wild strawberry species and could provide a rich source of genetic variations for cultivated strawberry improvement. Based on the 16 developed EST-SSR markers, we detected that the populations of *F. nilgerrensis* display abundant genetic diversity and significant genetic differentiation. High level genetic diversity suggests this species has strong capability to adapt to stressful environmental conditions, as well as to diseases. Therefore, the populations that exhibited higher genetic diversity suggests a higher level of utilization potential for resource conservation and selection of breeding materials. Although our study was based on a limited number of markers, our results should be considered in planning future conservation and research programs for the wild strawberry. In order to identify the candidate genes for important traits, future research should investigate genome-wide sequence variations of different populations by resequencing.

CRediT authorship contribution statement

Jie Lu: Methodology, Writing - original draft, Formal analysis. **Yichen Zhang:** Methodology, Writing - original draft, Formal analysis. **Xia Diao:** Resources, Methodology, Formal analysis. **Kun Yu:** Resources, Methodology, Formal analysis. **Xiongwei Dai:** Resources, Methodology. **Peng Qu:** Resources, Methodology. **M. James C. Crabbe:** Supervision, Writing - review & editing. **Ticao Zhang:** Writing - review & editing, Supervision, Project administration. **Qin Qiao:** Formal analysis, Writing - review & editing, Supervision, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This work is supported by grants from National Natural Science Foundation of China (32060085 & 31760082 to Q.Q., 32060237 & 31770408 to T.Z.), Applied Basic Research Project of Yunnan, 2019FB134 to Q.Q.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gene.2021.145791.

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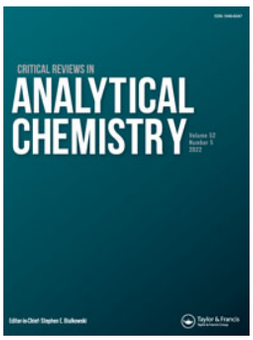
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Critical Reviews in Analytical Chemistry

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/batc20

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To cite this article: Zhimin Liu, Mei Quan Yang, Yingmei Zuo, Yuanzhong Wang & Jinyu Zhang (2021): Fraud Detection of Herbal Medicines Based on Modern Analytical Technologies Combine with Chemometrics Approach: A Review, Critical Reviews in Analytical Chemistry, DOI: 10.1080/10408347.2021.1905503

To link to this article: https://doi.org/10.1080/10408347.2021.1905503

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REVIEW ARTICLE

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Fraud Detection of Herbal Medicines Based on Modern Analytical Technologies Combine with Chemometrics Approach: A Review

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ABSTRACT

Fraud in herbal medicines (HMs), commonplace throughout human history, is significantly related to medicinal effects with sometimes lethal consequences. Major HMs fraud events seem to occur with a certain regularity, such as substitution by counterfeits, adulteration by addition of inferior production-own materials, adulteration by chemical compounds, and adulteration by addition of foreign matter. The assessment of HMs fraud is in urgent demand to guarantee consumer protection against the four fraudulent activities. In this review, three analysis platforms (targeted, non-targeted, and the combination of non-targeted and targeted analysis) were introduced and summarized. Furthermore, the integration of analysis technology and chemometrics method (e.g., class-modeling, discrimination, and regression method) have also been discussed. Each integration shows different applicability depending on their advantages, drawbacks, and some factors, such as the explicit objective analysis or the nature of four types of HMs fraud. In an attempt to better solve four typical HMs fraud, appropriate analytical strategies are advised and illustrated with several typical studies. The article provides a general workflow of analysis methods that have been used for detection of HMs fraud. All analysis technologies and chemometrics methods applied can conduce to excellent reference value for further exploration of analysis methods in HMs fraud.

KEYWORDS

Herbal medicines (HMs); fraud detection; analysis technologies; chemometrics methods

1. Introduction

Herbal medicines (HMs) have been used over thousands of years for maintaining human health. *Shen Nong Ben Cao Jing*, from its initial discovery in 2700 B.C, is the oldest systematic monograph to highlight the effect of traditional Chinese medicines in preventing and treating human diseases.[1,2] Taking into account the long historical medicinal effects, HMs in the global healthcare system had widely used in the terms of diagnosis, treatment as well as prevention of a vast range of diseases.[3,4] For instance, up to this point, HMs are what meeting the health care needs of 80% of the population in Asia, Africa, and Latin-America.[5,6] With the increasing demand for HMs and their premium prices, the contemporary phenomenon of HMs fraud gradually becomes more common. HMs fraud is divided into unintentional and deliberate fraud.[7] The unintentional fraud of HMs is mainly the result of the inaccurate collection, harvesting, and processing, whereas the deliberate fraud is carried out for economic purposes in order to reduce costs and improve the perceived quality.[8] In order to obtain more economic benefits, HMs fraud may pose a threat to the health of consumers.[9] For instance, the adulteration of *Stephania tetrandra* with *Aristolochia fangchi* caused 116 cases of aristolochic acid nephropathy (AAN) in China.[10] The adulteration of *Illicium verum* with *I. anisatum* resulted in a child having neurological and gastrointestinal problems.[11] The replacement of *Akebia quinata* in Longdan Xiegan Pill by *Aristolochiae Manshuriensis*, which lead to AAN and upper tract urothelial carcinomas.[12,13] Therefore, it is indispensable to seek reliable methods to monitor HMs fraud, which can ensure the huge economic value of HMs in treating diseases and avoid the huge threat to human health caused by fraud.

Before modern biological and chemical methods were developed, traditional methods gradually performed from both macroscopic and microscopic points to achieve qualitative and quantitative assessment of HMs.[14] Although they are simple and rapid, traditional authentication methods suffer from subjective differences in the qualitative evaluation.[15] Hence, recent momentums in the HMs fraud field tend to indicate that the use of traditional methods has fallen out in favor of modern analytical technologies which used the information of the secondary metabolites. The modern analytical technologies, including vibrational spectroscopy, nuclear magnetic resonance spectroscopy (NMR), mass hyphenated technologies, gas chromatography (GC), and liquid chromatography (LC), can divide into targeted, non-targeted, and non-targeted combined with targeted analytical technologies.

However, a stack of original analyte data consisting of useful and redundant chemical information was captured by different analytical techniques.[16] Hence, a pivotal problem responsible for hampering analysis is the presence of numerous data, which makes it difficult or incapable to explore

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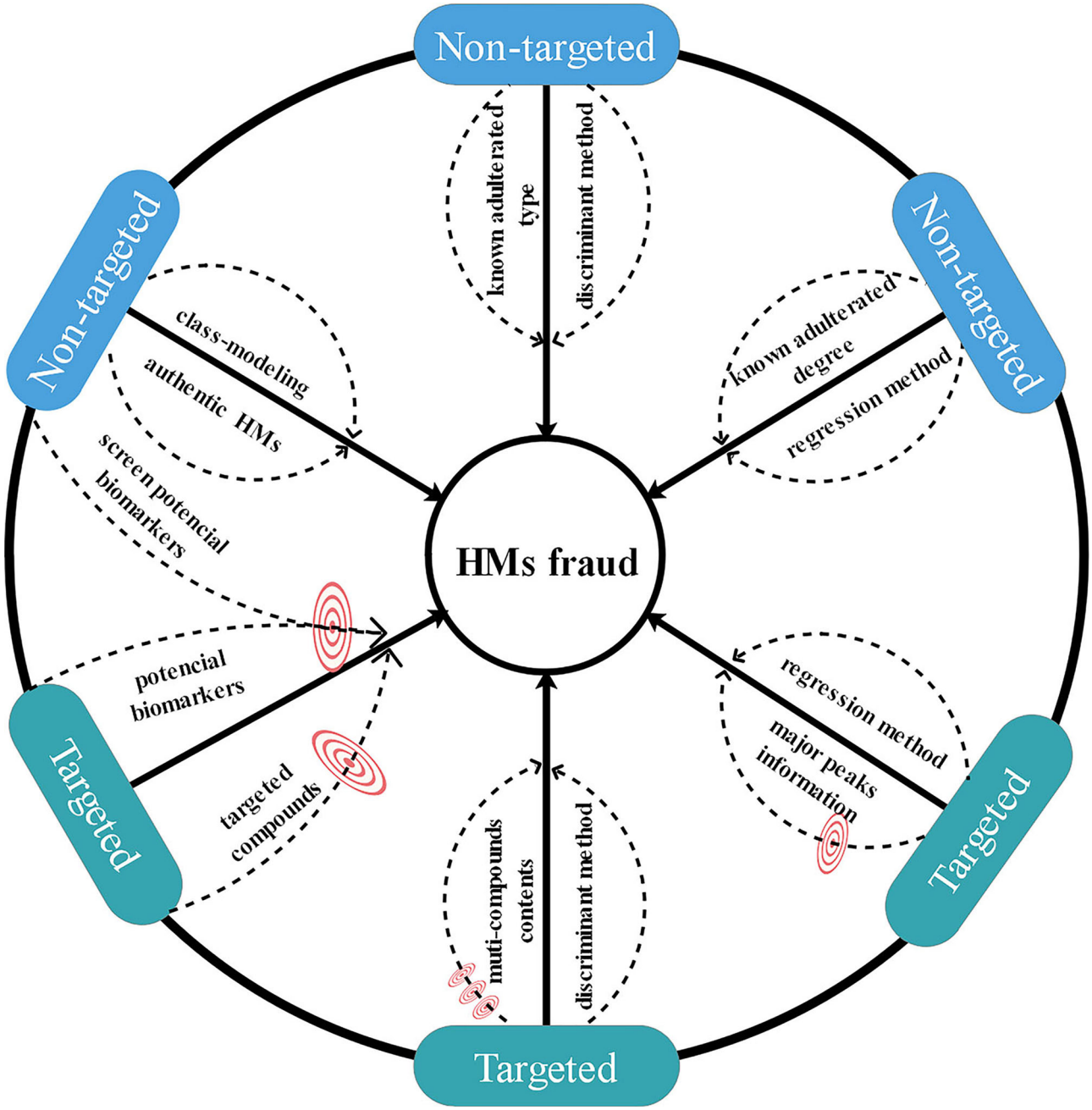


Figure 1. The integration of analysis technologies and chemistry methods to detect HMs fraud.

useful information only through univariate analysis (e.g., analysis of variance).[17] More contemporary, along with the evolution of computer science, chemometrics has been widely used, making data interpretation fast and accurate.[18] Chemometrics has already solved a vast range of analytical problems in HMs fraud, for example, a regression algorithm allows predicting the adulteration degree,[19] and a classification algorithm allows adulterated types to be identified.[10] Figure 1 showed the integration of analysis technologies and chemistry methods to detect HMs fraud.

2. Scope of this review

Some articles have reviewed the qualitative and quantitative assessment of HMs fraud through the use of different analytical technologies combined with chemometrics methods. Most reviews only focus on the comparison of different analytical techniques or different chemometrics methods. With the diversification of fraud types, these reviews have a narrowed application range in HMs fraud. The comparison between the analytical methods which were the combination of analytical techniques and chemometrics is thus required to identify HMs fraud. The choice of the analytical methods depend on the type of HMs fraud and the explicit objective of qualitative or quantitative analysis. Therefore, it is inevitable to put forward an integral and comprehensive evaluation of the analytical methods.

In that sense, this review provides the qualitative and quantitative application of analytical methods to assess HMs fraud, such as the recognition of the confused materials with

Table 1. Summary of applications of analysis methods in HMs fraud.

Analysis technology	The application number of analysis technology in HMs fraud detection	Analysis strategy	The application number of analysis strategy in HMs fraud detection
Targeted analysis	13	Targeted analysis	The dentification of counterfeits (2); Adulteration by addition of foreign matter (0); Adulteration by addition of inferior production-own materials (0); Adulteration by addition of chemical compounds (4)
		Targeted analysis combined with discrimine method	The dentification of counterfeits (2); Adulteration by addition of foreign matter (0); Adulteration by addition of inferior production-own materials (1); Adulteration by addition of chemical compounds (0)
		Targeted analysis combined with regression method	The dentification of counterfeits (0); Adulteration by addition of foreign matter (1); Adulteration by addition of inferior production-own materials (0); Adulteration by addition of chemical compounds (3)
Non-targeted analysis	58	Non-targeted analysis combined with discrimine method	The dentification of counterfeits (15); Adulteration by addition of foreign matter (9); Adulteration by addition of inferior production-own materials (3); Adulteration by addition of chemical compounds (5)
		Non-targeted analysis combined with class-modeling	The dentification of counterfeits (3); Adulteration by addition of foreign matter (3); Adulteration by addition of inferior production-own materials (3); Adulteration by addition of chemical compounds (2)
		Non-targeted analysis combined with regression method	The dentification of counterfeits (1); Adulteration by addition of foreign matter (7); Adulteration by addition of inferior production-own materials (2); Adulteration by addition of chemical compounds (5)
The combination of non-targeted and targeted analysis	2	The combination of non-targeted and targeted analysis	The dentification of counterfeits (1); Adulteration by addition of foreign matter (0); Adulteration by addition of inferior production-own materials (1); Adulteration by addition of chemical compounds (0)

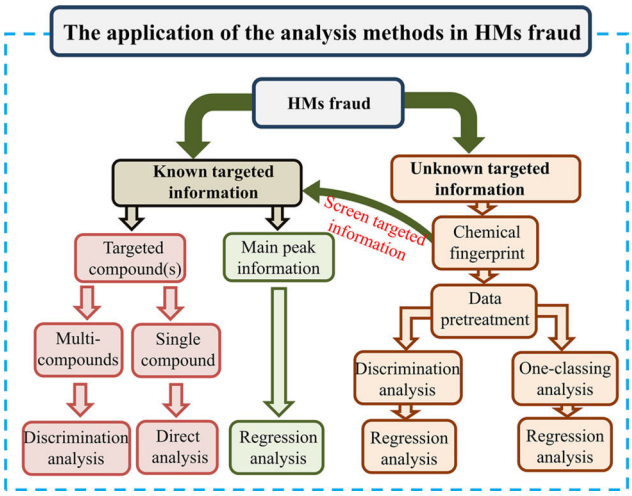


Figure 2. A general workflow of detection fraud based on analytical technologies combined with chemometrics approach.

different medicinal effects, chemical compounds adulteration, heterogenous adulteration. Qualitative analysis was used to identify the authenticity and adulteration types of HMs, and quantitative analysis was used to quantify the adulteration ratio. The following was organized as follows: Section 3 provided the description, advantages, and defects of the application of targeted, non-targeted and their combination. In addition, Table 1 summaried the applications of analysis techniques and methods in HMs fraud. The Section 4 was the workflow of chemometrics analysis, including preprocessing and feature extraction, outlier detection, qualitive analysis methods, and quantitative analysis methods. Moreover, the comparison of different analytical methods was also discussed in Section 4. Section 5 summarized the practical application of different analysis methods in HMs fraud. Furthermore, a general workflow of detection fraud based on analytical technologies combined with chemometrics approach was shown in Figure 2. Some conclusions were drawn in Section 6.

3. Analysis technologies for determination in HMs fraud

3.1. Non-targeted analysis technology

Non-targeted analysis technology is the most widely used platform in HMs fraud. This technology is normally making use of its global unbiased nature to profile detailed chemical information of the representative sample.[20,21] Numerous non-targeted analytical platforms are currently available, such as vibrational spectroscopy, hyperspectral imaging (HSI), mass spectrometry (MS)-based techniques, NMR spectroscopy, amongst others. Indeed, non-targeted analysis technology related to chemometrics has been gradually adopted to build classification and calibration models.[22] Compared with other non-targeted analytical techniques, vibrational spectroscopy techniques were the most used platform to detect HMs fraud due to the advantages of nondestructive, easy to use, rapid, and chemical-free. Most recent studies using vibrational spectroscopy aimed to assess the existence of HMs fraud, as reports involving *Gleditsia sinensis*,[19] cumin,[23] saffron,[24] and paprika powder.[25] Neverthless, they are suffering from unsatisfying interpretability.[26] As the different selection rules for vibration spectroscopy transitions and certain vibrations of the sample molecular symmetry, the information of certain vibrations between the vibration spectra is thus complementary.[27,28] There is the momentum of applying data fusion technology to improve the ability of spectral interpretation.[29,30] Multi-source vibrational fingerprint has been used to identify *Pinellia ternate* and *P. pedatisecta*.[31]

Notably, two dimensional correlation spectroscopy (2DCOS), aiming for improving the vibrational spectral resolution, has been proposed, which is of great important to solve the HMs fraud from the the image view.[32] In addition, vibration spectroscopy techniques are point-based scanning techniques that unable to allow one to detect the spatial information within HMs.[33] An important aspect responsible for

increasing attention about HSI is the simultaneous acquisition of spatial and spectral information.[34] One particular advantage is the capacity of trace detection of HSI in detection HMs fraud,[35] as reports involving *S. tetrandra*[10] and nutmeg.[36] It is known that vibrational spectroscopy only provides the information of functional groups, irrespective of the structures of chemical compounds.[37] With the aim of bypassing this drawback, MS has been developed to interpret the chemical composition information for identifying the differential compounds within HMs and their adulterants. Nevertheless, MS-based assays were usually needed separation of time dimension separation.[38] To overcome this problem, separation methods, such as chromatography and ion mobility spectrometry (IMS), have emerged one after another. Of particular concern is chromatography in the term of improving the efficiency of the MS detection. MS hyphenated with chromatographic separation system have been used to detect saffron adulterated with inferior production-own materials.[39] Furthermore, chromatographic fingerprint was also considered a powerful qualitative analysis technology. It has been used to detect *Ranae oviductus*[40] and *Polygonum multiflorum* root.[41] Nevertheless, due to the character of time-consuming and destructiveness, chromatography can cause considerable loss in the process of sample preparation.[42] With the propose to overcome the above-listed drawbacks, NMR has been developed to non-targeted analysis with higher throughput, reproducibility, and simpler sample preparation. More applications involving NMR include the detection of *Harpagophytum procumbens*,[43] culinary spices,[44,45] and saffron.[46] However, the character of low sensitivity limits the application of NMR, especially so if compared to MS-based technologies. In a study detecting the adulteration of saffron, the minimum detectable level of NMR is 20%.[47] In practically, non-targeted analysis suffers from poor reproducibility, unreliable quantitation. Therefore, the application of non-targeted analysis technology is limited. To eliminate these problems, alternative platforms such as targeted analysis and the combination of non-targeted and targeted analysis have been developed one after another.

3.2. Targeted analysis technology

Compared with non-targeted analysis technology, targeted analysis technology has the advantages of high specificity, high sensitivity, low complexity data processing, and wide dynamic range. This technology covers accurate identification or quantification of the pre-defined analytical targets.[48,49] The pre-defined analytical targets, such as characteristic compounds and chemical elements, are the basis for identifying the fraud because these targets are what makes HMs different from each other.[50] Given the multi-component property, targeted analysis technology combined with chemometrics method has been widely used to detect HMs fraud. Several targeted analysis platforms are available, such as chromatography, NMR, as well as inductively coupled plasma-based (ICP-based) techniques, among others. Chromatography is a powerful platform to achieve quantitative analysis of target compounds.[51] In this regard, GC and LC have different detection ranges according to the polar difference of the compounds. For example, regarding the volatile or semi-volatile metabolites, GC is unanimously acceptable method. In spite of being a high reproducibility method, GC suffers from lengthy sample pretreatment, and nonvolatile metabolites needed to be derivatized.[52] Thereby, there is little application in HMs fraud. In an attempt to overcome the above-listed problem, alternative technology, such as LC, has used to analyze polar and non-volatile adulterated chemical compounds without derivatization. Additionally, liquid chromatography (LC) is the most used platform in the detection of HMs fraud, as researches such as *Swertia chirayita*,[53] *Panax notoginseng*,[54] LC can also be hyphenated to different detectors, as diode array detector (DAD),[55,56] evaporative light scattering detector (ELSD),[57] and time of flight mass spectrometry (TOFMS).[58] Among them, of particular concern is MS-based LC analysis in HMs fraud. MS-based LC analysis can reliably evaluate the fraud of a great diversity of HMs. This includes, for instance, the detection of *P. ginseng*,[59] saffron,[60] and weight loss plant dietary supplements.[61] Although they are accurate and sensitive, MS-based LC analysis suffer from a requirement for complex sample pretreatment procedures.[62] To overcome this disadvantage, NMR has been developed to achieve nondestructive and automated analysis. Targeted NMR analysis is also widely used in HMs fraud, especially in the term of identification HMs spiked with Sudan dye, as reports involving paprika powder[62] and saffron.[63] In the quantitative process of target compounds, of practical concern is that NMR can simultaneously quantify different characteristic compounds in the micro-molar range.[64,65] On the other hand, it is apparent that the major disadvantage of this technology is the relatively low sensitivity, especially so if compared to MS technology.[66] It thus cannot identify the potential biomarkers with a lower content than the limit of detection (LOD). In contrast to character compounds, elements only indirectly provide information about adulteration of HMs.[50] The application of ICP-based techniques in HMs fraud detection is gradual emerging. It is apparent that the greatest advantage of ICP-based techniques is that they allow the exceptionally low concentration of elements to be quantified.[67] ICP-based techniques have been used to detect *Acorus calamus*.[68] Nevertheless, they are expensive, time-consuming, and environmental unfriendly.[69] Hence, the preferable targeted analysis technology should be selected according to the explicit analytical objective, the polarity of target compounds. In a word, the narrow range, significant cost of standards, and inability to identify unknown compounds are the major point to impede the application of targeted analysis technology. To overcome these disadvantages, some studies put forward the combination of non-targeted and targeted analysis technology to save costs and expedite testing.

3.3. The combination of non-targeted and targeted analysis technology

Dynamically fraud in HMs is the basis for avoiding HMs fraud testing, which makes targeted analysis gradually

becomes more difficult. Hence, it is impossible to monitor unknown hazardous compounds by using the targeted analysis. Non-targeted analysis technology affords a detailed profile of the representative sample, which demonstrates its feasibility for screening HMs fraud. Although non-targeted analysis technology obtains good performance, the uptake of them in routine surveillance lacks standardization and validation to guarantee reproducible and reliable results.[70] To overcome these disadvantages, there is a momentum to combine non-targeted with targeted analysis.[71] The combination of non-targeted and targeted analysis, such as NMR and LC-MS, is non-biased and has high specificity and sensitivity. Taking into account the application of NMR in routine life, the setting up of the comprehensive databases of HMs based on non-targeted analysis is the basis for the development of targeted analysis technology.[72] MS, such as orbitrap mass spectrometry and time-of-flight (TOF) mass spectrometry, served as screening methods, could provide high confidence detecting of molecular weights and chemical structures of small-molecules and macromolecular compounds and identify them through comparing with the in-house and on-line databases.[52,71,73] Furthermore, feature selection and extraction can be incorporated into the screening method for the identification of biomarkers. For example, feature selection and extraction have been used to determine the characteristic components for aiding the identification of *Ligusticum sinense*.[74] Once the characteristic components were identified, confirmatory testing was then operated by LC to detection the content of characteristic components through constructing standard curves for absolute quantification. With further research and development of analysis technologies, the application of the combination of non-targeted and targeted analysis in HMs fraud is also considerably increased. This has been used to discriminate *Lonicerae japonicae* flos[75] and *Calculus bovis*.[58]

4. Chemometrics methods

4.1. Preprocessing and feature extraction

The data from non-targeted analysis has in common is unwanted artifacts that make the data unsuitable for direct chemometrics analysis.[76] Data preprocessing is to remove detrimental artifacts to highlight the difference caused by adulterant.[77,78] In general, the choice of the preprocessing techniques depends on the specific artifacts to be dealt with. For example, in the case of the presence of noise, the Savitzky-Golay algorithm and wavelet transform has been proposed in an attempt to better obtain the data with a low level noise. Multiplicative effects caused by light-scatter and the discrepant size of particles can be solved by means of methods including standard normal variate (SNV), variable sorting for normalization (VSN) and multiplicative scatter correction (MSC), etc. Parametric time warping (PTW),[79] correlation optimized warping (COW)[80] are usually used to solve peak shifts generated by chromatographic and NMR. However, multiple artifacts presented in the non-targeted data may be multivariate.[81] In order to achieve high confidence identification of HMs fraud, multi-block data analysis, such as sequential preprocessing through orthogonalization (SPORT), is an emerging tool allowed one to fusion of multiple preprocessing, to identify the best preprocessing techniques and their combination.

In general, the preprocessed non-targeted data still hold co-linear and redundant information.[82] As can be seen from the model that the redundant information extremely reduces the performance of the model. This effect is more obvious in low-level data fusion strategy, since they only provided relative analysis through straightly concatenate the data from complementary.[83] Feature selection and extraction are thus followed performed to extract informative variables because this is what makes the data matrix more simple to build a reliable model or to perform mid-level fusion.[15] There are several ways to extract the feature of non-targeted data, such as variable importance in projection (VIP), the loadings plot, regression coefficients (RC). The number of variables is finally confirmed by calculating the area under receiver operating characteristic curve (AUC) of variable combination.[84] It is worth mention that, in the detection of HMs fraud, the largest RC may not consistent with the largest bands when the analyte peak is overlapped between HMs and their adulterant.[85]

4.2. Outlier detection

Outlier detection is a pivotal step in detection of HMs fraud and an optimal outlier detection is needed on the basis of type of outliers. Outliers are a major concern for non-targeted analysis, and yet they are usually away from well defined expected behavior, due of course to the reason that the outliers are either from a measurement error or the samples themselves.[86] In contrast to noises, outliers contain not only useless information but also exceptional information. Global outliers and collective outliers are the broadest categories in HMs fraud field. The common global outliers caused by experimental noises, the inconsistency of the sample preparation, and spectral acquisitions heavily impede the adulterant identification process, since they may completely change the overall trend of the non-targeted dataset.[87] In this respect, it is recommended that they should be excluded from the non-targeted dataset before modeling. Collective outliers may be a new type of HMs fraud, especially so if compared to global outliers. Hence, the best solution is keeping them to extract important information. Generally, the careful study of outliers is the basis for determining whether to keep or delete them.[88]

It may be noticed that the visualizing data is the basis for intuitively investigating the sources of abnormalities.[87] Principal component analysis (PCA) and soft independent modeling of class analogy (SIMCA) are the visual technologies with the greatest presence in outlier detection. PCA is performed to identify global outliers of non-targeted data by visualizing the clustering trends without prior knowledge of the adulterate type of HMs.[89] SIMCA assumes that the multivariate spaces from target class can be captured by prior analysis by PCA model.[90] Furthermore, the choices of optimal principal components (PCs) and the level of

significance (a) in SIMCA are the basis for determining the performance of the identification of the normal samples.[91] Recently, the ROC curve-based methodology have been proposed to choose the optimal number of PCs.[91] Once the PCs are confirmed, class frontiers based on a at the level of 0.05, are calculated by various class limit criteria to identify outliers.[92] In practice, Hotelling's T^2 versus Q residuals test is now the widely used technology to confirm the class frontiers.[93] The Hotelling's T^2 versus Q residuals have respectively represented the sum of the normalized squared scores and sum of squares of each sample in the visualizing error matrix.[94] As for the discriminant method, an important problem responsible for increasing concerns is the proper classification of samples, especially in the term of the identification of adulterated HMs not belonging to pre-defined classes.[95] Outlier detection can be incorporated into the discriminant method to detect the new type of HMs fraud.[96] With respect to this, the confidence interval of the estimated class value can be used as a new criterion to identify the collective outliers.[97] In conclusion, outlier detection can contribute to bringing a high-quality model for properly identifying the fraud.

4.3. Qualitative analysis methods

4.3.1. Unsupervised pattern recognition

Non-supervised technologies including similarity analysis and exploratory analysis are used to analyze the cluster information of the samples without prior information. Regarding similarity analysis, distance or similarity parameters are calculated to evaluate the degree of fingerprints similarity between standard and tested samples for the detection of HMs fraud.[98] PCA and hierarchical cluster analysis (HCA) are the exploratory analysis technologies with the greatest presence in detection of HMs fraud. PCA, as a data reduction analysis method, can decompose the preprocessed data set consisting of a mass of interrelated variables into several ordered, uncorrelated and orthogonal PCs that reflect most of the variation of the raw data set.[99,100] Additionally, the correlation of raw variables is the basis for achieving the dimension reduction.[101] HCA classifies HMs based on its similarity in space. The cluster exhibits high homogeneity in the real HMs group and high heterogeneity between the real group and an adulterated group of HMs.[102–104] Furthermore, the application of the combination of PCA and HCA in HMs fraud is very common due to their complementary result. For example, PCA is the first step to remove the invalid interference information and to detect the possible outliers, and then to conduct cluster information evaluation through HCA. As a case study, Gan et al.[40] used PCA and HCA to establish a discriminative model for chromatographic data of *R. oviductus* and its counterfeits, and the similarity was in the range of 0.779–0.980. It may be noticed that systematically classify samples cannot be realized, whilst solely used unsupervised techniques without supervised techniques.[105] Furthermore, unsupervised pattern recognition can superimpose to supervised pattern recognitions aiming to obtain better detection result.[106]

4.3.2. Multivariate classification model

Supervised classification methods, by means of optimizing internal parameters, provide useful information for the systematic classification of the types and adulteration degrees of an adulterant. As for the classification methods, discriminant and one-class model are representive appropriate tools. The main discriminant technologies applied in HMs fraud are linear discriminant analysis (LDA), quadratic discriminant analysis (QDA), partial least squares discriminant analysis (PLS-DA), k-nearest neighbors (k-NN) as well as support vector machines (SVMs), etc. Associated with discriminative methods, targeted analysis technology take the content of target compounds as input data to identify the HMs fraud. For example, it is an effective approach to identify HMs fraud through the content information of multiple components, especially in the term of the identification of the adulteration by addition of inferior production-own materials. Currently, the implementation of targeted analysis technology has two major barriers and limitations. Firstly, targeted analysis method cannot easily quantify biomacromolecules, such as starch.[107] Secondly, the characteristic compounds cannot be invariably presented in HMs fraud. Non-targeted analysis technology combined with discriminant methods is more suitable to identify the adulteration of biomacromolecules in HMs, especially so if compared to targeted analysis technology. Owing to the complicated and dynamic of adulterant, a single technology cannot fulfill the integral analysis of HMs fraud. The application of data fusion strategies fully utilize complementary information from non-targeted analysis methods to systematically profile the change of internal and external chemical caused by adulterate.[108] Both non-targeted and targeted analysis have their respective applicable scope, therefore it is necessary to select appropriate discriminant methods according to specific analysis objectives. Class-modeling analysis focus on the authentication problem and only authentic class is concerned.[95,109]A major algorithm of class-modeling is SIMCA. Concerning the establishment of the multivariate spaces, the little implementation of the content information of multi-components is due to the performance of class-modeling for known adulterated classes is lower than that of multi-class models. In contrast, non-targeted analysis technology combined with class modeling methods, is considered as a simple and feasible method to identify the authenticity of HMs. It is apparent that the advantage of non-targeted analysis technology is due to its macroscopic fingerprints, containing qualitative information about authentic properties of HMs. Additionally, apart from conventional adulterant, new fraud recipes that are unknown or unforeseen are also utilized for adulterating. With respect to this, class-modeling methods show splendid performance of authentication due to the establishment of the class frontiers of authentic HMs.[95,96] Nevertheless, the high variance of the respective class can heavily impede the authentic samples identification process. The discriminant method combined with outlier detection is generally considered to be a better model.

The implementation of classification methods in routine surveillance has several major barriers and limitations. The number of representative samples collected from each class could not be used to attain a robust model covering all natural variations, in the majority of studies. Besides, the sourcing of samples is also critical to the value of resultant data. For instance, the use of samples sourced from the internet and local retailer can result in an inaccurate classification model. Moreover, the matching degree of the adulteration process between experimental HMs and commercial HMs is also pivotal for determining whether laboratory verification methods can be applied to industrial products.[70]

4.4. Quantitative methods

Compared with classification methods, regression method, a quantitative strategy, is used to relate the properties of data to continuous, real-valued variables (e.g., adulteration ratio, the content of target compounds) through a linear or nonlinear multivariate correction method.[110] Linear class, including partial least squares regression (PLSR), principal component regression (PCR), and multivariate linear regression (MLR), and nonlinear class, including support vector regression (SVR), and artificial neural network (ANN), are the most commonly used multivariate correction methods. It may be noticed that PLS can minimize the overfitting issue in the identification of the adulterate degree.[111] Whilst PCR can provide more latent variables than PLS, both of them are now considered equal in the term of the overall calibration ability.[112] Targeted analysis cannot achieve quantitative analysis of target compounds under some circumstances, such as the interaction and the signal overlap between HMs matrices and reference compounds.[62] In this regard, targeted analysis combined with regression methods is suitable to achieve quantitative analysis through utilizing the major peaks of target compounds. Nevertheless, in the experiments of detecting concentration of target compounds, the obtained LOD values from targeted analysis technology may be higher than the minimum concentration of adulteration. As a case study, Hu et al.[62] established a linear regression model with the use of ^{1}H-NMR, and found that the LOD of ^{1}H-NMR for the detecting of Sudan I was 6.7 mg kg^{-1}. However, the minimum concentration of Sudan I abused in paprika powder is 2.8 mg kg^{-1}.[113] Thereby, the sensitivity of the targeted analysis methods is crucial to analyze the real samples in the market. These technologies have tremendous advantages in qualitative analysis, while the drawback can not directly use to predict the adulteration ratio. The application of regression method to non-targeted analysis technology available for indirectly quantify the adulteration ratio. It may be noticed that the type of adulterant from the HMs in the market is unknown.[114] Thereby, before the construction of the regression models, the discrimination model is used to recognize the specific information of adulteration. In contrast, the regression model, in combination with the one-class model, provide the accurate degree of adulterant, irrespective of its identity. In the experiments of detecting adulteration rate, the concentration gradient of adulteration is artificially set, which may be inconsistent with the real adulteration rate of HMs.[115] It may notice that the same HMs in different countries have different adulteration ranges. For instance, Brazil allows 10% starch to be added to the paprika power products.[116] Thereby, the detection of minimum detection concentration is crucial to validate the feasibility of indirectly quantitative of non-targeted analysis technology.

5. The application of the analysis methods in HMs fraud

5.1. The identification of counterfeits

Counterfeits that have a similar appearance and easy availability are usually used as HMs substitutes, due to the rising demands for HMs with high economic and pharmacological activity.[31] In some case, owing to their completely different efficacy in chemical composition and pharmacological action, the counterfeits may pose hazardous effects to the effectiveness or even human health.[117] For instance, black pepper mixed with papaya seeds is often sold in the market.[118] However, papaya seeds pose hazardous effects on the liver and stomach.[119] Due to the influence of exogenous factors, the appearance characteristics of adulterant species and the raw HMs appeared more similar, which caused confusion. For example, the processed tubers of *P. pedatisecta* can be used as a substitute for the processed tubers of *P. ternate*.[31]

Some examples regarding use of analysis methods for identification of counterfeits have been summarized in Table 2. In the case of the presence of the characteristic components between HMs and their counterfeits, the answer is found by the targeted analysis, which can directly confirm the authenticity of HMs. Shen et al.[120] used the content information of the anisatin signal to identify *I. verum* with *I. anisatum*. Koh et al.[127] established a targeted model for quantitative analysis of aristolochic acid I, which could be used to distinguish *S. tetrandra* and *A. fangchi*. In conclusion, targeted analysis technique can be used to distinguish HMs and their counterfeits with simple, rapid, and powerful interpretability. In the case that the characteristic components between HMs and adulterants have not been found, the combination of non-targeted and targeted analysis methods can confirm the authenticity of HMs with rapid, practical, simple, and sensitive. Zhang et al.[75] identified the differences of one of the iridoids content between *L. japonica* and *L. confusa* through comparing the fingerprints of them. On this basis, a targeted method for quantitative analysis of secologanic acid was established to identify the authenticity of HMs. Own to the considerable variability in the characteristic components, targeted analysis technology combine with discriminate methods has been demonstrated to be a great tool for the identification of a vast range of substitutes in HMs. Singh et al.[53] used the targeted methods to quantify gentiopicroside (GP), mangiferin (MF), sweroside (SW), and swertiamarin (SM). On this basis, the

Table 2. Summary of references applying analysis methods to identify counterfeits.

Year	Herbal medicine	Counterfeits	Detect technique	Chemometrics	Analysis method	Ref.
2012	*Eriocaulon buergerianum*	*E. faberi*, *E. sexangulare*, and *E. cinereum*	HPLC-DAD-ESI-MS^n	PCA	a	[55]
2012	*Illicium verum*	*I. anisatum*	DART-MS	NM	a	[120]
2013	*I. verum*	*I. anisatum*	HSI	PCA, PLS-DA	d	[117]
2014	*Harpagophytum procumbens*	*H. zeyheri*	^{1}H-NMR, UPLC-MS	PCA, OPLS-DA	d	[43]
2017	*Rheum palmatum*, *R. tanguticum*, and *R. officeinale*	Unofficial rhubarb	NIR, MIR	PLS-DA, SIMCA, SVM, ANN	c, and d	[108]
2017	*Ganoderma lucidum*	*Fomes igniarius*, *F. fomentarius*, *Phaeolus schweinitzii*, *Trametes versicolor*, *Coltricia perennis*	FT-NIR	OCPLS	c	[121]
2018	*Polygonum multiflorum* root	*Cynanchum auriculatum* root	UPLC	PCA, PLS-DA, SIMCA, SVM, CP-ANN	c, and d	[41]
2018	*F. cirrhosa*	*F. thunbergia*, *F. anhuiensis*, *F. hupehensis*	UPLC-ELSD	HCA, PCA	d	[57]
2018	*Stephania tetrandra*	*Aristolochia fangchi*	HSI	PCA, PLS-DA	d	[10]
2018	*Gleditsia Sinensis*	*Rosa multiflora*, *R. rugosa*	FT-NIR	LDA, SVM, BP-ANN	d	[22]
2018	*Corydalis yanhusuo*	*C. turtschaninovii*, *C. repens*, *C. watanabei*, *C. ambigua*	HPLC-DAD-Q-TOF-MS	PCA, PLS-DA	d	[122]
2018	*Ligusticum sinense* root	*L. Jeholens*, *L. acuminatum*, *L. pteridophyllum*, *Conioselinum vaginatum*, *Sinodielisa yunnanensis*, *Peucedanum terebinthaceum*, *Angelica sinensis*	UPLC-Q/TOF-MS	PCA, OPLS-DA	d	[74]
2018	*Astragalus mongholicus*	*A. lehmannianus*	SERS	PCA, LDA	d	[123]
2018	*Panax ginseng*	*Platycodon grandiflorum*, *Pueraria lobata*, *Codonopsis lanceolata*	HPLC, UPLC-DAD-ESI-IT-TOF-MS	NM	b	[59]
2019	*C. yanhusuo*	*C. decumbens*, *Typhonium flagelliforme*, *Dioscorea opposita*	NIR, MIR	PCA, PLS-DA, LDA, MWPLSDA	d	[124]
2019	*Acori Tatarinowii* Rhizoma	*Acori Calami* Rhizoma, *Anemones Altaicae* Rhizoma	UPLC-MS/MS	PCA, PLS-DA	d	[68]
2019	*Swertia chirayita*	*S. bimaculate*, *S. chordata*, *S. ciliate*, *S. paniculate*, *Halenia elliptica*	UPLC-PDA	PCA, HCA	b	[53]
2019	*P. notoginseng*	*Curcuma longa*, *Alpinia officinarum*, *Rhizoma curcumae*	NIR	PCA, HCA, PLS-DA, SVM, ELM, PCR, SVR, PLSR	d, and f	[125]
2019	*Lonicerae japonicae*	*L. macranthoides*, *L. hypoglauca*, *L. confusa*, *L. fulvotomentosa*	HPLC	NM	g	[75]
2019	*Crocus sativus*	*C. longa*, *Calendula officinalis*	GC	LDA, PLS-DA	d	[126]
2020	*Pinellia ternata*	*P. pedatisecta*	NIR, MIR	PLS-DA	d	[31]

Abbreviation: HPLC-DAD-ESI-MS^n, high-performance liquid chromatography with diode array detection and electrospray ionization tandem mass spectrometry; DART-MS, direct analysis in real time-MS; HSI, hyperspectral imaging; ^{1}H-NMR, nuclear magnetic resonance; UPLC-MS, ultra-high performance liquid chromatography coupled to mass spectrometry; NIR, near-infrared spectroscopy; MIR, mid-infrared spectroscopy; FT-NIR, Fourier transform near-infrared; HPLC-DAD-Q-TOFMS, high performance liquid chromatography-diode array detector -quadrupoletime-of-flight mass spectrometry; UPLC-Q/TOF-MS, ultra-high performance liquid chromatography-quadrupole time of flight-mass spectrometry; UPLC-DAD-ESI-IT-TOF-MS, ultraperformance liquid chromatography-diode array detector-electro-spray ionization-ion trap-time of flight-mass spectrometry; UPLC-PDA, ultra performance liquid chromatography-photodiode array detector; UPLC, ultra-high performance liquid chromatography; SERS, surface-enhanced raman; HPLC, high-performance liquid chromatography; UPLC-ELSD: ultra-high performance liquid chromatography-evaporative light scattering detector; PCA: principal component analysis; HCA: hierarchical cluster analysis; PLS-DA: partial least squares discriminant analysis; PLSR: partial least squares regression; OPLS-DA: orthogonal partial least squares-discriminant analysis; LDA: linear discriminant analysis; SVM: support vector machine; ANN: artificial neural network. BP-ANN: back propagation-artificial neural network. MWPLSDA, moving window partial least-squares discriminant analysis; SIMCA, soft independent modeling of class analogy; CP-ANN, counter-propagation artificial neural network; ELM, extreme learning machine; SVR, support vector regression; NM, no mention.

Analysis method: a, targeted analysis; b, targeted analysis combined with discrimine method; c, non-targeted analysis combined with class-modeling; d, non-targeted analysis combined with discrimine method; e, targeted analysis combined with regression method; f, non-targeted analysis combined with regression method; g, the combination of non-targeted and targeted analysis;

classification model which applied two chemical pattern recognition methods, named PCA and HCA, were designed to distinguish *S. chirayita* and its five possible substitutions. Qiao et al.[55] measured the content of all characterized compounds in *Eriocaulon buergerianum* and its three adulterating species. On this basis, PCA was applied by analyzing the data of semi-quantitative to explicitly identify *E. buergerianum*. In addition, due to the great difference in chemical composition between HMs and substitutes, non-targeted classification methods have a great application prospect. Sun et al.[31] successfully analyzed the authenticity of *P. ternate* by using PLS-DA to establish a discriminative model for two kinds of spectral information (NIR and MIR). Sun et al.[41] identified *Polygoni Multiflori* Radix and *Cynanchi Auriculati* Radix, established their chemical chromatographic fingerprints, and analyzed with SIMCA. The results indicate that the class-modeling can effectively distinguish *P. Multiflori* Radix and its counterfeits.

In conclusion, targeted analysis technology can be a sensitive and specific technology, grouping HMs and their

counterfeits based on differences in the content of biomarkers. But it has some limitations for the practical application. Non-targeted analysis technology combine with targeted analysis technology can allow the identification of biomarkers, followed by accuracy quantify.

5.2. Adulterate identification

5.2.1. Adulteration by addition of foreign matter

HMs can be adulterated using foreign matters with similar physical properties, such as color and granulometry. Perhaps the commonly used foreign matter is starch, which has low economic value.[128] It is worth mentioning that, some HMs contain endogenous starch. Starch, for example, is one of the main ingredients of Pueraria lobate and black pepper.[129,130] Therefore, to a certain extent, HMs adulterated with starch make them impossible for consumers to identify the adulteration by naked eyes, which greatly reduces the expected nutritional value of Chinese herbal medicine.[131] Starch also has a negative effect on the quality of HMs. For instance, starch can greatly reduce the content of curcumin and inhibit the biological activity of turmeric.[132,133] Furthermore, unscrupulous merchants also add other exogenous substances to HMs that can create health hazards. For example, the addition of yellow chalk powder to Turmeric powder can cause facial swelling and loss of appetite.[134]

Table 3 summarizes the references on detection of foreign matter adulteration by using analysis methods. In this type of adulteration, the introduction of adulterant can modify the overall chemical composition of HMs, which is reflected in the potential shift in the fingerprint.[148] The strategy of non-targeted discriminant is thus appropriate to identify the type of adulterate of the known foreign matters. In addition, it can also be used to identify the different concentrations of the same foreign matter. For example, Hu et al.[136] proposed a classification strategy with the use of fourier transform infrared (FTIR) spectroscopy combined with PCA and PLSDA to identify kudzu root samples with different adulteration degrees of cassava flour, and the accuracy of the prediction set was 97%. The results showed that non-targeted discriminant methods can quickly and effectively identify the labeled doping ratio of kudzu root samples. Another example is that Hu et al.[139] successfully classified the blend of different adulteration ratio of kudzu root by using fluorescence spectroscopy coupled with multilinear partial least squares discriminant analysis (N-PLS-DA) and multi-way principal components analysis (M-PCA). In contrast, prediction methods could directly predict the adulteration ratio of foreign matter. Nie et al.[149] used least-squares support vector machines (LS-SVM) and PLSR to establish a prediction model for three wavelength ranges of visible spectral data of *P. notoginseng* doped with corn flour, and the values of the correlation coefficient of prediction were 0.940, 0.939, and 0.867, respectively. Hence, in many studies, non-targeted

Table 3. Summary of references applying analysis methods to detect adulteration of foreign matter.

Year	Herbal medicine	Foreign matter	Detect technique	Chemometrics	Minimum detection concentration / LOD	Analysis method	Ref.
2015	Kudzu starch	Sweet potato starch, potato starche, and talcum powder	NIR	PLS-CM, OC-PLS	4% (plant starches), (talcum powder)	c	[135]
2017	Kudzu root power	Cassava powder	FT-IR	PCA, PLS-DA	2%	d	[136]
2017	Sichuan pepper power	Wheat bran, rice bran, corn flour, and rosin powder	NIR	PCA, PLSR	1%	d	[137]
2017	Shanyao power	Corn starch, and wheat starch	NIR	PCA, OPLS-DA, PLSR	10%	d, and f	[115]
2017	*Gastrodia elata*	*Polygonatum sibiricum* powder, taro starch, sweet potato starch, and potato starch	FT-NIR	PLSDA	1%	c	[138]
2018	Kudzu root power	Cassava flour	EEM	M-PCA, N-PLS-DA	2%	d	[139]
2018	Black pepper	Sorghum or Sichuan pepper	DRIFTS	PCA, GA-SVM, PLS-DA	5%	d, and f	[140]
2019	*P. notoginseng* powder	*Sophora flavescens* powder, and corn flour	NIR	PCA, PLSR	10%	f	[141]
2019	Turmeric powder	Corn starch	FT-NIR	PCA, PLSR	1%	f	[142]
2019	Paprika powder	Corn flour	NIR	LDA, PLSR	1%	d, and f	[143]
2019	Saffron	Corn stigma, chrysanthemum, and safflower	HPLC-MS/MS	NM	10%	a	[144]
2020	Paprika powder	Potato starch, acacia gum, and Annatto	NIR	PLS-DA, PLSR	NA	d, and f	[145]
2020	Black pepper, cumin	Cassava starch, and corn flour	NIR	PCA, MLR, PLSR	5%	d, and e	[23]
2020	Saffron	Lotus stamens, and corn stigmas	NIR	PLS-DA, PLSR	5%	d, and f	[146]
2020	*P. notoginseng*	Corn flour, and *S. flavescens* powder	NIR	SIMCA	12% (corn flour), 10% (*S. flavescens* powder)	c	[147]

Abbreviation: NIR, near-infrared spectroscopy; MIR, mid-infrared spectroscopy; FT-NIR, Fourier transform near-infrared; EEM, excitation-emission; DRIFTS, diffuse reflectance midinfrared Fourier transform; HPLC-MS/MS, high-performance liquid chromatography with tandem mass spectrometry; FT-IR, fourier transform-infra red; PLS-CM, partial least squares class model; PCA: principal component analysis; PLS-DA: partial least squares discriminant analysis; PLSR: partial least squares regression; OPLS-DA: orthogonal partial least squares-discriminant analysis; OC-PLS, one-class partial least squares; LDA, linear discrimenant analysis; N-PLS-DA, multi-way partial least squares discriminant analysis; M-PCA, multi-way principal component analysis; GA-SVM: genetic algorithm-support vector machine; MLR, multiple linear regression; NM, no mention.

Analysis method: a, targeted analysis; b, targeted analysis combined with discrimine method; c, non-targeted analysis combined with class-modeling; d, non-targeted analysis combined with discrimine method; e, targeted analysis combined with regression method; f, non-targeted analysis combined with regression method; g, the combination of non-targeted and targeted analysis.

discriminant methods and non-targeted prediction methods are constantly used in combination to address the issue of the type and degree of adulteration by the addition of foreign matter. For example, Ma et al.[115] used NIR combined with orthogonal partial least squares-discriminant analysis (OPLS-DA) and PLSR to identify and quantify the adulteration ratio of ultrafine granular powder of Shanyao. Another example is that Oliveira et al.[145] used PLS-DA and PLSR to establish a qualitative and quantitative model for NIR spectral data of paprika powder. In fact, the non-targeted analysis techniques combined with one-class strategy are best suited to identify HMs adulterated with foreign matter, since it defined the authentic properties of HMs to eliminate the influence of a vast range of unknown foreign matters. For instance, Lafeuille et al.[148] used microscopy and FT-IR in combination, along with PCA were used for detecting a broad range of potential foreign matters of black pepper. Furthermore, Xu et al.[135] used NIR combined with partial least squares class model (PLSCM) to establish a non-targeted method for the detection of kudzu starch with five potential adulterants. The strategy of non-targeted analysis technique combined with one-class classification thus is a practical, rapid tool to identify the known or suspected to exist specific foreign matters through establishing the authentic properties of HMs.

In summary, non-targeted analysis technology can be an efficient technique for identifying adulteration by foreign matter, grouping them based on differences in their fingerprint profile. Vibrational spectroscopy has allowed the development of nondestructive and rapid approach to determine foreign matter, such as corn flour, cassava powder, etc. PCA, PLS-DA, PLSR, and SIMCA are the suitable multivariate analyses in identification of adulteration by addition of foreign matter using spectroscopy.

5.2.2. Adulteration by addition of inferior production-own materials

In this group are inferior production-own materials, including spent powder and different medicinal parts of HMs, which are intentionally added to HMs to obtain an economic benefit. Examples of HMs with spent powder include nutmeg, black pepper, etc.[36,148] Furthermore, it is not uncommon for the different parts of HMs with less active ingredients or pharmacological activity added in the raw materials aiming to increase the volume and weight. For instance, the addition of stem and leaf powder in pure root powder of *P. notoginseng*.[54] Black pepper husk mixed with black pepper.[150] It is worth mentioning that it is complex to identify these adulterants since they are part of the same plant. Therefore, appropriate analytical strategies must be proposed to deal with the adulteration of different characteristics.

Different analysis methods utilized for detection of inferior production-own materials have been summarized in Table 4. In the identification of adulteration, targeted methods can be used to deal with the adulteration of biomarkers that existed in different medicinal parts. Given the multi-component property, targeted analysis technology, in combination with discrimination methods, is also appropriate. For instance, Li et al.[54] successfully analyzed nonvolatile characteristic components of *P. notoginseng* samples mixed with the different proportions of leaf and stem powder by using high-performance liquid chromatography (HPLC). Based on the content of 10 characteristic compounds, PCA and mahalanobis distance were used to classify the different adulteration degrees of stem and leaf powder. Additionally, non-targeted analysis combine with targeted analysis are also applicable to the identification of adulterants. For example, Qiu et al.[153] proposed a "commercial-homophyletic" comparison strategy to illustrate or preliminarily illustrate 164 compounds from five different parts of *P. ginseng*, of which 11 can be used as biomarkers. On this basis, the ANN model using the normalized ion intensity of biomarkers to exactly discriminate against different degrees of root adulterant with leaf. Regarding the non-targeted methods, the classification model is quick to identify adulterate. Kiani et al.[36] established a classification model with the use of HSI spectral data of nutmeg adulterated with inferior production-own materials combined with PLS-DA and ANN. Both ANN and PLS-DA showed good performance to detect adulteration at low levels. Furthermore, Wilde et al.[150] established NIR and FTIR of black pepper mixed

Table 4. Summary of references applying analysis methods to detect adulteration of inferiorproduction-own materials.

Year	Herbal medicine	Counterfeits	Detect technique	Chemometrics	Minimum detection concentration / LOD	Analysis method	Ref.
2015	Saffron	Saffron stamens, safflower, turmeric, and gardenia	^{1}H-NMR	PCA, OPLS-DA, O2PLS-DA	20%	d	[47]
2017	Saffron	Sativus stamens, calendula, safflower, turmeric, buddleja, and gardenia	DRIFTS	PLS-DA, PLSR	1% – 3.1%	d, and f	[151]
2019	Black pepper	Papaya seeds, chili, black pepper husk, pinheads, and defatted spent materials	NIR, FT-IR	PCA, OPLS-DA	10%	c	[150]
2019	Saffron stigmas	Stamens, and tepals	UPLC-ESI/QTOF-MS	OPLS-DA	5%	g	[39]
2019	Nutmeg	Pericarp, shell, and spent power	HSI	PCA, PLS-DA, ANN	5%	d	[36]
2020	Saffron stigmas	Exhaustively-extracted saffron stigmas, saffron stamens, hibiscus, curcuma, paprika, calendula flower, pomegranate fruit pee, and safflower	NIR	SIMCA, PLS-DA, PLSR	10 mg g^{-1}	c, and f	[24]
2020	*P. notoginseng* power	The stem and leaf powder	HPLC	PCA-MD		b	[54]
2020	Paprika	Spent paprika samples	FT-IR, NIR	OPLS-DA	50% (NIR), 40% (FTIR)	c	[152]

with defatted spent materials, black pepper husk as well as pinheads, and binary supervised OPLS-DA were used for non-targeted analysis. The results showed that the classification model based on FTIR and NIR data could quickly detect black pepper mixed with inferior production-own materials. In addition, the classification model and prediction model are often used in combination with a comprehensive qualitative and quantitative analysis of the adulterate of inferior production-own materials. Shawky et al.[24] used NIR combines with SIMCA and PLS-DA classification models to identify commercial adulteration of saffron stigma. The results showed that the SIMCA model could successfully authenticate the stigma of saffron, while PLS-DA could effectively identify the real pure samples at the lowest 10 mg g^{-1} adulterated samples. On this basis, 720 saffron samples mixed with eight adulterants were designed and one multivariate calibration method, named PLSR, was used to predict the quantitative amount of eight known adulterants.

Abbreviation: ^{1}H-NMR, nuclear magnetic resonance; DRIFTS: diffuse reflectance infrared Fourier transform spectroscopy; NIR, near-infrared spectroscopy; UPLC-ESI/QTOF-MS, ultra-high performance liquid chromatography coupled with electrospray ionization and quadrupole time-of-flight mass spectrometry; HIS, hyperspectral imaging; HPLC, high performance liquid chromatography; FT-IR, fourier transform-infra red; PCA: principal component analysis; PLS-DA: partial least squares discriminant analysis; PLSR: partial least squares regression; OPLS-DA: orthogonal partial least squares-discriminant analysis; O2PLS-DA, bidirectional modifications orthogonal partial least squares-discriminant analysis; ANN, artificial neural network; SIMCA, soft independent modeling of class analogy; PCA-MD, principal component analysis-mahalanobis distance; NM, no mention.

In summary, both non-targeted and targeted analysis technologies are suitable to identify adulteration by inferior production-own materials. Although the efficiency of targeted analysis technology is lower than that of non-targeted analysis technology. Targeted analysis technology can be used to detect inferior production-own materials with the same characteristic compounds as the real HMs. PCA-MD and PLS-DA are the efficient multivariate analyses for targeted analysis technology, especially in the term of identification of adulteration of inferior production-own materials.

5.2.3. Adulteration by addition of chemical compounds

HMs can be adulterated using chemical compounds that can make it appear more valuable. Various diseases and even death can be caused by chemical compounds, such as synthetic dyes, sildenafil and, tadalafil. Azo dyes and triphenylmethane dyes are the synthetic dyes with greatest presence in adulteration of chemical compounds.[116] The most commonly used azo dye in HMs are the Sudan dyes family, rhodamine B, metanil yellow, etc., which can make a serious threat to human health. For example, Sudan I, rhodamine B, and metanil yellow are considered to be a carcinogen. It is worth mentioning that, in some cases, certain official standards cannot recognize the adulteration by dyes. For instance, United States Pharmacopeia (USP) test cannot identify the *Hypericum perforatum* adulterated with dyes, making the detection of fraud even more complicated. Side effects of sibutramine contain insomnia, dizziness, headache as well as nausea, etc. Plenty of herbal supplements used to improve sexual performance are adulterated with sildenafil.[154] In addition, the addition of lead oxide to chili powder caused 60 people to be hospitalized.[155]

Table 5 summarizes the references on the chemical compounds adulteration detection by using analysis methods. Among them, targeted analysis is the rapid and sensitive platform with the greatest application for identifying of adulteration of chemical substances. As a case study, in order to determine dye in pepper products sensitively and quickly, Fu et al.[166] established a quantitative analysis model of rhodamine B (RhB) through HPLC. According to the excitation peak and emission peak of RhB, the constant wavelength interval was set to 25 nm, and the detection limit in chili powder is 1.3 ng g^{-1}. Cheng et al.[61] used MS hyphenated technique to successfully quantify abuse synthesis drugs in 120 batches of plant dietary supplements. The result indicated that nearly a quarter of supplements contained fluoxertine and sibutramine. In a similar way, the combination of ultraviolet with high-performance thin layer chromatography had also been used for quantitative illegal compounds in supplements. The quantitative indicted that among 52 batches of weight loss supplements, sibutramine was positive in 26 samples.[158] Regarding non-targeted methods, the classification model is a feasible, simple, and rapid method for the detection of adulteration. For example, Anibal et al.[159] used UV-visible spectroscopy combines with two classification techniques, named k-NN and PLSDA, for the identification of Sudan dyes in Culinary Spices samples at three concentration levels. Both of the two classification techniques had great performance, and none of the adulterated culinary spices were assigned as unadulterated. Dowlatabadi et al.[46] used the ^{1}H NMR to verify the authenticity of saffron, along with PCA and PLS-DA to establish a one-class model. The results showed that the model could successfully discriminate the authenticity of the samples with outstanding sensitivity and specificity. Additionally, non-targeted analysis combined with regression model, enables predicting the degree of adulteration. As a case study, Kar et al.[134] established the metanil yellow contents prediction model for turmeric powder by NIR. On this basis, a prediction model that applied two multivariate calibration methods, named PCR and PLSR, were designed. The result indicated that both the regression models could predict the adulteration degree which exceeds 0.3%. In a similar way, the content of Sudan dye in paprika powder was quantified by using FTIR combined with hybrid linear analysis (HLA).[167]

SERS, surface enhanced raman spectroscopy; PCA: principal component analysis; SIMCA: soft independent modeling of class analogy; PLS-DA: partial least squares discriminant analysis; PLSR: partial least squares regression; OPLS-DA: orthogonal partial least squares-discriminant

Table 5. Summary of references applying analysis methods to detect adulteration of chemical compounds.

Year	Herbal medicine	Counterfeits	Detect technique	Chemometrics	Minimum detection concentration / LOD	Analysis method	Ref.
2011	Chili foods	Sudan I, Sudan II, Sudan III, and Sudan IV	HPLC-DAD	NM	5.6 mg kg^{-1} (Sudan I), 4.2 mg kg^{-1} (Sudan II), 4.1 mg kg^{-1} (Sudan III),	a	[156]
2011	culinary spices	Sudan III, and Sudan IV	^{1}H NMR, ^{1}H NMR, UV-vis	PLS-DA	5.8 mg kg^{-1} (Sudan IV) 1.4 g kg^{-1} (Sudan III),	d	[45]
2011	Curry, turmeric, mild, and hot paprika	Sudan I, Sudan II, Sudan III, and Sudan IV	^{1}H NMR	PLS-DA	1.4 g kg^{-1} (Sudan IV) NM	a	[44]
2012	Paprika powders	Sudan I	Raman, FT-Raman, SERS	PCA	0.01 kg kg^{-1} (Raman, FT-Raman), 0.0036 kg kg-^{1} (SERS)	d	[157]
2013	Weight loss supplements	Sibutramine	HPTLC-UV	NM	0.3 mg (Sibutramine)	a	[158]
2014	Mild, hot, and smoked paprika	Sudan I, and Sudan IV	UV-vis	PCA, PLS-DA, k-NN	1 ppm	d	[159]
2015	Chili powders	Sudan I	NIR, and Raman	PLS-DA	0.25% (NIR), 0.88% (Raman)	d, and f	[160]
2016	Turmeric Powder	Metanil yellow	FT-Raman, and FT-IR	NM	1% (FT-Raman), 5% (FT-IR)	e	[161]
2017	Paprika powder	Sudan I	FT-IR	HLA	0.016%	f	[162]
2017	Paprika powders	Sudan I	SSNMR, and ^{1}H NMR	NM	128.6 mg kg^{-1} (SSNMR), 6.7 mg kg^{-1} (^{1}H NMR)	e	[62]
2017	Saffron	Safflower, tartrazine, and naphthol yellow	^{1}H NMR	PCA, PLS-DA	20% (tartrazine), 70% (naphthol yellow), 30% (Safflower)	d	[46]
2017	Weight loss plant dietary supplements	Ephedrine, methamphetamine, amfepramone, phenformin, clenbuterol, bupropion, fenfluramine, citalopram, paroxetine, fluoxertine, sibutramine, bisacodyl, and rimonabant	UPLC-LTQ-Orbitrap HRMS	NM	0.3 ng g^{-1} (ephedrine), 1ng g^{-1} (methamphetamine), 0.4 ng g^{-1} (amfepramone), 0.4 ng g^{-1} (Phenformin), 1ng g^{-1} (clenbuterol), 1ng g^{-1} (bupropion), 0.3 ng g^{-1} (fenfluramine), 1ng g^{-1} (citalopram), 1ng g^{-1} (paroxetine) 1ng g^{-1} (fluoxertine), 2ng g^{-1} (sibutramine), 0.3 ng g^{-1} (bisacodyl),	a	[61]
2018	Turmeric	Metanil yellow	NIR	PCR, PLSR	0.3 ng g^{-1} (rimonabant)0.3% (PCR, PLSR)	f	[134]
2018	Curry powder	Metanil yellow, and Sudan I	Raman	SMA	1%	e	[163]
2018	Paprika powder	Sudan I, Sudan IV, lead chromate, lead oxide, silicon dioxide, polyvinyl chloride, and gum arabic	FT-MIR	SIMCA	1% (Sudan I and Sudan IV), 3% (lead chromate), 3% (lead oxide), 5% (silicon dioxide), 10% (polyvinyl chloride), 10% (gum arabic)	c	[164]
2019	Paprika powder	Sudan I	Raman	PLSR, PLS-DA	0.5%	c, and f	[25]
2019	Sumac, hot chili, cumin, paprika, curry, turmeric	Sudan I, Sudan II, Sudan III, Sudan IV, Para Red, Orange G, and Red 7B	HPLC-DAD	NM	0.01 mg kg^{-1} (Sudan I, II, III, IV), 0.03 mg kg^{-1} (Sudan Red 7B), 0.02 mg kg^{-1} (Sudan Orange G)	a	[56]
2020	Turmeric	tartrazine and rice flour	UV imaging, NIR imaging	PCA, Bhattacharyya distance	5% (mixture of tartrazine and rice flour)	f	[165]

Abbreviation: HPLC-DAD, high-performance liquid chromatography with diode array detection; ^{1}H-NMR, nuclear magnetic resonance; FT-Raman, fourier transform Raman spectroscopy; SSNMR, solid-state nuclear magnetic resonance spectroscopies; FT-IR, fourier transform-infra red; FT-MIR, fourier transform mid-infrared spectroscopy.

Analysis method: a, targeted analysis; b, targeted analysis combined with discrimine method; c, non-targeted analysis combined with class-modeling; d, non-targeted analysis combined with discrimine method; e, targeted analysis combined with regression method; f, non-targeted analysis combined with regression method; g, the combination of non-targeted and targeted analysis.

analysis; k-NN: k-nearest neighbors; SMA, self-modeling mixture analysis; NM, no mention.

In the detection of adulteration of chemical compounds, targeted technology seems to be relatively mature and widely applicable technology. PLSR is the regression method with the greatest presence in quantitative analysis of chemical compounds. Although the simplicity of direct quantity performed by targeted analysis technology is lower than that of indirect quantity performed by targeted analysis technology combined with regression method, targeted analysis technique can achieve accurate quantitative analysis with low LOD and high sensitivity.

6. Conclusions

Taking into account the importance of HMs safety, HMs fraud, in the past decade, had become a main focal point for consumers, industry, and policymakers. Hence, the selection of analysis technologies together with chemometrics is a top priority in an attempt to be better implemented into routine use. Each analysis technique shows different applicability according to the characters of samples. Even at present, targeted analysis was still preferable methodology for fraud detection due to the advantages of sensitivity and specificity. Non-targeted analysis technology is the most use one due to its nondestructive, reproducibility, and easy sample preparation. The combination of non-targeted analysis and targeted analysis merging the advantages of both is the most worth further researched. In the application of the identification of counterfeits and adulterant, chemometrics methods are required to establish an effective model. The performance of the model may be affected by pre-processing, feature selection and extract, and modeling methods. Currently, the discriminate method is the multivariate analyses with the greatest presence in detection of HMs fraud. Further research and development of discriminate approach combined with outliers have the potential to become a powerful detection technology. Considering the thousands of adulterants in routine life, the class-modeling is a mainstream technology to identify the authentication of HMs. Regarding regression methods, it will need a long way in routine life to make LOD or the minimum detection concentration to meet the requirement of law. The integration of analysis technologies and chemometrics methods will offer a suitable method in HMs fraud. Although the use of this integration has proven their potential in the identification of counterfeits and adulterants, it is still necessary to develop on-site and real-time analysis technology and "user-friendly" chemometric methods, which are of large significant for the further application in routine life. Likewise, the establishment and development of databases that containing comprehensive information of HMs fraud are also critical to predict and combat HMs fraud.

Disclosure statement

The authors declare that there are no conflicts of interest.

Funding

This work was funded by the National Natural Science Foundation of China (31860584); the National Natural Science Foundation of China (31760360); the major science and technology projects in Yunnan Province (Grant number: 2018ZF010).

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Profiling of Phenolic Compounds of Fruit Peels of Different Ecotype Bananas Derived from Domestic and Imported Cultivars with Different Maturity

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Abstract: Banana is one of the most produced and consumed fruits in the world and its fruit peel accounts for about 40% of the total fresh quantity of ripe fruit, which is usually regarded as waste and poses serious environmental hazards. However, it is a promising source of natural bioactive compounds including phenolic compounds. Determination of the phenolic compounds in fruit peel from different cultivars and subgroups over a range of maturities provides convincing information for making full use of them. This study developed a sensitive and reliable analytical method—ultra-high performance liquid chromatography—coupled with electrospray ionization tandem mass spectrometry (UPLC-MS/MS) for measuring phenolic compounds in fruit peel from different ecotype cultivars and subgroups with different maturity. The results showed that quinic acid had the highest concentration ratio among the main phenolic compounds in the green/ripe peel of all banana cultivars; among all banana cultivars, the total phenolic compound contents of green banana peel were significantly higher than that of ripe banana peel; the total phenolic compound contents in the green/ripe fruit peel of non-dessert bananas were significantly higher than that of dessert bananas (green: non-dessert banana 1.48 ± 0.44 mg/g vs. dessert banana 0.97 ± 0.12 mg/g; ripe: non-dessert banana 0.26 ± 0.13 mg/g vs. dessert banana 0.19 ± 0.06 mg/g). These data provide a basis for the rational utilization of phenolic compound extractions from banana peel with huge biomass in the next step.

Keywords: banana; varieties; subgroup; ripeness; fruit peel; phenolic compounds; UPLC-MS/MS

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Citation: Zhang, J.; Wang, Y.; Yang, B.; Li, Y.; Liu, L.; Zhou, W.; Zheng, S.-J. Profiling of Phenolic Compounds of Fruit Peels of Different Ecotype Bananas Derived from Domestic and Imported Cultivars with Different Maturity. *Horticulturae* **2022**, *8*, 70. https://doi.org/10.3390/horticulturae8010070

Academic Editors: Jelena Popović-Djordjević and Luiz Fernando Cappa de Oliveira

Received: 15 November 2021
Accepted: 5 January 2022
Published: 12 January 2022

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1. Introduction

Banana is an important tropical and subtropical fruit crop [1], and is one of the most produced and consumed fruits in the world [2,3]. According to the latest data of Food and Agriculture Organization (FAO) in 2020, India was the world's largest banana producer, followed by China, with annual production reaching 30.46 and 12 million tons, respectively [4]. Banana peel accounts for about 40% of the total fresh quantity of ripe fruit [5], which is largely discarded in nature and becomes household and industrial food waste [5]. Usually, banana peel waste is indiscriminately dumped into the rivers, lakes, or low-lying landfills where it degrades slowly until it decays and forms methane and carbon dioxide, and other noxious gases such as hydrogen sulfide and ammonia that spread unpleasant odors, affect the nearby ecosystems [6], and cause serious environmental

damage [6–8]. Thus, banana peel waste is needed to recycle or reuse instead of discarding anyway or incinerating.

Banana peel, as agricultural waste, contains a large number of oligosaccharides, cellulose, and mineral elements [9], which is an ideal material for adsorbents and is widely used to reduce environmental pollutants, such as heavy metals, dyes, organic pollutants, nitrate, and arsenate in groundwater, pesticides, and various other gaseous pollutants [7,8]. Banana peel has recently been increasingly reported as a rich source of phenolic compounds [10], with total phenolic content ranging from 4.95 to 47 mg garlic acid equivalent/g dry matter (mg GAE/g DM). This level was 1.5–3 times higher than that recorded in the pulp [11]. These confer a wide range of biological and pharmacological properties with potential as antimutagenic, antiviral antithrombotic, hepatoprotective agents, as well as a food additive and signaling molecules because of strong natural antioxidant properties [12,13]. Many fruit peels' phenolic compounds have now been studied [14]. It is noted that gallic acid, quinic acid, protocatechuic acid, catechin, chlorogenic acid, caffeic acid, ferulic acid, and rutin are their main phenolic compounds [15], which are mainly associated with their antioxidant [16–19]. However, little research is available on the natural contents of main phenolic compounds in the banana peel of different cultivars and maturities for picking out banana peel with rich phenolic compounds for further efficient utilization. Thus, an extraction and analytical method is urgently needed to determine main phenolic compound contents in fruit peel from different banana cultivars and subgroups over a range of maturities.

To add value to the industrial waste from banana processing, an effective extraction would be beneficial to recover functional ingredients sustainably [19,20]. In order to obtain phenolic-rich extracts, conventional extraction methods with organic solvents are commonly used [21]. These extraction technologies are highly polluting, labor-intensive, solvent consuming, and time consuming, so it is necessary to develop green alternative methods [22]. The ultrasound assisted extraction (UAE) could be one of the most suitable techniques [23], as it is very simple to use, requires relatively inexpensive apparatus, reduces the volume of solvent [24], uses acoustic energy and solvents to enhance the release and diffusion of phenolic compounds from various matrices [25], and allows to use green extraction solvents with a high efficiency due to the mechanical effects that it produces in the cellular structure of the matrix [26,27]. Many analytical methods have been used to determine phenolic compound contents in fruit peel, but currently only UPLC-MS/MS could provide higher sensitivity, selectivity, shorter analysis time, and simultaneous determination of many phenolic compounds [28]. Therefore, in the study, we first proposed to use UPLC-MS/MS combining with ultrasonic extraction to simultaneous determine the main phenolic compound contents in banana peel and further explored the difference of phenolic compound contents in banana peel of different cultivars and subgroups with different levels of maturity.

The plant cultivar and maturity are the important factors that influence the synthesis and accumulation of biologically active substances in fruit peel. Thus, our work aimed to develop a sensitive and effective UPLC-MS/MS method combined with ultrasonic extraction for simultaneous determination of eight main phenolic compounds in the fruit peels of different banana ecotypes and subgroups, with different degrees of maturity. Quantification of main phenolic compounds across the fruit peels with different banana ecotypes and maturity will also provide valuable data on their contents, and will demonstrate which cultivar and maturity peel is used to extract individual phenolic compound might be meaningful. Our study contributes to finding new sources of phenolic compounds and laying the foundation for future utilization of banana peel. We could use the fruit peel extracts for the production of functional food, dietary supplements, or other innovative preparations with a specific biological effect.

2. Materials and Methods

2.1. Chemicals and Reagents

Standards (gallic acid, quinic acid, protocatechuic acid, catechin, chlorogenic acid, caffeic acid, ferulic acid, and rutin) were purchased from Sigma Company (Ontario, CA, USA). HPLC-grade methanol and acetonitrile (99.95%) were purchased from Fisher Scientific (Loughborough, UK). Formic acid (HPLC grade) was purchased from Sigma–Aldrich (St. Quentin Fallavier, France). Dimethyl sulfoxide (DMSO, analytical-reagent grade) was purchased from Gaoyao Chemical Reagent Co., Ltd. (Shanghai, China). Deionized water used throughout the study was purified using a Milli-Q water-purification system (Millipore, Bedford, MA, USA).

2.2. Preparation of Stock and Standard Solutions

Individual stock standard solutions of gallic acid, quinic acid, protocatechuic acid, catechin, chlorogenic acid, caffeic acid, ferulic acid, and rutin were prepared on a weight basis in pure methanol at 1.0 mg/mL. Next, 0.1 mL of each individual standard stock solution was accurately measured in a 10 mL amber volumetric flask, and the mixture was diluted with pure methanol to prepare a 100.0 μg/mL mixed standard stock solution. The standard stock solutions were sealed and stored at −80 °C.

The individual standard working solutions (100.0, 10.0, 5.0, 1.0 μg/m, 100.0, 10.0, and 1.0 ng/mL) and mixed standard working solutions (1.0, 0.5 μg/mL, and 200.0, 100.0, 50.0, 20.0, and 10.0 ng/mL) were prepared by diluting the individual standard stock solutions and mixed standard stock solution with pure methanol, respectively. The individual standard working solutions and mixed standard working solution were stable for 1 week at −20 °C.

2.3. Conditions of Ultra-High-Performance Liquid Chromatography–Tandem Mass Spectrometry

The analytical system was UPLC-MS/MS analyses. The model of mass spectrometer (MS) consisted of a Qtrap 6500+ (Applied Biosystems, New York, NY, USA) with an electrospray ionization (ESI) source for molecule ionization. The optimized ESI temperature was set at 500 °C, ionspray voltage at −4500 V for negative mode. The curtain gas and collision gas pressure were 36 and 10 psi, respectively. All qualitative and quantitative data in this study were acquired using MRM mode. A UPLC model 20A (Shimadzu, Kyoto, Japanese) consisted of a binary pump, degasser, and auto-sampler. Peak areas of each constituent were automatically integrated using analyst software. The analytical column was Agilent Zorbax Extend-C_{18} (2.1 × 100 mm, 3.5 μm; Agilent Technologies, Santa Clara, CA, USA). The binary mobile phase contained both methanol (solvent A) and 0.2% (v/v) aqueous formic acid (solvent B), which was pumped at a flow rate of 0.3 mL/min for a total run time of 9 min [29]. The injection volume was 5.0 μL, and the column temperature was 35 °C.

2.4. Samples Collection

Banana samples were imported from the Alliance of Bioversity International and CIAT, the Agrobiodiversity and Banana Research Team of Agricultural Environment and Resources Institute, Yunnan Academy of Agricultural Sciences, then tissue culture and rapid propagation were performed. Afterwards, 10 banana germplasm resources were planted in the experimental base of Xishuangbanna, Yunnan (21°54′13″ N; 100°48′25″ E). The 10 banana ecotypes were, respectively, Pahang, Inkira, Kazirakwe, Brazilian, Reke No. 2, Reke No. 4, Nantianhuang, Baodaojiao, Zhongjiao No. 8, and Yunjiao No. 1. During sampling, the third comb of banana was collected from top to bottom, with 3 repeating bases, one for each cultivar in each base, and the middle 4–6 fruit fingers of each comb of banana shall be continuously taken for measurement and statistics.

Banana germplasms are classified into different subgroups according to clusters of phenotypically similar populations of a particular species. The 10 banana ecotype cultivars in the study were divided into three subgroups based on their origin and genetic relationship:

the Malaccensis subgroup, the Mutika–Lujµgira subgroup, and the Cavendish subgroup. Pahang in the Malaccensis subgroup was the wild banana species from Malaysia, Inkira, and Kazirakwe in the Mutika–Lujµgira subgroup were the cultivated cooked bananas from Africa, and the other seven banana cultivars, in the Cavendish subgroup, were all the cultivated and domesticated cultivars which were commonly grown in China with high yields and the highest degree of commercialization. The first two subgroups belong to non-dessert banana, which includes non-edible banana and boiled banana, and the last subgroup is the dessert banana which includes fresh banana.

2.5. Sample Extraction

According to the color chart of banana maturity, seven ripe-green bananas and full-ripe bananas accelerated with Ethrel were harvested. A knife was used to separate the peel from the pulp to obtain a weighed sample of 100.0 g of fresh fruit peel. After washing and slicing, we put the slices in an oven at 70 °C and dried them for 9–10 h until they were dry. They were then weighed and pulverized, and sieved through a 60-mesh sieve to make a powder for use. Samples of accurately weighed 1.0 g of banana peel powder were placed in a 50 mL centrifuge tube, and 45 mL of 30% acetonitrile was added, and vortexed for 10 s. Then ultrasonic extraction was conducted for 40 min, centrifugation at 5000 rpm/min for 10 min, and then 15 mL of supernatant in a new centrifuge tube was taken. We repeated the above operation 3 times. Finally, 45 mL of the supernatant was obtained, vortexed to mix, and passed through 0.22 µm microporous membrane to obtain the test solution.

2.6. Determination of Fruit Agronomic Traits, the Proportion and the Moisture Content of the Peel in Different Green/Ripe Banana Ecotypes

Six fruit agronomic characters of green and ripe banana were measured, including fruit finger girth, fruit finger length, peel thickness, fresh weight of pulp, fresh weight of peel, and fruit finger fresh weight. The girth of fruit finger refers to the circumference of the middle position of fruit finger. The fruit finger length refers to the length from the top of the fruit along the outer edge of the curved surface to the base of the fruit handle. Then, we determined the proportion of the fresh green fruit peel and dry weight of fruit peel of 10 banana cultivars. The proportion of fresh green fruit peel was the percentage of fresh green fruit peel fresh weight/green banana fresh weight, the dry weight ratio of banana peel refers to the percentage of dried banana peel weight/fresh banana peel weight. The proportion of dry weight of the peel can be used to explain the water content of the peel. The smaller the dry weight is, the higher the water content is. The data indexes of banana samples were determined six times.

2.7. Statistical Analysis

For the quantitative analysis of phenolic compounds in banana peel, we measured 10 samples for each banana cultivar and calculated the average value. The validation experiment was performed in six replicates. Experimental data are represented as means ± SD and $p < 0.05$ was considered to be statistically significant. Data were calculated by one-way analysis of variance (ANOVA) and analyses were performed using SPSS 26.0. Probability values of less than 5% were considered to be significant.

3. Results and Discussion

3.1. Optimization of Multiple Reaction Monitoring (MRM) Parameters

At first, in the mass spectrum system, the Q1 quadrupole mass spectral analyses in full scan mode (m/z 100–300) were conducted to search in ion mode and precursor ions to search in the MRM method [30]. This was respectively obtained by ESI in the negative and positive ion mode. Eight kinds of phenolic compounds could display the deprotonated ion peak ($[M-H]^-$) in the negative ion mode and the protonated ion peak ($[M+H]^+$) in the positive ion mode. All the phenolic compounds showed a stronger response in the negative ion mode than that in the positive ion mode, which was the same as the result of

Fan et al. [31]. Thus, the negative ion mode was selected for the MRM method, and the deprotonated ion in ($[M-H]^-$) for the eight compounds was selected as the precursor ion. The results are shown in Table 1. After selecting precursor ions in the Q1 quadrupole mass spectra, the product ion scan mode was applied for fragment ions in the Q3 quadrupole mass spectra. One precursor ion and two main ion fragments were selected and optimized with collision energy (CE) and declustering potential (DP) voltage for each compound in the MRM mode [32]. All the optimized MRM parameters are shown in Table 1.

Table 1. The optimized parameters of multiple reaction monitoring (MRM) mode (n = 6) [33].

Compounds	Molecular Weight	Retention Time (min)	Ionization Mode	Precursor Ions	Product Ions	DP (V)	CE (eV)
Gallic acid	170.12	2.68 ± 0.15	$[M-H]^-$	169.0	**124.9**	−20	−21
					125.0	−20	−21
Quinic acid	192.17	3.92 ± 0.15	$[M-H]^-$	191.0	**85.0**	−30	−28
					93.0	−30	−27
Protocatechuic acid	154.12	4.03 ± 0.10	$[M-H]^-$	152.9	**109.0**	−30	−23
					108.9	−30	−18
Catechin	290.27	6.76 ± 0.21	$[M-H]^-$	289.1	**109.0**	−30	−30
					203.1	−30	−27
Chlorogenic acid	354.31	6.96 ± 0.23	$[M-H]^-$	353.1	**191.1**	−30	−24
					179.0	−30	−19
Caffeic acid	180.15	7.23 ± 0.20	$[M-H]^-$	179.1	**135.1**	−30	−21
					134.1	−20	−34
Ferulic acid	194.19	7.78 ± 0.21	$[M-H]^-$	193.0	**134.0**	−20	−23
					178.0	−20	−16
Rutin	610.51	7.85 ± 0.18	$[M-H]^-$	609.2	**300.1**	−30	−51
					271.1	−30	−74

Note: Among the product ions, those marked in bold were quantitative ions, and those not marked in bold were qualitative ions.

3.2. Optimization of Separation Conditions

To completely separate and preserve the eight analytes on the column, we referred to the reported methods [34], and an Agilent ZORBAX Extend-C_{18} column was selected for UPLC. In UPLC, polar compounds showed good retention on this column, and the column can tolerate a wide pH range. In order to obtain the best separation of target analytes, we tested the feasibility of different mixtures of solvents (such as methanol-water and acetonitrile-water) using various buffers (such as 0.05%, 0.1%, 0.2% formic acid solution and 1, 2, 3 mM of ammonium formate solution) with different flow rates and column temperatures, to optimize the separation condition in the C_{18} column. It was determined that both acetonitrile and methanol can elute these eight compounds. Eight compounds were found to be difficult in separating well in acetonitrile, although the elution capacity of acetonitrile was higher than that of methanol. Therefore, we selected methanol for the organic phase. In addition, the peaks of gallic acid and quinic acid were always trailing in ammonium formate buffer or 0.1% formic acid-1 mM ammonium formate solution. Finally, we concluded that the amine salt was not suitable for the determining the peak shape of phenolic compounds.

The acid formulation in solution as an aqueous phase appears to be a better choice due to better resolution and good peak shape. We investigated the effect of adding 0.05%, 0.1%, and 0.2% (v/v) formic acid on the sensitivity of each analyte in the aqueous phase. This improved separation made the peak shape more symmetrical and increased the retention time of the target analytes. The maximum response of the eight target analytes was attained with 0.2% formic acid, and the peaks and separation effects of each compound were enhanced. Therefore, methanol−0.2% (v/v) formic acid was deemed suitable for these compounds after testing. In order to achieve effective separation of the eight phenolic compounds, the gradient elution program was optimized by adjusting the mobile phase ratio and analysis time. Finally, the optimum liquid chromatographic conditions for

separating eight phenolic compounds were established. The gradient elution program was as follows: 0–4% A for 1 min, 4–15% A for 2 min, 15–55% A for 1 min, 55–60% A for 1.5 min, 60–90% A for 0.5 min, 90–4% A for 1 min, and 4% A for 2 min. Mass ion chromatograms of the phenolic compound solutions are shown in Figure 1. Their retention times are shown in Table 1.

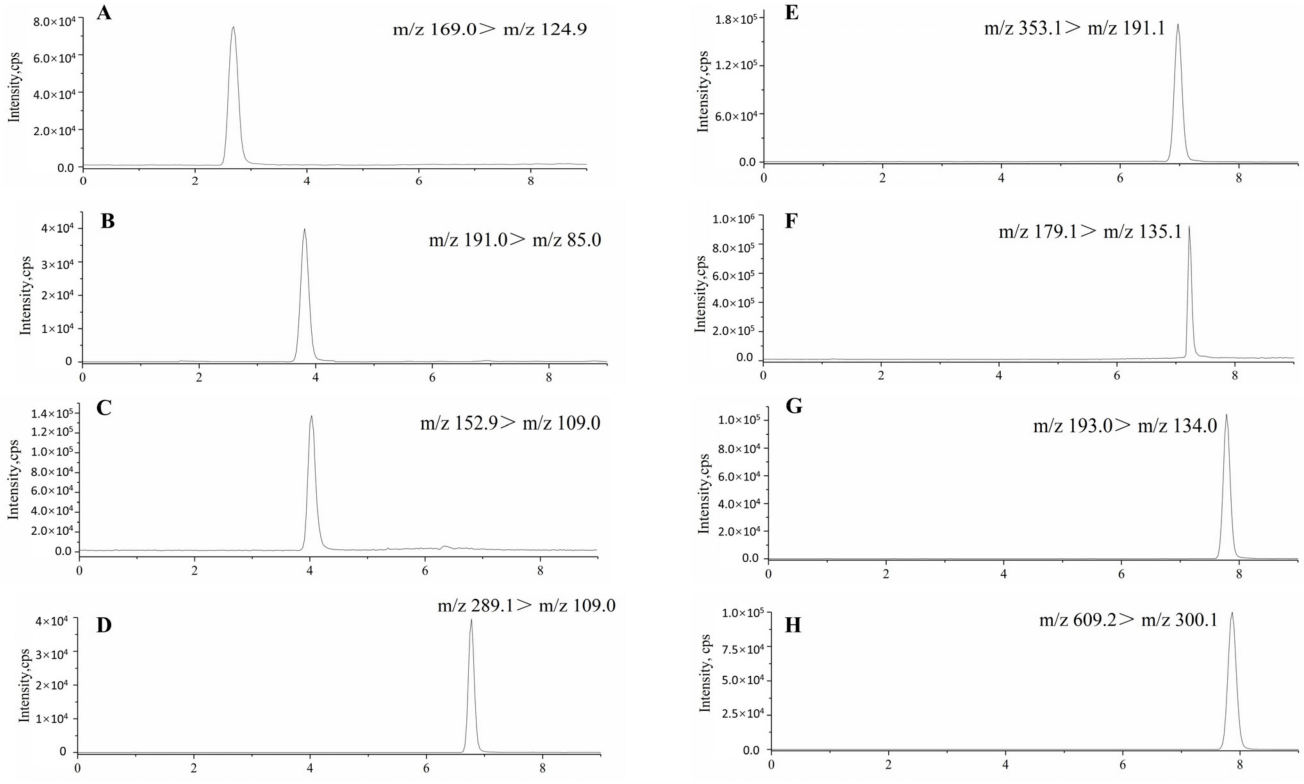


Figure 1. MRM chromatography of gallic acid (**A**), quinic acid (**B**), protocatechuic acid (**C**), catechin (**D**), chlorogenic acid (**E**), caffeic acid (**F**), ferulic acid (**G**), and rutin (**H**) was spiked at 10 ng/mL concentration in methanol solution.

3.3. Optimization of Extraction Conditions

Jimenez et al., indicated that UAE had a great potential to improve the extraction of bioactive compounds from natural products [35]. In order to obtain better extraction yield, the main 4 factors were optimized in an ultrasonic extraction method based on a single factor experiment, using specified acetonitrile concentration (30%, 50%, 70%), extraction times (40, 60, 90 min), solid–liquid ratios (1:15, 1:30, 1:45 g/mL), and the numbers of extraction (1, 2, 3 times). On this basis, a four-factor three-level orthogonal experiment L_9 (3^4) was designed (Table 2). The influence of the four factors on the extraction of phenolic compounds from banana peel was as follows: the number of extraction > solid–liquid ratio > extraction time > acetonitrile concentration. The optimal extraction process was as follows: the number of extraction was 3 times, the solid–liquid ratio 1:45 g/mL, extraction time 40 min, and the acetonitrile concentration 30%.

3.4. Method Validation of UPLC-MS/MS Analysis

The developed method was validated by determining the linearity, recovery, accuracy, stability, precision (intra-day precision and inter-day precision), limit of detection (LOD), and limit of quantification (LOQ) as established by the European Union and the Food and Drug Administration [36]. An external standard method was used for quantification.

Table 2. Orthogonal test and measurement results.

Experiment Number	Factors				Results
	Acetonitrile Concentration (A)	Extraction Time (B)	The Numbers of Extraction (C)	Solid-Liquid Ratio (D)	
1	1	1	1	1	2.34
2	1	2	2	2	3.64
3	1	3	3	3	4.48
4	2	1	2	3	3.83
5	2	2	3	1	4.07
6	2	3	1	2	2.45
7	3	1	3	2	4.38
8	3	2	1	3	2.60
9	3	3	2	1	3.11
K1	10.46	10.55	7.39	9.52	
K2	10.35	10.31	10.58	10.47	
K3	10.09	10.04	12.93	10.91	
k1	3.49	3.52	2.46	3.17	
k2	3.45	3.44	3.53	3.49	
k3	3.36	3.35	4.31	3.64	
Range	0.13	0.17	1.85	0.47	
Factor priority order	C > D > B > A				
Excellent level	A1	B1	C3	D3	
Excellent combination	A1B1C3D3				

Note: 1, 2, and 3 in the columns of A, B, C, and D, respectively, represent the acetonitrile concentration (30%, 50%, 70%), extraction time (40, 60, 90 min), and the numbers of extraction (1, 2, 3 times) and the solid–liquid ratio (1:15, 1:30, 1:45 g·mL^{-1}) of each of the four single factor levels.

3.4.1. Linearity Range, Limits of Detection (LOD), and Limits of Quantification (LOQ)

The calibration curves of eight phenolic compounds were constructed by plotting each of the peak-area ratios (y) of targets to IS versus the corresponding concentration (x) of targets at different levels using the weighted ($1/x^2$) least squares linear regression model. The linear range was selected according to the concentrations of real samples. All analytes showed good linear regression in the range from 1 to 1000 μg/L with the correlation coefficients no less than 0.9992. The LOD and LOQ were calculated as the concentration for each analyte with signal/noise ratio (SNR) at 3 and 10, respectively. The LOD and LOQ for 8 analytes were in the range of 0.10–1.00 and 0.30–3.00 μg/L, respectively (Table 3).

Table 3. Regression equation, correlation coefficient, linear range, limit of detection (LOD), and limit of quantitation (LOQ) of banana phenolic compounds.

Compounds	Linear Equation	Correlation Coefficients (r^2)	Dynamic Range (μg/L)	LOD (μg/L)	LOQ (μg/L)
Gallic acid	$Y = 1.15 \times 10^4x - 2.38 \times 10^4$	0.9992	1–200	0.8	2.5
Quinic acid	$Y = 3.57 \times 10^3x - 3.19 \times 10^2$	0.9999	1–1000	0.8	3.0
Protocatechuic acid	$Y = 1.46 \times 10^4x - 1.36 \times 10^4$	0.9993	1–200	0.6	2.0
Catechin	$Y = 2.57 \times 10^3x - 6.24 \times 10^3$	0.9994	1–200	0.8	3.0
Chlorogenic acid	$Y = 1.31 \times 10^4x - 3.27 \times 10^4$	0.9999	1–200	0.5	1.5
Caffeic acid	$Y = 4.03 \times 10^4x + 2.14 \times 10^5$	0.9998	1–200	0.1	0.3
Ferulic acid	$Y = 7.71 \times 10^3x - 4.07 \times 10^3$	0.9996	2–200	1.0	3.0
Rutin	$Y = 9.04 \times 10^3x - 2.73 \times 10^4$	0.9995	1–500	0.5	1.5

3.4.2. Recoveries and Relative Standard Deviations

In the recovery experiment, the working standard solutions were spiked at low, medium, and high concentration levels (50%, 100%, and 150% of the measured amount of each component of the sample) [37] for each compound into the samples separately and then processed and analyzed following the procedure articulated in Section 2.5. The recov-

eries of all analytes in banana peel varied between 86.5–94.9% with the relative standard deviations (RSD) lower than 11.0% (data only shown for medium level) (Table 4).

Table 4. Recovery of phenolic compounds in the peel of 10 banana cultivars (n = 6).

Compounds	Original Quantity μg/g	Added Scalar Quantity μg/g	Average Recovery/%	Standard Deviation	RSD/%
Gallic acid	2.50	1.25 2.50 3.75	92.2	10.1	11.0
Quinic acid	1000	500 1000 1500	91.7	7.9	8.6
Protocatechuic acid	4.00	2.00 4.00 6.00	94.9	9.0	9.4
Catechin	1.69	1.00 2.00 3.00	87.5	5.1	5.8
Chlorogenic acid	50	25 50 75	86.5	3.3	3.8
Caffeic acid	<LOQ	/	/	/	/
Ferulic acid	3.00	1.50 3.00 4.50	87.0	7.5	8.6
Rutin	50.00	25.00 50.00 75.00	86.7	7.6	8.8

3.4.3. Precision, Repeatability, and Stability

The precision of the method was expressed by intra-day and inter-day RSDs. Intra-day and inter-day precision of the analytical method were measured and assessed by injecting six replicates at low, medium, and high concentrations of the reference compounds on the same day and three consecutive days. The precision was excellent, showing RSD values of intra-day and inter-day variations within 3.7% and 4.1%, respectively. Six samples from the same batch were extracted and analyzed to evaluate the method repeatability. The RSD values of repeatability were lower than 5.5%. The solution stability was analyzed with injecting a sample solution at 0, 2, 4, 8, 12, and 24 h at room temperature. The stability tests showed the analytes were stable enough at room temperature for 24 h (Table 5).

Table 5. Precision, stability, and repeatability of phenolic compounds in 10 banana cultivars (n = 6).

Compounds	Precisions		Stability (RSD%)	Repeatability (RSD%)
	Intra-Day (RSD%)	Inter-Day (RSD%)		
Gallic acid	2.8	3.7	1.7	5.5
Quinic acid	3.3	4.1	2.3	1.7
Protocatechuic acid	2.2	2.9	1.5	1.5
Catechin	2.8	3.2	1.6	1.7
Chlorogenic acid	3.7	3.9	3.0	0.4
Caffeic acid	/	/	/	/
Ferulic acid	1.9	3.9	1.7	3.9
Rutin	1.2	3.6	2.6	3.7

The results indicated the method developed was sensitive, precise, and accurate for simultaneous quantitation of the 8 phenolic compounds in the banana peel samples.

3.5. Determination of Fruit Agronomic Traits of Different Green/Ripe Ecotype Bananas

Among the six agronomic traits (see Section 2.6) of green and ripe banana, Pahang was both significantly lower than the other nine cultivars, $p < 0.001$ (Tables S1 and S2 in Supplementary Materials). There were significant differences in fruit agronomic characters between green and ripe banana among all cultivars. The fruit agronomic character index provided the data basis for the ample utilization of banana peel resources in the future.

3.6. Determination of the Proportion of Peel and the Moisture Content of Peel in Different Green/Ripe Ecotype Bananas

The proportion of fresh green fruit peel of Pahang was 2–3 times of that of the other nine cultivars (Pahang 93.7 ± 8.6% vs. other nine cultivars 43.0 ± 6.0%). Similarly, Pahang had the highest proportion in ripe fruit peel (Pahang 80.7 ± 7.8% vs. other nine cultivars 38.4 ± 5.6%). The dry matter of ripe fruit peel of Pahang was also the highest (Pahang 28.4 ± 3.7% vs. other nine cultivars 9.3 ± 3.2%). The results showed that the peel proportion and dry matter content of the Pahang was significantly higher than those of other cultivars (Table S3 in Supplementary Materials); therefore, Pahang can be used as a dominant cultivar for resource utilization of pericarp.

3.7. Quantitative Analysis of Main Phenolic Compounds in the Banana Peel

3.7.1. Quantitative Analysis of Main Phenolic Compounds in Green Banana Peel

In the green fruit peel extracts of 10 banana ecotype cultivars, three phenolic compounds were dominant: quinic acid, chlorogenic acid, and rutin. The quinic acid content in green fruit peel of Pahang was the highest, at 177 mg/100 g DW (dry weight is about the dried material). In addition, the chlorogenic acid contents of Pahang and Kazirakwe were similar, and significantly higher than the other cultivars, $p < 0.001$. Furthermore, the rutin content in Pahang was 4–200 times higher than that in the other nine cultivars. The content of ferulic acid in 10 banana cultivars was in the middle level, and its content in Pahang was the highest, which was significantly higher than the other nine cultivars, $p < 0.001$. However, among the 10 banana cultivars, the contents of gallic acid, protocatechuic acid, and catechin were very low. Among them, the gallic acid contents of Pahang and Reke No.4 were the highest in the green banana peel, which were significantly higher than that of the other cultivars, $p < 0.001$. The protocatechuic acid content of Pahang was significantly higher than that of other cultivars, $p < 0.001$. The catechin content of Pahang was 10 times higher than that of the other nine cultivars (Table 6). Traces of caffeic acid were detected but in very low amounts in green banana peel; therefore, they were not quantified. There are significant differences in the contents of phenolic compounds in the green banana peel, especially Pahang, the green peel of which can be used as a high-quality raw material for extracting phenolic compounds. Furthermore, green banana peels could be exploited in food and pharmaceutical industries for their high contents in phenolic compounds.

3.7.2. Quantitative Analysis of Main Phenolic Compounds in Ripe Banana Peel

Quinic acid and rutin were the most abundant phenolic compounds in the fruit peel of fully ripe banana cultivars. The quinic acid contents in Zhongjiao No.8, Inkira, and Kazirakwe were the highest, but that in Pahang was the lowest, which was about 10 times lower. Among the 10 banana cultivars, Kazirakwe had the highest rutin content, followed by Pahang and Inkira. Additionally, the ferulic acid content in Pahang was the highest, which was significantly higher than the other nine cultivars, $p < 0.001$. The content of protocatechuic acid in Baodaojiao was the highest, followed by Pahang and Reke No.2. However, among the 10 banana cultivars, the contents of gallic acid, catechin, and chlorogenic acid were very low in ripe banana peel (Table 7). Traces of caffeic acid were detected but in very low amounts in ripe banana peel; therefore, they were not quantified.

Table 6. Determination of main phenolic compounds in green fruit peel of 10 banana cultivars (μg/g).

Cultivar	Gallic Acid	Quinic Acid	Protocatechuic Acid	Catechin	Chlorogenic Acid	Ferulic Acid	Rutin	Total Phenolic Compound Content
Pahang	3.92 ± 0.07 ^{b}	1722.50 ± 35.97 ^{a}	4.16 ± 0.06 ^{a}	10.15 ± 0.16 ^{a}	72.63 ± 1.99 ^{b}	27.93 ± 0.47 ^{a}	235.30 ± 6.12 ^{a}	2076.60 ± 40.49 $_{a}$
Inkira	1.27 ± 0.04 ^{h}	955.60 ± 5.49 ^{e,f,g}	0.92 ± 0.02 ^{j}	0.76 ± 0.11 ^{i}	55.96 ± 1.01 ^{d}	1.23 ± 0.36 ^{h}	14.08 ± 0.42 ^{c}	1029.82 ± 6.21 ^{e}
Kazirakwe	1.30 ± 0.16 ^{g,h}	1195.80 ± 10.73 ^{c}	1.04 ± 0.02 ^{i}	1.28 ± 0.09 $^{c, d}$	72.71 ± 1.76 $_{a,b}$	1.49 ± 0.24 ^{f,g,h}	53.00 ± 1.25 ^{b}	1326.61 ± 11.29 $_{b}$
Brazilian	2.02 ± 0.11 ^{e}	907.45 ± 8.58 ^{h}	2.22 ± 0.03 ^{e}	0.93 ± 0.08 ^{g,h}	2.71 ± 0.55 ^{i,j}	1.51 ± 0.08 $_{e, f,g,h}$	3.87 ± 0.19 ^{g,h,i}	920.71 ± 8.04 ^{i}
Reke No.2	0.73 ± 0.11 ^{j}	952.65 ± 5.11 ^{f,g}	3.86 ± 0.07 ^{b}	0.64 ± 0.03 ^{j}	4.23 ± 0.34 ^{h}	1.16 ± 0.21 ^{i}	1.09 ± 0.08 ^{j}	964.34 ± 4.83 $_{f,g,h,i}$
Reke No.4	4.01 ± 0.12 ^{a}	1488.75 ± 40.67 ^{b,c}	2.25 ± 0.03 $_{d,e}$	1.39 ± 0.09 ^{b}	16.81 ± 0.15 $_{e,f}$	1.57 ± 0.14 $_{d,e,f,g,h,i,j}$	3.15 ± 0.08 ^{h,i}	1218.15 ± 40.60 $_{c}$
Nantianhuang	2.03 ± 0.09 ^{d,e}	939.85 ± 16.43 ^{g}	1.91 ± 0.01 ^{f}	1.22 ± 0.08 ^{d}	5.24 ± 0.04 ^{g,h}	2.60 ± 0.08 ^{b}	6.60 ± 0.25 ^{d,e,f}	959.45 ± 16.49 $_{g,h,i}$
Baodaojiao	2.75 ± 0.09 ^{c}	1026.85 ± 33.35 ^{d}	2.69 ± 0.06 ^{c}	1.01 ± 0.07 ^{e,f,g}	2.58 ± 0.41 ^{j}	1.49 ± 0.11 ^{g}	2.80 ± 0.10 ^{i}	1040.17 ± 33.34 $_{d,e}$
Zhongjiao No.8	1.54 ± 0.12 ^{f}	852.15 ± 3.29 ^{i}	1.36 ± 0.03 ^{h}	0.87 ± 0.05 ^{h}	59.95 ± 1.12 ^{c}	1.15 ± 0.39 ^{j}	5.84 ± 0.12 ^{e,f}	922.86 ± 4.18 ^{h,i}
Yunjiao No.1	1.00 ± 0.07 ^{i}	768.95 ± 29.74 ^{j}	1.76 ± 0.05 ^{g}	0.93 ± 0.04 ^{f,g,h}	16.41 ± 0.87 ^{f}	1.80 ± 0.08 ^{c,e}	4.54 ± 0.18 $_{f,g,h,i}$	795.39 ± 28.94 ^{j}

Statistical note: Means (n = 3) within a column followed by different letters are significantly different at $p < 0.05$ according to the Tukey's honestly significant difference (HSD) multiple range test. Means with superscripts having the same letter are not significantly different.

Table 7. Determination of main phenolic compounds in ripe fruit peel of 10 banana cultivars (μg/g).

Cultivar	Gallic Acid	Quinic Acid	Protocatechuic Acid	Catechin	Chlorogenic Acid	Ferulic Acid	Rutin	Total Phenolic Compound Content
Pahang	0.40 ± 0.02 ^{i,j}	26.93 ± 0.43 ^{j}	5.47 ± 0.07 ^{b}	0.92 ± 0.14 $_{d,e,f,g}$	1.14 ± 0.00 ^{b,c}	14.27 ± 0.50 ^{a}	21.97 ± 0.73 ^{c}	71.10 ± 0.97 ^{j}
Inkira	0.71 ± 0.02 ^{g}	290.05 ± 0.60 ^{b}	2.10 ± 0.04 ^{f}	1.02 ± 0.12 ^{b,c,d}	1.04 ± 0.02 ^{c}	2.49 ± 0.41 ^{b}	37.47 ± 0.61 ^{b}	334.88 ± 0.60 ^{b,c}
Kazirakwe	0.53 ± 0.06 ^{h}	279.20 ± 4.07 ^{c}	1.33 ± 0.03 ^{j}	0.94 ± 0.16 $_{c,d,e,f,g}$	0.81 ± 0.01 ^{d}	1.49 ± 0.21 ^{f,g,h,i}	76.75 ± 2.32 ^{a}	361.05 ± 5.02 ^{a}
Brazilian	0.73 ± 0.04 $^{f, g}$	162.60 ± 4.98 ^{g}	1.87 ± 0.03 ^{g}	0.71 ± 0.15 ^{i,j}	0.52 ± 0.00 $_{f,g,h,i,j}$	1.39 ± 0.16 ^{h,i}	2.10 ± 0.11 ^{h,i}	169.91 ± 5.02 ^{g}
Reke No.2	1.80 ± 0.03 ^{b}	124.59 ± 0.25 ^{i}	4.59 ± 0.06 ^{c}	0.82 ± 0.14 $_{e,f,g,h,i,j}$	0.45 ± 0.01 ^{i,j}	1.53 ± 0.19 $_{e,f,g,h,i}$	3.09 ± 0.22 ^{f,g,h}	136.87 ± 0.34 ^{i}
Reke No.4	0.98 ± 0.04 ^{e}	167.25 ± 4.24 ^{f}	1.75 ± 0.02 ^{h}	0.70 ± 0.12 ^{j}	0.48 ± 0.01 $_{g,h,i,j}$	0.92 ± 0.17 ^{j}	0.92 ± 0.10 ^{j}	173.00 ± 4.17 ^{f,g}
Nantianhuang	0.39 ± 0.05 ^{j}	177.30 ± 1.49 ^{e}	1.65 ± 0.04 ^{i}	0.72 ± 0.13 ^{h,i,j}	0.47 ± 0.00 ^{h,i,j}	1.55 ± 0.14 $_{d,e,f,g,h,i}$	1.91 ± 0.14 ^{i,j}	183.99 ± 1.50 ^{e,f}
Baodaojiao	2.88 ± 0.08 ^{a}	209.05 ± 6.00 ^{d}	12.25 ± 0.17 ^{a}	0.78 ± 0.15 $_{g,h,i,j}$	0.42 ± 0.01 ^{j}	1.46 ± 0.17 ^{g,h,i}	2.27 ± 0.11 ^{g,h,i}	229.11 ± 5.93 ^{d}
Zhongjiao No.8	1.13 ± 0.06 ^{c}	315.23 ± 4.07 ^{a}	2.41 ± 0.06 ^{e}	1.69 ± 0.06 ^{a}	2.95 ± 0.48 ^{a}	1.34 ± 0.02 ^{i}	6.56 ± 0.21 ^{d}	331.29 ± 4.39 ^{c}
Yunjiao No.1	1.04 ± 0.05 ^{d,e}	129.50 ± 0.99 ^{h}	2.58 ± 0.04 ^{d}	0.78 ± 0.14 $_{f,g,h,i,j}$	0.53 ± 0.01 $_{e,f,g,h,i,j}$	1.78 ± 0.22 $_{c,d,e,f,g}$	4.41 ± 0.19 ^{e}	140.62 ± 1.08 ^{h,i}

Statistical note: Means (n = 3) within a column followed by different letters are significantly different at $p < 0.05$ according to the Tukey's honestly significant difference (HSD) multiple range test. Means with superscripts having the same letter are not significantly different.

Among the green/ripe peel of the banana cultivars selected in our study, quinic acid had the highest concentration ratio among the main phenolic compounds. The above results verified that the type and content of phenolic compounds in banana peel were varied depending on the variety of banana [11,16]. Similarly, research by Bashmil et al., showed that the highest phenolic compound contents were found in the ripe Ducasse peel, unripe Ladyfinger peel, ripe Plantain peel, and unripe Monkey peel, which contained 1.32, 1.15, 0.87, and 0.79 mg GAE/g, respectively [38]. Baskar et al. [39] also found that among the nine local cultivars from Tamil Nadu, India, Poovan cultivar had less total phenolic compound content (0.39 mg catechol equivalent (CE)/g), but higher level of flavonoids (22.83 mg rutin equivalent (RE)/g) in comparison with other cultivars, such as Sevvazhai, Nendran, and Rasthali. Passo Tsamo et al. [40] reported that rutin was dominant in the peel of all plantain cultivars and the dessert cultivar Gros Michel. However, rutin was low in the hybrids (F568) and was even not detected in the dessert cultivar Grand Nain. In addition, kaempferol-3-O-rutinoside was predominant only in the peel of hybrids banana, but not in other cultivars [41].

In our study, the contents of the main phenolic compounds and the composition proportion of each compound in the green/ripe banana peel of each cultivar were different, so the peel of each cultivar had its own characteristics. We should therefore determine the utilization mode suitable for the peel of each cultivar according to our quantitative analysis results of phenolic compounds. Therefore, extracting phenolic compound from fruit peel improves the utilization of banana peel. The banana peel residues used as compost especially as a source of P/K, thus, reduce the pollution of peel waste.

3.7.3. Comparison of Main Phenolic Compound Contents between Green Fruit Peel and Ripe Fruit Peel

Whether in the green fruit peel or ripe fruit peel, the contents of quinic acid and rutin were the highest among the eight phenolic compounds. In the 10 banana cultivars, the quinic acid content of green fruit peel was 3–66 times higher than that of the ripe fruit peel. For rutin, only the Pahang had a significantly higher content in green fruit peel than in its ripe fruit peel, which was about 10 times higher, and yet in other banana cultivars, the content of green fruit peel was equivalent to that in ripe fruit peel. In addition, the chlorogenic acid content in green fruit peel was 10–70 times that in ripe fruit peel among the 10 banana ecotype cultivars. The ferulic acid content in the green fruit peel of Pahang was twice that in the ripe fruit peel. For gallic acid and catechin, their contents in the green banana peel of Pahang were significantly higher than that of ripe banana peel, but there was no significant difference among other cultivars. The maturity of fruit has been reported to significantly affect phenolic compound content of the peel [16,42]. Among all banana cultivars, the total phenolic compound contents of green fruit peel were significantly higher than that of ripe banana peel (Figure 2). It is consistent with the results found by Fatemeh et al., that total phenolic content was found to decrease during the ripening process [42]. Over-ripened peel had 52% less, while ripe peel had 15–45% less phenolic content than that in the green peel [43]. Many individual bioactive compounds such as naringin, rutin, norepinephrine, dopamine, and L-dopa were reduced significantly when the peel changed from green to yellow [44]. Vu et al., also found the peel without ethylene treatment had a relatively higher phenolic content and antioxidant power than those of the peel treated by ethylene at maturity stages 5–7 [45]. Therefore, depending on the purpose of its potential usage, the stage of maturation must be considered when selecting of the peel material: Green banana peel is a better source of phenolic compounds compared to ripe ones, and banana cultivar and maturity can be screened according to the results determined in this study, when extracting phenolic compounds from banana peel resources in the future. These findings will help a more rational utilization of the huge biomass of banana peel in the future.

3.8. Comparison of Phenolic Compound Contents and Fruit Agronomic Traits among the Three Subgroups

In the study, we found that the total phenolic compound content in the green banana peel of the Malaccensis subgroup was significantly higher than that in other subgroups, $p < 0.01$. In the ripe banana peel, the content of total phenolic compound in Mutika–Lujµgira subgroup was the highest (Figure 2). Furthermore, the total phenolic compound contents in the green/ripe fruit peel of the non-dessert banana were significantly higher than that of dessert banana (green: non-dessert banana 1.48 ± 0.44 mg/g vs. dessert banana 0.97 ± 0.12 mg/g; ripe: non-dessert banana 0.26 ± 0.13 mg/g vs. dessert banana 0.19 ± 0.06 mg/g). In particular, in the Malaccensis subgroup, the total phenolic compound content in its green fruit peel was the highest (2.08 ± 0.04 mg/g) (Table 8), in which the proportion of fruit peel was significantly higher (93.7 ± 8.6%) than the other two subgroups (total mean 43.0 ± 6.0%) and the water content was very low (Table S3 in Supplementary Materials); thus, it was a high-quality source for extracting phenolic compounds from pericarp. However, studies found the dessert bananas (e.g., 'Ney Poovan') and cooking bananas (e.g., 'Tiparot') had the highest concentrations of phenolic compounds, mainly in ripe fruit (stage 5-yellow with green) [46]. This was mainly due to the differences

in cultivar and maturity from our study. The comparison of phenolic compound content and fruit agronomic traits among different subgroups may provide a useful guide for phenolic compound utilization from the banana peel in the future.

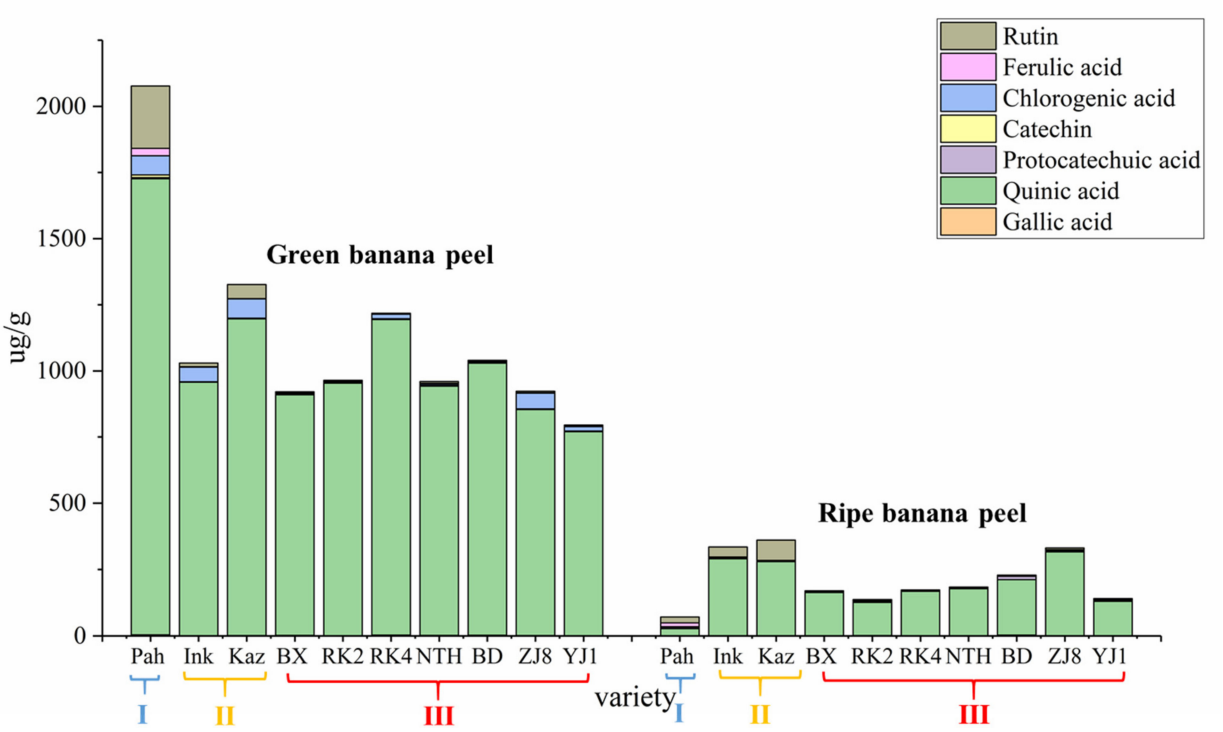


Figure 2. The accumulation histogram of total phenolic compound content in green fruit peel and ripe fruit peel of 10 banana cultivars. Note: Pah: Pahang, Ink: Inkira, Kaz: Kazirakwe, BX: Brazilian, RK2: Reke No. 2, RK4: Reke No. 4, NTH: Nantianhuang, BD: Baodaojiao, ZJ8: Zhongjiao No. 8, YJ1: Yunjiao No. 1; Mark "I" stands for Malaccensis subgroup; mark "II" stands for Mutika–Lujµgira subgroup; mark "III" stands for Cavendish subgroup.

Table 8. Comparison of the total phenolic compound content in three banana subgroups with different maturity.

Classification	Subgroups	Green Banana Peel	Ripe Banana Peel
		Total Phenolic Compound Content µg/g	Total Phenolic Compound Content µg/g
Non-dessert banana	Malaccensis	2076.60 ± 40.49	71.10 ± 0.97
	Mutika–Lujµgira	1178.21 ± 148.68	347.97 ± 13.56
Dessert banana	Cavendish	974.44 ± 122.84	194.97 ± 62.58

4. Conclusions

In conclusion, banana peel contains a substantial number of phenolic compounds and has a high antioxidant potential. The results showed that among the green/ripe peel of the banana cultivars selected in our study, quinic acid had the highest concentration ratio among the main phenolic compounds. Obviously, the content of phenolic compounds in green fruit peel was significantly higher than that in ripe fruit peel. The obtained data can be useful to select banana peel materials with high phenolic compounds for better utilizing as many dietary supplements, food additives, drug formulations, and so on. As an inexpensive source of phenolic compounds, there remains great potential for future studies on the recovery of these compounds and their utilization. In addition, health benefits of banana peel crude extract and their individual compounds, along with its

potential applications as natural preservatives or functional food ingredients, requires further in-depth investigation.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae8010070/s1, Table S1: Fruit agronomic traits of different green banana ecotypes; Table S2: Fruit agronomic traits of different ripe banana ecotypes; Table S3: The proportion of green/ripe banana peel and the water content of the peel of 10 banana cultivars.

Author Contributions: Conceived and designed the experiments, S.-J.Z., W.Z. and J.Z. Performed the sampling and carried out the sample preparation, Y.W., B.Y., Y.L. and L.L. Analyzed the data and wrote the paper, J.Z. Revised the manuscript, S.-J.Z. and W.Z. Supervision, S.-J.Z. and W.Z. Project administration, S.-J.Z. and J.Z. Funding acquisition, S.-J.Z. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by National Natural Science Foundation of China (NSFC32161143001), Yunling Scholar Programme of Yunnan Provincial Government (YNWR-YLXZ-2018-018).

Data Availability Statement: All the data are available in manuscript file and Supplementary Materials.

Acknowledgments: We are grateful to Junjun Jin, Fan Gao, Chuping Tan, and Junjie Tan for their kind help during implementing field experiments. The authors acknowledge Vincent Johnson of Green Quills and the Alliance of Bioversity International and CIAT for his editorial review.

Conflicts of Interest: The authors declare no conflict of interest.

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Genome-Wide Analyses of Tea Plant Stress-Associated Proteins (SAPs) Reveal the Role of *CsSAP12* in Increased Drought Tolerance in Transgenic Tomatoes

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Abstract: Plant stress-associated proteins (SAPs) contain A20/AN1 zinc finger domains and are involved in plant response to abiotic stresses. In this study, we aimed to explore the biological function of tea plant CsSAPs. A total of 14 *CsSAP* genes were identified in the tea plant genome using a reference genome database (*Camellia sinensis* var. *sinensis*). The CsSAPs were divided into the following two groups: Group I, containing one AN1 domain and/or one A20 domain; and Group II, containing two AN1 domains and/or two C2H2 domains. The sequence alignments and conserved domains analysis indicated that the CsSAPs were highly structurally conserved in terms of amino acid sequence and protein structure. The *CsSAPs* showed different transcript levels in spatio-temporal expression and in response to cold and drought stress in tea plants. Furthermore, the expression of *CsSAP12* was considerably upregulated under drought stress. The overexpression of *CsSAP12* in transgenic tomatoes showed increased tolerance to drought stress compared with the wild type. Altogether, the results showed that *CsSAP12* might be involved in drought stress. Thus, *CsSAP12* might be a target gene in genetic engineering to improve drought tolerance in tea plants.

Keywords: SAP gene family; tea plant; *CsSAP12*; expression analysis; functional analysis; drought stress

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Citation: Fan, S.-C.; Li, C.; Li, S.-H.; Tang, J.; Shi, H.-D.; Yang, T.-M.; Liang, M.-Z.; Liu, D.-D. Genome-Wide Analyses of Tea Plant Stress-Associated Proteins (SAPs) Reveal the Role of *CsSAP12* in Increased Drought Tolerance in Transgenic Tomatoes. *Horticulturae* **2022**, *8*, 363. https://doi.org/10.3390/horticulturae8050363

Academic Editor: Krishna Bhattarai

Received: 7 March 2022
Accepted: 20 April 2022
Published: 21 April 2022

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

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1. Introduction

Environmental stresses, including biotic and abiotic stresses, severely affect plant growth, development, and even survival. However, plants have evolved complex molecular mechanisms for regulating their gene expressions in response to adverse natural environments that could minimize stress damage by altering their growth and development, and physiological and biochemical responses to stress [1]. Stress-associated proteins (SAPs) are a class of newly discovered zinc finger proteins that act as a key factor in the plant response to abiotic stresses [2].

The A20, AN1, or both A20/AN1 zinc finger domains are contained in the plant SAP family members [3]. The A20 zinc finger domain protein was first identified in human vascular endothelial cells, which is tumour necrosis factor alpha (TNFA)-inducible protein, and its C-terminus has at least one C2C2 zinc finger domain [4–6]. The protein AN1, was the first identified protein encoded by the RNA of the maternal hemisphere of *Xenopus laevis*, has conserved sequences that can be divided into two categories [7]. The first category is CX2CX9-12CX1-2CX4CX2HX5HXC (X stands for any amino acid, C stands for cysteine, and H stands for histidine), which is usually present without the A20 domain, and the corresponding gene contains introns in its sequence [8]. The second category is CX4CX9-12

CX4CX2HX5HXC [9], which contains the A20 domain and non-introns in its sequence. A total of 8, 27, 15 and 24 SAPs, belonging to the first type of AN1 zinc-finger structure, are present in rice, cotton, desert poplar (*Populus euphratica*), and apple, respectively [10–12].

The SAP members have been induced by several challenges and found to play a significant role in improving tolerance to abiotic stress [11,13]. The OSISAP1/OsSAP1 is the first stress-associated protein studied, which was stimulated by drought, salt, and cold [11]. In *Arabidopsis*, *AtSAP5* could improve salt tolerance and drought resistance, and the analysis of transgenic *Arabidopsis* using gene chips indicated that the expression of many genes associated with stress had increased [14]. Furthermore, the SAP genes were associated with biotic and abiotic stresses in *Artemisia annua*, tomato, rice and banana [15–19]. In tomato, *SlSAP3* and *SlSAP4* expression increased basic resistance in *Pseudomonas syringae* pv. tomato DC3000 and necrotrophic fungus *Botrytis cinerea*, respectively [20]. Additionally, the SAP gene is associated with the regulation of signal transduction and hormone synthesis. The overexpression of *OsDOG* (*OsiSAP11*) and *OsZFP185* (*OsiSAP4*) resulted in dwarf phenotypes, decreased gibberellic acid (GA) contents, and insufficient cell elongation [21]. Furthermore, *OsZFP185* down-regulates the expression of genes associated with abscisic acid (ABA) biosynthesis and interferes with ABA-mediated tolerance to high salt, drought, and cold. The SAP genes are also associated with the regulation of plant development. Overexpression of *OsZFP185* (*OsiSAP4*) in rice brings about a dwarfism phenotype, a decrease in endogenous GA_3 and ABA content, and negative regulation of the expression of several genes bound up with ABA biosynthesis [21]. Furthermore, SAPs with E3 ubiquitin ligase activity have a hand in the regulation of redox sensors and/or regulators of gene expression under abiotic stress. In *Arabidopsis*, *AtSAP5* acts as an E3 ubiquitin ligase to participate in drought tolerance [22].

The genome-wide analysis of SAP genes in plants has helped understand their biological functions. In this study, the TPIA database (http://tpia.teaplant.org/, accessed on 24 March 2020) was used to excavate and identify *CsSAP* genes (*CsSAPs*). Furthermore, *CsSAPs* were comprehensively and systematically analysed in tea, including their conserved domains, protein and gene structures, phylogenetic relationships, cis-acting elements, and transcript levels under cold and drought stresses. Finally, *CsSAP12* was isolated from tea and was transformed into tomatoes to identify its function. This study provides an analysis of the biological functions of CsSAPs under abiotic stresses in tea plant with a solid theoretical basis and reference.

2. Materials and Methods

2.1. Identification of CsSAPs in Tea Plant

Protein sequences of tea plant were downloaded from the published TPIA database to construct a local blast database. Both A20 (A20-likezinc finger, PF01754) and AN1 conservative domains (AN1 zinc finger, PF01428) were downloaded from the Pfam database (http://pfam.xfam.org/, accessed on 24 March 2020). The software HMMER 3.1 (http://hmmer.org/, accessed on 24 March 2020) was used to identify the candidate *CsSAP* genes from the tea plant protein sequences. The candidate genes were confirmed using NCBI (https://www.ncbi.nlm.nih.gov/, accessed on 24 March 2020) and Pfam (http://pfam.xfam.org/, accessed on 24 March 2020) for further confirmation of the A20 and/or AN1 conserved domains.

2.2. Evolutionary Analysis of Tea Plant CsSAPs

The evolutionary tree analysis software MEGA 6 (http://www.megasoftware.net, accessed on 24 March 2020) was used to analyse the evolution of SAPs using the maximum likelihood (ML) approach (execution parameters: position correction, paired deletions, and guidance (1000 repetitions).

2.3. Gene Structure and Genome Localization of CsSAP Genes

The data on intron, exon, and genomic mapping of *CsSAPs* were acquired from the TPIA database. The introns and exons in the *CsSAPs* of tea plant were mapped according to their location in the TPIA database.

2.4. Amino Acid Sequences and Conserved Elements of CsSAPs

Both the A20 and AN1 domains of tea plant *CsSAPs* were analysed using the software DNAMAN 5.0 and WebLogo 3 (http://weblogo.threeplusone.com/, accessed on 24 March 2020). The online software SWISS-MODEL was used to analyse the 3D structure of the A20 domain (AN1 topology; PDB ID: 2KZY) and the AN1 domain (AN1 topology; PDB ID: 1WFP) of *CsSAPs* for model matching (http://swissmodel.expasy.org/, accessed on 24 March 2020). The 3D structure was visualized using RasTop software (http://www.geneinfinity.org/rastop/, accessed on 24 March 2020).

2.5. Cis-Acting Elements in Promoters

To assess assumed cis-acting elements in the promoter of *CsSAPs* in tea plant, we isolated the genome sequences 1500 bp upstream of the translation start codon of *CsSAPs*. We then predicted putative cis-acting elements based on the Plant CARE database (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/, accessed on 25 March 2020).

2.6. Plant Materials, Growth Conditions and Stress Treatments

Fully-expanded leaves from tea (*C. sinensis* var. *Assamica* cv. Yunkang10) that were cultured in a greenhouse were collected and included as the control group. These plants were grown in a greenhouse, and drought stress treatment was not initiated until these plants were about 50 cm high. Type YK-10 planted in a climate incubator for 15 days without water was used as samples for evaluating the effects of water deficits. The sampling schedule involved the harvesting of mature leaves from drought-treated YK-10 from the intermediate node on days of 0, 3, 5, 7 and 15 after treatment. All samples were immediately frozen in liquid nitrogen and stored in a refrigerator at −80 °C.

2.7. The qRT-PCR Analysis of CsSAPs

We extracted total RNA from the frozen leaves of tea plant using RNAprep pure Plant Kit (TIANGEN; Beijing, China). In total, 2 μg of RNA was collected for synthesizing the first strand of cDNA through the use of a PrimeScript™ II 1st Strand cDNA Synthesis Kit (TaKaRa; Shanghai, China). For the qRT-PCR assays, reverse transcription was performed with 1 μg of total RNA from each sample, followed by amplification of 1 μL of the product. We performed qRT-PCR assays with 20 μL of reaction mixtures containing 10 μL of SYBR® Premix Ex Taq™ (TaKaRa; Beijing, China). The sequences of primers used for qRT-PCR are listed in Table S1. The ΔCt values were calculated by using *CsActin* or *SlActin* as the endogenous control. The relative fold change in gene expression of samples was calculated using the $2^{-\Delta\Delta Ct}$ method [23]. The qRT-PCR conditions were pre-denaturation at 95 °C for 30 s; followed by 35 cycles of 95 °C for 5 s, 60 °C for 20 s, and 72 °C for 15 s. The qRT-PCR was also used to calculate the copy number of foreign genes, and *SlGRX1* was selected as the single copy reference gene [24,25].

2.8. Vector Construction, Plant Transformation and Drought Stress Treatment

The open reading frame (ORF) of *CsSAP12* was amplified by PCR, which consisted of denaturation at 94 °C for 5 min; followed by 25 cycles of 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 60 s. The cDNA of *CsSAP12* was cloned into pBWA(V)KS plant transformation vectors containing the kanamycin resistance gene. Tomato transformations were performed as previously described [26]. The transgenic plants were screened and confirmed by kanamycin-resistance and PCR using a primer pair specific to the CsSAP12 primers (Table S1). Additionally, tomato seeds were screened on MS culture medium containing

kanamycin to identify transgenic plants. Therefore, MDA content, oxygen free radical content, and antioxidant enzyme activities were determined using T3 generation plants.

The transgenic tomato and wild-type plants were planted for 3 months before being used for drought stress in a greenhouse. Drought stress treatment was performed for 20 days without water. The plants were analysed using the determination of physiological indices, such as leaf relative water content, MDA content, oxygen free radical content, and antioxidant enzyme activities.

2.9. Measurement of Physiological Indices

For the measurement of leaf relative water content, leaves were weighed using a balance (Suzhou Science Instrument Co., Ltd., Suzhou, China) and recorded as fresh weight (FW). The leaves were dried to a constant weight at 100 °C in an oven (Shanghai Badh Machinery Equipment Co., Ltd., Shanghai, China). Next, the leaves were weighed as dry weight (DW). The calculation formula of leaf water content was as follows:

$$\text{Leaf water content} = (\text{FW} - \text{DW})/\text{FW} \times 100\%.$$

The accumulation assay of two Reactive Oxygen Species (ROS), hydrogen peroxide (H_2O_2) and oxygen free radicals (O_2^-), MDA content, and activities of antioxidant enzyme, including SOD (EC: 1.15.1.1), CAT (EC: 1.11.1.6), and POD (EC: 1.11.1.7) were performed using reagent kits of A064-1-1, A052-1-1, A003-1, A001-1, A007-1-1 and A084-3-1 (Jiancheng Bioengineering Institute, Nanjing, China), respectively.

The osmotic potential (Ψ_w) was calculated according to the formula: $\Psi_w = -iCRT$; *i*, dissociation coefficient ($CaCl_2$ = 2.60), the concentration of $CaCl_2$ solution comparable to the water potential of the plant material was found by varying the weight of the plant material. C, isotonic concentration (mol/L). R, Gas constant 0.008314 LMPa/(mol·K), T, Kelvin temperature.

3. Results

3.1. Identification of CsSAPs in Tea Plant

In total, 14 CsSAPs were obtained from the proteins of tea plant. The gene name, ID, conserved domains, protein length, molecular weight, theoretical pI, and chromosome location of the *CsSAPs* are listed in Table 1. The genomic DNA sequences of the *CsSAPs* ranged from 435 bp to 9197 bp, whereas the length of the coding DNA sequence of the *CsSAPs* ranged from 435 bp to 1737 bp. Furthermore, the CsSAPs contained different types of zinc-finger domain, i.e., both CsSAP6 and CsSAP7 contained the AN1-AN1 domain, whereas CsSAP11 contained only an AN1 domain, 11 other CsSAPs contained the A20-AN1 domain. Multiple sequence alignment analysis showed that the A20 and AN1 domains and multiple amino acid sites were highly conserved (Figure 1).

Sequence logos were then produced, further indicating that these domains were highly conservative at every residue position (Figure 2a,b). Furthermore, by modelling and analysing the A20 and AN1 domains of the *CsSAPs* using the SWISS-MODEL online software, the homologous models of the A20 and AN1 domains are shown in Figure 2c,d, respectively. The results showed clearly that the A20 and AN1 domains of the *CsSAPs* work together with the A20 domain of ubiquitin receptor ZNF216 and the zf-AN1 domain of *Arabidopsis* F5O11.17 protein (PDB ID: 2KZY.1.A, 44.7% sequence identity for residues 10–48; 1WFP.1.A, 53.2% sequence identity for residues 107–153), suggesting that the A20 and AN1 domains of the *CsSAPs* were highly conserved.

3.2. Analysis of CsSAPs Structure and Conservative Elements

The evolution, exon-intron structure, and conserved domains were analysed to explore the structural variability of the SAP family genes. An evolutionary analysis of 14 CsSAPs showed that they can be separated into two groups: Group I, including one AN1 domain and/or one A20 domain; and Group II, including two AN1 domains and/or other domains (Figure 3a). Gene structure analysis results showed that *CsSAP1-CsSAP5*, *CsSAP8*, *CsSAP9*,

CsSAP11, and *CsSAP14* had no introns, *CsSAP7* and *CsSAP10* had only one intron, and *CsSAP6* contained two introns (Figure 3a). Conservative domain analysis showed that all the CsSAPs contained A20 and/or AN1 conserved domains (Figure 3b). In Group I, *CsSAP5*, *CsSAP8*, *CsSAP9*, *CsSAP11* and *CsSAP14* had no introns, and CsSAP7 and CsSAP10 contained A20 and AN1 conserved domains. CsSAP6 and CsSAP7 in Group II contained two AN1 conserved domains.

Table 1. Properties of *CsSAPs* identified from tea plant genome.

Gene Name	Gene ID	Zinc-Finger Domain	CDS Length (bp)	Protein Length (aa)	Molecular Weight (kDa)	No. of Introns	Theoretical pI	Scaffold Location
CsSAP1	TEA003271.1	A20-AN1	519	173	18.31	0	7.72	Scaffold622: 1698779:1699297:+
CsSAP2	TEA007661.1	A20-AN1	522	174	17.45	0	7.51	Scaffold560: 754898:755419:+
CsSAP3	TEA007758.1	A20-AN1	483	161	17.06	0	8.14	Scaffold390: 736618:737100:+
CsSAP4	TEA008252.1	A20-AN1	516	172	18.14	0	7.44	Scaffold2348: 1266811:1267326:+
CsSAP5	TEA009516.1	A20-AN1	492	164	17.53	0	7.76	Scaffold2009: 46003:46494:−
CsSAP6	TEA013656.1	AN1-AN1	1737	579	63.76	4	9.66	Scaffold942: 1448206:1457402:−
CsSAP7	TEA013686.1	AN1-AN1	573	191	20.92	1	8.52	Scaffold4677: 68010:69805:+
CsSAP8	TEA014231.1	A20-AN1	465	155	17.24	0	8.91	Scaffold3002: 9737:10201:+
CsSAP9	TEA016255.1	A20-AN1	507	169	17.96	0	8.46	Scaffold435: 1131711:1132217:+
CsSAP10	TEA016540.1	A20-AN1	636	212	22.52	1	7.92	Scaffold1761: 717585:722550:−
CsSAP11	TEA016572.1	AN1	507	169	18.75	0	9.11	Scaffold1761: 663031:663537:−
CsSAP12	TEA021384.1	A20-AN1	498	166	17.85	0	8.08	Scaffold4125: 306796:307293:+
CsSAP13	TEA023579.1	A20-AN1	492	164	17.64	0	8.7	Scaffold5358: 143051:143542:+
CsSAP14	TEA025409.1	A20-AN1	435	145	15.69	0	8.6	Scaffold2300: 157553:157987:+

'+' and '−' indicate that the gene is located in the forward and reverse strand, respectively.

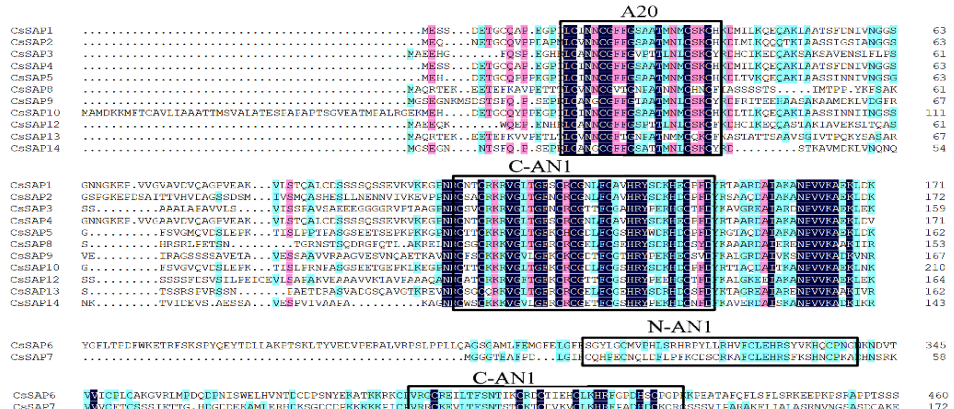


Figure 1. Multiple alignments of the A20/AN1 domain in tea plant CsSAPs. The conserved domains are shown in boxes, identical amino acids are shown with a dark blue background (similarity: dark blue = 100%; pink > 75%; cyan > 50%).

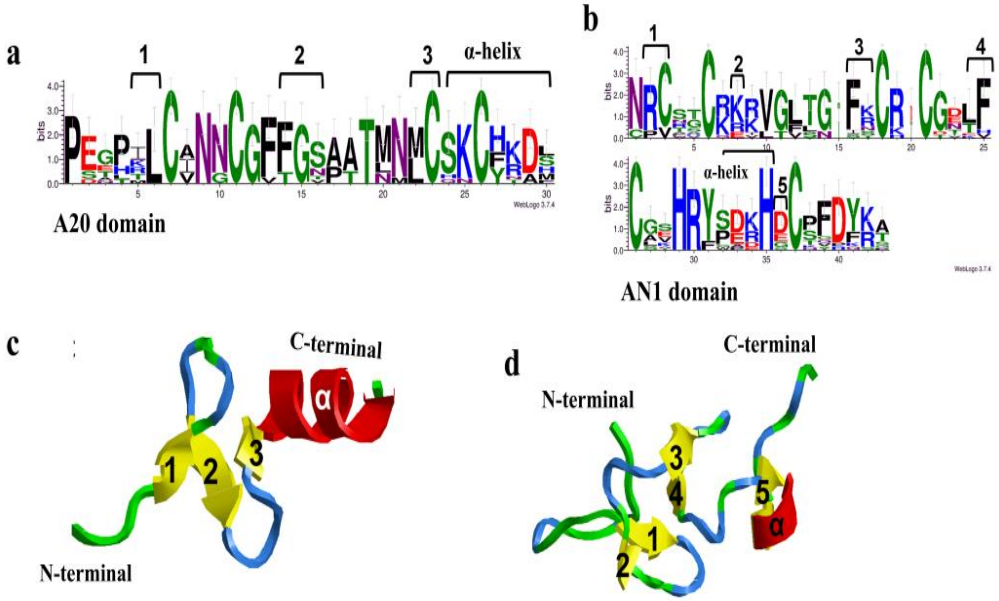


Figure 2. (**a**) Sequence logos of A20 domain in the CsSAPs. (**b**) Sequence logos of the AN1 domain in the CsSAPs. (**c**) Three-dimensional tertiary structural model of the A20 domain (PDB ID: 2KZY.1.A); and (**d**) three-dimensional tertiary structural model of the AN1 domain (PDB ID: 1WFP.1.A). The logos of the A20 and AN1 domains were obtained through multiple alignments of the CsSAPs. In each stack, the symbol height represents the relative frequency of each amino acid at that position; numbers 1–5 represent β-sheets in A20 and AN1 domains; α-helices are red, β-sheets (numbers 1–5) are yellow, and strands are blue/green.

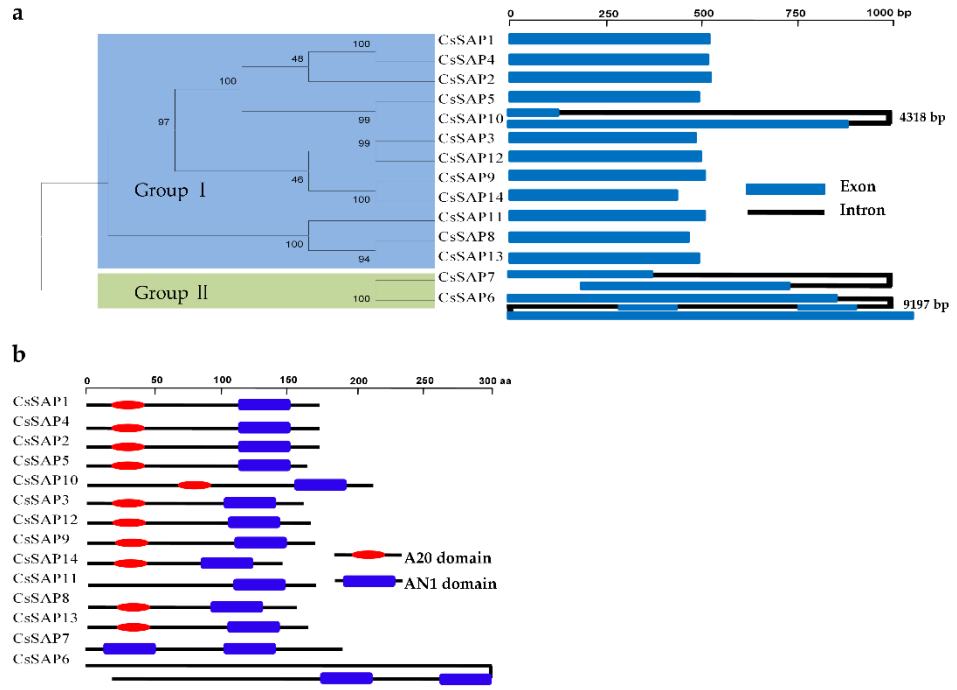


Figure 3. Sequence analysis of *CsSAPs*. (**a**) Phylogenetic relationships and Gene structure analysis; (**b**) analysis of the conserved domains for CsSAPs in tea plant. The phylogenetic tree of full-length amino acid sequences was constructed using MEGA software.

3.3. Phylogenetic Analysis of CsSAPs

To examine the evolutionary relationships among plant SAPs in tea plant and other plants, such as *Actinidia chinensis* and *Vitis vinifera*, full-length protein sequences encoded

by 118 SAP genes from 20 species were used to construct unrooted phylogenetic trees. The evolutionary analysis of *CsSAPs* showed that they were classified into following two groups: CsSAP1-CsSAP5 and CsSAP8-CsSAP14 belonged to Group I, containing an AN1 domain and/or an A20 domain; CsSAP6 and CsSAP7 belonged to Group II, containing two AN1 domains and/or other domains (Figure 4). Group I was classified into two subgroups (Ia–Ib): Ia, containing one AN1 domain and one A20 domain, including CsSAP1-5, CsSAP8-10 and CsSAP12-14; and Ib, containing only one AN1 domain, CsSAP11. Group II can be divided into three subgroups (IIa–IIb): IIa, containing two AN1 domains and two C2H2 domains, including CsSAP6; IIb, two AN1 domains, including CsSAP7.

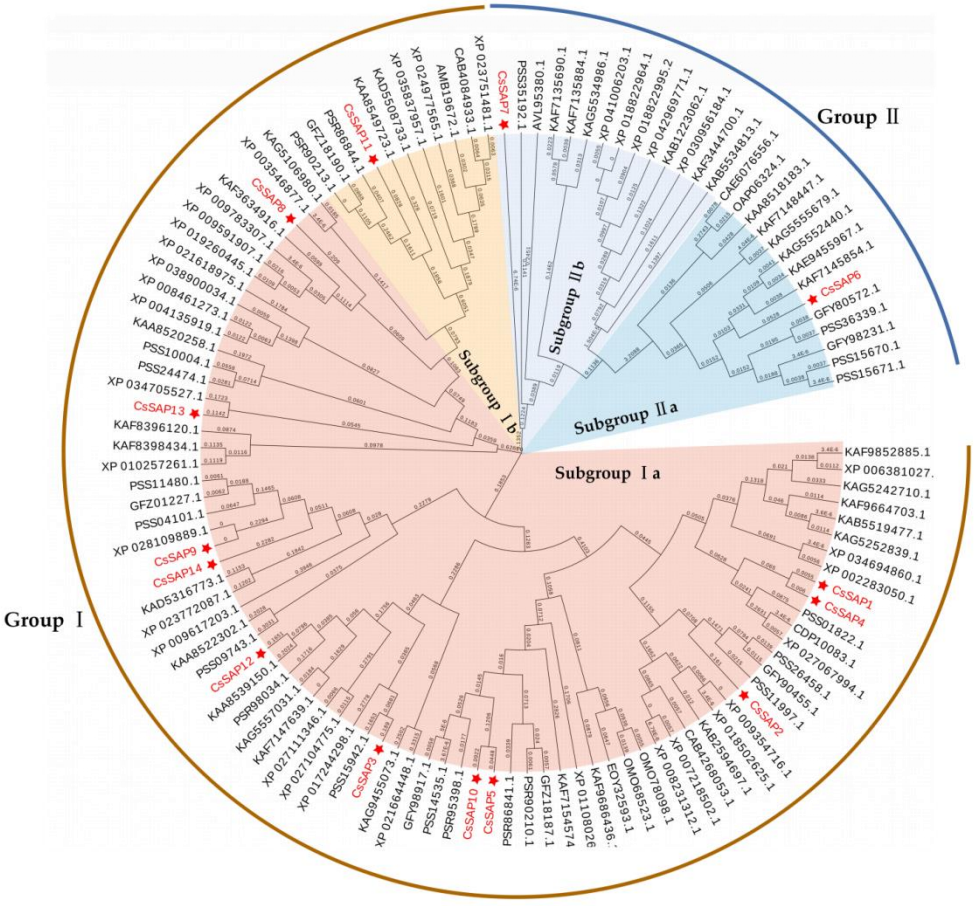


Figure 4. Phylogenetic analysis of 118 SAPs from 20 species of plants. The unrooted NJ tree was constructed using the full-length amino acid sequences. Ia, Ib, IIa, IIb indicates four subgroups. 14 CsSAPs in tea were showed in red and plus stars.

3.4. Functional Prediction of CsSAPs

To identify the likely cis-regulatory elements (CREs) of *CsSAPs*, we isolated genomic sequences 1500 bp upstream of the start codon from *CsSAPs*. A total of 100 CREs belonging to 10 typical types of abiotic and biotic response elements or transcription factors binding sites were obtained in 14 promoters. These were related to responses to hypoxia, cold, drought, pathogens, trauma, and hormones (Table 2). Most *CsSAPs* promoters contained the anaerobic response element, ethylene-responsive element, and W-box element. Additionally, some hormone-related CREs, such as ABA, salicylic acid, methyl jasmonate, and ethylene, were also found in *CsSAPs* gene promoters. The results showed that the CREs of *CsSAPs* played a significant role in the stress responses of plant and might be associated with the circadian clock, cell differentiation, and morphogenesis regulation.

Table 2. The cis-acting elements analysis of *CsSAPs*.

Cis-Acting Elements	ABRE	ARE	CGTCA	ERE	LTR	MBS	TCA	TC-Rich Repeat	W-Box
Stress to Response	**ABA**	**Hypoxia**	**MeJA**	**Ethylene**	**Chilling**	**Drought**	**SA**	**Defence**	**Pathogen**
CsSAP1		0/5		1/0		1/0	0/1		1/0
CsSAP2		0/2	1/1				1/0		
CsSAP3	1/1	3/0	1/0			0/1			2/0
CsSAP4	2/0	2/2	1/0			1/0		1/0	
CsSAP5	1/0	1/1	0/1						1/0
CsSAP6		0/1	1/0	1/2					0/1
CsSAP7	1/1	1/0	1/1	0/1			1/0		1/0
CsSAP8	1/1	0/1	0/1			1/0	0/1		
CsSAP9	2/1	1/2							1/0
CsSAP10		1/1		2/1			0/1		2/1
CsSAP11		0/3	0/1	0/2	0/1	2/0			1/0
CsSAP12	0/1		1/0				1/0		0/1
CsSAP13		1/0	0/1	3/2					
CsSAP14	0/1		1/0					1/0	1/2

ABRE (ABA response element), ARE (anaerobic response element), CGTCA (MeJA-responsiveness), ERE (ethylene-responsive element), LTR (low-temperature response element), MBS (MYB binding site involved in drought response), TCA (salicylic acid response element), TC-rich repeat (defence and stress responsiveness), and W-box (elicitation; wounding and pathogen responsiveness; binding site of WRKY type transcription factors); digits represent the number of regulatory elements on the positive and negative chain. The blank area indicates no corresponding cis-acting element in any chain of the promoter.

3.5. Expression Pattern of CsSAPs in Tea Plant

Identifying the expression patterns of *CsSAPs* in different tissues can help us understand their functions. Real-time quantitative PCR results showed that *CsSAP1*, *CsSAP2*, *CsSAP6*, *CsSAP13* and *CsSAP14* were highly expressed in roots and leaves, *CsSAP3* and *CsSAP8* were expressed strongly in stems, and *CsSAP4*, *CsSAP5*, *CsSAP7*, *CsSAP9*, *CsSAP10*, *CsSAP11* and *CsSAP12* were highly expressed in stems and flowers (Figure 5a). In addition, the relative expressions of *CsSAPs* were determined in tea plants under drought stress. Under 5 days of drought treatment, the expression of *CsSAP9* and *CsSAP12* increased significantly, whereas the transcripts of *CsSAP4* was significantly reduced. The expression of the other *CsSAPs* showed no obvious changes (Figure 5b).

3.6. CsSAP12 Overexpression Enhances Drought Tolerance in Transgenic Tomato

Transcript analysis showed that *CsSAP12* was significantly accumulated under drought stress. The full-length cDNA of the *CsSAP12* obtained from tea plant was 498 bp, which encoded a predicted protein containing 165 amino acid residues with a calculated molecular mass of 17.86 kDa and an isoelectric point of 8.44. It contained both A20 and AN1 zinc finger domains and its amino acid sequence showed a similarity of up to 53.27% with that of *SlSAP4* (Figure S1), which corresponded to drought stress in tomato plants (*Solanum lycopersicum*) [27].

To further identify its function, *CsSAP12* was transformed into tomatoes. After detection, 12 transformants were identified and confirmed with obviously increased levels of *CsSAP12* transcripts; it could not be observed in wild type (WT) (Figure 6a). In transgenic tomato seedlings, the copy number of *CsSAP12* was 1.39 times that of the reference gene, *SlGRX1*, indicating that *CsSAP12* was a single copy gene in transgenic tomatoes (Figure S2). We then selected transgenic tomatoes with high *CsSAP12* expression to assess their potential function when responding to drought stress.

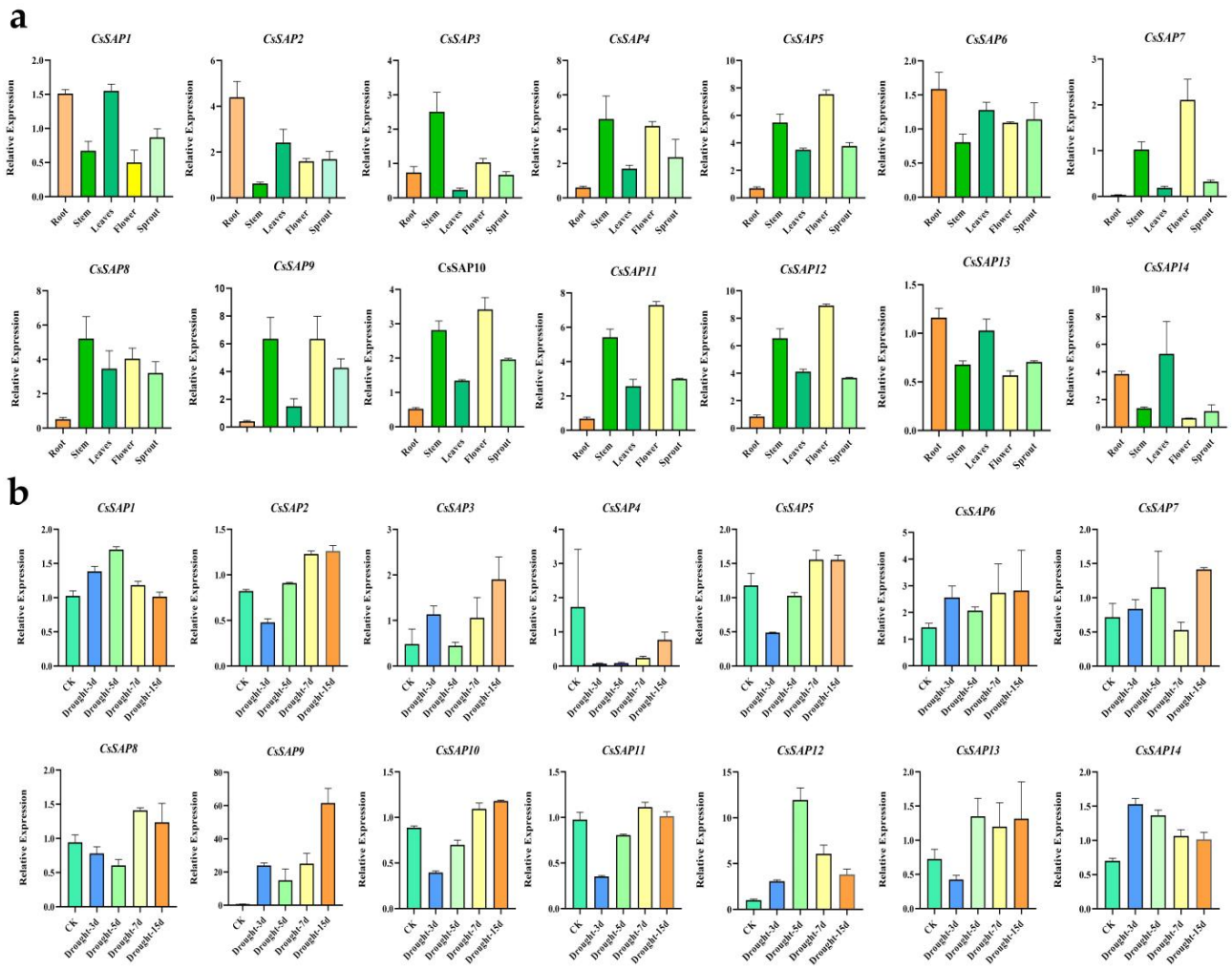


Figure 5. Tissue-specific expression and drought response of 14 *CsSAPs*. (**a**) Expression patterns of *CsSAPs* in different tissues of tea plant. (**b**) Expression patterns of *CsSAPs* in response to drought stress.

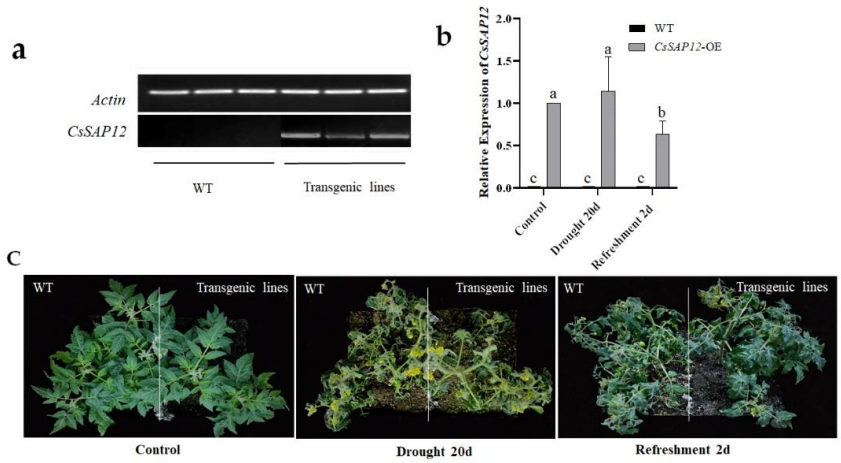


Figure 6. Identification of transgenic tomato lines under drought stress. (**a**) Semi-quantitative PCR analysis of the *CsSAP12* expression level. (**b**) Relative expression of *CsSAP12* in leaves of WT and transgenic plants. (**c**) Phenotypes of WT and transgenic lines under drought stress. The values are expressed as the mean ± standard deviation, and different letters of the alphabet represent significant differences calculated by Duncan's multiple range test ($p < 0.05$).

The WT tomato and *CsSAP12*-overexpressed seedlings showed similar growth characteristics without any treatment, and there were no obvious changes in seeds germination, leaf development, flower fruit, and leaf development between transgenic and wild tomatoes (Figure S3). After 20 days of drought stress, the green leaves of WT plants were wilted and subsequently turned yellow and drooped significantly; *CsSAP12*-overexpressed transgenic tomatoes displayed slightly wilted leaves but without drooping (Figure 6b). Furthermore, the WT plants did not recover normal development when rehydration began (2 days of refreshment), the transgenic tomato leaves recovered to upright growth (Figure 6c), and the expression of *CsSAP12* in the transgenic lines treated with drought stress was significantly higher (p-value < 0.05) than that under 2 days of refreshment (Figure 6b), suggesting that the resistance of transgenic tomatoes to drought was increased due to the increased transcript level of *CsSAP12*.

3.7. Measurement of Physiological Indices of CsSAP12 Transgenic Plants

In a further effort to appraise the growth conditions of *CsSAP12* transgenic lines under drought stress, leaf relative water content, malondialdehyde (MDA) content, and oxygen free radical content (O_2^-) were evaluated in WT and transgenic tomatoes. The leaf relative water content of transgenic lines was remarkably higher than that of WT under drought stress and after 2 days of refreshment (Figure 7a). The MDA content and oxygen free radical content of WT were significantly higher than those of the transgenic lines. After 2 days of refreshment, the MDA and oxygen free radical content of both WT and transgenic lines decreased rapidly (Figure 7b,c).

Moreover, antioxidant enzyme activities, including peroxidase (POD), catalase (CAT), peroxidase (POD), and superoxide dismutase (SOD) were also measured. Compared with WT after 20 days of drought, CAT, POD, and SOD activities in the transgenic lines were 1.37-, 1.29- and 1.47-times higher than those of WT, respectively. The antioxidant enzyme activities of WT and transgenic lines after 2 days of refreshment displayed a sharp downward trend compared with that of WT and transgenic lines after 20 days of the drought-contrasting trend; the CAT, POD, and SOD activities of the transgenic lines were 1.74-, 1.16- and 1.82-times greater than those of the WT (Figure 7d).

Furthermore, to identify the physiological differences between the WT and transgenic tomatoes at the early stage of drought stress, osmotic potential and two Reactive Oxygen Species (ROS), hydrogen peroxide (H_2O_2) and oxygen free radicals (O_2^-) were also detected using WT and transgenic tomatoes under 7 days drought stress. The results showed that the osmotic potential of *CsSAP12* transgenic plants was much lower than that of wild plants after 7 days of drought and 2 days of refreshment (Figure 7e). Both hydrogen peroxide and oxygen free radicals accumulated mostly in leaves, and they were decreased obviously in the WT and transgenic tomatoes after 7 days of drought (Figure 7f,g). Interestingly, no obvious changes were found in the hydrogen peroxide contents of stems between the WT and *CsSAP12* transgenic tomatoes, while the content of oxygen free radicals in *CsSAP12* transgenic plants was lower than that of the WT. After 2 days of refreshment, the hydrogen peroxide and oxygen free radical content were decreased in both the WT and transgenic lines. Moreover, the hydrogen peroxide content showed no obvious changes in roots and stems of WT and transgenic tomatoes, but oxygen free radical contents of roots were lower in *CsSAP12* transgenic lines than in the WT (Figure 7f,g).

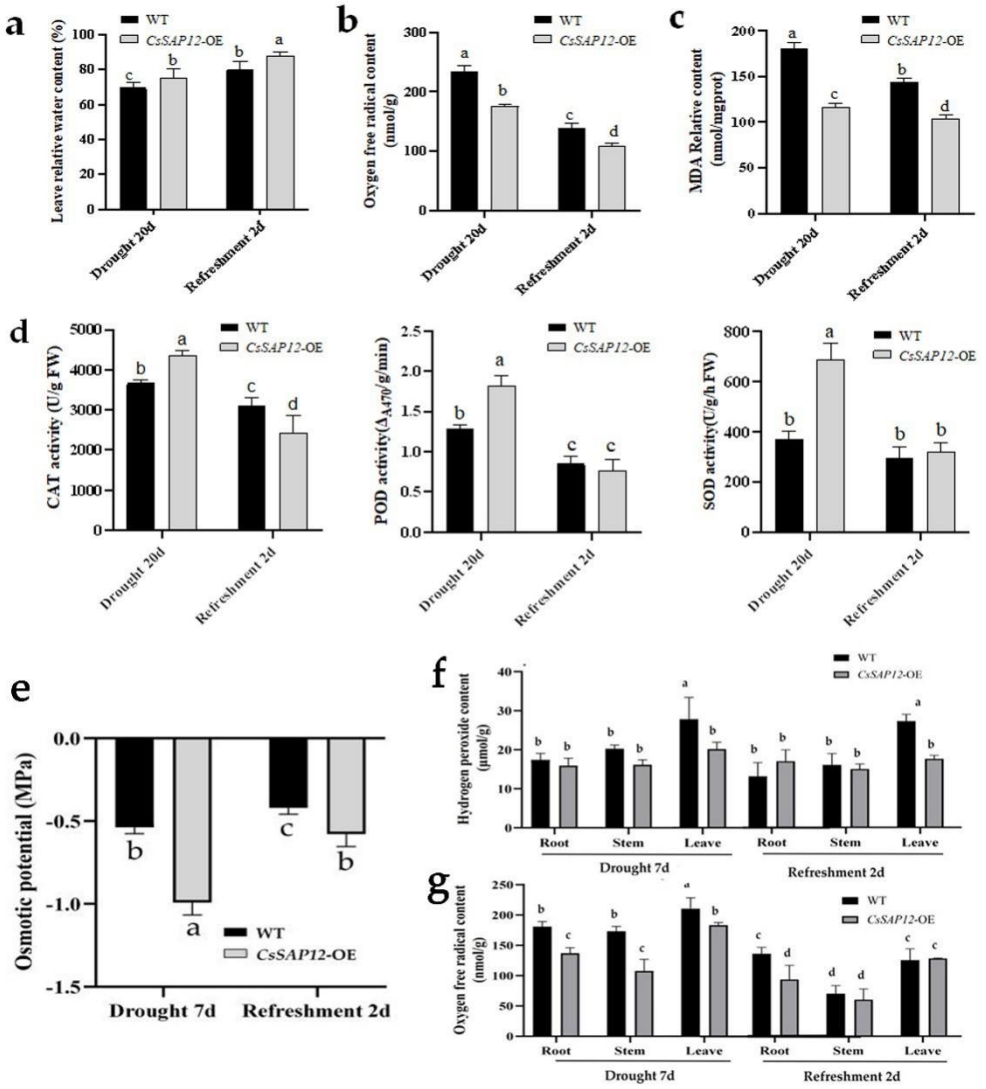


Figure 7. Stress physiological indices and antioxidant enzyme activities of wild types (WT) and *CsSAP12*−overexpressed transgenic lines under drought stress. (**a**) Relative water content of leaves. (**b**) Oxygen free radical content. (**c**) MDA relative content. (**d**) Activities of antioxidant enzyme (CAT, POD, SOD) in WT and transgenic lines under drought stress and refreshment assay. (**e**) Osmotic potential. (**f**) Hydrogen peroxide and oxygen free radical content (**g**) of different tissues in WT and transgenic lines. The values are expressed as the mean ± standard deviation, and different letters of the alphabet a−d suggest significant differences calculated by Duncan's multiple range test ($p < 0.05$), error bars represent standard errors.

4. Discussion

Recently, along with the discovery and sustained development of gene sequencing and sequence assembly technology, the preparation of high-quality profiles of plant genomes provides great convenience in the study of plant-specific agronomic traits, the acceleration of plant breeding, an increase in production, and the ability to resist biotic and abiotic stresses [28]. Tea, one of the world's most important beverage crops, has a variety of secondary metabolites that are good for the human body, and has economic, medical, and cultural significance [29,30]. As the quality of tea genome profiles has improved, it provides great opportunities and platforms for identifying tea family genes [27,28]. Recently, several tea plant gene families have been identified, such as GRAS, LEA, HSP, WOX, WRKY, bZIP, and LBD [31–39]; however, reports about the tea plant SAP gene family are not available. The SAP gene families have been identified and analysed in rapeseed, cotton maize, tomato and *Arabidopsis* (Table 3). The numbers of SAP gene family members in rapeseed and cotton

are 57 and 37, respectively, whereas the numbers of SAP gene family members in maize, tomato, and *Arabidopsis* are 11, 13 and 14 [3,40,41]. In this study, 14 *CsSAPs* were identified by searching the tea plant genome. Compared with the number of SAP gene families in other species, the tea plant *CsSAP* gene family has fewer members. As reported in previous studies, gene replication, which includes a segment, tandem and genome-wide replication, is essential for the diversification of gene functions and the rearrangement and expansion of the genome [42,43]. The tea genome emerged in two rounds of whole-genome duplication (WGD) events 30–40 million years ago and 90–100 million years ago, respectively [32]. These WGD events and subsequent paralogous repeats impacted considerably on the copy number of secondary metabolite-related genes of tea plant. However, because of the lack of chromosome localization information, we could not specifically analyse whether members of the SAP gene family participated in gene replication events. With developments in the accuracy and assembly of tea genome sequencing, the promotion and replacement of search and analysis software, and the discovery of variable shear, the aforementioned problems will be overcome, and other SAP gene family members will be determined. Furthermore, the zinc finger structures of the SAP gene family are not the same in different plants. The number of A20 zinc finger structures among members of the SAP gene family of rapeseed, apple, and rice were 7, 3, and 1, respectively. The A20-A20-AN1 zinc-finger structure was also found in the *Arabidopsis* and rapeseed SAP gene families [40,44]. Altogether, the loss or increase in zinc-finger type genes in these genomes is essential for the complex enzyme activity of these plants.

Table 3. Numbers of SAP gene family members in various species.

Plant Species	A20-AN1	A20-A20-AN1	A20	AN1	AN1-AN1	AN1-AN1-C2H2	AN1-AN1-C2H2-C2H2	Total Number
Camellia sinensis	11	0	0	1	2	0	0	14
Arabidopsis thaliana	10	0	0	1	1	1	1	14
Brassica napus	32	0	7	9	5	4	0	57
Gossypium hirsutum	28	0	0	3	4	0	2	37
Malus domestica	23	0	3	0	3	0	1	30
Medicago truncatula	11	0	0	2	1	0	2	16
Oryza sativa	11	1	1	3	1	0	1	18
Populus euphratica	15	0	0	0	2	0	1	18
Solanum lycopersicum	9	0	0	1	2	0	1	13
Zea mays	8	0	0	1	1	0	1	11

The SAP gene family members of *Arabidopsis*, rice, tomato, and cotton are classified into five groups [32,41,43], while in apple (*Malus domestica* Borkh.), they are divided into the following two groups: Group I, with an A20 domain and an AN1 domain; and Group II, including two AN1 domains [5]. In the present study, we performed an evolutionary analysis of tea plant SAPs and compared them with 118 SAP genes from 20 species. The evolutionary analysis of tea SAPs showed that tea SAPs can be classified into the two groups: Group I (A20-AN1 type) and Group II (AN1 type). A typical feature of SAP in each plant is the absence of introns. Among the 12 *CsSAP* members of Group I, 11 *CsSAP*s were found to contain no introns, whereas all Group II-type *CsSAPs* contained introns. The 11 *CsSAPs* with no popular intron genes indicated the ancient origins of the SAP genes and their close relationship with the rapid accumulation of transcripts because of a reduction in post-transcriptional processes.

Plant SAP genes can be quickly induced by several abiotic stresses. The *OsiSAP1*/*OsSAP1* gene can be upregulated by high salt, aridity, chill, ABA, submergence, and mechanical

wounding induction [44]. The *OsSAP9* gene is upregulated responding to cold, heat, PEG6000, and other stresses [45,46]. The *OsiSAP8* gene is upregulated by salt, heat, cold, ABA, desiccation, heavy metals, submergence, and wounding [47]. Similar results induced by abiotic stress have also been found in *Aeluropus littoralis*, *Arabidopsis*, maize, cotton, *Populus euphratica*, *Brassica napus*, and apple [8,47–53].

The expression patterns of 14 SAP genes in tea plants were comprehensively analysed in this study. Among the 14 genes, *CsSAP12* was, not only highly expressed in various tissues of plants, but its expression increased in drought conditions. Drought stress allowed the formation of hydrogen peroxide and oxygen free radical in plants, and could cause MDA accumulation because of membrane lipid peroxidation, indicating that antioxidant activity in plants can improve plant tolerance to different stress factors [53]. The overexpression of *CsSAP12* can effectively reduce osmotic potential in leaves and the content of hydrogen peroxide, oxygen free radical in different tissues, promoting lower MDA content and higher leaf water content. And after drought treatment, the anti-oxidase activity of *CsSAP12*-overexpressed transgenic lines was higher than wild species and rapidly reduced after rehydration; MDA content and oxygen free radical content in *CsSAP12*-overexpressed transgenic lines were lower than in WT lines, while the relative water content of leaves was even higher, suggesting that transgenic tomatoes had better drought tolerance than that of wild-type tomatoes.

5. Conclusions

In this study, 14 *CsSAP* genes were identified in tea plant. We studied the function of *CsSAP* genes in apple growth and development using bioinformatics, gene expression, and functional analysis. Functional characterization showed that *CsSAP12* might play an important role in salt stress. It will provide a basis for future studies to comprehensively and comparatively analyse the functional characteristics of CsSAP to deeply investigate aspects of drought stress in tea.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae8050363/s1. Figure S1: Protein sequence alignment of *SlSAP4* (GenBank accession number: XP 019066408) and *CsSAP12* by Clustalx; A20 and AN1 domains are indicated at the top. Figure S2. The qRT-PCR amplification curve of *CsSAP12* and *SlGRX1*. Figure S3. Phenotypes of *CsSAP12* over-expressed transgenic and wildtype tomatoes. Table S1: Application of primers used for PCR and qRT-PCR.

Author Contributions: Data curation, S.-C.F.; formal analysis, H.-D.S.; funding acquisition, D.-D.L. and M.-Z.L.; methodology, S.-C.F., C.L. and S.-H.L.; resources, M.-Z.L.; software, S.-C.F., J.T. and T.-M.Y.; supervision, D.-D.L.; writing—original draft, S.-C.F.; writing—review and editing, D.-D.L. and M.-Z.L. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Natural Science Foundation of China (Grant No. 31660566), and The Open Fund of the State Key Laboratory of Yunnan Provincial Key Laboratory of Tea Science (Grant No. 2021YNCX002).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data used for the analysis in this study are available within the article and Supplementary Materials.

Acknowledgments: We thank TopEdit (www.topeditsci.com, accessed on 25 March 2020) for its linguistic assistance during the preparation of this manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

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贵州农业科学 2019,47(4):33～35
Guizhou Agricultural Sciences

[文章编号]1001－3601(2019)04－0118－0033－03

复合肥施用量对云薯 902 农艺及产量性状的影响

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[摘 要]为玉溪市马铃薯生产提供科学依据，在玉溪农业职业技术学院农林系农场进行了起垄栽培不施肥(CK)，施三元复合肥(N∶P∶K=15∶15∶15)50 kg/667m²、100 kg/667m²和 150 kg/667m²对马铃薯新品种云薯 902 农艺及产量性状的影响试验。结果表明：与 CK 相比，施用三元复合肥有利于云薯 902 植株生长和产量性状的提高。其中，以云薯 902 施用三元复合肥 100 kg/667m²的效果最好，产量高达 2 189 kg/667m²，纯收益为 883.50 元/667m²。

[关键词]马铃薯；云薯 902；三元复合肥；农艺性状；产量
[中图分类号]S532 [文献标识码]A

Effects of Application Rate of NPK Compound Fertilizer on Agronomic and Yield Traits of Yunshu 902

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Abstract: To provide a scientific basis for potato production in Yuxi City, the experiment on the agronomic and yield traits of the new potato variety Yunshu 902 was carried out in the agriculture and forestry farm of Yuxi Agricultural Vocational-Technical College. Four levels were set, including no fertilization(0 kg/667m²), normal fertilization(50 kg/667m²), high fertilizer(100 kg/667m²) and super high fertilizer(150 kg/667m²). Results: Compared with CK, the application of ternary compound fertilizer was beneficial to the growth and yield of Yunshu 902. Among them, the application of ternary compound fertilizer 100 kg/667m² with Yunshu 902 was the best, the yield was as high as 2 189 kg/667m², and the final net income was 883.50 Yuan/667m².

Key words: potato; Yunshu 902; NPK compound fertilizer; agronomic traits; yield

马铃薯是我国重要的粮食作物和蔬菜作物，种植面积居我国粮食作物第四位。云南是马铃薯生产大省，产量居我国前列[1]。作物生长需要适宜的氮磷钾复合肥才能较好地生长，获得较高的产量[2]。目前，在马铃薯种植过程中主要采用不同配比的复合肥作基肥，不再作追肥，因此开展马铃薯复合肥用量试验具有一定的现实意义。

氮肥对马铃薯的影响较大，不施用氮肥其产量和商品性均会明显下降[3]。马铃薯是喜钾作物，钾肥在马铃薯生产中能提高膨大速度，从而提高产量[4]。磷肥是马铃薯必需的大量元素，多施磷肥可增加薯块表皮光滑程度，提高其商品性，从而提高品质[5]。有机肥是作物栽培的优质肥料，增施有机肥能显著增加马铃薯的产量，在生产实践中可结合当地情况合理施用有机肥[6]。微生物菌肥不仅可以改善土壤的物理性质，还为马铃薯的生长提供全价养分和有益微生物[7]。农作物生长过程或生命活动中需要多种营养元素，且每种营养元素不能相互代替[8]。以上试验均是基于某种养分对马铃薯生长方面的影响，关于复合肥对马铃薯的生产效应国内已有一些相关研究报道[9-11]。马铃薯对肥料的需求量较大，在试验中注意测试土壤肥力，作好对比，才能得到较好的结果；对马铃薯进行氮磷钾肥效试验[12]，有利于指导当地马铃薯生产。为此，笔者等在玉溪市参照“3414”肥料试验[13-14]，选取氮磷钾三要素相同配比的复合肥(15∶15∶15)，研究不同复合肥用量对马铃薯农艺及产量性状的影响，以筛选出适宜复合肥用量，为玉溪市马铃薯生产提供参考。

1 材料与方法

1.1 试验材料

1.1.1 马铃薯与肥料品种 供试马铃薯为云南省农业科学院经济作物研究所选育的马铃薯新品种云薯 902，2016 年通过云南省农作物品种审定委员会

[收稿日期] 2018-11-20
[基金项目] 国家重点研发计划项目(2017YFD0101906)；云南省马铃薯种业研究示范型国际科技合作基地项目(2017IB040)；玉溪市农业科技专项“玉溪市马铃薯新品种引种及配套栽培技术研究”(2060403)
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审定，播种前进行切块，切块后沾草木灰后播种。供试肥料为从罗马尼亚进口的硫酸钾型复合肥（N∶P∶K=15∶15∶15）。

1.1.2 试验地概况 试验于2017年12月26日在玉溪农业职业技术学院农林系农场进行，该农场位于东经102°29′53.18″、北纬24°14′39.25″，海拔1 650 m。试验地土壤为红壤土，肥力中等水平，播种前土壤养分由云南省农业科学院检测：全氮1.67 g/kg、水解氮88.13 mg/kg、有效磷74.94 mg/kg、速效钾263.93 mg/kg、有机质25.91 g/kg，pH 7.76。

1.2 试验设计

设不施肥（CK，处理1），施复合肥50 kg/667m²（处理2）、100 kg/667m²（处理3）和150 kg /667m²（处理4）等共4个处理，每个处理3次重复，共12个小区，随机排列。小区面积10 m²（10 m×1 m），起垄双行栽培，垄内行宽20 cm，株距30 cm，小区种植密度为60株，栽培密度4 000株/667m²左右。复合肥和杀虫剂锌硫磷（2.5 kg/667m²）于起垄栽培时施入土壤中。

1.3 观测项目及测定指标

1.3.1 生长期观测 出苗时间以播种后出苗为准，观察时间为播种后45 d，当出苗率达到50%以上时为出苗期。开花期以开花率达到50%以上时间为准，并记录花色情况。对于叶色、单株羽状叶数、茎粗、株高、单株分枝数等性状在收获前完成观测。

1.3.2 收获期指标测定 收获时测定马铃薯的株高、茎粗、单薯重和单株重等指标，分2次对各小区进行测产，每次对各小区随机挖取5株对地上茎叶和地下薯块进行称量，最后按2次各重复小区测产的平均数确定小区产量，折算单产，并对试验结果进行统计分析。

2 结果与分析

2.1 不同处理马铃薯的出苗率

从表1看出，处理1～4的小区平均出苗数分别为59株、60株、59株和50株，施复合肥50 kg/667m²和100 kg/667m²的马铃薯出苗均正常，但施复合肥150 kg/667m²的出苗率明显降低。说明，以施基肥栽培马铃薯，在加大复合肥施用量时会抑制马铃薯的出苗数，因此在生产中复合肥施用量不宜过大。

表1 不同处理马铃薯的出苗情况

Table 1 Potato seedling emergence of different treatment

处理 Treatment	栽培数/株 Tubers planted	平均出苗数/株 Number of emergence	出苗率/% Emergence rate
1(CK)	60	59	98
2	60	60	100
3	60	59	98
4	60	50	83

2.2 不同处理马铃薯的植株与产量性状

从表2可看出，不同处理马铃薯的叶色、花色、单株羽状叶数、茎粗、株高、单株分枝数、薯块重、地上茎叶重与地下薯块重比（T/R）与产量的变化情况。

2.2.1 叶色与单株羽状叶数 处理1～4马铃薯植株的叶色分别为黄绿色、浅绿色、绿色和深绿色，平均单株羽状叶数分别为15.7个、18.6个、24.3个和34.0个。即随着复合肥用量增加，马铃薯的叶色逐渐加深、平均单株羽状叶数逐渐增加。表明，施用复合肥对马铃薯植株生长有促进作用，但过多施用复合肥会导致地上茎叶生长太旺盛。

2.2.2 茎粗与株高 处理1～4马铃薯植株的茎粗分别为3.6 cm、4.2 cm、5.0 cm和5.2 cm，平均株高分别为37.7 cm、49.0 cm、57.9 cm和51.7 cm。即随着复合肥用量增加，马铃薯的茎粗逐渐增加、株高先增加后降低。表明，施用复合肥能促进马铃薯的茎粗增大，但超量施用复合肥不利于植株株高生长，且茎秆有小叶增多的趋势。

2.2.3 茎基分枝与地上茎叶重 处理1～4马铃薯植株的平均茎基分枝数分别为3.2个、3.1个、3.6个和3.9个，平均地上单株茎叶重分别为0.20 kg、0.45 kg、0.48 kg和0.59 kg。即随着复合肥用量增加，马铃薯的平均茎基分枝数、地上单株茎叶重逐渐增加。表明，施用复合肥能促进马铃薯茎基部分枝和茎叶生长。

表2 不同处理马铃薯的植株性状与产量

Table 2 Plant characters and yield of potatoes under different treatment

处理 Treatment	叶色 Leaf color	花色 Flower color	羽状叶数/(个/株) Number of pinnate leaves	茎粗/cm Stem diameter	株高/cm Plant height	茎基分枝/(个/株) Number of branches	地上茎叶重量/(kg/株) Weight of Aboveground stem and leaves	薯块重/(kg/株) Tuber weight	T/R	产量/(kg/667m²) Yield
1(CK)	黄绿色	白色	15.7	3.6	37.7	3.2	0.20	0.41	0.49	1 364 Bc
2	浅绿色	白色	18.6	4.2	49.0	3.1	0.45	0.61	0.74	2 013 Ab
3	绿色	白色	24.3	5.0	57.9	3.6	0.48	0.67	0.72	2 189 Aa
4	深绿色	白色	34.0	5.2	51.7	3.9	0.59	0.66	0.89	2 178 Aa

注：产量列不同大小写字母分别表示差异极显著（$P<0.01$）和显著（$P<0.05$）。

Note: Different capital and lowercase letters in the yield column indicate 1% and 5% significant levels respectively.

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2.2.4　**薯块重与产量**　处理1～4马铃薯的平均单株薯块重分别为0.41 kg、0.61 kg、0.67 kg和0.66 kg，薯块产量分别为1 364 kg/667m²、2 013 kg/667m²、2 189 kg/667m²和2 178 kg/667m²。即随着复合肥用量增加，马铃薯单株薯块重、薯块产量均先升高后下降，表明适量施用复合肥能促进马铃薯地下薯块的生长，超量施肥用复合肥易造成马铃薯地上部分徒长，降低马铃薯地下薯块的养分供应，薯块产量反而下降。

2.2.5　**T/R值**　处理1～4马铃薯的T/R值分别为0.49、0.74、0.72和0.89。表明，不施复合肥马铃薯有早衰表现，适量施复合肥的茎叶生长与地下薯块生长较协调，超量施复合肥茎叶徒长，从而影响地下薯块产量。

2.3　不同处理马铃薯的产值

从表3看出，处理1～4马铃薯的产值分别为2 046.00元/667m²、3 019.50元/667m²、3 283.50元/667m²和3 267.00元/667m²，利润为2 046.00元/667m²、2 819.50元/667m²、2 883.50元/667m²和2 667.00元/667m²。扣除劳动力、种薯和肥料成本，处理1～4马铃薯的纯收益分别为46.00元/667m²、819.00元/667m²、883.50元/667m²和667.00元/667m²。其中，以处理3的纯收益最高，为883.50元/667m²，表明马铃薯施复合肥100 kg/667m²的效益最好。

表3　不同处理马铃薯的成本与收益情况

Table 3　Costs and benefits per of potatoes under different treatment　元/667m²

处理 Treatment	复合肥成本 Cost of compound fertilizer	产值 Output value	利润 Profit	利润较CK± Profit compared with CK	劳动力与种薯成本 Cost of both seed tubers and labor force	纯收益 Net income
1(CK)	0.00	2 046.00	2 046.00	0.00	2 000.00	46.00
2	200.00	3 019.50	2 819.50	773.50	2 000.00	819.00
3	400.00	3 283.50	2 883.50	837.50	2 000.00	883.50
4	600.00	3 267.00	2 667.00	621.00	2 000.00	667.00

注：复合肥为4.00元/kg，马铃薯薯块售价为1.50元/kg，劳动力成本为1 500.0元/667m²，种薯费为500.00元/667m²。

Note: Compound fertilizer 4.00 yuan/kg, potato tuber 1.50 yuan/kg, labor cost 1 500.0 yuan/667m², seed tuber 500.00 yuan/667m².

3　结论与讨论

试验结果表明，施用一定量的复合肥，有利于促进马铃薯云薯902的植株生长和薯块产量增加；复合肥用量100 kg/667m²的马铃薯产量为2 189 kg/667m²；复合肥用量150 kg/667m²的产量为2 178 kg/667m²，即复合肥施用量过高也不利于产量提高，与陈莹[15]的研究结果一致。因为，复合肥施用量过高易造成马铃薯地上部分徒长，不利于地下薯块膨大。试验土壤有效钾含量较高，虽然使用三元复合肥的氮磷钾比例(15∶15∶15)相对较高，但对喜钾作物马铃薯的影响不大。

综上所述，马铃薯云薯902在起垄栽培时施用三元复合肥(N∶P∶K＝15∶15∶15)100 kg/667m²可获得最高产量和经济效益，其最高产量达2 189 kg/667m²，纯收益为883.50元/667m²。该结果对玉溪市及云南省生态适应性相一致的地区，种植云薯902施用三元复合肥具有指导作用。

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分子植物育种，2020 年，第 18 卷，第 16 期，第 5488-5494 页
Molecular Plant Breeding, 2020, Vol.18, No.16, 5488-5494

研究报告
Research Report

适宜转蓝色基因的仙客来受体品种筛选

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摘　要　受体材料的生理环境是蓝色花卉转基因育种成功的重要因素之一。为了筛选出适宜进行蓝色转基因工程的仙客来受体品种，本研究以 12 个不同类型的仙客来花瓣为材料，对其二氢黄酮醇 4- 还原酶利用二氢杨梅素(dihydromyricetin, DHM)生成飞燕草素苷的能力、总黄酮含量、液泡 pH 值进行检测，结合灰色关联分析方法对各指标进行综合分析。结果表明：仙客来‘条纹荷花’利用二氢杨梅素生成飞燕草素苷的量最多达 39.01 μg/g，其花瓣 pH 也最高为 6.82，仙客来‘八重玫瑰’的总黄酮含量最多达 2 507.64 μg/g；由这 3 个值组成灰色关联分析的参考品种参数，计算出加权关联度最高的品种为仙客来‘八重玫瑰’，其关联度为 0.840 8。本研究从不同类型仙客来品种中筛选出了二氢黄酮醇 4- 还原酶特异性催化能力强、总黄酮含量和液泡 pH 值高的品种，可以为蓝色仙客来的转基因育种提供材料参考。

关键词　仙客来(*Cyclamen persicum*), 蓝色花, 转化受体, 灰色关联分析

The Selection of Cyclamen (*Cyclamen persicum*) Cultivars for Being Transformed Blue Genes

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DOI: 10.13271/j.mpb.018.005488

Abstract　In order to screen out the cyclamen receptor varieties suitable for blue transgenic engineering. We used 12 different types of cyclamen petals as materials, and detected the ability of DFR to generate delphinidin by dihydromyricetin (DHM), the total flavonoid content of petals, the pH of the vacuole. The gray correlation analysis method was used to comprehensively analyze each index. The results showed that the contents of delphinidin produced by cyclamen 'Strip lotus' using DHM was up to 39.01 μg/g, and its petal pH was also up to 6.82. The total flavonoid content of cyclamen 'Double rose' was up to 2 507.64 μg/g. These three values is composed of the reference sequence of gray correlation analysis. And the species with the highest weighted correlation is calculated as the cyclamen 'Double rose' with a correlation degree of 0.840 8. The varieties with strong DFR specificity, total flavonoid content and high vacuolar pH were screened from different types of cyclamen varieties, which could provide reference for transgenic breeding of blue cyclamen.

Keywords　*Cyclamen persicum*, Blue flower, Genetic transformation receptor, Grey correlation analysis

仙客来(*Cyclamen persicum* Mill.)是报春花科仙客来属植物，原产于地中海地区，为多年生球茎花卉(张芹等, 2005)。市售的园艺品种仙客来的自然花期是初冬至晚春时节，其花姿奇异、颜色绚丽多彩，是

基金项目：本研究由云南大学农学学科项目建设(C176210302)和教育部部省合建重点项目子项目“云南几种主栽花卉新品种选育及绿色栽培技术研发与示范”(C176280108)共同资助
引用格式：He J.Y., Li R.N., Yin H., Wu X.W., and Liu H.Y., 2020, The selection of cyclamen (*Cyclamen persicum*) cultivars for being transformed blue genes, Fenzi Zhiwu Yuzhong (Molecular Plant Breeding), 18(16): 5488-5494 (何佳越, 李瑞娜, 尹红, 吴学尉, 刘海英, 2020, 适宜转蓝色基因的仙客来受体品种筛选, 分子植物育种, 18(16): 5488-5494)

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圣诞、元旦及春节的热门装饰盆花。

花色作为观赏植物育种的重要目标，目前尽管通过各种育种方式极大的丰富了园艺仙客来的花色，但仍有待培育蓝色色系的仙客来品种。花色素苷是决定花呈色的重要色素，飞燕草素苷作为其中之一是花呈现蓝紫色的关键组分(Tanaka et al., 2010)。合成飞燕草素苷的关键前体是 DHM (二氢杨梅素)，F3'5'H (类黄酮 −3'5'− 羟化酶)可以使黄酮或二氢黄酮醇的 C3'5' 位置上羟基化生成 DHM，DFR (二氢黄酮醇 4−还原酶)则能够以 DHM 为底物生成飞燕草素苷。向仙客来中转入 *F3'5'H* 基因，使仙客来能够生成 DHM 从而合成蓝色的飞燕草素苷是培育蓝色仙客来的有效途径之一。但由于不同植物品种的 DFR 对底物选择有特异性(Nakayama et al., 1997)，*F3'5'H* 基因导入受体植物后生成的 DHM 能否转化成飞燕草素苷取决于该植物的 DFR 特异性催化。因此，本研究内容之一是对仙客来花瓣进行 DHM 饲喂后检测飞燕草素苷生成情况来评价仙客来的 DFR 特异性催化能力。

蓝色花除了和飞燕草素苷的含量有关外，还受到黄酮类物质和液泡 pH 值的影响。黄酮可以与花青素苷作用起到增色效应并且可以使花青素苷在紫外可见光区的吸收光谱中发生红移，视觉上增强花色的蓝紫色效果(Tanaka, 2006)。花青苷有几种不同的结构表现不同的颜色且共存于花瓣中，花色烊阳离子、无水醌式碱、甲醇假碱和查耳酮分别呈现红色、蓝色、无色和浅黄色，当液泡 pH 值较高时，醌式碱的比例会上升，有利于花色表现蓝色。Katsumoto 等(2007)通过测定类黄酮和液泡 pH 值，在 169 个月季备选品种中筛选适宜受体品种，导入蓝色基因后得到了蓝色月季；Nielsen 等(2005)在 16 个长春花品种中同样通过测定类黄酮和液泡 pH 值来筛选长春花的转蓝色基因受体品种。因此，本研究除了将仙客来的 DFR 催化能力作为选择指标，还将仙客来的总黄酮含量和液泡 pH 值纳入考量，以期选择出的受体品种在转入蓝色基因后能够表现出理想的蓝色花色性状。

使用灰色关联分析法对测得的各项指标进行综合分析比对，筛选出能高效转化 DHM、总黄酮含量和花瓣液泡 pH 值都较高的仙客来品种，为转入 *F3'5'H* 等蓝色相关基因培育蓝色仙客来提供参考。

1 结果与分析

1.1 仙客来花瓣经 DHM 培养后的颜色变化

12 个品种的仙客来花瓣经含飞燕草素苷前体物 DHM 的培养基培养 24 h 后有 4 个品种的花瓣出现了较为明显的颜色变化(图 1)。其中仙客来‘条纹荷花’表现为粉色条纹增多增粗；仙客来‘中国红’、‘八重玫瑰’和‘蕾丝贝贝 A’均发生颜色变化，经 RHSCC (英国皇家园艺学会比色卡)比色表明其分别从 Vivid-Red 44A 变为 Strong-Red 46A、Light-Pink 49C 变为 Moderate-Pink 51D、Moderate Purplish-Pink 54D 变为 Deep Purplish-Pink 54B。经过 DHM 饲喂培养的花瓣发生了颜色变化，说明这些供试品种的 DFR 能够利用 DHM 作为底物进行色素合成。

1.2 仙客来花瓣利用 DHM 能力、总黄酮含量及液泡 pH 值检测

检测仙客来花瓣经 DHM 培养生成的飞燕草素苷含量、花瓣总黄酮含量和液泡 pH 值(表 1)。无添加 DIM 培养的 12 个仙客来品种花瓣未检出飞燕草素苷生成，经添加 1 mg/mL DHM 培养后 有 8 个品种花瓣检出飞燕草素苷。能够检出飞燕草素苷表明这 8 个品种的 DFR 能够特异性催化 DHM 生成飞燕草素苷，但催化效果各个品种间有差异。效果最好的品种是仙客来‘条纹荷花’，其次是‘中国红’、‘八重玫瑰’和‘蕾丝贝贝 A’，飞燕草素苷生成量均在 20 μg/g 以上，也导致了花瓣颜色明显变化，与 RHSCC 比色观察结果一致。另外 4 个品种由于飞燕草素苷生成量过低，肉眼较难观察出颜色变化。无飞燕草素苷生成的仙客来品种中有 3 个花色为白色或浅黄色。仙客来‘宫灯’的花瓣进行培养后色素发生降解，花瓣呈透明水渍状，无法进行飞燕草素苷生成含量检测。

各个品种仙客来的总黄酮含量差异分析表明(表 1)，总黄酮含量较高的仙客来花色特点是大多为浅色，含量达 2 400 μg/g 以上，仙客来‘八重玫瑰’的总黄酮含量最高为 2 507.64 μg/g。仙客来花瓣液泡的 pH 值范围在 5.7~6.9 之间，其中白色和浅黄色的仙客来液泡 pH 值最低在 6.0 以下，最高的是仙客来‘条纹荷花’为 6.82。

1.3 灰色关联分析辅助选择仙客来的适宜转蓝色基因品种

由于仙客来‘宫灯’、‘梦幻白’、‘蕾丝贝贝 B’和‘浅黄’这 4 个品种在经 DHM 培养后未检出飞燕草素苷，所以不参与灰色关联分析。根据各品种仙客来总黄酮含量、液泡 pH 值及经 DHM 培养后生成的飞燕草素苷含量数据(表 1)设计出参考品种的最佳

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图 1 经 DHM 培养后仙客来花瓣的颜色变化

注: 对照组为不添加 DHM 的 1/4MS 培养基培养 24 h, 实验组为含 DHM 1 mg/mL 的 1/4MS 培养基培养 24 h

Figure 1 The color change after incubation of petal segments in blank and DHM

Note: The control group was cultured in 1/4MS medium without DHM for 24 h, and the experimental group was cultured in 1/4MS medium containing DHM 1 mg/mL for 24 h

参数是飞燕草素苷生成量为 39.01 μg/g，总黄酮含量 2 507.64 μg/g，花瓣液泡 pH 值为 6.82。将各个仙客来品种与参考品种进行灰色关联分析(表 2)。其中关联序排第 1 的仙客来品种‘八重玫瑰’与参考品种的关联度最高为 0.840 8，表明其各项指标综合后与参考品种最相似，最适合作为蓝色转基因仙客来的受体品种。

2 讨论

前人对蓝色花基因工程开展了一定的研究，仙客来的园艺品种和月季、百合、菊花等花卉一样由于缺乏 *F3'5'H* 基因不能有效生成 DHM，因而不能合成蓝色的飞燕草素苷(Tanaka, 2006)。所以这些植物的花色中没有蓝色色系，也很难通过常规育种方式培育出蓝色花。有研究将 *F3'5'H* 基因转入到月季(Katsumoto et al., 2007)、康乃馨(Fukui et al., 2003)等植物中，以期培育出蓝色花品种，但最终花色表现大都不尽如人意，多为紫红色或粉紫色。原因之一可能是在飞燕草素苷的合成路径中，这些植物的 DFR 不能有效催化 *F3'5'H* 基因生成的 DHM。目前主要采用两种方式解决这个问题，一是在转入 *F3'5'H* 基因的同时转入可以高效催化 DHM 的 *DFR* 基因，但来源于不同植物的 *DFR* 基因也需要经过筛选才能使用(Shimada et al., 2001)；二是在该植物的不同品种中筛选可以高效催化 DHM 的品种。于凯丽等(2018)用 DHM 饲喂培养切花菊花瓣筛选出蓝色关键基因转化菊花受体‘南农绯玉’；Brugliera 等(2013)给花瓣添加DHM 后检测到了飞燕草素苷的合成，花色由粉红色变成了紫红色，表明该试验品种的 DFR 可以催化 DHM。

试验发现白色或浅黄的仙客来品种无法利用 DHM 生成飞燕草色苷，可能和白色品种不能在花瓣中有效积累花青苷有关(Katsumoto et al., 2007)。此外，花色为浅粉色、白色和浅黄色的仙客来总黄酮含量较高，但其中白色和浅黄的仙客来液泡 pH 值偏低。因此，最佳受体品种在粉色色系品种中选择比较合适，这与 Katsumoto 等(2007)在筛选蓝色转基因月季

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表 1 各品种仙客来总黄酮含量, 液泡 pH 值及经 DHM 培养后生成的飞燕草素苷含量

Table 1 Content of total flavonoids in cyclamen, pH value of vacuoles and delphinidin content after incubation with DHM

序号 No.	品种 Variety	RHSCC 颜色描述 RHSCC color description	飞燕草素苷含量(μg/g) Delphinidin contents (μg/g)	总黄酮含量(μg/g) Total flavonoid contents (μg/g)	pH 值 pH value
1	条纹荷花 Stripe lotus	深粉 50B Deep-Pink 50B	39.01±2.34 a	695.35±26.09 d	6.82
2	中国红 China red	鲜红 44A Vivid-Red 44A	30.34±0.95 b	1 626.11±23.97 b	6.37
3	宫灯 Gong deng	中粉 49B Moderate-Pink 49B	−	1 547.45±29.61 b	6.45
4	八重玫瑰 Double rose	浅粉 49C Light-Pink 49C	25.99±3.19 c	2 507.64±83.69 a	6.51
5	维多利亚 Victoria	浅粉 39D/鲜红 45A Light-Pink 39D/Vivid-Red 45A	10.90±0.60 d	2 494.52±109.61 a	6.34
6	梦幻粉 Dream-pink	粉白 49D/深粉 52C Pale-Pink 49D/Deep-Pink 52C	9.08±2.55 d	901.18±31.49 c	6.01
7	梦幻红 Dream-red	大红 50A Strong-Red 50A	4.97±2.01 e	778.66±9.73 d	6.45
8	梦幻白 Dream-white	−	−	2 488.67±117.61 a	5.93
9	蕾丝贝贝 A Lace babe-A	中粉紫 54D Moderate Purplish-Pink 54D	22.46±2.59 c	975.13±33.35 c	6.53
10	蕾丝贝贝 B Lace babe-B	深粉紫 64D Deep Purplish-Pink 64D	−	2 492.17±107.44 a	6.08
11	蕾丝贝贝 C Lace babe-C	浅粉紫 65D Pale Purplish-Pink 65D	4.49±0.54 e	343.65±8.64 e	6.62
12	浅黄 Light yellow	浅黄 20D/中粉 49B Pale-Yellow 20D/Moderate-Pink 49B	−	2 484.70±108.39 a	5.79

注: −: 未检出; 有喉部色的花瓣颜色描述为: 花瓣主色/花喉部色; 同一列数据后不同小写字母代表 0.05 差异水平

Note: −: Not detected; The color of the petals with throat color is described as: main color of flower petals/flower throat color; Different lowercase letters after the same column of data represent 0.05 difference level

受体品种时的选择类似。张萍等(2010)认为筛选蓝色转基因受体品种应选择矢车菊色素含量低的品种，在花色素苷的合成途径中作用于矢车菊素苷的 F3'H (类黄酮 −3'− 羟化酶)会与 F3'5'H 竞争作用底物，影响蓝色花色表达。本研究得出的关联序第 2 的品种花色为红色，一般红色花的矢车菊色素含量较高，F3'H 活力较强(张萍等, 2010)。因此，如果要使用该品种作为蓝色转基因受体品种可以通过 RNA 干扰技术先抑制 *F3'H* 基因的表达。

通过对各品种仙客来 DFR 催化 DHM 能力、总黄酮含量、液泡 pH 值的综合分析，认为粉色的仙客来‘八重玫瑰’最适宜作为蓝色仙客来的转基因受体品种，将适宜来源的 *F3'5'H* 基因转化到该品种中有望得到较为理想的蓝色色系仙客来。

3 材料与方法

3.1 试验材料

供试花瓣来源于 12 个不同花色花型的仙客来品种(图 2)，种植于云南大学农学院植物生长室。2019 年 3 月采集相同时期的完全显色并展开的花朵作为试验材料。

3.2 仙客来花色测定及花瓣 DHM 饲喂培养

使用 RHSCC 对 12 个仙客来品种进行花色测定，花瓣若有白色区域只测定其他有色部分；同一花瓣有过渡色的选取花瓣中部测色；花朵喉部颜色单独测色，用花瓣主色/花喉部色表示。确定各品种花色编号后，对各品种花瓣进行 DHM 饲喂培养。DHM 为分析纯，购自昆明云科生物科技有限公司。结合

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表 2 仙客来各指标无量纲化及与参考品种的关联

Table 2 Non-dimensionalization of each indicator and correlation analysis with reference varieties

品种 Variety	指标无量纲化 Non-dimensionalization of indicators			关联度 Correlation	关联序 Correlation order
	飞燕草素苷 Delphinidin	总黄酮 Total flavonoid	pH		
条纹荷花 Stripe lotus	2.12	0.54	1.06	0.694 1	3
中国红 China red	1.65	1.26	0.99	0.741 1	2
八重玫瑰 Double rose	1.41	1.94	1.01	0.840 8	1
维多利亚 Victoria	0.59	1.93	0.98	0.657 0	4
梦幻粉 Dream-pink	0.49	0.70	0.93	0.361 0	6
梦幻红 Dream-red	0.27	0.60	1.00	0.299 0	7
蕾丝贝贝 A Lace babe-A	1.22	0.76	1.01	0.540 5	5
蕾丝贝贝 C Lace babe-C	0.24	0.27	1.02	0.222 8	8

注: 指标无量纲化以“1”为所有样本均值水平

Note: The indicator is dimensionless with "1" as the mean value of all samples



图 2 12 个不同花色花型仙客来品种

注: 1: 条纹荷花; 2: 中国红; 3: 宫灯; 4: 八重玫瑰; 5: 维多利亚; 6: 梦幻粉; 7: 梦幻红; 8: 梦幻白; 9: 蕾丝贝贝 A; 10: 蕾丝贝贝 B; 11: 蕾丝贝贝 C; 12: 浅黄

Figure 2 12 Cyclamen varieties with different flower colors and types

Note: 1: Stripe lotus; 2: China red; 3: Gong deng; 4: Double rose; 5: Victoria; 6: Dream-pink; 7: Dream-red; 8: Dream-white; 9: Lace babe-A; 10: Lace babe-B; 11: Lace babe-C; 12: Light yellow

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Brugliera 等(2013)的体外 DHM 饲喂培养，预实验发现仙客来花瓣在液体培养中色素容易降解及 DHM 在培养过程容易析出，所以采用固体培养基添加 DHM 方法进行饲喂。固体培养基成分为 1/4MS+6 g/L 琼脂+15 g/L 蔗糖+90 mg/L 柠檬酸钠+25 mg/L 硝酸银，pH 调至 6.0；DHM 试验组在此基础上添加 1 mg/mL DHM。将仙客来完全显色花朵经过 75%酒精擦拭消毒后，切取颜色分布均匀的中部花瓣 200 mg，每个品种取 3 片，置于培养基中，花瓣切口完全没入培养基。将其放置于 20℃、湿度 90%、光照强度 5 000 lx 的培养箱中持续培养 24 h。各品种每个处理设 3 次重复。培养结束记录颜色变化。

3.3 仙客来花瓣 DHM 饲喂培养后的飞燕草素苷提取及测定

飞燕草素苷的提取以于凯丽等(2018)在菊花花瓣中使用的方法为基础，有一定调整。将两组经培养的花瓣从培养基中取出，滤纸擦干后分别取 200 mg 置于研钵中，加入 2 mL 盐酸甲醇(V:V=15:85)充分研磨后转入离心管，再取 0.5 mL 盐酸甲醇润洗研钵将残渣一起转入离心管；将离心管置于冰上避光浸提 0.5 h，加入 10%甲酸水溶液 1.5 mL，经 12 000 r/min 离心 10 min 后取上清液用 0.22 μm 微膜过滤备用。

仙客来经培养花瓣的飞燕草素苷测定参照褚云霞等(2014)在矮牵牛中使用的方法。飞燕草素苷标准品购自上海源叶生物科技有限公司，分析仪器为赛默飞超高液相色谱仪(UltiMate™ 3000 UHPLC+)，色谱柱为 Zorbax SB C18，流动相 A 相为色谱级乙腈，B相为 10%的甲酸，检测波长为 530 nm。

3.4 仙客来花瓣总黄酮及液泡 pH 值测定

仙客来花瓣总黄酮的提取及测定以任梅娟和卢永昌(2018)对密花翠雀花中使用的方法为基础，有一定调整。将 12 个品种的仙客来花瓣各 200 mg 放入研钵，加入 2 mL 甲醇中充分研磨后转入离心管，4℃避光浸提 24 h；经离心机 12 000 r/min 转速离心 5 min。吸取上清液并加入等量的 60%乙醇和 0.5 mL 5%亚硝酸钠摇匀后静置 6 min；加入 0.5 mL 10%硝酸铝溶液摇匀后静置 6 min；加入 4 mL 4%氢氧化钠溶液和 3 mL 60%乙醇，摇匀后静置 15 min 备用。芦丁标准对照品购自昆明云科生物科技有限公司，分析仪器为 METASH UV-8000 紫外可见光分光光度计，检测波长为 510 nm。

仙客来花瓣液泡 pH 值测定：各品种取 1 g 花瓣加入液氮充分研磨后用滤纸过滤掉残渣，所得细胞液使用 METTLER TOLEDO S220-Micro Seven-Compact 台式 pH 计测定 pH 值。

3.5 结合灰色关联度理论对各仙客来品种进行综合评价

由于飞燕草素苷、总黄酮和 pH 值的直接数据差异过大，不便于同时比较，将其用平均值进行无量纲化以后数值均会在 1 左右，进行灰色关联分析也需要将指标进行无量纲化(吴丹等, 2019)。若无量纲化后的数值大于 1 表明该指标在 12 个仙客来品种中比平均值高，小于则反之，可以直观的看出各个品种各个指标的优劣。灰色关联分析所需的参考品种参数由供试品种的各指标最佳值构成。对仙客来的无量纲化后的各项指标赋权为：DFR 利用 DHM 的能力即生成的飞燕草素苷含量系数为 0.4，总黄酮含量系数为 0.4，液泡 pH 值为 0.2。最后应用灰色系统的关联度分析(于萍和李克, 2011)计算供试品种与参考品种的加权关联度，由关联序确定最佳仙客来转蓝色基因受体品种。

3.6 数据处理

使用 Canon EOS M6 相机进行拍照记录，Microsoft Excel 2010 和 SPSS 20 (Statistical Product and Service Solutions)进行数据处理分析。

作者贡献

何佳越是本研究的实验设计者和实验研究的执行人，完成数据分析，论文初稿的写作；李瑞娜和尹红参与实验设计和试验结果分析；吴学尉和刘海英是项目的构思者及负责人，指导实验设计、数据分析、论文写作与修改。全体作者都阅读并同意最终的文本。

致谢

本研究由云南大学农学学科项目建设(C176210-302)和教育部部省合建重点项目子项目“云南几种主栽花卉新品种选育及绿色栽培技术研发与示范”(C176280108)共同资助。

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山地农业生物学报 38(5):070~074,2019
Journal of Mountain Agriculture and Biology

8 个北方甘薯品种在云南的种植表现

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摘 要: 在甘薯生产过程中,选择优良品种是首要问题。试验通过对来自中国北方的运薯 650、运薯 728、运徐紫 20、运薯 66、运薯 716、运薯 606、晋薯 9 号、济薯 26 等 8 个甘薯品种和来自南方的广薯 87 进行种植对比试验,对 9 个品种的 40 个农艺指标进行观测,从定性、定量及干物重率等方面进行分析。结果表明:大部分品种表现良好,其中以运薯 728、济薯 26 表现较佳;运薯 728 和济薯 26 平均每亩产量分别为 5764 kg、5500 kg,其薯块干物重率分别为 25.5%、28.8%。在云南鲜食甘薯种植区域,推荐选择运薯 728 和济薯 26 这两 2 个品种。

关键词: 北方甘薯;品种;种植
中图分类号: S531 **文献标识码:** A
文章编号: 1008-0457(2019)05-0070-05 **国际 DOI 编码:** 10.15958/j.cnki.sdnyswxb.2019.05.012

Planting Performance of 8 North Wweet Potato Varieties in Yunnan

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Abstract: In the cultivation process of sweet potato, selecting the best variety is the primary issue. We tested 35 agronomic indicators of north sweet potato varieties (Yunshu650, Yunshu728, Yunxuzi20, Yunshu66, Yunshu716, Yunshu606, Jinshu9 and Jishu26), comparing with south sweet potato varieties Guangshu87, and also analyzed their qualitative, quantitative and dry matter weight (%) of sweet potato. The results showed that most varieties of sweet potato performed well. The two varieties of the Yunshu728 and Jishu26 had higher average yield with 5764 kg/667m^2 and 5500 kg/667m^2, and the dry matter weight percentages (%) of the two potato cake were 25.5% and 28.8%, respectively. We recommended to plant these two varieties, Yunshu728 and Jishu26, in planting area of fresh sweet potato in Yunnan.

Key words: north sweet potato; variety; plant

甘薯是一种重要的农作物。四百多年前引进中国后,曾对我国的经济、社会产生了重要的影响。甘薯由于有较强的“腹胀感”,能有效帮助人们减少过多食物的摄入,减少肥胖,维护人民的健康,已由过去单纯的粮食产品转变为现在的保健食品[1]。中国是世界上最大的甘薯生产国,近年来年均种植面积在 450 万 hm^{2}左右,约占世界甘薯种植面积的45.0%,总产约 1.0 亿 t,约占世界甘薯总产

收稿日期: 2019-05-08;**修回日期:** 2019-07-08
基金项目: 山西省科技成果转化引导专项二批项目“专用型甘薯品种引进及轻简化栽培技术示范”(201604D132051);山西省农业科学院生育种工程项目“现代农业产业技术体系 CARS-10-C2”(17yzgc007)。
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量的 80%[2]。由于历史原因，我国的甘薯研发中心主要分布在北方。山东省是我国甘薯研究和生产都比较发达的省份，山西是我国较早进行甘薯研究和种植的地方[3-4]。如何充分发挥这些研发中心的作用，促进我国南方的甘薯生产，是一个重要的问题。云南是中国甘薯种植较早的省份之一，栽培历史悠久，筛选高产优质的甘薯品种是云南甘薯产业发展面临的重要任务[5-6]。历史上，北京 553、徐薯 18 曾在云南的甘薯生产中发挥重要作用。前人在进行甘薯优良品种引试筛选中，也发现一些北方品种能直接使用[12-13]。近期，结合“科技入滇”项目，我们引进大量的甘薯新品种和轻简化栽培技术，取得了一些积极的成果[1,9]，为引进甘薯品种奠定理论基础。

1　材料和方法

1.1　试验品种

2018 年 6 月从北方的山西省和山东省引进运薯 650 号、运薯 728 号、运徐紫 20 号、运薯 66 号、运薯 716 号、运薯 606 号、晋薯 9 号、济薯 26 号等 8 个甘薯品种，并以南方的广东省引进的广薯 87 号作对照，共 9 个品种作为试验材料。

1.2　试验设计与方法

2018 年 6 月 10 日在玉溪农业职业技术学院农场进行整地，6 月 15 日进行移栽，试验采用随机区组设计，小区面积 10 m^2，小区长 10 m，小区宽 1 m，采用起垄栽培，重复 3 次。垄距 1 m，株距 0.30 m，采用单垄单行斜插栽培，小区栽培薯苗 33 株，每亩栽薯苗 2200 株左右。每亩施 15∶15∶15 复合肥 50 kg，施毒辛 5 kg 防地下害虫，于起垄前一次性施入。薯苗移栽后一周后，对其进行覆黑膜。在整个生长过程中，各品种肥水管理均一致。

1.3　农艺性状及表型数据采集

薯苗移栽后每天进行 1 次浇水，连续浇水一周，保证薯苗成活。薯苗成活后于前期和中期对其进行除草。在中期对各品种生长势进行观察记录，后期于 2018 年 11 月 15 日对各品种的茎蔓长等 34 个农艺性状进行观察记录，收获后对茎长、茎粗、茎基部分枝、茎叶产量、薯块产量及干物重率 6 个性状进行观测，共计 40 个性状指标。各品种产量采用挖取测定，每个重复选取 5 株进行地上和地下产量的测量，重复 3 次，共测量 15 株，计算出单株的平均产量，再按每亩估算出亩产量。干物重率用烤箱对各品种取鲜重 100 g 进行烘干称量检测，测定各品种的干物重率，每个品种取样重复 3 次。最后，对各品种产量结果采用 DPS 统计分析软件进行分析。

2　结果与分析

2.1　各品种农艺性状分析

通过对运薯 650、运薯 728、运徐紫 20、运薯 66、运薯 716、运薯 606、晋薯 9 号、济薯 26 和广薯 87 等 9 个甘薯品种进行对比试验，从各品种农艺性状来看（表 1），在农艺性状中，除皮层厚度、单株结薯数和薯蒂长 3 个数量性状外，其余 26 个性状按甘薯观测标准进行定性观测记录。从薯块皮层厚度来看，广薯 87 和晋薯 9 号分别为 1.5 mm、1 mm，较薄；运薯 66 为 2 mm，运薯 650 为 4 mm，较厚；其余各品种均表现为 3 mm，居中。从薯块单株结薯数来看，运薯 650 为 3 个，结薯较少；运薯 716 结薯数较多，为 20 个；其余各品种结薯数为 5～6 个，结薯表现为中等。从薯块薯蒂长（cm）来看，薯蒂长较长的是运薯 650、运薯 716、晋薯 9 号、济薯 26、广薯 87 号 5 个品种，其薯蒂长在 10 cm 以上，其余各品种的薯蒂长在 4.5～7.5 cm 之间。从薯块缺陷来看，济薯 26、广薯 87、运薯 66 的薯块较为光滑，运薯 728 和运薯 716 有浅裂沟，其余各品种有浅条沟；从地下薯块熟食口感情况来看，广薯 87、济薯 26、运薯 728、运薯 66、晋薯 9 号、运薯 606 这 6 个品种的口感相对要好，而运徐紫 20、运薯 650、运薯 716 这 3 个品种的口感要差一些。

2.2　各品种茎蔓数量性状基本情况分析

从各品种茎蔓部分农艺性状来看（表 2），在茎长方面，运徐紫 20、济薯 26 是长蔓品种，蔓长显著高于其他品种；运薯 66 号、广薯 87 号、运薯 716、运薯 650 号为短蔓品种，其余品种为中蔓品种，其中长蔓品种与对照等短蔓品种间均达到极显著差异；在茎粗方面，运薯 728 茎粗达 5.3 cm，与对照等其他品种的茎粗之间均达到极显著差异；在地上茎基分枝方面，地上茎基分枝在 6～10 个的是运薯 728、运徐紫 20 号、运薯 66 号、运薯 606 号和普薯 9 号 5 个品种，属于中等分枝品种，而广薯 87 号、运薯 650 号和运薯 716 号的地上茎基分枝在 14～17 个之间，属于多分枝品种，而济薯 26 地上茎基分枝为 4 个，属于分枝少的品种，各品种分枝之间的差异情况详见表 2。甘薯的茎长、茎粗和分枝指标代表植株的长势，特别是茎粗和分枝两个指标。

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表 1 甘薯各品种 34 个农艺性状基本情况表

Tab. 1 34 agronomic traits of the tested sweet potato varieties

性状	运薯 650	运薯 728	运徐紫 20	运薯 66	运薯 716	运薯 606	晋薯 9 号	济薯 26	广薯 87
顶叶色	绿紫色	绿紫色	绿紫色	绿紫色	绿紫色	绿紫色	绿紫色	绿色	绿色
叶色	墨绿色	绿色	绿色	绿色	绿色	绿色	绿色	绿色	绿色
叶形	心形	心形	枫叶形	心形	心形	心形	心形	心形	尖五角
叶缘类型	全缘	全缘	缺刻	全缘	全缘	全缘	全缘	全缘	缺刻
缺刻深浅	无	浅	深	无	浅	无	无	无	深
缺刻类型	单缺刻	单缺刻	复缺刻	单缺刻	单缺刻	单缺刻	单缺刻	单缺	复缺刻
中裂片形状	三角形	三角形	半椭圆	椭圆	三角形	半椭圆	三角形	椭尖	椭尖
叶尖形状	叶尖锐	叶尖锐	叶尖锐	叶尖锐 acuity	叶尖锐	叶尖锐	叶尖锐	叶尖锐	叶尖锐
叶正主脉色	绿色	紫绿色	绿色	绿色	绿色	绿色	浅绿色	绿色	绿色
叶正侧脉色	绿色	绿色	绿色	绿色	绿色	绿色	浅绿色	绿色	绿色
叶背主脉色	紫色	绿色	绿色	绿色	绿色	绿色	绿色	紫色	绿色
叶背侧脉色	紫色	绿色	绿色	绿色	绿色	绿色	绿色	紫色	绿色
正脉基色	紫色	紫色	绿色	绿色	绿色	绿色	浅绿色	紫色	紫色
背脉基色	紫色	绿色	紫色	绿色	绿色	紫绿色	紫绿色	紫色	紫色
茎端绒毛	少	少	少	少	少	极少	少	中	中
茎色	绿色	绿色	紫绿色	绿色	绿色	紫绿色	紫红色	绿色	绿色
薯块形状	短纺锤	短纺锤	短纺锤	短纺锤	纺锤形	长纺锤	长纺锤	短纺锤	长纺锤形
薯皮色	紫红色	紫红色	紫色	紫红色	紫红色	紫红色	红色	紫红色	浅红色
皮层色	紫红色	橘红色	紫色	白色	紫色	白色	紫色	黄色	紫黄色
皮层厚(mm)	4	3	3	2	3	3	1	3	1.5
薯肉主色	橘黄色	橘黄色	紫色	白色	橘黄色	白色	黄白色	黄色	黄色
薯肉次色	黄白色	黄色	紫色	白色	黄色	白色	黄色	黄白色	白色
薯肉次色分布	均匀	不均匀	不均匀	均匀	不均匀	均匀	均匀	均匀	均匀
结薯习性	集中	集中	集中	集中	集中	集中	较集中	集中	集中
单株结薯数(个)	3	5	6	6	20	6	5	5	6
薯蒂长(cm)	13	7.5	6	5	12	4.5	10	15	18.3
薯块缺陷	浅条沟	浅裂沟	浅条沟	无	有裂沟	浅条沟	浅条沟	无	无
薯皮光滑度	光滑	光滑	光滑	光滑	粗糙	较光滑	光滑	较光滑	光滑
薯块整齐度	不整齐	整齐	不整齐	整齐	整齐	一般	一般	整齐	一般
株型	半直立	半直立	葡萄	半直立	葡萄	葡萄	半直立	半直立	半直立
茎长度情况	短	中	长	短	长	长	长	中	中
长势	中	强	强	强	强	强	强	强	强
开花否	否	开	开	开	开	否	否	开花	否
熟食口感	中	好	中	好	中	好	好	好	好

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表 2　甘薯各品种 3 个数量性状基本情况

Tab. 2　The basic table of 3 quantitative traits (stem length,stem diatmeter,stem basal branch) of the tested sweet potato varieties

品种	茎长(cm)	茎粗(cm)	茎基分枝(个)
运薯 650	155. 2cB	1fD	17. 1aA
运薯 728	181. 4bcB	5. 3aA	7. 8bcCD
运徐紫 20	276. 3aA	2deCD	7. 7bcCD
运薯 66	140cB	2. 9bcBC	6. 9bcD
运薯 716	149. 1cB	2. 7cdBC	14. 1aAB
运薯 606	209. 5bAB	3. 6bB	9. 3bBCD
晋薯 9 号	218. 1bAB	2. 7cdBC	9. 5bBCD
济薯 26	270aA	1. 8eCD	4. 3cD
广薯 87	145cB	3. 6bB	13. 7aABC

注:表中茎粗为近地面茎蔓的周长,表中大、小写字母分别表示差异的极显著(1%)与显著(5%)。

2. 3　各品种产量及干物重率的分析

各品种产量与干物重率来见表 3。在地上茎叶产量方面,运薯 650 号、运薯 728 号、运徐紫 20 号、运薯 66 号、运薯 716 号、运薯 606 号、晋薯 9 号、广薯 87 号、济薯 26 号每亩产量分别为 2680. 0 kg、2420. 0 kg、1760. 0 kg、1320. 0 kg、2860. 0 kg、2640. 0 kg、1958. 0 kg、2200. 0 kg、1041. 0 kg,产量较高的是运薯 650 号、运薯 728 号、运薯 716 号、运薯 606 号、广薯 87 号这 5 个品种,其地上茎叶产量每亩在 2 t 以上,属于茎叶繁茂品种;运徐紫 20 号、运薯 66 号、晋薯 9 号、济薯 26 号 4 个品种的地上茎叶产量每亩低于 2 t;通过方差分析及多重比较,可以看出,各品种与济薯 26 号之间达到极显著差异。运薯 650 号、运薯 728 号、运徐紫 20 号、运薯 66 号、运薯 716 号、运薯 606 号、晋薯 9 号、济薯 26 号、广薯 87 号每亩产量分别为 3300. 0 kg、5764. 0 kg、2860. 0 kg、3300. 0 kg、5060. 0 kg、3740. 0 kg、3520. 0 kg、5500. 0 kg、2904. 0 kg。产量较高的是运薯 728 号、济薯 26 号和运薯 716 号 3 个品种,其地下薯块产量每亩在 5 t 以上,属于高产品种;产量较低的是运徐紫 20 号和广薯 87 号,其每亩产量为 2860. 0 kg、2904. 0 kg,接近 3 t;而运薯 650 号、运薯 66 号、运薯 606 号、晋薯 9 号 4 个品种的地下薯块产量每亩在 3300. 0 ~3740. 0 kg 之间,产量居中;在试验的 9 个品种中,特别是运薯 728 号和济薯 26 号这 2 个品种,它们与运薯 650 号、运徐紫 20 号、运薯 66 号、运薯 606 号、晋薯 9 号、广薯 87 号 6 个品种之间的产量差异均达到极显著,属于优势高产品种。在薯块干物重率方面,运薯 650 号、运薯 728 号、运徐紫 20 号、运薯 66 号、运薯 716 号、运薯 606 号、晋薯 9 号、济薯 26 号、广薯 87 号分别为 23. 1%、25. 5%、28. 7%、27. 5%、21. 7%、29. 8%、29. 6%、28. 8%、24. 5%,薯块干物重率较高的是运徐紫 20 号、济薯 26 号、运薯 66 号、运薯 606 号、晋薯 9 号 5 个品种,薯块干物重率在 27. 5% ~29. 8% 之间,属于高干物重率品种;薯块干物重率较低的是运薯 650 号和运薯 716 号两个品种,薯块干物重率在 21. 7% ~23. 1% 之间,属于干物重率较低品种;而运薯 728、广薯 87 号的薯块干物重率为 25. 5%、24. 5%,其薯块干物重率居中;运徐紫 20 号、济薯 26 号、运薯 66 号、运薯 606 号、晋薯 9 号 5 个品种的干物重率与其余品种间均达到极显著差异。根据王文质等人[10]的研究成果,甘薯干物重率高的品种,共淀粉含量也较高,其口感一般情况下也较好。

表 3　甘薯各品种产量与干物重率情况表

Tab. 3　The yield and dry matter weightws of the tested sweet potato varieties

品种	茎叶产量 ($kg/667m^2$)	薯块产量 ($kg/667m^2$)	干物重率
运薯 728	2346. 7abcAB	5764aA	25. 5bcBCD
济薯 26	1041. 3cB	5500abAB	28. 8aAB
运薯 716	2933. 3aA	4986. 7abcABC	21. 7dD
运薯 606	2566. 7abAB	3813. 3bcdABC	29. 8aA
晋薯 9 号	1965. 3abcAB	3512. 7cdABC	29. 6aAB
运薯 650	2698. 7aAB	3373. 3cdBC	23. 1cdD
运薯 66	1261. 3bcAB	3314. 7cdBC	27. 5abABC
广薯 87	2126. 7abcAB	2896. 7dC	24. 5cdCD
运徐紫 20	1833. 3abcAB	2786. 7dC	28. 7aAB

注:表中大、小写字母分别表示差异的极显著(1%)与显著(5%)。

3　结论与讨论

3. 1　某些农艺性状的观测问题

通过对省外引进的运薯 650、运薯 728、运徐紫 20 号、运薯 66、运薯 716、运薯 606、晋薯 9 号、济薯 26 和广薯 87 等 9 个甘薯品种比较试验。从定性的农艺性状方面来看,顶叶色在中期进行观察比较明显,由于受低温的影响,效果要差一些。本次由于在后期观察,各品种的顶叶色差异不大。在叶形

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方面，各品种的叶形存在差异，这是正常的现象，而同一品种在不同部位也发现不同类型的叶片，这是红薯某些品种的特性[11]。在薯蒂长方面，由于薯蒂长在收获中会被切断，因此给测量带来一定的难度，在测量薯蒂长时的数据准确性问题有待于改进。

从优良甘薯品种几个商品性关键的性状来看，在薯块产量较高的运薯 728、济薯 26 号和运薯 716 号 3 个品种中，运薯 728 和运薯 716 的薯肉色为橘黄色，而济薯 26 号为黄色；运薯 728 和济薯 26 号的薯形为短纺锤形，且表皮光滑，而运薯 716 为长短纺锤形，且表皮粗糙有裂沟。由表 1 和表 3 综合可以看出，运薯 728 和济薯 26 号两个品种的商品性要好，且熟食口感好，综合其干物重率来看，其淀粉含量在中上水平，而运薯 716 的商品性要差一些，熟食口感也要差一些。所以，运薯 728 和济薯 26 号比对照及其他品种有较好的优势，有一定的推广价值。

3.2 有关产量性状方面的观测问题

在地上茎叶产量方面，由于受生态因素及病虫害等的影响，各品种茎叶生长的繁茂时间有一定差异，所以观测结果也会存在一定的差异，但总体情况还是能看出地上产量的基本情况[12]。在地下薯块产量方面，由于采取每小区以一定数量来测产估算每亩的产量，因此，数据准确性也会有一定的差异，但总体情况还是能看出地下产量的基本情况。从地上产量与地下产量的关联度方面来看，一般情况下，地上茎叶产量高的品种，地下薯块产量也较高，本次试验基本反映出这一规律[13-14]。关于各品种薯块干物重率方面，在测试时，关键注意烘烤时的烘干标准，统一标准称量，观测结果才具有准确性[15]。

选择甘薯优良品种的关键指标是地下薯块产量，从试种的 9 个品种来看，产量较高的是运薯 728 号、济薯 26 和运薯 716 号 3 个品种，其每亩的产量均超过 5 t，特别是运薯 728 号和济薯 26 号 2 个品种的产量与对照及其它品种间均达到极显著差异，且品质也较好。

通过对省外提供的运薯 650 号、运薯 728 号、运徐紫 20 号、运薯 66 号、运薯 716 号、运薯 606 号、晋薯 9 号、济薯 26 号和广薯 87 号等 9 个甘薯品种进行种植对比，从定性、定量及干物重率等重要指标分析表明，在云南鲜食甘薯种植区域，推荐选择运薯 728 号和济薯 26 号 2 个品种来种植，其商品性、产量、熟食口感及干物重百分率等品质较好，有一定的市场潜力。

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云南大学学报(自然科学版), 2020, 42(1):187~193 DOI: 10.7540/j.ynu.20190199
Journal of Yunnan University: Natural Sciences Edition

东云'Corderoyi'的EMS离体诱变及突变体筛选

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摘要: 对多肉植物东云系'Corderoyi' 进行不同浓度和浸泡时间的甲基磺酸乙酯(EMS)诱变处理, 研究最适合的处理方法、获得不同类型突变体. 实验中以'Corderoyi'的花梗为外植体进行不同浓度 EMS 浸泡处理, 再诱导分化培养, 观察表型变异, 进行突变体筛选. 结果表明'Corderoyi'外植体诱变的最佳处理是 0.05% EMS 溶液浸泡 6 h, 外植体致死率为 64.86%, 突变率可以达到 14.50%. 研究筛选得到了 4 类表型变异稳定、具有观赏价值的多肉植物突变体, 可以丰富其种质资源, 为景天科多肉植物的诱变提供参考.

关键词: 多肉植物; 甲基磺酸乙酯; 离体诱变; 表型变异
中图分类号: S682.33　　**文献标志码:** A　　**文章编号:** 0258−7971(2020)01−0187−07

东云'Corderoyi'是景天科(Crassulaceae)拟石莲属(*Echeveria*)多肉植物, 是通过东云(*Echeveria agavoides*)系列的野生种杂交选育得到的优良园艺品种. 植株整体呈莲座状, 茎极短, 肉质叶片肥厚、光滑无粉, 叶色翠绿, 低温和强光照的条件下叶尖处叶缘变红, 具有较高的观赏价值. 为了进一步丰富东云系品种资源, 本研究采用化学诱变剂甲基磺酸乙酯(EMS)诱变的方法对'Corderoyi'进行离体诱变. EMS 诱变可以导致嘌呤、嘧啶的转化突变[1]和染色体缺失[2]. EMS 可以直接处理植物材料, 也可以与组织培养结合处理愈伤组织, 具有突变频率高、突变类型广和对染色体伤害低等优点[3], 已用于多种植物的诱变育种. Jander[4] 直接用 EMS 浸泡拟南芥种子得到了抗除草剂的突变体. 薛惠丹和李周岐[5] 用 EMS 处理花椒愈伤组织, 通过分化培养获得了具有形态学差异的花椒诱变植株. EMS 诱变在农作物上应用较多, 小麦[6]、水稻[7] 和玉米[8]等通过 EMS 诱变获得了相应的抗性或品质好的突变体. 化学诱变方法在多肉植物育种上的相关报道尚不多见. 周海成等[9] 以冰灯玉露愈伤组织为材料, 置于添加 EMS 的 MS 培养基上培养得到了部分形态变异个体; 吴正景等[10] 通过叠氮化钠处理玉扇的愈伤组织诱导了叶序变异的个体; 王俐等[11]利用秋水仙素诱导库拉索芦荟得到了多倍体植株. 目前为止, 还未见景天科拟石莲属多肉植物的诱变育种报道. 本研究以'Corderoyi'的花梗为外植体, 采用 EMS 诱变和组织培养相结合的方法, 对外植体进行不同浓度和浸泡时间的 EMS 处理, 再通过组织培养进行诱导分化, 旨在快速获得多种株型、叶形和叶色发生明显变异和具有更多观赏价值的突变体. 研究结果除了能够丰富东云系多肉植物的市场品种, 也可以为景天科其它多肉植物的诱变提供参考.

1　材料与方法

1.1　供试材料　东云'Corderoyi'的花梗作为外植体诱变材料, 植株购自云南省昆明市斗南花卉交易市场.

1.2　培养基组成及培养条件　通过预实验得到适宜'Corderoyi'花梗培养的条件. 愈伤培养基: 以 MS 为基础培养基添加植物生长调节剂 6-苄氨基腺嘌呤(6-BA)2.0 mg/L, 萘乙酸(NAA)0.20 mg/L, 蔗糖 30 g/L 作为碳源, 琼脂 6 g/L 作为固化剂; 分化阶段: 植物生长调节剂配比为 6-BA 2.0 mg/L,

收稿日期: 2019-04-12;　接受日期: 2019-09-19;　网络出版日期: 2019-11-26
基金项目: 云南大学农学学科项目建设(C176210302); 云南省校合作项目(C176280108).
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NAA 0.10 mg/L. 培养基 pH=5.8, 培养温度(25±2) ℃, 茎段愈伤组织诱导前期黑暗培养 7 d, 其余阶段光照强度为 30 ~ 40 μmol·m^{-2}·s^{-1}, 光照时间 12 h/d. 在云南大学农学院组培室进行诱导分化培养.

1.3 诱变处理 将 EMS 加入 pH=7.0 的磷酸缓冲液中, 配置成质量分数为 0.05%、0.10%、0.15%、0.20% 的处理液, 处理时间设置为 2 h 和 6 h, 共 8 个实验组. 对照为不加 EMS 的磷酸缓冲液分别处理 2 h 和 6 h. 将外植体花梗进行流水冲洗 1 h 后, 在超净工作台上进行诱变处理, 取处理液各 50 mL 于烧杯中, 将花梗没入溶液中处理相应时间, 处理完成后洗掉花梗表面残余处理液后备用. 诱变处理于 2017 年 6 月在云南大学农学院组培室进行.

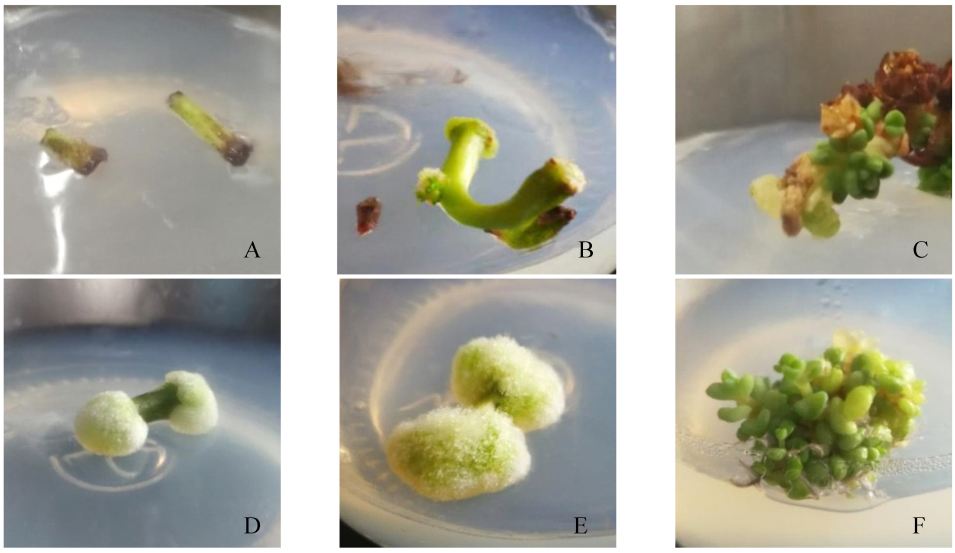
1.4 无菌外植体制备及接种 将诱变处理后的花梗置于无菌瓶中, 75% 酒精摇晃浸洗 10 s 后用无菌水冲洗; 再用 2% 次氯酸钠浸洗 12 min 并摇动数次后洗净. 灭菌完成后将花梗切成约 1 cm 茎段, 无菌滤纸吸干水分接种于培养基中, 每个处理接种 10 瓶, 每瓶 3 个外植体, 3 次重复. 待外植体产生愈伤组织后将其增殖培养至约为外植体的 5 倍大小, 再将各组愈伤转接至分化培养基, 在分化培养基中诱导出芽后培养至幼苗生根.

1.5 表型测定 诱变材料分化出苗后, 选择根系健康与大小一致的植株炼苗移栽, 使用泥炭、蛭石和珍珠岩质量比为 3:1:1 的混合基质栽培放置于植物生长室中; 各植株日常管理相同, 观察植株表型性状. 表型鉴定分两次: 直径 1 cm 左右的组培苗鉴定和培养 60 d 时的植株鉴定. 组培苗仅观察对比株型和叶色等性状, 不作筛选分类; 栽培 60 d 后分类, 选取各种类型代表植株, 观察株型, 统计叶长、叶宽、叶厚、叶色和叶尖等性状. 1 a 后对比各性状观察突变体是否稳定.

1.6 数据统计处理及分析 外植体接种培养后, 观察外植体褐化死亡情况、愈伤组织发生及分化情况, 外植体分化出苗后观察统计突变率. 致死率为各组 EMS 处理后的‘Corderoyi’花梗转接至愈伤组织诱导培养基 7 d 时外植体整体褐化变色的数量与接种的外植体总数的百分比; 愈伤组织诱导率为发生体积约有 0.5 cm^3 愈伤组织的外植体数与接种的外植体总数的百分比; 愈伤组织分化率为发生 1 个芽以上的愈伤组织数与接种的愈伤组织总数的百分比; 突变率为具有形态变异的再生苗数与再生苗总数的百分比. 使用 Canon EOS M6 相机对组培苗、移栽 60 d 及 1 a 后的植株拍照, 并测定记录表型性状, 使用 SPSS20 软件进行数据分析.

2 结果

2.1 诱变处理对‘Corderoyi’外植体成活和突变的影响 ‘Corderoyi’花梗经各组 EMS 处理后转接至愈伤组织诱导培养基 7 d 时统计的致死率结果表明, 在处理过的外植体中, 部分外植体在培养过程中逐渐褐化(图 1A), 未处理的外植体则保持绿色



A: 经 EMS 处理的外植体褐化; B: 未经 EMS 处理的外植体; C: 外植体花梗结节处直接产生不定芽; D: 外植体产生白色致密的愈伤组织; E: 愈伤组织松散变绿; F: 愈伤组织分化出芽.

图 1 外植体花梗经 EMS 诱变后生长情况

Fig. 1 Growth of explant pedicel induced by EMS

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(图 1B). EMS 诱变剂对外植体的成活有显著的影响, 各 EMS 处理的外植体致死率均显著高于对照组(表 1). 在相同浓度的 EMS 处理中, 浸泡 6 h 处理组的致死率均显著高于浸泡 2 h 的处理组; 同一浸泡时间在不同的处理浓度中, 外植体致死率随着浓度增大而上升, 表明 EMS 浓度和处理时间均对外植体的成活产生影响. 在浸泡 2 h 的处理中相邻浓度梯度间差异不显著, 但在浸泡 6 h 的处理中不同浓度间的致死率均有显著差异, 表明 EMS 的浓度差异效果会随处理时间增加而增强. 处理 3 和处理 8 的致死率分别为 64.86% 和 41.27%, 已接近半致死量. 待外植体分化出苗后, 对比处理组和对照组的苗形态得到突变率, 对照组再生苗没有发生形态变异. 突变率和致死率总的变化趋势类似, EMS 浓度越高、处理时间越长, 突变率越高, 但其中处理 9 的突变率低于处理 7, 处理 7 的突变率最高为 17.78%(表 1). 接近半致死的处理 3 和 8 的突变率差异显著, 分别为 14.50% 和 12.22%.

表 1　EMS 诱变处理后‘Corderoyi’花梗的致死率和突变率

Tab. 1　Fatality and mutation rate of 'Corderoyi' pedicel treated by EMS

w(EMS)/%	浸泡2 h			浸泡6 h		
	处理号	致死率/%	突变率/%	处理号	致死率/%	突变率/%
0.00	1	0.00 h	0.00 f	1	0.00 h	0.00 f
0.05	2	9.30±3.61 g	4.44±1.92 e	3	64.86±3.61 d	14.50±3.75 b
0.10	4	20.64±1.28 fg	5.56±1.92 de	5	72.83±13.55 c	15.56±1.92 b
0.15	6	27.82±6.01 ef	7.78±5.09 d	7	77.27±19.99 b	17.78±5.09 a
0.20	8	41.27±7.94 e	12.22±1.92 c	9	85.62±13.40 a	14.44±1.92 b

表中不同字母表示数据间存在显著性差异(P<0.05).

2.2　EMS 诱变处理对‘Corderoyi’外植体愈伤组织诱导和分化的影响　接种 10 d 后一部分外植体切口膨大开始产生愈伤组织, 还有一部分外植体在培养 15 d 后花梗结节处开始出现不定芽(图 1C). 各处理组培养 30 d 后均逐渐产生明显的愈伤组织, 但各处理组的愈伤组织数量有差异. 较早产生的愈伤组织逐渐变成白色致密的晶体绒毛状(图 1D), 也有部分愈伤组织逐渐变为绿色松散的形态(图 1E). 总的情况是未经 EMS 处理过的外植体发生愈伤组织的时间较早, 生长质量较优. 各处理对‘Corderoyi’花茎外植体的愈伤组织诱导率也有一定影响(表 2), 对照组的愈伤组织诱导率显著高于各处理组. 当 EMS 质量分数在 0.05% ~ 0.15% 范围时, 相同浓度下浸泡 2 h 的愈伤组织诱导率要显著高于浸泡 6 h 的一些处理, 表明用较低浓度 EMS 处理时, 增加浸泡时间会增强对愈伤组织的诱导抑制; 但当 EMS 质量分数达到 0.2% 时, 浸泡时间对愈伤组织诱导没有显著影响, 诱导率均降至 3% 水平. 在浸泡 6 h 的处理下, 不同浓度 EMS 处理组的愈伤组织诱导率都极低, 高于 0.05% 组的诱

表 2　诱变处理对‘Corderoyi’的愈伤诱导率和分化率影响

Tab. 2　Effects of different treatments on callus induction rate and differentiation rate of Corderoyi

w(EMS)/%	浸泡2 h			浸泡6 h		
	处理号	诱导率/%	分化率/%	处理号	诱导率/%	分化率/%
0.00	1	17.38±3.12 a	82.00±1.23 a	1	17.28±3.12 a	82.00±1.23 a
0.05	2	14.53±2.52 b	79.18±2.41 ab	3	5.91±2.01 d	58.33±1.76 c
0.10	4	13.00±2.28 bc	72.21±2.70 b	5	4.28±0.44 e	57.12±2.67 cd
0.15	6	9.41±2.33 c	40.00±1.50 e	7	3.91±0.73 f	30.55±2.43 f
0.20	8	3.10±0.71 fg	18.67±2.33 g	9	3.25±0.52 fg	12.47±1.55 g

表中不同字母表示数据间存在显著性差异(P<0.05).

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导率均低于 3%. 将各组愈伤组织增殖培养至足够数量转接到分化培养基, 30 d 后开始有愈伤组织分化出芽(图 1F), 60 d 后统计的分化率各组间差异也较大(表 2). 0.05%EMS 浸泡 2 h 处理组与对照组的分化率 82% 相近, 没有显著差异, 其余各组的分化率均显著低于对照组. 0.05% 和 0.10% 的 EMS 浓度组之间的分化率没有显著差异, 当质量分数高于 0.15% 时, 分化率显著下降; 当 EMS 质量分数达到 0.2% 时, 浸泡时间的差异对愈伤组织分化没有显著影响, 分化率最低在 10%水平. 接近半致死的处理 3 和 8 的分化率差异显著, 分别为 58.33% 和 18.67%.

2.3 'Corderoyi'组培苗突变体观察 比较诱变后的'Corderoyi'组培苗与对照植株的形态, 发现部分植株的叶形和叶色等性状发生了明显变异(图 2). 叶片有窄薄型、宽厚型和狭长型等, 叶尖有急尖和钝尖两种类型; 叶色主要是叶缘出现浅色镶边、叶冠变黄和整体变黄. 通过致死率、愈伤诱导率、分化率、组培苗表型性状的综合比较分析, 0.05%EMS 溶液处理 6 h 为最佳诱变条件.



A B C D

A: 对照植株; B: 丛生、株型松散、叶片变薄和变尖的诱变植株; C: 镶边、叶冠变黄的诱变植株; D: 整体变黄的诱变植株.

图 2 'Corderoyi'诱变组培苗与对照植株形态比较

Fig. 2 Morphological comparison between'Corderoyi' induced tissue culture seedlings and control plants

2.4 'Corderoyi'突变体筛选 将'Corderoyi'组培苗移栽培养 60 d 后观察, 整体变黄的植株类型(图 2D)不能成活, 其他形态变异的再生苗均能正常生长. 经性状表型数据统计分析后, 筛选出 4 种类型的突变体(图 3, 表 3). 与对照植株相比, Ⅰ类变异株的的叶片宽度和厚度分别达 2.0 cm 和 0.45 cm, 分别比对照增加 29.19% 和 40.63%; 叶片的长宽比显著降低, 叶片整体更加圆润饱满, 属典型的宽厚型叶片; 叶尖由急尖变为钝尖. Ⅱ类变异株的长势快, 株型变大, 平均叶长达 3.47 cm; 叶片长宽比值增加至 2.51, 属典型的狭长型叶片; 叶片整体呈现翠绿色, 老叶叶缘有较窄的淡黄镶边, 中心幼叶镶边比例增大. Ⅲ类变异株的植株相对较小, 叶色变浅, 叶缘淡黄色镶边明显, 叶片显著变薄, 叶尖由急尖变为钝尖. Ⅳ类变异株在所有变异植株类型中最小, 长势最慢, 叶片呈灰绿色, 叶表面覆盖稠密短绒毛, 叶片极薄, 叶缘也有明显的淡黄色镶边. 所有诱变苗移栽后与相应的组培苗阶段比较, 株型更紧凑, 叶形和叶色的变化比较稳定.

2.5 'Corderoyi'突变体性状表现的稳定性 盆栽 1 a 后, 各类变异植株的表型基本稳定(图 4). Ⅰ类变异仍为宽厚型, 宽度及厚度约为对照植株的两倍, 叶色、叶缘及叶尖与 60 d 时的表现一样. Ⅱ类变异仍为金边狭长型; Ⅲ类变异的长势较慢, 叶型株型变化不大, 仅是叶片厚度增加, 浅黄的叶缘区域面积最大; Ⅳ类变异与 60 d 的植株比较, 厚度发生了很大变化, 但长宽比稳定, 叶色仍是四类变异中颜色最浅的一类. 通过横向及纵向比较结果表明这四类变异很可能是突变体.

3 讨论

目前, 多肉植物的育种方式主要采用传统的杂交育种方法, 耗时长、效率低、不能快速满足市场多样性发展的需求. 本研究采用了 EMS 诱变和组培技术结合的方法, 对'Corderoyi'进行了诱变研究, 得到了 4 类突变体.

多肉植物的化学诱变所产生的变异主要包括株型、叶色和染色体倍性等变异. 玉扇的扇形株型经诱变后变为旋生莲花状株型, 叶色经诱变后由翠绿色变为黄白色和黄绿色[10]; 库拉索芦荟经诱变后产生了多倍体[11]. 本研究使用 EMS 诱变东云'Corderoyi'也产生了株型变异和类似的叶色变异,

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A 叶形变异对比



B 植株整体对比

图 3　突变体植株与对照植株栽培 60 d 后的形态对比

Fig. 3　Morphological comparison between variant plants and control plants for 60 days after transplanting

表 3　‘Corderoyi’突变体的叶形态表现

Tab. 3　Leaf phenotype of ‘Corderoyi’ mutants

变异类型	叶长/cm	叶宽/cm	叶长宽比	叶厚/cm	叶色	叶尖
CK	2.69±0.25 b	1.61±0.12 b	1.67±0.03 bc	0.32±0.01 b	翠绿	急尖
Ⅰ类	2.72±0.18 b	2.08±0.20 a	1.31±0.04 d	0.45±0.02 a	翠绿	钝尖
Ⅱ类	3.47±0.35 a	1.38±0.09 c	2.51±0.11 a	0.39±0.01 ab	翠绿	急尖
Ⅲ类	1.92±0.09 c	1.28±0.15 dc	1.50±0.09 c	0.27±0.01 c	淡绿	钝尖
Ⅳ类	1.71±0.11 c	0.86±0.06 d	1.98±0.01 b	0.12±0.01 d	灰绿	钝尖

表中不同字母表示数据间存在显著性差异(P<0.05).



图 4　4 类变异植株移栽 1 a 后的表型

Fig. 4　The phenotype of four variants for one year after transplanting

并且部分变异植株的株型、叶片长宽厚均大于对照植株, 表皮气孔大而稀疏, 可能发生了染色体倍性变异. 影响植物 EMS 离体诱变效果的因素主要包括植物种类及取材部位、EMS 浓度、处理时间和方式等[12]. 周海成等[9]以冰灯玉露的愈伤组织在 0.1% EMS 培养基中培养, 4 d 时获得了理想的半致死效果, 最后得到了 1.5% 发生形态变异的突变体, 但存在愈伤组织成活率低, 较难分化再生苗

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的问题. 吴正景等[10]以含 0.1 mmol/L 叠氮化钠的培养基培养玉扇愈伤组织 30 d, 再转入无叠氮化钠培养基培养 60 d 得到了 77.1% 的突变率, 但能够正常发育的变异芽只有 6.0%, 变异类型主要是叶序变异. 王俐等[11]用 0.06% 秋水仙素处理库拉索芦荟再生苗 12 h, 获得了 50% 的多倍体植株. 使用诱变剂直接处理愈伤组织步骤较为复杂, 所用培养基需要采用抽滤灭菌的方式灭菌, 污染率较高. 本研究选用的诱变材料是多肉植物'Corderoyi'的离体花梗, 表面光滑的花梗相对其它部位灭菌操作更容易, 污染较少; 花梗诱变处理后再灭菌接种, 培养基可采用高温高压彻底灭菌以降低污染风险. 秋水仙素的诱变效率较高, 但产生的主要变异是染色体倍性变异, 叠氮化钠处理多肉植物产生的变异类型也较为有限, 所以本研究选用了 EMS 作为诱变剂. 不同浓度的 EMS 和不同处理时间对'Corderoyi'外植体致死率、愈伤组织诱导率和分化率等表现有显著影响. EMS 浓度越高、浸泡时间越长, 则'Corderoyi'外植体的致死率越高, 诱导率和分化率越低, 对材料的伤害越大, 这与甘蔗的 EMS 诱变研究结果一致[13]. EMS 浓度较低时, 不同处理时间对'Corderoyi'外植体的影响有显著差异; 当 EMS 达到一定浓度时, 处理时间对结果没有显著影响. 相对而言, EMS 浓度对'Corderoyi'的诱变影响更大, 这与冬瓜[14]、金花菜[15]EMS 诱变的结论相似.

植物的育种目标不同, 突变体的筛选方式也有所差异. 抗性育种选择主要是通过病菌毒素筛选获得[16], 品质育种则是对 M_2 种子进行品质分析[17]. 'Corderoyi'作为观赏植物, 其育种目标之一是实现观赏性状多样化, 因此, 本研究中采用的突变体筛选方式是直接观察诱变植株的表型性状. 由于表型变异易受环境影响, 所以本研究对所得的突变体进行了一年的跟踪观察. 总体上来说, 变异性状比较稳定.

综上, EMS 诱变'Corderoyi'的最佳条件为 0.05% EMS 浸泡外植体花梗 6 h, 具有 14.50% 的突变率, 可以为景天科其它多肉植物的育种提供一种更高效简便的方法参考. 本研究获得的 4 类突变体与对照比较, 首先是叶片的表型产生差异, 叶缘与对照相比没有红色叶尖及叶尖旁的红色叶缘, 但有稳定的浅黄色叶缘, 特别是Ⅱ和Ⅲ类变异叶缘颜色更明显, 可以丰富其观赏性状; 叶形突变体中的宽厚型植株, 叶片宽度和厚度约为对照的 2 倍; 叶色方面Ⅳ类突变体覆盖有稠密短绒, 叶色表现为灰绿色甚至呈现浅蓝色, 可进一步提升'Corderoyi'的观赏价值. 其次是长势表现, 所有突变体长势均要好于对照; Ⅰ和Ⅱ类突变体, 所有阶段皆优于对照; Ⅲ和Ⅳ类突变体相对Ⅰ和Ⅱ类突变体较差, 但后期长势也优于对照. 对于生长相对缓慢的多肉植物来说, 长势快的突变体形成品种后更利于市场化生产繁殖.

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EMS mutagenesis *in vitro* and mutant screening of *Echeveria agavoides* ‘Corderoyi’

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Abstract: In order to determine the optimum treatment method and obtain different types of mutants, *Echeveria agavoides* ‘Corderoyi’ were treated by ethyl methyl sulfonate (EMS) under different concentrations and soaking time in this study. The pedicels of ‘Corderoyi’ were treated by EMS immersion as explants, and differentiation was induced by tissue culture. Phenotypic difference was observed to screen the mutants. The results showed that the optimum treatment for ‘Corderoyi’ explant mutagenesis was at 0.05% EMS immersion for 6 hours. The fatality rate and mutation rate of explants were 64.86% and 14.50%, respectively. Four types of mutants with stable phenotypic variation and ornamental value were screened. It could enrich their germplasm resources and serve as reference for the mutagenesis of other Crassulaceae plants.

Key words: succulent plant; the ethyl methane sulfonate; mutagenesis *in vitro*; phenotypic variation

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中国农学通报 2020,36(34):142-147
Chinese Agricultural Science Bulletin

不同加工工艺下‘紫娟’茶品质的比较

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摘 要:为研究不同加工工艺对‘紫娟’茶品质的影响,以‘紫娟’鲜叶为原料,分别利用烘青绿茶、晒青绿茶、红茶、白茶及黑茶的加工工艺制作成5种‘紫娟’茶,检测其主要品质成分的含量并进行系统分析。结果表明,不同加工工艺下,同一品种‘紫娟’鲜叶制得的茶样所含化学成分的含量差异较大。其中,‘紫娟’烘青绿茶中花青素含量最高,晒青绿茶次之;儿茶素的含量随着发酵程度的加深而降低。此外,在黑茶中没食子酸含量最高,在白茶中茶氨酸含量最高,而咖啡碱在红茶中的含量最高。由此可见,不同加工工艺对‘紫娟’茶的主要品质化学成分影响较大,其中‘紫娟’烘青绿茶的多酚类物质含量最为丰富。

关键词:茶;‘紫娟’;加工工艺;品质;化学成分
中图分类号:S571.1 **文献标志码:**A **论文编号:**casb20200100066

The Quality of ‘Zijuan’ Tea Under Different Processing Techniques: A Comparative Analysis

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Abstract: To study the effect of different processing techniques on the quality of ‘Zijuan’ tea, we tried to make five kinds of ‘Zijuan’ tea samples by taking fresh leaves of ‘Zijuan’ as raw materials and adopting the processing techniques of baked green tea, sundried green tea, black tea, white tea and dark tea, and tested the contents of main quality-related chemical constituents and made a systematic analysis. The results showed that different processing techniques could make the contents of chemical constituents significantly different in the tea samples that were made from the fresh leaves of the same variety of ‘Zijuan’. The content of anthocyanin in the baked green tea was the highest, followed by that in the sundried green tea. With the advancing of fermentation, the content of tea polyphenol and catechin declined. Additionally, the dark tea had the highest content of gallic acid among all the tea samples, the white tea had the highest content of theanine, while black tea had the highest caffeine. Therefore, different processing techniques have significant influence on the contents of main quality-related chemical constituents of ‘Zijuan’ tea, and the content of polyphenols in the baked green tea is the most abundant.

Keywords: tea; ‘Zijuan’; processing techniques; quality; chemical constituents

0 引言

茶与可可、咖啡并称为当今世界三大不含酒精的饮料[1-2],而茶的消费量已跃居世界首位。云南是茶树的主要起源中心,其中大叶种茶树是在云南特殊生态环境条件下繁衍的、具有自身独特个性的栽培品种,也是中国著名的茶树良种,其内含物丰富,含量高于中、小叶种[3-4]。

‘紫娟’为山茶科(Theaceae)山茶属(*Camellia*)茶种(*Camellia sinensis*)中的普洱茶变种(*C. sinensis* var. *assamica*)植物,由云南省农业科学院茶叶研究所于

基金项目:云南大学教育部部省合建重点项目“茶叶产品质量安全控制技术及健康功能产品研发”(C176280106)。
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收稿日期:2020-01-20,**修回日期:**2020-03-22。

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1985年在云南大叶群体品种中发现的一株紫色茶树的基础上选育而成，因该茶树具有紫芽、紫叶和紫茎，特取名为‘紫娟’[5-7]。‘紫娟’内含物质丰富，尤其花青素含量远高于普通大叶种。此外，‘紫娟’中的花青素和儿茶素都属于茶叶多酚类物质，是‘紫娟’茶最重要的功效成分。由于花青素比其他大叶种丰富，‘紫娟’茶在口感上稍显苦涩，但其对人体有众多保健功效，因而具有重要的科学研究价值和开发空间，在生产中可通过不同深加工技术将其制作为茶叶相关的衍生品以充分挖掘其潜在价值，延长产业链。

茶叶按照加工工艺可以分为6种基本茶类（绿茶、白茶、黄茶、乌龙茶、红茶、黑茶）[8]，加工工艺的差异导致茶叶品质化学成分的组成和含量均不相同，从而使得茶呈现特有的品质风味。茶叶的品质风味主要归因于其多样的化学成分，有茶多酚、生物碱、氨基酸等。其中，茶多酚主要包括儿茶素、花青素和酚酸等；生物碱主要包括咖啡碱、茶碱和可可碱；氨基酸主要包括茶氨酸、天冬氨酸、谷氨酸等[9]。这些成分共同决定着茶叶的品质。

目前，大多数的研究集中在普通大叶种以及中、小叶种的不同鲜叶加工而成的不同茶类，进行其茶多酚等化学成分的对比研究，普遍发现多酚含量与茶叶发酵程度密切相关，发酵度越低，多酚类成分含量越高[2,10-11]。此外，在用同一种鲜叶原料制作的红茶和绿茶中，绿茶抗氧化活性明显优于红茶[12]，说明不同加工工艺对茶叶品质的影响巨大。‘紫娟’茶具有明显的保健功效，但是市场上‘紫娟’茶的种类较为单一，而且熟知度不高，并没有发挥出其独特的优势，因此‘紫娟’茶新品及其衍生品的开发可充分利用其独特的价值以最大程度延长其产业链。现阶段有关‘紫娟’的研究主要局限在‘紫娟’绿茶和红茶品质化学成分含量的比较分析，而对其利用价值鲜有报道。本研究通过比较5种不同加工工艺‘紫娟’茶的品质差异，探讨其主要品质成分的变化，旨在为‘紫娟’茶的合理开发提供科学参考，为研制相关‘紫娟’茶新产品和深加工产品提供理论依据。

1 材料与方法

1.1 试验时间、地点

试验于2019年4—11月在云南大学农学院和云南民族大学民族医药学院实验室进行。

1.2 材料与试剂

1.2.1 样品 参试5种‘紫娟’茶的鲜叶原料采自云南省普洱市江城县整董镇，采摘标准为一芽二叶，然后由普洱天福生物科技发展有限公司按照工厂化生产流程加工制作而成。晒青绿茶和烘青绿茶参考国家标准GB/T 14456.2—2018制作、白茶和黑茶参考国家标准GB/T 22111—2008制作、红茶参考国家标准GB/T 35810—2018制作[13-15]。

1.2.2 标准品 茶多酚、茶氨酸、咖啡碱、儿茶素单体（儿茶素、表儿茶素、表没食子儿茶素、表没食子儿茶素没食子酸酯、表儿茶素没食子酸酯）、没食子酸，购自北京普天同创生物科技有限公司，纯度均≥98%。

1.2.3 试剂 氧化镁，天津市风船化学试剂科技有限公司，分析纯；甲醇，广州彼西络科技有限公司，色谱纯；碳酸钠，天津市风船化学试剂科技有限公司，分析纯；硫酸亚铁，西陇科学股份有限公司，分析纯；酒石酸钾钠，西陇科学股份有限公司，分析纯；磷酸二氢钾，西陇科学股份有限公司，分析纯；磷酸氢二钠，西陇科学股份有限公司，分析纯；乙腈，广州彼西络科技有限公司，色谱纯；冰乙酸，上海麦克林生化科技有限公司，色谱纯；盐酸，天津市风船化学试剂科技有限公司，分析纯；无水乙醇，成都市科隆化学品有限公司，分析纯。

1.3 主要仪器

天喜M150A型小型磨粉机，浙江天喜网络科技有限公司；WGL-125B鼓风干燥箱，天津市泰斯特仪器有限公司；LEETD XZ-1B旋片式真空泵，温岭市力拓机电有限公司；SHIMADZU LC-2030高效液相色谱仪，岛津公司；Agilent LC-1200高效液相色谱仪，安捷伦科技有限公司；泰斯特DK-98-Ⅱ电热恒温水浴锅，天津市泰斯特仪器有限公司；Eppendorf 5418R离心机，德国艾本德股份公司；METASH UV-8000型分光光度计，上海元析仪器有限公司；Sartorius BSA223S分析天平，德国赛多利斯公司。

1.4 试验方法

1.4.1 茶样制备 5种‘紫娟’茶的基本加工工序分别为：（1）晒青绿茶，鲜叶→摊青→杀青→揉捻→干燥（晒干）→毛茶；（2）烘青绿茶，鲜叶→摊青→杀青→揉捻→干燥（烘干）→毛茶；（3）白茶，鲜叶→萎凋→干燥→毛茶；（4）红茶，鲜叶→萎凋→揉捻→发酵→干燥→毛茶；（5）黑茶，鲜叶→摊青→杀青→揉捻→渥堆→干燥→毛茶。

分别取‘紫娟’晒青绿茶、烘青绿茶、白茶、红茶和黑茶各30 g，磨碎，用30目筛网过筛后储存于带盖棕色玻璃瓶中，存放于阴凉干燥处。

1.4.2 测定方法

（1）水分和干物质含量测定采用直接干燥法，分别参照GB 5009.3—2016[16]和GB/T 8303—2013[17]进行。

（2）水浸出物和灰分含量测定分别参照GB/T 8305—2013[18]和GB 5009.4—2016[19]进行。

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（3）茶多酚含量测定采用分光光度法，参照GB/T 8313—2002[20]进行，并作修改。配制浓度为500 μg/mL的茶多酚标准品储备溶液，分别吸取0.0、0.2、0.4、1.0、2.0、3.0 mL标准品储备溶液于10 mL容量瓶中，再分别加入2 mL超纯水和2.5 mL酒石酸亚铁溶液，最后用pH 7.5的磷酸盐缓冲溶液定容至刻度线，配制成浓度分别为0、10、20、50、100、150 μg/mL的系列标准工作液。用10 mm比色皿，于波长540 nm处测定吸光度，绘制标准曲线。

称取1.0 g磨碎试样，置于50 mL锥形瓶中，用30%的乙醇溶液加至刻度线，于沸水浴中浸提20 min（每5 min摇一次），浸提后用30%的乙醇溶液补至刻度线，滤液移入500 mL容量瓶中，冷却后用水定容至刻度，混匀，准确吸取试液1 mL，过0.45 μm滤膜，注入10 mL容量瓶中，加水2 mL和酒石酸亚铁溶液2.5 mL，用pH 7.5的磷酸盐缓冲溶液定容至刻度线，用10 mm比色皿，于波长540 nm处测定吸光度。

（4）花青素含量测定采用分光光度法，参照文献[21-22]中的方法进行。

（5）儿茶素和没食子酸含量测定采用HPLC法，儿茶素含量的测定参照GB/T 8313—2018[23]和文献[24]中的方法进行，没食子酸含量的测定参照GB/T 30483—2013[25]和文献[24]进行，略作修改。

色谱条件：Agilent C_{18}色谱柱（粒径5 μm，250 mm×4.6 mm），流动相A为9%乙腈-1.5%冰乙酸溶液，流动相B为80%乙腈-1.5%冰乙酸溶液，流动相流速1.0 mL/min，柱温35℃，紫外检测波长278 nm，进样量10 μL，梯度条件为梯度洗脱，程序见表1。

表1 HPLC检测儿茶素和没食子酸的梯度程序

时间/min	流动相体积分数配比/%	
	A相	B相
0.00	100	0
10.00	100	0
25.00	70	30
35.00	70	30
40.00	100	0

（6）茶氨酸含量测定采用HPLC法，参照GB/T 23193—2017[26]和文献[27]中的方法进行，略作修改。

色谱条件：Agilent C_{18}色谱柱（粒径5 μm，250 mm×4.6 mm），流动相A为0.05%乙酸水，流动相B为甲醇，流动相流速1.0 mL/min，柱温35℃，紫外检测波长274 nm，进样量10 μL。HPLC检测茶氨酸的梯度程序见表2。

表2 HPLC检测茶氨酸的梯度程序

时间/min	流动相体积分数配比/%	
	A相	B相
0.00	94	6
5.00	94	6
8.00	92	8
10.00	90	10

（7）咖啡碱含量测定采用HPLC法，参照文献[28]中的方法进行，略作修改。

色谱条件：Agilent C_{18}色谱柱（粒径5 μm，250 mm×4.6 mm），流动相A为0.5%乙酸水，流动相B为甲醇，流动相流速1.0 mL/min，柱温35℃，紫外检测波长203 nm，进样量10 μL。HPLC检测儿茶素的梯度程序见表3。

表3 HPLC检测咖啡碱的梯度程序

时间/min	流动相体积分数配比/%	
	A相	B相
0.00	95	5
10.00	80	20
20.00	80	20
25.00	95	5

1.5 数据统计分析

利用SPSS 18.0软件对试验数据进行统计分析。采用单因素ANOVA对多个独立样本的两两均数进行差异性分析($P<0.05$)。

2 结果与分析

2.1 ‘紫娟’茶中主要多酚类成分含量分析

茶多酚是茶叶中重要的活性物质，是多酚类物质的总称。对‘紫娟’茶中主要多酚类成分含量的检测（表4）发现，烘青绿茶的茶多酚含量显著高于其他茶样，为26.33%；晒青绿茶次之；黑茶最低，为5.16%左右。总体由高到低表现为烘青绿茶、晒青绿茶、白茶、红茶、黑茶。

表4 ‘紫娟’茶中主要多酚类成分的含量

样品	茶多酚/%	花青素/(mg/g)	没食子酸/%	儿茶素/%
晒青绿茶	26.03±0.83a	7.07±0.23b	0.02±0.01d	14.48±0.41a
烘青绿茶	26.33±0.96a	7.77±0.35a	0.04±0.01d	15.34±0.46a
白茶	16.03±0.58b	3.20±0.06c	0.12±0.01c	8.24±0.26b
红茶	12.44±0.46c	2.60±0.07c	0.26±0.03b	5.06±0.27c
黑茶	5.16±0.05d	0.16±0.01d	0.44±0.04a	2.33±0.10d

注：同列不同小写字母表示差异显著，$P<0.05$，下同。

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为检测茶多酚中变化较大的物质，还对花青素、没食子酸及儿茶素的含量进行测定（表4~5）。结果表明，烘青绿茶的花青素含量(7.77 mg/g)显著高于其他茶样；其次为晒青绿茶(7.07 mg/g)；白茶(3.20 mg/g)和红茶(2.60 mg/g)花青素的含量显著低于绿茶；黑茶中花青素的含量最低，仅有0.16 mg/g。通过对比没食子酸的含量发现，黑茶中具有较高的没食子酸(0.44%)，显著高于其他茶样。此外，烘青绿茶的儿茶素总量为15.34%，显著高于其他茶样；晒青绿茶的儿茶素总量为14.48%，仅次于烘青绿茶；而发酵度最高的黑茶儿茶素总量最低，仅有2.33%。对儿茶素的5种主要单体物质[儿茶素(catechin，C)、表儿茶素(epicatechin，EC)、表没食子儿茶素(epigallocatechin，EGC)、表儿茶素没食子酸酯(epicatechin gallate，ECG)和表没食子儿茶素没食子酸酯(epigallocatechin gallate，EGCG)][29-31]进行检测，其中，‘紫娟’烘青绿茶和晒青绿茶的EGCG含量最高，分别为8.02%和7.22%，分别占所对应绿茶总儿茶素比例的52.28%和49.86%，远高于其他茶样的儿茶素单体含量；其次为ECG，在‘紫娟’晒青绿茶和烘青绿茶中的含量分别为3.80%和3.44%，而在5种加工工艺‘紫娟’茶中C的含量普遍较低。

2.2 ‘紫娟’茶中其他化学成分含量分析

‘紫娟’茶之所以拥有优秀的品质，除了与含有丰富的多酚类物质有重要关系外，和游离氨基酸、咖啡碱等品质化学成分也是密不可分的。此外，茶叶的水分含量、干物质含量、水浸出物含量以及总灰分含量也与其品质和经济价值有直接关系。以上均是衡量茶叶营养品质的重要指标，因此测定这些成分的含量变化是评价茶叶品质所必需的[32-33]。

通过对5种不同加工工艺下的‘紫娟’茶其他化学成分测定分析（表6）发现，5种茶样的含水量均小于12.00%，其中，黑茶含水量显著高于其他茶样，为10.90%；其次为白茶，含量为10.03%；红茶含水量最低为8.33%；其余茶样含水量差异不明显，均在8.00%~9.00%之间。晒青绿茶、烘青绿茶和红茶的干物质含量差异不大，并且显著高于白茶和黑茶，其中，黑茶的干物质含量最低；黑茶的总灰分含量最高，为5.74%，而晒青绿茶的总灰分含量最低，为3.70%，其余茶样总灰分均在3.00%~5.00%之间，总体表现为晒青绿茶＜白茶＜烘青绿茶＜红茶＜黑茶。晒青绿茶和烘青绿茶的水浸出物含量显著高于其他3种茶样。茶氨酸是特征性氨基酸且含量最高，白茶的茶氨酸含量最高，为2.47%，滋味鲜爽，香气足；黑茶茶氨酸含量最低，为0.77%，白茶茶氨酸含量约是黑茶的3.2倍。红茶的咖啡碱含量最高，为5.04%，与白茶差异不显著；晒青绿茶和烘青绿茶的咖啡碱含量差异不显著分别为4.32%和4.17%；黑茶的咖啡碱含量最低，仅有1.92%。

3 结论

本研究以5种不同加工工艺下的‘紫娟’茶作为研究对象，通过品质成分的对比，探讨不同加工工艺下‘紫娟’茶品质的差异。

（1）5种加工工艺‘紫娟’茶的茶氨酸、咖啡碱以及多酚类的含量差异较大，‘紫娟’绿茶中花青素含量较高，具有独特的保健功能，但其苦涩的口感较为明显；

表5 ‘紫娟’茶中儿茶素成分含量 %

样品	C	EC	EGC	EGCG	ECG	儿茶素
晒青绿茶	0.44±0.02a	1.47±0.03b	1.56±0.05a	7.22±0.20b	3.80±0.18a	14.48±0.41a
烘青绿茶	0.43±0.01a	1.82±0.05a	1.62±0.07a	8.02±0.18a	3.44±0.19a	15.34±0.46a
白茶	0.11±0.02b	0.49±0.01c	0.53±0.03b	4.23±0.14c	2.88±0.12b	8.24±0.26b
红茶	0.11±0.01b	0.37±0.02d	0.41±0.01b	2.81±0.16d	1.36±0.11c	5.06±0.27c
黑茶	0.04±0.01c	0.08±0.02e	0.02±0.01c	1.33±0.17e	0.86±0.15d	2.33±0.10d

表6 ‘紫娟’茶中其他化学成分的含量 %

样品	水分	干物质	水浸出物	总灰分	茶氨酸	咖啡碱
晒青绿茶	8.67±0.12c	91.33±0.12a	49.90±0.81a	3.70±0.20d	2.10±0.03b	4.32±0.06b
烘青绿茶	8.97±0.04c	90.88±0.19a	50.20±0.38a	4.02±0.07b	2.07±0.05b	4.17±0.13b
白茶	10.03±0.24b	89.97±0.24b	34.92±0.41c	3.82±0.08c	2.47±0.02a	4.75±0.06a
红茶	8.33±0.23c	91.67±0.23a	37.58±0.39b	4.49±0.18a	1.10±0.03c	5.04±0.11a
黑茶	10.90±0.49a	89.10±0.49b	29.98±0.50d	5.74±0.08a	0.77±0.08d	1.92±0.21c

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相比之下，‘紫娟’白茶的茶氨酸和咖啡碱含量较高，使其口感鲜爽微甜；‘紫娟’红茶和‘紫娟’黑茶发酵程度深，也具有自身的独特滋味和功效。

（2）5种加工工艺‘紫娟’茶在干物质含量上的差异相对不太明显，‘紫娟’红茶的水分含量最低，在贮藏过程中最不容易发霉变质；‘紫娟’晒青绿茶的总灰分最低，而‘紫娟’烘青绿茶的水浸出物含量显著高于其他茶样，随着发酵程度的加深，‘紫娟’茶水浸出物的含量呈下降趋势。

（3）综合来看，‘紫娟’绿茶的表现相对优于其他茶样，‘紫娟’的特征成分花青素在烘青绿茶中含量最高，可采用速溶茶粉、茶膏等多种形式充分发挥其利用价值，为‘紫娟’茶的深加工提供了参考。

4 讨论

多酚类物质是茶叶具有抗氧化等功效的主要成分，主要包括儿茶素类、黄酮类、花色素、酚酸及缩酚酸类等，另外，茶叶中还含有氨基酸、生物碱等有效成分。云南大叶种茶树的叶片海绵组织丰厚，茶多酚等内含物比中叶种和小叶种都要丰富，且滋味浓郁，这也是大叶种适宜作为普洱茶制作原材料的原因之一[34]。‘紫娟’作为大叶种中的稀有品种，从外形到内质均与普通大叶种有明显的区别，其新鲜嫩梢的芽、叶、茎均为紫色，内含物质丰富，花青素含量远高于普通大叶种，具有巨大的应用潜力[35]，然而目前以‘紫娟’为原料的深加工产品依然较少。市场上较为常见的茶叶加工品多以其他茶类为主，如以普洱茶为原料开发的速溶茶，基本保持了原普洱茶的品质风格，且其茶多酚含量具有明显的优势，保健功能更好[36]；以优化的工艺制备的速溶绿茶，不仅提高了速溶绿茶中茶多酚的溶出率，而且有效地保留了原始茶汤口感和色泽[37]；采用普洱茶科学制作的普洱茶膏，保留了普洱茶的精华[38-39]。另外，还有诸多以茶叶为原料研制的保健饮料，如玫瑰红茶复合饮料[40]、柑橘红茶复合饮料[41]以及黑茶饮料[42]等；利用绿茶的抗氧化功效，还开发制备了绿茶面膜[43]、绿茶洗发露[44]等产品，极大地丰富了茶叶的深加工产品类型。综合来看，‘紫娟’茶的开发空间巨大，譬如，研发以‘紫娟’茶为原料的固态速溶茶和茶膏，还有‘紫娟’茉莉绿茶和玫瑰红茶等花茶，还可研发以‘紫娟’茶提取物为基础的洗护用品等，均可丰富‘紫娟’茶产品的类型，拓展‘紫娟’茶的市场，提升其利用价值。

‘紫娟’鲜叶从采摘到制作的过程中，不同的加工工序会引起化学成分的变化，前人研究主要集中在‘紫娟’绿茶和红茶方面，发酵方式会对‘紫娟’茶的品质造成较大的影响，绿茶在花青素和儿茶素成分上保留较多，抗氧化功效突出，而红茶经过发酵后产生了儿茶素衍生物，如茶黄素和茶红素等，而花青素含量则明显下降[35,45-46]，随着发酵程度的加深，其优势明显减弱[47]，显然以‘紫娟’鲜叶制作的绿茶品质较佳[48-49]。本研究与以上结果保持一致，并在此基础上增加了对‘紫娟’白茶和‘紫娟’黑茶的相关研究，更加全面地掌握了不同加工工艺下‘紫娟’茶的品质差异。试验结果表明，‘紫娟’绿茶的确有着很大的潜在利用价值，特别是烘青绿茶。本试验所采用的材料为不同加工工艺的‘紫娟’茶，根据试验结果可以明确不同加工工艺下其品质的差异，但需要进一步结合加工后茶叶品质成分的动态变化，综合分析加工过程中的每一道工序对‘紫娟’茶品质造成的差异，深入探讨‘紫娟’茶在不同领域的深加工利用，不仅可以为‘紫娟’茶在加工条件上的优化提供理论支撑，还可以为拓展‘紫娟’深加工产品市场提供科学参考。

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云南大学学报(自然科学版), 2020, 42(1):194~200 DOI: 10.7540/j.ynu.20190295
Journal of Yunnan University: Natural Sciences Edition

咖啡园土壤酸缓冲能力及其影响因素

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摘要: 为探明咖啡园土壤酸缓冲能力及其影响因素, 采用酸碱滴定法对云南省德宏州芒市和保山潞江坝两个主产区 7 个咖啡园土壤不同初始 pH 值(pH=4.44 ~ 5.04)进行分析. 结果表明: 供试土壤酸碱缓冲容量(pHBC)在 13.8 ~ 25.0 mmol·kg^{-1}. 从敏感性分级看, 6 个样点对酸碱敏感, 1 个样点稍敏感, 说明供试土壤缓冲酸变化的能力较弱. pHBC 与有机质、电导率、交换性 H^+、交换性酸、阳离子交换量和最大持水量呈极显著正相关(p>0.01, n=21), 采用逐步回归对 pHBC 与土壤基本理化性质的因果关系进行分析, 表达式为 y=0.190x_1+0.327 x_2+1.297, r^2=0.823, 其中 x_1 为电导率, x_2 为阳离子交换量, 表明电导率和阳离子交换量对 pHBC 贡献最大. 应采用合理施肥, 调整土壤理化性质等措施以提高咖啡园土壤酸缓冲能力, 为优质咖啡生产创造良好的土壤生态环境.

关键词: 酸碱缓冲容量; 阳离子交换量; 咖啡园土壤
中图分类号: S714.5 **文献标志码:** A **文章编号:** 0258−7971(2020)01−0194−07

我国酸化土壤面积占国土面积的 40% 以上, 在南方地区尤为严重[1]. 土壤酸化是外源输入 H^+ 引起土壤酸中和能力下降的过程[2], 其发生原因有自然酸化、酸沉降、豆科植物固氮和氮肥施用等. 几十年的集约化种植, 我国农田土壤显著酸化, pH 值平均降低约 0.50 个单位[3], 下降速度高于洛桑试验站 Park Grass 长期定位试验[4]. 土壤酸化导致 Al^{3+}、Mn^{2+}等金属离子对植物的毒害及 Ca^{2+}、Mg^{2+} 等营养元素的缺乏, 造成作物减产[5-7]. 土壤严重酸化后, 缓冲游离 H^+的能力下降, 会进一步引起地下水体酸化. 土壤 pH 缓冲容量、阳离子交换量等是常用的土壤酸敏感性指标[8-9], 也是土壤酸化预防、控制和修复的理论基础.

土壤对酸或碱的缓冲能力是重要的土壤质量评价指标. 要评价土壤的酸化过程以及预测酸化趋势, 需要对土壤酸缓冲能力深入研究. 目前, 国内外对不同的土壤类型, 如茶园土[8]、水稻土[10]、潮土[11]、橡胶土[12-13]、小麦土[14], 以及不同施肥条件和不同土壤层次的酸缓冲性能[15], 均有报道, 而云南热区咖啡种植红壤酸缓冲特性鲜有研究. 热区是云南重要的经济作物带, 西南生态安全屏障的前沿, 也是少数民族聚集地和贫困地区. 云南省咖啡种植面积和咖啡产量约占全国的 98% 以上, 是我国最主要的咖啡产地[16]. 研究表明 pH 值为 5.5 ~ 6.5 的弱酸性土壤更适宜咖啡生长[17], 但近年来云南咖啡主栽区土壤已有 pH 值低于 5.0 的报道[18]. 在大力发展经济作物种植过程中, 不合理的栽培管理可能会使酸性土壤酸化加剧, 进而影响土壤适耕性与生态环境等. 本研究以云南咖啡红壤为典型材料开展热区酸性土壤酸缓冲特性研究, 以期为该区域防治土壤酸化管理技术体系改进提供参考.

1 材料与方法

1.1 土样采集区概况 土壤采集点位于云南咖啡主产地——保山市隆阳区潞江坝和德宏傣族景颇族自治州芒市. 潞江坝位于云南省西部, 为典型的亚热带干热河谷气候(干热区), 光照充足, 终年无霜. 芒市毗邻保山, 属南亚热带季风气候(湿热区), 地处低纬高原, 热量丰富, 气候温和. 在潞江坝和芒市共采集 7 个不同初始 pH 值咖啡土壤, 土样经风干、磨细过筛后备用. 供试土壤基本理化性质见表 1.

1.2 土壤采集与分析 依据种植年限, 采集种植

收稿日期: 2019-05-27; 接受日期: 2019-09-20; 网络出版日期: 2019-11-26
基金项目: 国家自然科学基金(41661071); 国家重点研发计划(2018YFD0201100); 云南省重大科技专项计划(2018ZG015).
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表 1　供试土壤基本理化性质

Tab. 1　Soil properties of the coffee planting soils

编号	采样地点		有机质/($g\cdot kg^{-1}$)	全氮/($g\cdot kg^{-1}$)	有效磷/($mg\cdot kg^{-1}$)	速效钾/($mg\cdot kg^{-1}$)	电导率/($\mu S\cdot cm^{-1}$)	土壤酸碱度
1	芒市	(24°21′46″N, 98°27′5″E)	23.6+1.20 a	0.45+0.07 a	123+3.76 b	365+0.30 b	49.6+0.80 b	5.04+0.01 d
2	潞江坝	(25°2′49″N, 98°50′5″E)	53.0+2.34 e	1.10+0.09 c	91.0+6.69 a	159+1.00 a	63.5+1.14 d	4.44+0.01 a
3	潞江坝	(25°1′56″N, 98°50′26″E)	40.7+0.54 c	0.98+0.03 c	129+2.00 b	508+1.22 d	67.4+1.01 de	4.85+0.04 c
4	芒市	(24°21′59″N, 98°26′52″E)	29.6+2.09 b	0.63+0.09 ab	213+9.23 d	374+20.2 b	56.1+2.13 c	4.72+0.02 b
5	芒市	(24°21′55″N, 98°25′27″E)	33.7+0.10 b	0.60+0.02 a	85.3+7.28 a	162+3.86 a	40.8+2.11 a	4.86+0.07 c
6	芒市	(24°18′13″N, 98°32′49″E)	47.4+0.62 d	0.80+0.02 b	104+6.28 a	513+28.0 d	80.1+1.30 f	4.67+0.03 b
7	潞江坝	(25°1′58″N, 98°49′35″E)	33.4+1.21 b	0.52+0.03 a	176+2.22 c	438+32.6 c	71.2+0.56 e	4.63+0.03 b

编号	采样地点		交换性H^+/($cmol\cdot kg^{-1}$)	交换性1/3Al^{3+}/($cmol\cdot kg^{-1}$)	交换性酸/($cmol\cdot kg^{-1}$)	阳离子交换量/($cmol\cdot kg^{-1}$)	最大持水量 w/%
1	芒市	(24°21′46″N, 98°27′5″E)	0.05+0.01 a	0.23+0.01 a	0.28+0.01 a	11.3+1.35 b	31.8+0.67 a
2	潞江坝	(25°2′49″N, 98°50′5″E)	3.73+0.13 e	0.57+0.08 b	4.30+0.05 e	15.3+1.68 c	38.7+0.68 d
3	潞江坝	(25°1′56″N, 98°50′26″E)	2.27+0.01 d	0.88+0.01 c	3.15+0.01 d	8.86+0.38 ab	35.7+0.13 c
4	芒市	(24°21′59″N, 98°26′52″E)	0.32+0.01 b	1.30+0.10 d	1.62+0.10 b	7.25+0.19 a	32.2+0.18 ab
5	芒市	(24°21′55″N, 98°25′27″E)	2.07+0.03 d	1.05+0.02 cd	3.12+0.01 d	14.9+0.34 c	33.6+0.58 ab
6	芒市	(24°18′13″N, 98°32′49″E)	4.99+0.13 f	0.52+0.03 ab	5.51+0.10 f	22.5+1.52 d	39.4+0.62 d
7	潞江坝	(25°1′58″N, 98°49′35″E)	0.82+0.11 c	1.13+0.22 cd	1.95+0.12 c	9.95+0.46 ab	34.0+0.85 bc

不同采样点同一列不同字母表示处理间差异达到显著水平(p<0.05)

5 ~ 20a 能代表当地生态条件咖啡土壤. 在 2017 年咖啡采摘结束后, 在典型咖啡样地按 S 形取样法取 10 个点作 1 个混合样, 每块样地重复采集 3 次, 采集深度 0 ~ 20 cm 耕层. 带回实验室自然风干、研磨, 过筛备用.

全氮: 凯氏定氮法; 有机质: 低温外热重铬酸钾-容量法; 速效钾: 乙酸铵提取-火焰光度计法; 有效磷: 碳酸氢钠浸提-钼蓝比色法; 电导率: 电导率仪 DDS-307A 测定; 土壤 pH: pH 仪(inolabpH7300)测定; 交换性酸(氢、铝): 氯化钾交换-中和滴定法; 阳离子交换量: 乙酸铵浸提、离心, 定氮法; 交换性盐基离子: 乙酸铵交换-中和滴定法.

1.3　土壤酸碱缓冲容量测定　参照成杰民[19]及李源环等[20]的方法, 取 13 只烧杯, 每只烧杯中称取 4.00 g 土, 分别依次加入 0、0.25、0.5、1.0、2.0、4.0、6.0 mL 0.1 $mol\cdot L^{-1}$ 的 HCl; 以及与 HCl 等量、等浓度的 NaOH. 加入无 CO_2 蒸馏水使各烧杯总体积达 20.00 mL(液土比 5 mL ∶ 1 g). 摇匀, 日间歇摇动 3 ~ 4 次, 恒温 25 ℃ 放置 72 h, 最后一次摇动静置 2 h 后测定 pH. 以 pH 为纵坐标, 酸碱加入量为横坐标, 建立线性方程如公式 (1):

$$pHBC = 1/[a], \tag{1}$$

式中, pHBC 为试验结束时的酸碱缓冲容量, a 为斜率. 根据酸碱缓冲容量对土壤酸敏感性进行分级, 缓冲容量<10 $mmol\cdot kg^{-1}$, 对酸最敏感, 极易受酸害; 缓冲容量 10 ~ 20 $mmol\cdot kg^{-1}$, 对酸敏感, 易受酸害; 缓冲容量 20 ~ 40 $mmol\cdot kg^{-1}$, 对酸稍敏感, 稍易受酸害; 缓冲容量>40 $mmol\cdot kg^{-1}$, 对酸不敏感, 不易受酸害[20-21].

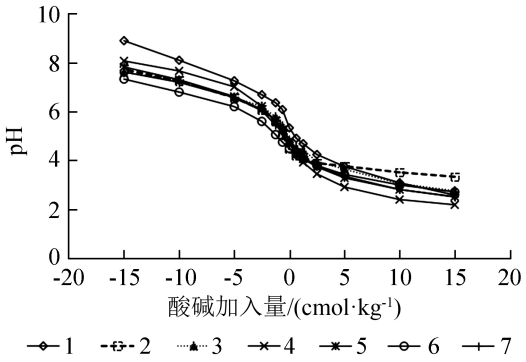
1.4　数据处理与分析　数据采用 Excel 2010 和 SPSS 13.0 进行数据整理、方差分析与多重比较.

2　结果与分析

2.1　咖啡土壤酸碱缓冲曲线　土壤酸碱缓冲曲线是土壤对酸碱缓冲能力变化的最直观表现, 曲线斜率越小则表明土壤在该 pH 范围内对酸碱的缓冲能力越强; 反之, 斜率越大则表明土壤的缓冲能力越弱[22]. 潞江坝及芒市的 7 个土壤的酸碱缓冲曲线

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变化趋势相似,在 pH=2.0 ~ 10.0 范围内,土壤的酸碱缓冲曲线均呈明显的 S 形,但不同土壤酸缓冲能力存在差异(图 1). 其中随酸碱滴定量的增加,pH 值变化呈减弱趋势集中在 pH>6 和 pH<3 时,即该 pH 范围土壤对酸和碱的缓冲能力强. 供试土壤在 pH 在 3 ~ 6 之间滴定曲线近似一条直线,与酸性土壤上的研究结论基本相一致[12, 23].



正值代表酸加入量,负值代表碱加入量

图 1 不同咖啡园土壤的酸碱滴定曲线

Fig. 1 Titration curves for different coffee planting soils. Positive value means the amount of acid addition, negative value means the amount of base addition

2.2 咖啡土壤酸碱缓冲容量 土壤酸碱缓冲容量是指使土壤改变一个单位 pH 所需要的酸量[24]. 有研究结果表明,在突跃范围内,可把土壤酸碱缓冲曲线视为直线,即土壤 pH 与酸碱的加入量呈线性相关[25].

为准确得到酸碱滴定曲线在 pH 突跃范围内的直线方程,本试验选取酸、碱滴定量分别为 0、0.625、1.25 cmol·kg^{-1} 的 5 个点,该 5 个点拟合直线的截距和斜率见表 2. 经 F 检验,酸碱滴定量与 pH 呈显著和极显著线性相关,说明拟合直线成立,斜率具有实际意义. 供试土壤的酸碱缓冲容量在 13.8 ~ 25.0 mmol·kg^{-1},其中 6 号样点酸碱缓冲容量最高(25.0 mmol·kg^{-1}). 从敏感性分级看,6 个样点对酸碱敏感,稍敏感的样点有 1 个,说明供试土壤缓冲酸变化的能力较弱.

2.3 酸碱缓冲容量与土壤理化性质间的相关性 酸碱缓冲容量与土壤理化性质相关分析结果见表 3. 在 pH=3.0 ~ 6.0 的范围内,pHBC 与有机质、电导率、交换性 H^+、交换性酸、阳离子交换量和最大持水量呈极显著正相关(p<0.01, n=21),与速效钾呈显著正相关(p<0.01, n=21),与土壤全氮、有效磷、pH、交换性 Al^{3+}没有相关关系(p>0.05, n=21),即随着有机质、交换性 H^+、阳离子交换量等的含量的增加,土壤酸碱缓冲容量也随之增加. 采用逐步

表 2 土壤酸缓冲曲线在突跃范围内的直线拟合方程及缓冲容量

Tab. 2 Linear fitting equation and buffer capacity of soil acid buffer curve in jump range

土壤编号	$y=a+bx$			酸碱缓冲容量/(mmol·kg^{-1}·pH unit^{-1})	酸敏感性
	a	b	r^2		
1	5.47	−0.73	0.957**	13.8+0.41 a	敏感
2	4.70	−0.61	0.918*	16.4+0.48 cd	敏感
3	5.00	−0.58	0.941**	17.4+0.61 de	敏感
4	4.74	−0.69	0.995**	14.5+0.13 ab	敏感
5	4.80	−0.66	0.984**	15.1+0.20 bc	敏感
6	4.52	−0.40	0.993**	25.0+0.58 f	稍敏感
7	4.77	−0.55	0.996**	18.1+0.28 e	敏感

x为酸碱的加入量,y为对应土壤的pH

回归对 pHBC 与土壤基本理化性质的因果关系进行分析,表达式为 $y=0.190x_1+0.327x_2+1.297$, $r^2=0.823$, 其中 x_1 为电导率, x_2 为阳离子交换量.

3 讨论

本研究结果表明土壤在 pH=3.0 ~ 6.0 的范围内,其 pHBC 与有机质、电导率、交换性 H^+、交换性酸、阳离子交换量和最大持水量呈极显著正相关,与土壤全氮、有效磷、pH、交换性 Al^{3+}没有相关关系(p>0.05, n=21). 土壤阳离子交换量是土壤胶体所能吸附的各种阳离子的总量,土壤胶体可通过吸附土壤溶液中的 H^+从而对酸起到缓冲作用. 因此交换性阳离子是土壤产生缓冲性的主要原因之一,阳离子交换量越大,土壤可以吸附更多进入土壤的 H^+,缓冲性能就越强[26-27]. 电导率是表征土壤可溶性盐总量的指标[28-29],在一定低浓度范围内,土壤养分离子的有效性随土壤溶液浓度增高而加大[30],即在一定范围内,土壤盐分含量与土壤供肥能力可能存在某种相关关系,进而影响土壤酸缓冲能力. 因此,作者认为在一定范围内,电导率可以作为土壤酸缓冲能力的表征. 土壤有机质与 pHBC 存在极显著正相关关系,与前人的研究结果一致[31-33]. 这与有机质中的腐殖质具有较强吸附性和阳离子交换能力有关,因为腐殖质含有大量负电荷,对土壤溶液中的阳离子具有较强的吸附作用,从而提高了土壤酸碱缓冲容量[33-34]. 土壤交换性 H^+含量越高,表明土壤胶体吸附的 H^+越多,即土壤

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表 3　咖啡园土壤酸碱缓冲容量与基本理化性质之间的相关性

Tab. 3　Pearson correlation coefficient of pHBC values and the selected soil properties

	有机质	全氮	有效磷	速效钾	电导率	土壤 pH	交换性 H^+	交换性 $1/3Al^{3+}$	交换性酸	阳离子交换量	最大持水量
酸碱缓冲容量	0.586**	0.276	−0.207	0.496*	0.800**	−0.377	0.758**	−0.124	0.766**	0.662**	0.745**
有机质		0.792**	−0.467*	−0.095	0.581**	−0.715**	0.885**	−0.167	0.889**	0.543**	0.922**
全氮			−0.369	−0.110	0.359	−0.540*	0.646**	−0.114	0.651**	0.191	0.728**
有效磷				0.399	0.115	−0.011	−0.626**	0.527*	−0.539*	−0.651**	−0.571*
速效钾					0.663**	0.228	0.006	−0.010	0.003	−0.055	0.046
电导率						−0.490*	0.536*	−0.095	0.539*	0.321	0.657**
土壤							−0.501*	−0.179	−0.564*	−0.237	−0.644**
交换性 H^+								−0.310	0.976**	0.811**	0.943**
交换性 $1/3Al^{3+}$									−0.097	−0.457*	−0.297
交换性酸										0.744**	0.920**
阳离子交换量											0.713**

*表示相关达到显著水平(p<0.05); **表示相关达到极显著水平(p<0.01)

具有更高的酸碱缓冲容量, 这与前人研究结果相同[22]. 但也有土壤缓冲容量与交换性 H^+含量呈显著负相关的报道[8, 20], 这可能是土壤类型以及土壤缓冲体系的不同所导致. 有报道表明土壤酸缓冲容量与土壤 pH 呈显著正相关[26, 35-36], 但本研究结果显示两者之间无相关关系, 这与雷永程等[37] 研究结果相似. 土壤 pH 值是土壤活性酸浓度的反映, 但影响土壤缓冲性能的是土壤胶体吸附的 H^+, 即交换性酸, 因此土壤酸碱缓冲容量与土壤在某一阶段的 pH 值可能没有必然的联系. 本研究表明土壤酸碱缓冲容量与交换性 Al^{3+}之间也没有相关关系, 这与李源环等[20] 研究结果相似, 但也有 pHBC 与土壤交换性 Al^{3+}呈显著正相关的报道[22], 需要对土壤中复杂的缓冲作用机制作进一步研究.

Nelson[38] 认为土壤在不同 pH 环境条件下对酸的缓冲能力不同, 本研究所得结果与之类似, 土壤酸碱缓冲曲线在 pH=3.0 附近发生突变. 根据 Ulrich[39] 对土壤缓冲体系的划分, 供试土壤可能是阳离子交换缓冲体系(pH=4.2 ~ 5.0)起主要作用, 也就是当外源 H^+输入时, 土壤固液相间会发生 H^+与交换性阳离子的交换, 从而降低土壤活性酸度. 由于中国南方大部分地区酸性土壤 pH 在 3.5 ~ 6.0 之间, 因此本研究的测定和方法具有实际意义.

本研究中, 酸碱缓冲曲线在 pH=3.0 附近发生突变, 即表示缓冲体系由初级体系转变为次级体系. 有研究表明红壤对酸沉降的缓冲作用以次级缓冲体系为主, 主要通过矿物风化过程来消耗输入进土壤的 H^+[40]. 由矿物风化是个不可逆的过程, 随着 H^+的不断输入矿物被不断的腐蚀消耗, 致使土壤的酸碱缓冲容量不断减小, 因此造成土壤的长久持续性酸化[35]. 近年来, 随着土壤改良剂的快速发展, 不少研究表明在土壤中施用生物炭能显著提高土壤 pH 值[41-42], 改善有机碳和交换性盐基离子等土壤酸敏感性指标[43-45]. 因此, 在咖啡种植时, 配施生物炭等改良剂是否能够有效改善咖啡土壤酸缓冲能力, 以及合适的配施比例有待进一步的研究.

4　结　论

供试土壤的酸碱缓冲容量在 13.8 ~ 25.0 $mmol \cdot kg^{-1}$. 土壤酸碱缓冲性能受多种因素共同影响, 本试验相关分析表明有机质、电导率、交换性 H^+、交换性酸、阳离子交换量和最大持水量、速效钾与酸碱缓冲容量 pHBC 关系密切, 回归分析表明电导率和阳离子交换量对 pHBC 贡献最大.

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Soil acid buffer capacity and the affecting factors of coffee planting field

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Abstract: Soil acid buffer performance was studied with the coffee planting soils from Mangshi and Lujiangba, major producing area of Yunnan Province. To study pH buffer capacity and influencing factors, 7 coffee planting soil samples were collected with different initial pH values (pH=4.44 ~ 5.04). The results showed that the pH buffer capacities (pHBC) ranged from 13.8 mmol·kg^{-1} to 25.0 mmol·kg^{-1}. Based on the sensitivity classification, 6 samples showed sensitive and 1 sample showed slightly sensitive to acid and alkali changes, which implied weak acid buffer performance of the studied soils. Statistics analysis suggested that there was a significant positive correlation between pHBC and organic matter, conductivity, exchangeable H^+, exchangeable acid, cation exchange capacity and maximum water holding capacity (p<0.01, n=21). The relationship between pHBC and the soil basic physical and chemical properties was analyzed by stepwise regression, which indicating that EC and CEC were the main factors affecting pHBC. In order to improve acid buffering performance of coffee planting soils and create a good soil ecological environment for high-quality coffee production, reasonable application of fertilizer and adjustment of soil physical and chemical properties should be adopted.

Key words: pH buffer capacity; CEC value; coffee planting soil

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文章编号:1001 - 4829(2019)11 - 2550 - 07 DOI:10.16213/j.cnki.scjas.2019.11.011

云南不同产地及品种小粒种咖啡豆化学及卫生指标比较

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摘 要:【目的】云南是生态环境最多样的省份,因受生长环境的影响各地出产的咖啡豆品质不同,研究云南不同产地咖啡豆的品质特点可为其合理种植及利用提供指导。【方法】以云南保山和普洱 2 个主产区的不同海拔、不同品种小粒种咖啡豆样品共 50 份为材料,对其主要品质指标(包括水分、灰分、水浸出物、咖啡因、总糖、蛋白质、粗脂肪、粗纤维和重金属砷、铅、镉的含量共 11 项)进行比较研究。【结果】2 个产地咖啡豆的主要化学指标(水浸出物、总糖、粗脂肪、粗纤维含量)和卫生指标(重金属砷、铅、镉含量)均达到行业标准 NY/T 604-2006 和地方标准 DB53/T 154.5-2006,而且水浸出物含量、总糖含量、粗脂肪含量和粗纤维含量显著高于地方标准;在品质特点方面,保山出产的卡蒂姆咖啡豆水分含量较低而水浸出物、总糖和粗脂肪含量较高,普洱出产的卡蒂姆咖啡豆灰分、咖啡因和粗纤维含量较低而蛋白质含量较高;咖啡豆的品质因品种和产地而不同,而海拔与除蛋白质之外的其余品质指标相关均不显著。【结论】云南保山和普洱所产的咖啡豆均为优质咖啡豆,品质有异但各具特色,各地应根据品种特性和环境条件合理进行选择和开发利用。

关键词:小粒种咖啡;化学指标;卫生指标;比较研究

中图分类号:F713 **文献标识码:**A

Comparison of Chemical and Health Indexes of *Coffea arabica* Bean from Different Producing Areas and Varieties in Yunnan

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Abstract 【Objective】Yunnan was the most diverse ecological environment province in China. The quality of coffee beans from different producing areas was different because of the influence of growing environment. The study on the quality characteristics of coffee beans from different producing areas could provide guidance for their reasonable planting and utilization. 【Method】Total 50 samples of coffee raw beans from different altitudes and varieties of two main producing areas Baoshan and Pu'er, Yunnan, were used as materials to compare their main quality indexes (including water content, ash content, water extract, caffeine, total sugar, protein, crude fat, crude fiber, and heavy metals As, Pb, Cd of total 11 indexes). 【Result】The main chemical indexes (contents of ash, water extract, caffeine, total sugar, crude fat and crude fiber) and health indexes (contents of heavy metals As, Pb and Cd) of the coffee beans from the two producing areas met the industry standard NY/T 604-2006 and local standard DB53/T 154.5-2006, and the contents of water extract (30.53 %), total sugar (10.61 %), crude fat (12.83 %) and total sugar (29.15 %) were significantly higher than the local standards. However, their quality characteristics were different: the Catimor coffee beans produced from Baoshan had lower water content and higher water extract, total sugar and crude fat content than from Pu'er, and the Catimor coffee beans produced from Pu'er had lower ash, caffeine and crude fiber content and higher protein content than from Baoshan. The quality of coffee beans varied with varieties and producing areas, and altitude had no significant correlation with other quality indexes except protein. 【Conclusion】The coffee beans from Baoshan and Pu'er of Yunnan were all high-quality coffee beans, and they have their respective advantages. Different coffee producing areas should reasonably select varieties for planting and utilizing according to varieties characteristics and environmental conditions.

Key words: *Coffea arabica*; Chemical indexes; Health indexes; Comparative study

收稿日期:2019 - 03 - 12

基金项目:云南省建立农科教相结合新型农业社会化服务体系试点项目"云咖啡"产品单元子项目"咖啡标准与质量控制技术"(2014NG004)

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【研究意义】咖啡是茜草科(Rubiaceae)咖啡属(*Coffea* genus)常绿乔木,原产非洲中北部,现已遍及全球 76 个国家和地区,被列为世界三大饮料之首[1-3]。全球栽种的主要以小粒种咖啡(*Coffea*

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arabica L.) 为主,约占咖啡总面积的 80 %[1-2,4]。中国种植咖啡的省份有云南、海南、广东、广西和四川等,其中云南是全国最大的咖啡种植和出口基地,种植面积和产量均占全国的 99 %[1,3]。云南省规模化种植咖啡的地区主要分布在热区,其中以普洱、保山、德宏和临沧为主,种植面积约占全省的 80 % 以上; 由于具有适宜小粒种咖啡生长的地理气候优势,咖啡豆产量高、品质优,咖啡豆在国内外市场具有较强的竞争力[6-8]。云南各地的地理气候差异较大,出产的咖啡豆品质有所不同。为充分利用云南省多样的地理气候优势和生产更加优质的咖啡豆原料,研究不同产区咖啡豆的品质特点及其影响因素,可为小粒种咖啡的合理种植和开发利用提供指导。【前人研究进展】前人研究表明,咖啡豆品质受诸多因素影响[9-11],其中以品种、产地和环境的影响最大[12-16]。张洪波等[12]研究不同咖啡品种的杯品质量,结果表明除卡蒂姆品种因含有部分中粒种咖啡基因而杯品质量一般,其余的小粒种咖啡品种的杯品质量均较高; 黄家雄等[13]研究云南不同产地咖啡豆的理化指标,结果不同产地的咖啡豆因受气候和土壤等因素的影响,在感观、物理、化学和卫生指标上均存在一定差异; 李晓霞等[14]和黄家雄等[15]研究不同海拔的小粒种咖啡品质,结果表明随着海拔的升高、咖啡的品质提高; 李锦红等[16]研究不同纬度出产的小粒种咖啡品质,结果表明高纬度地区的咖啡品质比低纬度的好。【本研究切入点】很多因素都会影响到咖啡的品质,如品种、产地、环境等; 前人研究仅从单因素入手,没有考虑 2 个以上因素共同作用对品质的影响。【拟解决的关键问题】以保山和普洱 2 个产区不同海拔、不同品种的小粒种咖啡生豆样品为材料,对其主要品质指标进行分析比较,研究不同因素对咖啡品质的影响以及不同产区咖啡豆的品质特点,以期为小粒种咖啡的合理布局提供科学依据。

1　材料与方法

1.1　试验材料

供试样品来自云南保山和普洱的咖啡园,均为当年采摘的咖啡生豆; 每份样品采集鲜果 5 kg,经脱皮、发酵脱胶、清洗、自然干燥、脱壳后制成咖啡米。保山样品品种为卡蒂姆 Catimor 系列,来源于潞江镇(北纬 24°48′,东经 98°45′) 海拔 500 ~ 1480 m 的不同村寨,共 20 份样品; 普洱样品来源于普洱茶树良种场(北纬 22°75′,东经 100°97′,海拔 1340 m) 的不同品种,共 30 份样品。具体信息见表 1。

1.2　试验方法

供试咖啡生豆送农业部农产品质量监督检验测

表 1　不同产地和品种咖啡豆样品

Table 1　Coffee bean samples of different varieties from different producing areas

产地 Location	品种 Varieties	海拔(m) Altitude	编号 No.
保山 Baoshan	卡蒂姆	500	1
	卡蒂姆	570	2
	卡蒂姆	570	3
	卡蒂姆	630	4
	卡蒂姆	650	5
	卡蒂姆	730	6
	卡蒂姆	780	7
	卡蒂姆	810	8
	卡蒂姆	870	9
	卡蒂姆	900	10
	卡蒂姆	920	11
	卡蒂姆	1000	12
	卡蒂姆	1020	13
	卡蒂姆	1150	14
	卡蒂姆	1270	15
	卡蒂姆	1330	16
	卡蒂姆	1360	17
	卡蒂姆	1380	18
	卡蒂姆	1380	19
	卡蒂姆	1480	20
普洱 Pu'er	卡杜埃 44	1340	21
	50 号	1340	22
	DTARZ293	1340	23
	热选 1 号	1340	24
	T5157	1340	25
	50 号	1340	26
	德宏单株	1340	27
	蒙多诺沃	1340	28
	154 号	1340	29
	铁皮卡	1340	30
	49 号	1340	31
	热选 2 号	1340	32
	55 号	1340	33
	51 号	1340	34
	瑞丽 48 号	1340	35
	矮卡	1340	36
	T5157	1340	37
	Pt	1340	38
	DTARZ46	1340	39
	思茅单株	1340	40
	191 号	1340	41
	161 号	1340	42
	黄果皮 1	1340	43
	155 号	1340	44
	35-50 号	1340	45
	卡杜埃 44	1340	46
	DT30	1340	47
	78667	1340	48
	NY002	1340	49
	卡蒂姆	1340	50

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试中心(昆明)分析其主要化学和卫生指标,包括水分、灰分、水浸出物、咖啡因、总糖、蛋白质、粗脂肪、粗纤维和重金属砷、铅、镉的含量共 11 项指标,各项指标均按相应国家标准进行检测分析。

1.3 数据处理

原始数据经 Excel 2007 整理后,采用 DPS 14.50 进行分析。

2 结果与分析

2.1 水分

由表 2 可以看出,2 个产地的咖啡豆样品水分含量在 6.1 % ~18.3 %、平均为 10.43 %;其中保山的卡蒂姆 Catimor 系列样品水分含量较低,在 6.1 % ~11.8 %、平均为 9.23 %,符合 NY/T 604-2006

表 2 不同产地和品种咖啡豆的化学和卫生指标

Table 2 The chemical and heath indexes of coffee bean of different varieties from different producing areas

编号 No.	水分(%) Water content	灰分(%) Ash content	水浸出物(%) Water extract	咖啡因(%) Caffeine	总糖(%) Total sugar	蛋白质(%) Protein	粗脂肪(%) Crude fat	粗纤维(%) Crude fiber	砷(mg/kg) As	铅(mg/kg) Pb	镉(mg/kg) Cd
1	10.4	3.9	34.4	1.15	11.06	14	12.68	24.8	0.02	未检出	0.012
2	9.3	3.9	33.4	1.2	10.5	14.7	13.23	28.57	0.042	未检出	0.017
3	8.5	4.2	32.8	1.18	10.36	14.2	12.27	23.06	未检出	未检出	0.013
4	9.9	3.4	34	1.16	11.73	13.2	12.4	25.14	未检出	未检出	0.04
5	6.1	3.7	33.4	1	10.61	12.9	14.49	29.93	未检出	未检出	未检出
6	10.4	3.6	31.7	1.12	11.14	13.6	12.66	24.05	未检出	未检出	未检出
7	8.7	4.3	32.9	1.18	10.72	15.1	12.28	27.99	未检出	未检出	0.01
8	9.2	4	32.1	1.04	10.32	13.7	12.81	25.43	未检出	未检出	0.013
9	11.8	4.2	35.6	1.11	10.07	14.9	12.38	28.35	0.042	未检出	0.016
10	9.6	3.7	32	1.11	10.35	12	14.27	29.21	0.017	未检出	未检出
11	8.5	3.7	32.8	1.1	10.96	14.4	12.74	28.66	0.05	未检出	0.004
12	11.7	3.4	31.2	1.31	10.76	13.3	14.17	28.43	0.034	未检出	0.088
13	9.7	3.8	31.2	1.06	10.9	12.7	13.59	29.34	未检出	未检出	0.012
14	7.8	3.9	34	1.22	10.75	13.3	13.53	29.12	0.04	未检出	未检出
15	7.8	3.5	32.4	1.06	11.11	12.4	15.47	26.91	0.066	未检出	0.004
16	8.7	3.8	33.6	1.17	10.94	12.3	12.89	29.85	0.036	未检出	未检出
17	9.2	3.9	34	1.11	10.72	13.2	13.01	30.91	0.012	未检出	0.014
18	8.9	3.3	32.5	0.93	11.2	11	13.79	24.98	0.02	未检出	未检出
19	8.6	3.6	32	1.07	10.81	11.3	14.06	25	未检出	未检出	未检出
20	9.8	4.1	33.2	1.18	10.94	13.2	13.65	28.6	未检出	未检出	0.004
平均	9.23**##	3.80**##	32.96##	1.12**##	10.80##	13.27##	13.32##	27.42##	0.03##	0##	0.02
21	10.3	3.5	29.9	0.97	10.95	11.9	11.94	30.38	未检出	未检出	未检出
22	12.9	3.4	27.2	0.88	10.98	11.4	11.92	30.15	未检出	未检出	未检出
23	11.4	3.5	30	0.94	10.68	10.8	13.31	29.99	未检出	未检出	0.005
24	16.2	3.4	28.6	0.87	10.56	9.99	10.52	31.66	未检出	未检出	未检出
25	15.3	3.5	27	1.09	10.6	11.8	12.61	30.54	0.101	未检出	0.006
26	10.9	3.3	28.5	1.02	10.54	11.1	13.3	30.2	未检出	未检出	0.006
27	12.1	3.4	28.4	0.88	10.84	9.02	11.5	31.49	未检出	未检出	未检出
28	11.7	3.4	28.7	1.04	10.16	10.6	12.98	31.14	未检出	未检出	未检出
29	12.2	3.6	29.6	1.05	10.89	11.9	13.55	29.19	未检出	未检出	未检出
30	12.4	3.4	26.9	1.09	10.08	10.6	10.85	31.49	0.05	未检出	0.01
31	14.3	3.5	28.8	1.12	9.8	10.6	11.74	29.7	0.076	未检出	未检出
32	18.3	3.5	27.6	1.03	10.46	10.6	11.43	29.34	0.012	未检出	0.014
33	10.4	3.3	28.9	1.05	10.17	10.8	13.37	31.11	0.164	未检出	未检出

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续表 2 Continued table 2

编号 No.	水分(%) Water content	灰分(%) Ash content	水浸出物(%) Water extract	咖啡因(%) Caffeine	总糖(%) Total sugar	蛋白质(%) Protein	粗脂肪(%) Crude fat	粗纤维(%) Crude fiber	砷(mg/kg) As	铅(mg/kg) Pb	镉(mg/kg) Cd
34	7.8	3.6	29.5	1.02	10.11	10.9	12.68	30.34	0.055	未检出	0.006
35	13.3	3.3	26.9	0.83	9.51	10.6	13.02	31.68	0.04	未检出	未检出
36	7.4	3.3	31.3	1.08	10.35	12	12.15	32.05	0.058	未检出	0.011
37	14.5	3.2	26.8	0.87	9.5	10.9	12.19	31.06	0.01	未检出	0.008
38	7.2	3.6	31.2	0.95	11.01	11	10.87	25.73	未检出	未检出	0.008
39	10.5	3.8	29.4	1.02	10.52	11.1	13.45	30.42	0.033	未检出	0.005
40	7.7	3.4	29.2	0.93	10.26	11	13.06	31.11	未检出	未检出	0.005
41	7.2	3.6	30.2	1.04	10.86	11.7	12.64	31.79	0.02	未检出	0.004
42	10.6	3.3	28.2	0.93	10.39	11.2	12.95	32.61	0.068	未检出	0.011
43	7.5	3.7	31.1	1	10.89	11.9	13.17	29.41	未检出	未检出	未检出
44	8	3.8	30.7	0.95	11.04	11.5	14.13	30.89	0.012	未检出	0.006
45	16.2	3.6	26	1	9.97	11.5	11.61	27.98	0.024	未检出	未检出
46	10.5	3.5	29.5	1.07	11.12	11	13.23	30.92	0.022	未检出	0.008
47	7.4	3.6	31.8	1.27	10.52	12.4	12.91	29.03	0.026	未检出	未检出
48	10.9	3.9	29	1.07	11	10.8	12.08	29.77	未检出	未检出	未检出
49	11.2	2.9	27.2	0.85	10.5	11.8	13.24	31.33	0.048	未检出	0.007
50	10.6	3.7	29	1.02	10.29	12.5	12.78	27.64	0.022	未检出	未检出
平均	$11.23^{\#}$	$3.48^{**\#\#}$	$28.90^{\#\#}$	$1.00^{**\#\#}$	$10.49^{\#\#}$	11.16	$12.51^{\#\#}$	$30.34^{\#\#}$	$0.05^{\#\#}$	$0^{\#\#}$	0.01
NY/T 604-2006	≤12.0	≤5.5	/	≥0.8	/	/	/	/	≤0.5	≤0.5	/
DB53/T154.5-2006	≤12.5	≤4.2	≥20.0	≤1.2	≥9.0	≥11.0	≥5.0	≤35.0	≤0.5	≤0.5	/

注: * 代表与 NY/T 604-2006 比较的结果,* 表示差异显著($P<0.05$),** 表示差异极显著($P<0.01$);#代表与 DB53/T 154.54-2006 比较的结果,#表示差异显著($P<0.05$),##表示差异极显著($P<0.01$)。

Note: * means the results compared with NY/T 604-2006; * means significant difference ($P<0.05$); ** means highly significant difference ($P<0.01$). # means the results compared with DB53/T 154.54-2006; # means significant difference ($P<0.05$); # means highly significant difference ($P<0.01$).

表 3　保山咖啡豆的化学指标与海拔的相关分析

Table 3　Correlation analysis between chemical indexes and altitude of Baoshan's coffee bean

	水分 Water content	灰分 Ash content	水浸出物 Water extract	咖啡因 Caffeine	总糖 Total sugar	蛋白质 Protein	粗脂肪 Crude fat	粗纤维 Crude fiber	砷 As	铅 Pb	镉 Cd
相关系数	-0.11	-0.22	-0.14	-0.18	0.16	-0.62^{**}	0.44	0.32	-	-	-

注: ** 表示差异极显著($P<0.01$)。

Note: ** means the difference was highly significant ($P<0.01$).

≤12.0 % 的标准,也符合 DB53/T 154.5-2006 ≤12.5 % 的标准;虽然不同海拔咖啡豆样品的水分含量差异较大,但相关分析显示水分含量与海拔高度之间的相关不显著(表 3);普洱的采自海拔 1340 m 茶树良种场的 30 个不同品种的样品水分含量较高,在 7.2 % ~18.3 %、平均为 11.23 %;不同品种之间的差异较大,铁皮卡 Typica、瑞丽 48 号、热选 1 号、热选 2 号和 T5157 等 11 个品种的水分含量超过 12.0 %,其中瑞丽 48 号、热选 1 号、热选 2 号和 T5157 等 8 个品种的水分含量超过 12.5 %,水分含量低于卡蒂姆 Catimor 品种的有矮卡、卡杜埃 Catuai、DTARZ46 等 12 个品种。采自保山 1330 m、普洱 1340 m 的同一品种卡蒂姆 Catimor 样品的水分含量分别为 8.7 %、10.6 %,2 个产地的咖啡豆水分含量除了与产地有关外,还与咖啡豆鲜果的采摘时期和采后处理有较大关系。

2.2　灰分

由表 2 可以看出,2 个产地的咖啡豆样品灰分含量在 2.9 % ~4.3 %、平均为 3.61 %;其中保山的卡蒂姆 Catimor 系列样品灰分含量较高,在 3.3 %

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~4.3 %、平均为 3.80 %，符合 NY/T 604-2006≤5.5 %的标准，但按 DB53/T 154.5-2006≤4.2 %的标准，则有 1 份采自 780 m 的咖啡豆样品灰分为 4.3 %；虽然不同海拔咖啡豆样品的灰分含量差异较大，但相关分析显示灰分含量与海拔高度之间的相关不显著(表 3)；普洱的采自海拔 1340 m 茶树良种场的 30 个不同品种的样品灰分含量较低，在 2.9 % ~3.9 %、平均为 3.48 %，所有样品都符合 NY/T 604-2006≤5.5 %的标准，同时也符合 DB53/T 154.5-2006≤4.2 %的标准，而且品种之间差异较大，除 DTARZ46、155 号和 T8667 3 个品种外其余品种的灰分含量均低于卡蒂姆 Catimor。采自保山 1330 m 和普洱 1340 m 的同一品种卡蒂姆 Catimor 样品的灰分含量分别为 3.8 %和 3.7 %，说明相同品种和相近海拔下，产地对咖啡豆灰分含量的影响较小。

2.3 水浸出物

由表 2 可以看出，2 个产地的咖啡豆样品水浸出物含量在 26 % ~35.6 %、平均为 30.53 %，极显著高于 DB53/T 154.5-2006≥20 %的标准；其中保山的卡蒂姆 Catimor 系列样品水浸出物含量较高，在 31.2 % ~35.6 %、平均为 32.96 %；虽然不同海拔咖啡豆样品的水浸出物含量差异较大，但相关分析显示水浸出物含量与海拔高度之间的相关不显著(表 3)；普洱的采自海拔 1340 m 茶树良种场的 30 个不同品种的样品水浸出物含量较低，在 26 % ~31.8 %、平均为 28.9 %，品种之间差异较大，水浸出物含量高于卡蒂姆 Catimor 品种的有矮卡、卡杜埃 Catuai、DTARZ46、DTARZ293 等 13 个品种。采自保山 1330 m、普洱 1340 m 的同一品种卡蒂姆 Catimor 样品的水浸出物含量分别为 33.6 %、29 %，说明相同品种和相近海拔下，产地对咖啡豆水浸出物含量的影响较大，保山的咖啡豆水浸出物含量高于普洱。

2.4 咖啡因

由表 2 可以看出，2 个产地的咖啡豆样品咖啡因含量在 0.83 % ~1.31 %、平均为 1.05 %，均高于 NY/T 604-2006≥0.8 %的标准；其中保山的卡蒂姆 Catimor 系列样品咖啡因含量较高，在 0.93 % ~1.31 %、平均为 1.12 %，2 份采自 1000 和 1150 m 样品的咖啡因含量超过 DB53/T 154.5-2006≤1.2 %的标准；虽然不同海拔咖啡豆样品的咖啡因含量差异较大，但相关分析显示咖啡因含量与海拔高度之间的相关不显著(表 3)；普洱的采自海拔 1340 m 茶树良种场的 30 个不同品种的样品咖啡因含量较低，在 0.83 % ~1.27 %、平均为 0.99 %，1 份采自 DT30 样品的咖啡因含量超过 DB53/T 154.5-2006≤1.2 %的标准，而且品种之间差异较大，共有瑞丽 48 号、热选 1 号、DTARZ293、卡杜埃 Catuai 等 13 个品种的咖啡因含量低于卡蒂姆 Catimor。采自保山 1330 m、普洱 1340 m 的同一品种卡蒂姆 Catimor 样品的咖啡因含量分别为 1.17 %、1.02 %，说明相同品种和相近海拔下，产地对咖啡豆咖啡因含量的影响较大，保山的咖啡豆咖啡因含量高于普洱。

2.5 总糖

由表 2 可以看出，2 个产地的咖啡豆样品总糖含量在 9.5 % ~11.73 %、平均为 10.61 %，均高于 DB53/T 154.5-2006≥9.0 %的标准；其中保山的卡蒂姆 Catimor 系列样品总糖含量较高，在 10.07 % ~11.73 %、平均为 10.79 %；虽然不同海拔咖啡豆样品的总糖含量差异较大，但相关分析显示总糖含量与海拔高度之间的相关不显著(表 3)；普洱的采自海拔 1340 m 茶树良种场的 30 个不同品种的样品总糖含量较低，在 9.5 % ~11.12 %、平均为 10.49 %，品种之间差异较大，共有卡杜埃 Catuai、DTARZ293、热选 1 号、DTARZ46、热选 1 号和矮卡等 20 个品种的总糖含量高于卡蒂姆 Catimor。采自保山 1330 m、普洱 1340 m 的同一品种卡蒂姆 Catimor 样品的总糖含量分别为 10.94 %、10.29 %，说明相同品种和相近海拔下，产地对咖啡豆的总糖含量有一定影响，保山的咖啡豆总糖含量高于普洱。

2.6 蛋白质

由表 2 可以看出，2 个产地的咖啡豆样品蛋白质含量在 9.02 % ~15.1 %、平均为 12.01 %；其中保山的卡蒂姆 Catimor 系列样品蛋白质含量较高，在 11.0 % ~15.1 %、平均为 13.27 %，符合 DB53/T 154.5-2006≥11.0 %的标准，而且相关分析显示蛋白质含量与海拔高度之间呈极显著负相关($r = -0.62$，$P = 0.003$)(表 3)；普洱的采自海拔 1340 m 茶树良种场的 30 个不同品种的样品蛋白质含量较低，在 9.02 % ~12.5 %、平均为 11.16 %，其中 DTARZ293、热选 1 号、热选 2 号、蒙多诺沃 Mundo-Novo、铁皮卡 Typica 等 12 个品种的咖啡样品的蛋白质含量低于 11.0 %，而且品种之间差异较大，所有 29 个品种的蛋白质含量均低于卡蒂姆 Catimor。采自保山 1330 m 和普洱 1340 m 的同一品种卡蒂姆 Catimor 样品的蛋白质含量分别为 12.3 %和 12.5 %，说明相同品种和相近海拔下，产地对咖啡豆蛋白质含量的影响较小。

2.7 粗脂肪

由表 2 可以看出，2 个产地的咖啡豆样品粗脂肪含量在 10.52 % ~15.47 %、平均为 12.83 %，极显著高于 DB53/T 154.5-2006≥5.0 %的标准；其中

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保山的卡蒂姆 Catimor 系列样品粗脂肪含量较高，在 12.27 % ~15.47 %、平均为 13.32 %；虽然不同海拔咖啡豆样品的粗脂肪含量差异较大，但相关分析显示粗脂肪含量与海拔高度之间的相关不显著(表 3)；普洱的采自海拔 1340 m 茶树良种场的 30 个不同品种的样品粗脂肪含量较低，在 10.52 % ~14.13 %、平均为 12.51 %，而且品种之间差异较大，共有 DTARZ46、DTARZ293、瑞丽 48 号、蒙多诺沃 MundoNovo 等 14 个品种的粗脂肪含量高于卡蒂姆 Catimor。采自保山 1330 m、普洱 1340 m 的同一品种卡蒂姆 Catimor 样品的粗脂肪含量分别为 12.89 %、12.78 %，说明相同品种和相近海拔下，产地对咖啡豆粗脂肪含量的影响较小。

2.8　粗纤维

由表 2 可以看出，2 个产地的咖啡豆样品粗纤维含量在 23.06 % ~32.61 %、平均为 29.17 %，符合 DB53/T 154.5-2006 ≤35 % 标准；其中保山的卡蒂姆 Catimor 系列样品粗纤维含量较低，在 23.06 % ~30.91 %、平均为 27.42 %；虽然不同海拔咖啡豆样品的粗纤维含量差异较大，但相关分析显示粗纤维含量与海拔高度之间的相关不显著(表 3)；普洱的采自海拔 1340 m 茶树良种场的 30 个不同品种的样品粗纤维含量较高，在 25.73 % ~32.61 %、平均为 30.34 %，而且品种之间差异较大，只有 Pt 一个品种的粗纤维含量低于卡蒂姆 Catimor。采自保山 1330 m、普洱 1340 m 的同一品种卡蒂姆 Catimor 样品的粗纤维含量分别为 29.85 %、27.64 %，2 个产地咖啡豆粗纤维的含量不同会导致同一条件下焙炒的咖啡豆色调不同。

2.9　卫生指标

由表 2 可以看出，2 个产地的咖啡豆样品中有 21 份样品的砷含量在检出限 0.01 之下而未检出，其余 29 份检出的样品砷含量在 0.01 ~0.164 mg/kg，显著低于 NY/T 604-2006 ≤0.5 mg/kg 和 DB53/T 154.5-2006 ≤0.5 mg/kg 的标准；2 个产地的咖啡豆样品的铅含量均在检出限 0.03 之下均未检出，显著低于 NY/T 604-2006 ≤0.5 mg/kg 和 DB53/T 154.5-2006 ≤0.5 mg/kg 的标准；2 个产地的咖啡豆样品中有 21 份样品的镉含量在检出限 0.003 之下而未检出，其余 29 份检出的样品镉含量在 0.004 ~0.088 mg/kg，虽然 NY/T 604-2006 和 DB53/T 154.5-2006 未规定生咖啡豆中镉的含量标准，但新制定的绿色食品咖啡行业标准 NY/T 289-2012 规定镉含量≤0.5 mg/kg，因此供试的所有咖啡豆样品都符合绿色食品咖啡的标准。

3　讨　论

咖啡由于其独特的品味、口感和功效而成为全球最受欢迎的非酒精类饮料之一，咖啡属中以小粒种咖啡品质最好，这主要源自其不同的化学成分及含量。研究表明，小粒种咖啡的咖啡因约占豆重的 1.0 % ~1.4 %，而中粒种咖啡的咖啡因约占豆重的 2.4 % 以上[17]；小粒种咖啡的脂类约占豆重的 13 % ~17 %，比中粒种咖啡高出 60 %[18]；小粒种咖啡的蔗糖约占豆重的 6 % ~9 %，是中粒种咖啡的 2 倍，因此小粒种咖啡的风味和口感远优于中粒种咖啡[19-21]。

云南由于独特的自然气候和地理优势，不仅是全国最大的咖啡豆生产基地，而且出产的咖啡豆品质优越，为优质小粒咖啡制品的加工原料[6-8]。在云南省普洱市思茅区的一些村，咖啡成为主要的经济作物[22]。咖啡豆的品质受很多因素的影响[9-11]，包括品种、产地、纬度、海拔、土壤及栽培管理等[12-16]，因此不同产地的咖啡豆品质存在差异。保山、普洱所产的咖啡豆，主要化学和卫生指标均达到行业和地方标准，而且水浸出物含量、总糖含量、粗脂肪含量和粗纤维含量显著高于地方标准，咖啡因含量较高，因此云南出产的小粒种咖啡香味更浓、甜度更高、提神醒脑功效更强[1]，均为优质咖啡豆；但 2 个产地咖啡豆的品质特点有所不同，就卡蒂姆 Catimor 品种而言，保山出产的咖啡豆具有更低的水分含量以及更高的水浸出物、总糖和粗脂肪含量，普洱出产的咖啡豆具有更低的灰分、咖啡因和粗纤维含量以及更高的蛋白质含量，这与张洪波等[12]和黄家雄等[13]的研究结果类似。就卡蒂姆 Catimor 品种而言，保山和普洱 2 个不同产地对其产出的咖啡生豆的灰分、蛋白质、粗脂肪含量和卫生指标影响较小，但对咖啡生豆的水分、水浸出物、咖啡因、总糖和粗纤维含量影响较大，保山出产的卡蒂姆 Catimor 比普洱的甜度更高、烘焙色调略深，与李锦红等[16]研究的高纬度地区相类似。就普洱产地而言，不同品种之间品质的差异较大，李学俊等[11]的研究也有类似结果；卡蒂姆 Catimor 与其他品种相比，除具有较高的蛋白质、灰分含量和较低的粗纤维含量，其余指标居中；小粒种咖啡 155 号品种的粗脂肪、总糖和水浸出物含量很高而咖啡因含量较低，P1 和 154 号品种的粗脂肪含量很高、咖啡因含量也较高，DTARZ293 品种的粗脂肪含量较高而咖啡因含量较低，这些品种的品质、品味口感也各异，各具特色。

4　结　论

保山和普洱是云南小粒种咖啡种植的主要产

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区，生产的均为优质咖啡豆，主要化学和卫生指标均达到行业和地方标准，而且水浸出物、总糖、粗脂肪和粗纤维含量显著高于地方标准；但它们的品质因产地和品种而不同，而海拔与除蛋白质之外的其余品质指标相关均不显著；2 个产地的咖啡豆各有优点，各地应根据品种特性和环境条件合理进行选择和开发利用。

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云南大学学报(自然科学版), 2020, 42(2):374~381 DOI: 10.7540/j.ynu.20190301
Journal of Yunnan University: Natural Sciences Edition

不同外源物质喷施对干旱胁迫下‘云麻1号’苗期生理的影响

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摘要: 为了探明不同外源物质处理对干旱胁迫下工业大麻苗期的影响. 研究以‘云麻1号’为试验材料, 通过盆栽控水法模拟干旱胁迫, 研究不同质量浓度(200、500、800 mg/L 和 1 200 mg/L)的 Vc、甜菜碱、$CaCl_2$ 和赤霉素喷施处理下对工业大麻苗期抗旱的生理效应. 结果表明: 与对照相比, 干旱胁迫处理使‘云麻1号’苗期的叶绿素、脯氨酸和可溶性糖的含量降低, 使 SOD 和 POD 酶活性也降低. 外源物质 Vc、甜菜碱、$CaCl_2$ 和赤霉素处理均可提高大麻苗的叶绿素、脯氨酸和可溶性糖的含量, 同时也提高 SOD 和 POD 酶活性, 从而在一定程度上缓解干旱胁迫对幼苗的伤害. 根据隶属函数评价结果得出, ‘云麻1号’分别在喷施 500 mg/L Vc 和 $CaCl_2$ 时生理抗旱特性较好, 在喷施 200 mg/L 甜菜碱和赤霉素时缓解效果也较理想. 4 种外源物质缓解效果依次为 Vc >$CaCl_2$ >赤霉素 >甜菜碱. 研究结果揭示了干旱胁迫下大麻幼苗对外源物质喷施的生理响应, 为外源物质处理提高工业大麻抗旱性的研究提供参考依据.

关键词: 工业大麻; 干旱胁迫; 苗期; 外源物质; 生理特性
中图分类号: S563.3 **文献标志码:** A **文章编号:** 0258-7971(2020)02-0374-08

干旱是影响作物生长发育的重要环境因素之一, 已成为影响我国农业生产最严重的制约因素. 干旱所导致的作物减产, 超过其它环境胁迫所造成减产的总和[1]. 解决这一问题的途径, 除了选择耐旱品种外, 目前, 比较简单有效的抗旱栽培方式是施用外源物质来提高作物对干旱逆境的耐受力, 改善作物干旱条件下的生长发育状况.

影响植物抗旱性的外源物质种类很多, 不同外源物质对于提高植物抗旱性的作用机制有所不同[2]. 甜菜碱是植物组织中的渗透调节物质, 可在逆境胁迫下大量积累, 能在渗透胁迫环境下稳定多种生物大分子的结构和生理功能, 对植物维持呼吸作用和光合作用的正常进行具有重要的生理意义[3]. Vc 作为一种普遍存在于植物组织中的抗氧化物质, 可以在干旱等逆境情况下还原超氧物自由基, 提高植物体内水分含量, 从一定程度上调节酶的活性, 保护细胞膜[4]. Ca^{2+}对于调节植物生理过程也意义重大, 它可以使细胞膜和细胞壁的稳定性在逆境胁迫下得以较好的维持[5]. 赤霉素作为重要植物激素, 在植物抗旱方面具有重要作用, 可诱导与抗逆性形成相关的基因表达和蛋白质的合成[6].

大麻(*Cannabis sativa* L.)又叫火麻、汉麻、线麻等, 是大麻科(Cannabaceae)大麻属的一年生草本植物[7], 是最古老的栽培作物之一. 工业大麻是指嫩茎叶中四氢大麻酚(THC)质量分数<0.3%, 不具备制毒条件的品种类型[8]. 工业大麻具有多方面的经济利用价值, 除了传统的纺织、造纸、制药、食品应用外, 近年来, 进一步扩大到汽车、建筑、能源、军需以及化工等方面[9]. 干旱易造成大麻不出苗或出苗率低下, 植株生长缓慢、矮小, 个体发育不一致, 最终严重影响大麻的产量和品质. 所以, 大麻的抗旱研究是实现工业大麻高产优质发展的迫

收稿日期: 2019-05-28; 接受日期: 2019-11-15; 网络出版日期: 2020-02-11
基金项目: 现代农业产业技术体系建设专项(CARS-16-E15); 云南省基础研究计划项目(2015FB108).
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切需要.

目前, 国内外有关喷施不同外源物质对工业大麻苗期干旱胁迫缓解的研究还未见报道. 因此, 本试验以工业大麻品种‘云麻 1 号’为试验材料, 研究不同浓度的甜菜碱、Vc、$CaCl_2$ 和赤霉素喷施处理对干旱胁迫的大麻苗期生理指标的影响, 筛选出适宜的浓度, 应对大麻生产中出现的干旱问题提供基础依据.

1　材料与方法

1.1　材料　供试材料为云南省推广工业大麻品种‘云麻 1 号’, 由云南省农业科学院经济作物研究所工业大麻育种课题组提供.

1.2　方法

1.2.1　试验方法　采用盆栽称重控水法模拟干旱胁迫处理. 每盆(上、下直径分别为 18.5, 14.0 cm, 高 16.5 cm)分别装入 500 g 培养基质(菜园土-腐殖质质量比 1∶1), 均匀撒播 30 粒种子(3 次重复), 然后每盆覆盖基质 50 g, 待苗长至 5 cm 高时进行间苗, 每盆定苗 10 株. 在此期间正常浇水并做好除草工作. 待大部分植株长到 20 cm 左右时, 开始称重(盆和植株的质量)控水模拟干旱胁迫(50% 基质含水量, 下同). 干旱处理 1 周后用不同质量浓度(200、500、800 mg/L 和 1 200 mg/L)赤霉素(GA_3)、甜菜碱(GB)、维生素 C(Vc)和氯化钙($CaCl_2$)对大麻植株从上到下进行喷施, 隔 1 天喷施 1 次, 共喷施 3 次. 以正常浇水为对照 1(CK_1), 以干旱胁迫(不喷施外源物质)为对照 2(CK_2).

1.2.2　指标测定　喷施结束 15 d 后, 取植株的第 3 对真叶(从顶部展开向下数)作为取样测定样本, 每个处理 3 次重复. 叶绿素含量采用浸泡法测定, 可溶性糖含量测定采用硫代巴比妥酸法测定[10]; 脯氨酸含量采用磺基水杨酸法测定[11]; 过氧化物酶(POD)活性采用愈创木酚法测定, 超氧化物歧化酶(SOD)活性采用氮蓝四唑法测定[12].

1.3　数据分析　利用隶属函数法, 分别对喷施不同外源物质处理对干旱胁迫下‘云麻 1 号’苗期生理影响进行综合评价. 每个样品各项指标的具体隶属函数值计算公式为:

$$X_u = (X - X_{min}) / (X_{max} - X_{min}), \quad (1)$$

式中, X 为参试样品某一指标的测定值, X_{max}、X_{min} 分别为所有试样中该指标的最大值和最小值. 最后把每个试样各项指标隶属函数值累加, 取其平均值.

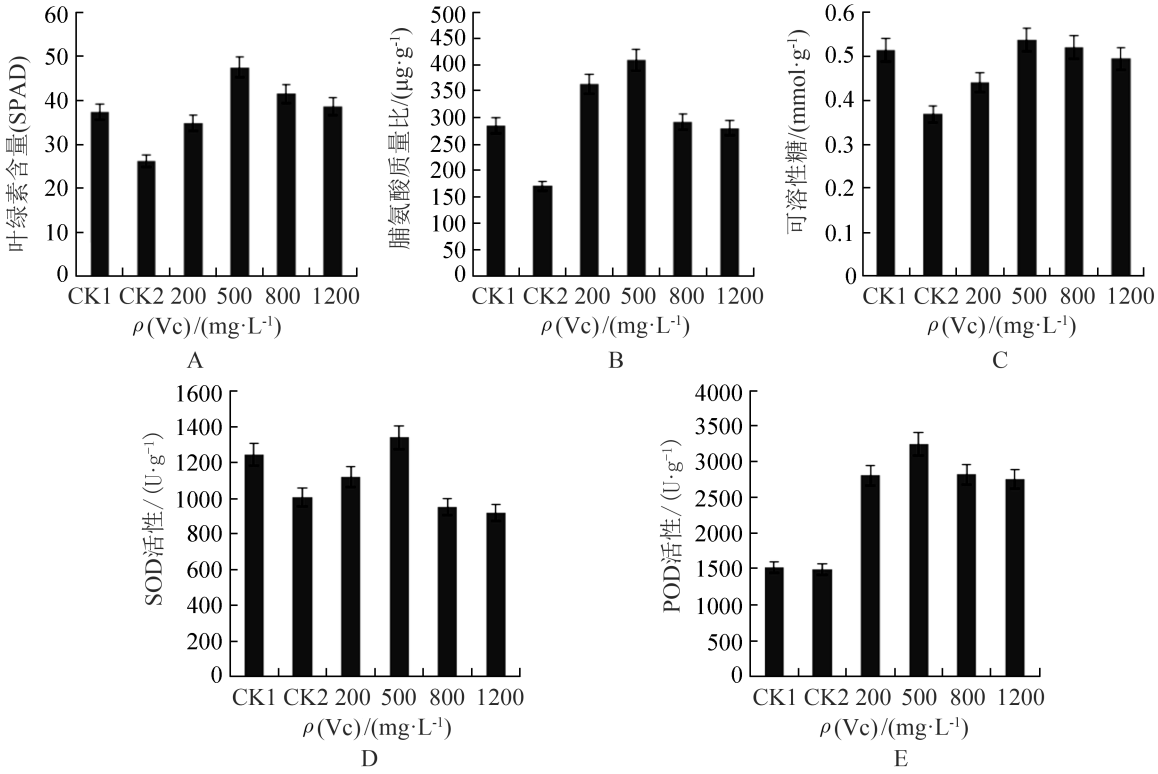


图 1　Vc 处理对干旱胁迫下‘云麻 1 号’苗期生理指标的影响

Fig. 1　Effects of Vc treatments on physiological indexes of ‘Yunma 1’ seedlings under drought stress

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2 结果与分析

2.1 Vc 对干旱胁迫下大麻苗期生理指标的影响

如图 1A 所示,干旱胁迫条件下,‘云麻 1 号’的叶绿素含量降低. 喷施不同浓度的 Vc,‘云麻 1 号’叶绿素含量呈先增后减的趋势,且上升幅度较大,在 500 mg/L Vc 处理时叶绿素含量最高. 与 CK_1 相比,干旱胁迫减少了‘云麻 1 号’渗透调节物质脯氨酸和可溶性糖含量的积累. Vc 喷施处理下‘云麻 1 号’的脯氨酸和可溶性糖含量随着处理浓度的增加而呈先增后减趋势,且在 500 mg/L 时达到峰值(图 1B、1C). 与 CK_1 相比,干旱胁迫严重影响了‘云麻 1 号’的氧化酶系统,使得 SOD、POD 活性下降. 不同浓度 Vc 喷施处理后 SOD 和 POD 活性均呈现先增后减的趋势,在 500 mg/L 时酶活性最高(图 1D、1E).

2.2 $CaCl_2$ 对干旱胁迫下大麻苗期生理指标的影响

如图 2A 所示,喷施不同质量浓度的 $CaCl_2$ 对‘云麻 1 号’叶绿素含量均有不同程度的提高,且随着质量浓度的增加,先增后减,在 500 mg/L $CaCl_2$ 处理时叶绿素含量最高. 干旱胁迫使得‘云麻 1 号’的脯氨酸和可溶性糖的含量降低,喷施 $CaCl_2$ 条件下,‘云麻 1 号’的脯氨酸和可溶性糖的含量随着浓度的增加而呈先增后减趋势,并在 500 mg/L 时达到峰值(图 2B、2C). 干旱胁迫处理下,‘云麻 1 号’的 SOD和 POD 活性降低,$CaCl_2$ 处理后酶活性均为先增后减趋势. 在质量浓度为 500 mg/L 时酶活性最好,对‘云麻 1 号’干旱胁迫的缓解作用最好(图 2D、2E).

2.3 甜菜碱对干旱胁迫下大麻苗期生理指标的影响

如图 3A 所示,与 CK_2 相比,喷施低质量浓度(200 mg/L)的甜菜碱时,‘云麻 1 号’叶绿素含量较高,随着甜菜碱浓度的增加,叶绿素含量呈明显的下降趋势. 与 CK_2 相比,喷施甜菜碱有效的增加了大麻幼苗脯氨酸和可溶性糖含量,促进大麻对干旱环境的适应,随着甜菜碱浓度的增加,‘云麻 1 号’脯氨酸和可溶性糖含量呈下降趋势,在 200 mg/L 时可溶性糖含量最高,且高于 CK_1(图 3B、3C). 甜菜碱喷施处理后,SOD 和 POD 活性有不同程度的提高. 随着喷施浓度的增加,‘云麻 1 号’的 SOD 和 POD 活性呈下降趋势. 在低质量浓度 200 mg/L 时,‘云麻 1 号’的酶活性最高(图 3D、3E).

2.4 赤霉素对干旱胁迫下大麻苗期生理指标的影响

由图 4A 可看出,赤霉素对‘云麻 1 号’的叶绿

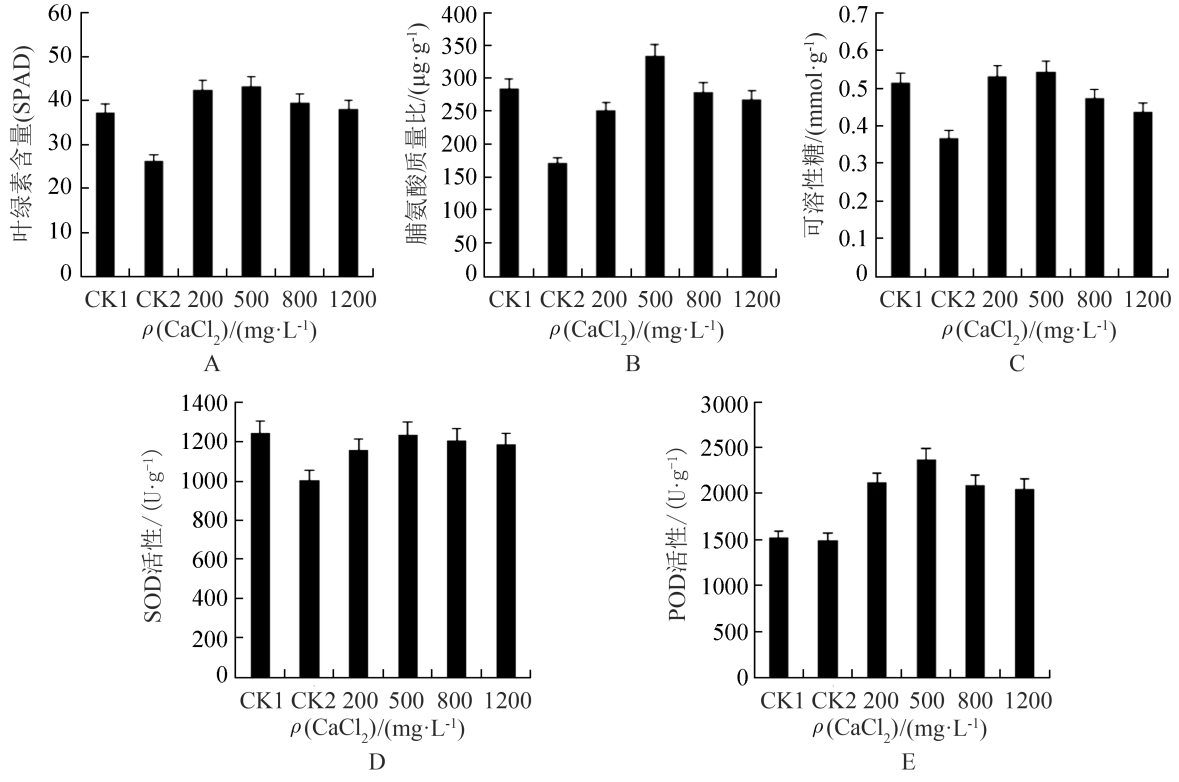


图 2 $CaCl_2$ 处理对干旱胁迫下‘云麻 1 号’苗期生理生化指标的影响

Fig. 2 Effects of $CaCl_2$ treatments on physiological indexes of ‘Yunma 1’ seedlings under drought stress

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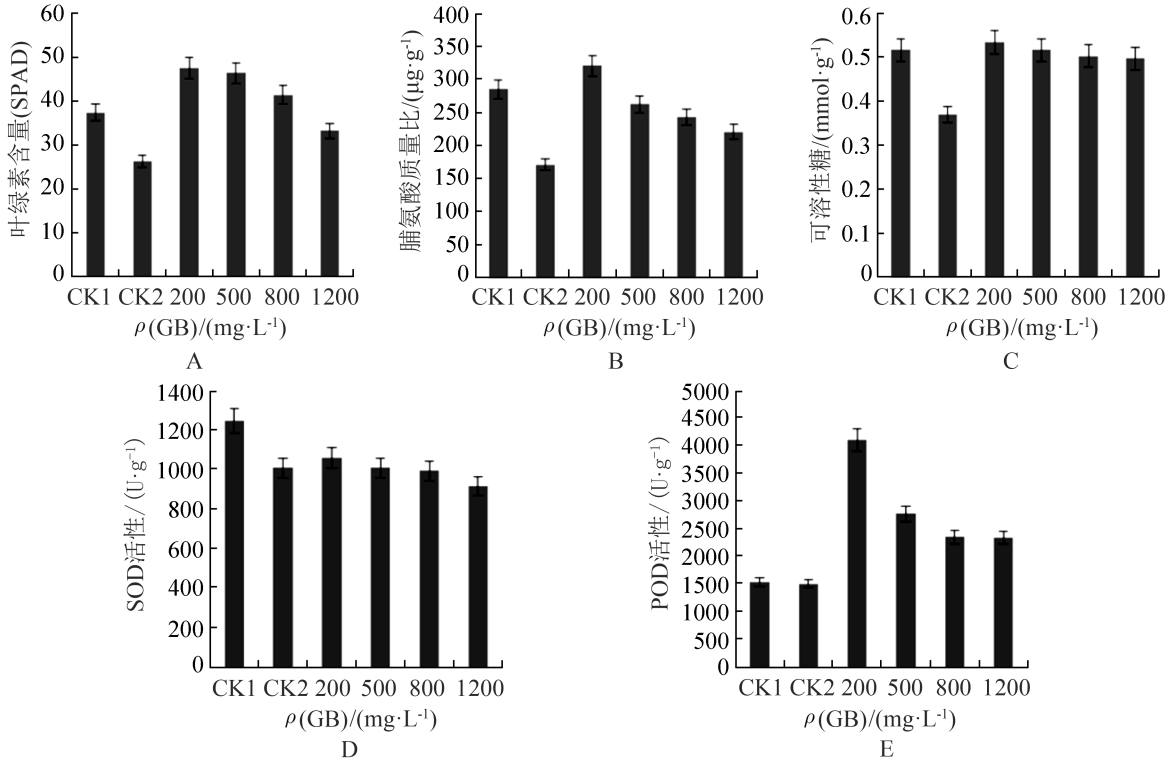


图 3　甜菜碱(GB)处理对干旱胁迫下‘云麻 1 号’苗期生理指标的影响

Fig. 3　Effects of betaine treatments on physiological indexes of ‘Yunma 1’ seedlings under drought stress

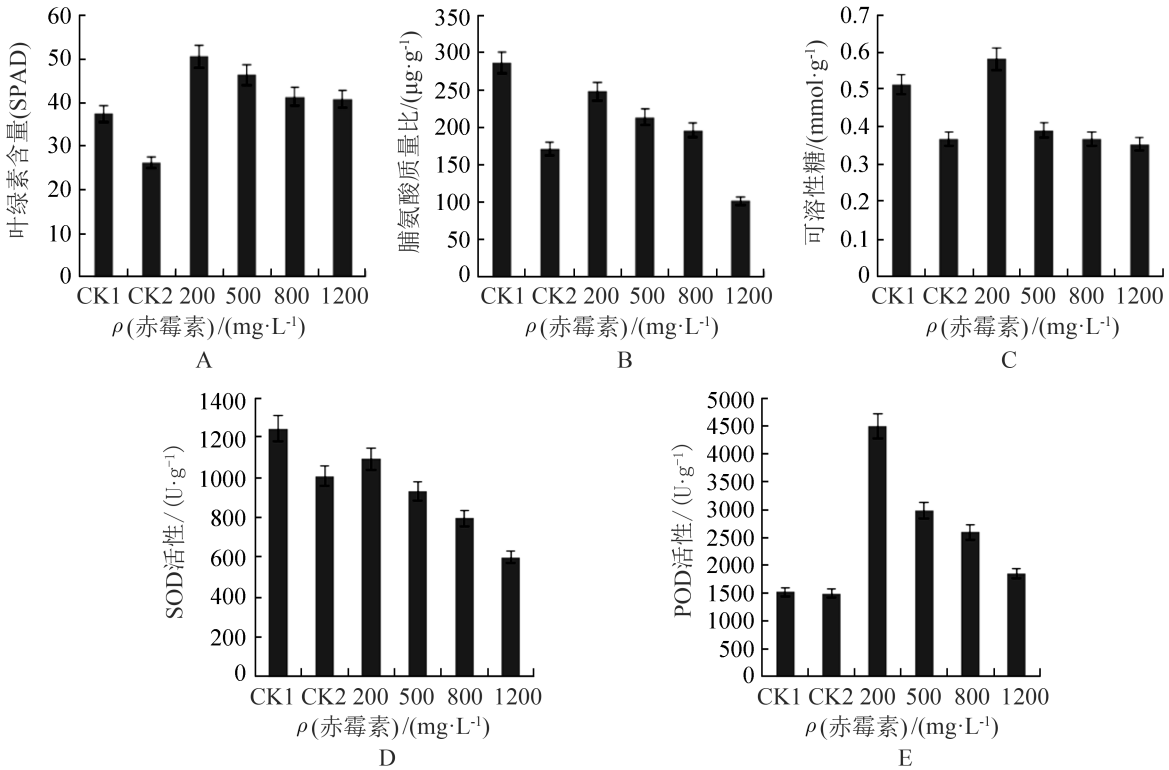


图 4　赤霉素处理对干旱胁迫的‘云麻 1 号’苗期生理指标的影响

Fig. 4　Effects of gibberellin treatments on physiological indexes of ‘Yunma 1’ seedlings under drought stress

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素含量有不同程度的影响,随处理浓度的升高而快速降低,喷施 200 mg/L 赤霉素时,‘云麻 1 号’叶绿素含量最高. 赤霉素处理下,‘云麻 1 号’的脯氨酸和可溶性糖含量不同程度的提高,在 200 mg/L 时可溶性糖含量最高(图 4B、4C). 如图 4D 和 4E 所示,低浓度的赤霉素处理增加了‘云麻 1 号’的酶活性,随着赤霉素浓度的增加,SOD、POD 酶活性呈下降趋势. 在赤霉素质量浓度为 200 mg/L 时,‘云麻 1 号’的酶活性最高,此时 POD 活性甚至显著高于正常对照(图 4D、4E).

2.5 外源物质对大麻苗期干旱的缓解效果综合评价 采用隶属函数法分别对干旱胁迫下不同浓度外源物质喷施处理对大麻苗期 5 个生理生化指标的影响进行全面、综合的评价,比较不同浓度外源物质处理对大麻苗期抗旱性影响的大小. 隶属函数分析结果表明(表 1),‘云麻 1 号’分别在喷施 500 mg/L Vc 和 $CaCl_2$ 时生理特性最好. ‘云麻 1 号’在分别喷施 200 mg/L 甜菜碱和赤霉素时效果最好. 不同外源物质适宜浓度下的综合评价 Vc>$CaCl_2$>赤霉素>甜菜碱.

表 1 不同外源物质对干旱胁迫下‘云麻 1 号’生理影响的评价

Tab. 1 Effect evaluation of exogenous substances on the physiological index of ‘Yunma 1’ under drought stress

处理	隶属函数值					平均值	排序
	叶绿素质量比	脯氨酸质量比	可溶性糖质量比	SOD活性	POD活性		
CK_1	0.521 1	0.478 0	0.864 6	0.771 9	0.013 0	0.529 7	11
CK_2	0.000 0	0.000 0	0.000 0	0.203 7	0.000 0	0.040 7	18
Vc_{200}	0.403 5	0.809 6	0.422 6	1.000 0	0.749 8	0.677 1	7
Vc_{500}	1.000 0	1.000 0	1.000 0	1.000 0	1.000 0	1.000 0	1
Vc_{800}	0.714 5	0.506 0	0.896 4	0.069 2	0.756 0	0.588 5	10
$Vc_{1\ 200}$	0.579 1	0.458 3	0.745 2	0.000 0	0.720 4	0.500 6	14
Ca_{200}	0.950 8	0.489 8	0.930 0	0.634 4	0.710 3	0.743 1	5
Ca_{500}	1.000 0	1.000 0	1.000 0	0.967 7	1.000 0	0.993 5	2
Ca_{800}	0.780 8	0.659 4	0.598 7	0.842 3	0.684 2	0.713 1	6
$Ca_{1\ 200}$	0.697 7	0.589 6	0.395 0	0.752 7	0.635 6	0.614 1	9
GB_{200}	1.000 0	1.000 0	1.000 0	0.430 1	1.000 0	0.886 0	4
GB_{500}	0.948 4	0.612 5	0.893 7	0.277 2	0.487 9	0.643 9	8
GB_{800}	0.712 2	0.483 2	0.813 5	0.230 6	0.325 0	0.512 9	13
$GB_{1\ 200}$	0.327 0	0.335 6	0.775 5	0.000 0	0.322 5	0.352 1	16
GA_{200}	1.000 0	0.793 0	1.000 0	0.764 6	1.000 0	0.911 5	3
GA_{500}	0.829 0	0.606 9	0.161 2	0.514 6	0.493 5	0.521 0	12
GA_{800}	0.621 8	0.513 5	0.062 7	0.304 2	0.364 4	0.373 3	15
$GA_{1\ 200}$	0.594 9	0.000 0	0.000 0	0.000 0	0.117 8	0.142 5	17

3 讨论

干旱作为一种非生物胁迫影响了植物生理和代谢的各个方面,能够抑制植物的光合作用,改变渗透调节物质的含量以及植物的酶活性[13]. 本研究发现,长期干旱胁迫导致‘云麻 1 号’苗期的叶绿素含量下降,而渗透调节物质脯氨酸和可溶性糖的含量也显著降低,同时,SOD 和 POD 酶活性也降低.

研究表明,外源物质处理可以影响植物在干旱胁迫下的生理活动,最终提高其干旱抗性. 其中,叶面喷施质量浓度为 40 mg/L Vc,有效地提高了干旱胁迫下朝天椒幼苗叶片的 POD 活性,促进脯氨酸

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等调节物质的积累,增强了朝天椒在开花期的耐旱性[14]. 叶面喷施 10 mmol/L $CaCl_2$ 有效增加了烤烟幼苗叶片上类囊体膜的电子传递能力,提高了烤烟幼苗叶片的光合作用,也提高光能转换速率[15]. 外源 10 mmol/L $CaCl_2$ 的施用显著降低干旱胁迫对苜蓿幼苗的伤害[16]. 喷施 200 mmol/L 甜菜碱提高了石榴叶片的叶绿素含量,同时提高了可溶性糖和游离脯氨酸含量,使石榴幼树渗透调节能力提高[17]. 在干旱胁迫处理下,喷施甜菜碱后,叶绿素和可溶性蛋白含量有增长的现象,同时,增加了脯氨酸和可溶性糖的含量,抗氧化酶活性也显著增强. 干旱胁迫下,喷施低浓度甜菜碱有助于番茄抵御干旱[18]. 喷施甜菜碱能够减少细胞膜结构的损伤,提高叶片叶绿素含量,提高苹果幼树抗旱性[19]. 外源施用 100 mg/L 赤霉素提高了苜蓿幼苗的抗旱性[16].

本研究发现,外源物质 Vc、甜菜碱、$CaCl_2$ 和赤霉素处理均可提高大麻苗的叶绿素、脯氨酸和可溶性糖的含量,同时也提高 SOD 和 POD 酶活性,从而在一定程度上缓解干旱胁迫对幼苗的伤害. 但是,不同外源物质处理的适宜浓度不同,研究表明‘云麻 1 号’分别在喷施 500 mg/L Vc 和 $CaCl_2$ 时生理抗旱特性较好,在喷施 200 mg/L 甜菜碱和赤霉素时缓解效果较理想,此时可有效缓解干旱胁迫对大麻苗的伤害.

综上所述,外源物质处理确实可以对干旱胁迫下大麻苗期的生理性状产生影响,提高大麻的抗旱性,但其所表现的抗旱机理不明,有待进一步研究分析. 同时,本研究中只是对‘云麻 1 号’的处理结果,对于其他工业大麻品种的外源物质的选择和适宜的处理浓度,仍需进一步分析. 本研究是在外源物质对干旱胁迫下大麻种子萌发影响研究的基础上[20],进一步对大麻苗期抗旱性的影响研究. 研究结果将完善外源物质处理对大麻在不同生长时期干旱缓解处理,也为工业大麻抗旱研究提供了参考.

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Li G J, Li X, Wang Q, et al. Promoting effect of soaking with different exogenous substances on seeds germination of hemp 'Bamahuoma' under stress[J]. Journal of Yunnan University: Natural Sciences Edition, 2018, 40(5): 1 034-1 041.

Physiological effects of different exogenous substances spraying on seedlings of 'Yunma 1' under drought stress

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Abstract: In order to explore the effects of different exogenous substances on the seedling stage of industrial hemp under drought stress, 'Yunma 1' was used as experimental material. Drought stress was simulated by pot water control method. The physiological responses of Vc, betaine, $CaCl_2$ and gibberellin spraying treatments with different concentrations (200, 500, 800 and 1 200 mg/L) to drought resistance of industrial hemp seedlings were studied. The results showed that drought stress reduced the contents of chlorophyll, proline and soluble sugar, and

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the activities of SOD and POD in ‘Yunma 1’ seedlings compared with the control. Exogenous substances such as Vc, betaine, $CaCl_2$ and gibberellin could increase the contents of chlorophyll, proline and soluble sugar in seedlings, as well as the activities of SOD and POD, thus alleviating the damage of drought stress to seedlings to a certain extent. According to the evaluation result of membership function, ‘Yunma 1’ had better physiological drought resistance when being sprayed 500 mg/L Vc and $CaCl_2$ respectively, and had better mitigation effect being sprayed with 200 mg/L betaine and gibberellin. The alleviating effects of four exogenous substances were Vc > $CaCl_2$ > Gibberellin > Betaine. The results reveal the physiological response of hemp seedlings to exogenous substances spraying under drought stress, and provide a reference for improving drought resistance of industrial hemp by exogenous substance treatment.

Key words: *Cannabis sativa* L.; Drought stress; Seedling stage; Exogenous substances; Physiological characteristics

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云南大学学报(自然科学版), 2020, 42(2):393~400 DOI: 10.7540/j.ynu.20190454
Journal of Yunnan University: Natural Sciences Edition

黄毛草莓愈伤组织诱导条件优化

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摘要: 本研究分别以黄毛草莓叶片和茎尖生长点为外植体进行愈伤组织的诱导, 并通过比较茎尖愈伤组织在不同培养基以及不同植物激素组合下的生长情况, 为建立黄毛草莓高效快速的繁殖体系, 筛选愈伤组织诱导的最适培养基奠定基础. 结果表明, 与 1/2MS 培养基相比, MS 培养基作为基础培养基(0.4 mg/L TDZ+0.2 mg/L 6-BA 条件下)诱导的愈伤组织效果最佳; 以 MS 为基本培养基, 通过 4 因素 3 水平 $L_9(3^4)$正交实验研究不同浓度组合的 4 种植物激素对黄毛草莓愈伤组织诱导率的影响. 结果表明 2,4-D 对黄毛草莓叶片愈伤组织的诱导影响最大. 黄毛草莓叶片愈伤组织诱导的最优培养基为 MS+0.4 mg/L TDZ+0.2 mg/L 6-BA+0.15 mg/L 2,4-D+0.2 mg/L NAA; NAA 对黄毛草莓茎尖愈伤组织的诱导影响最大, 黄毛草莓茎尖愈伤组织诱导的最优培养基为 MS+0.2 mg/L TDZ+0.6 mg/L 6-BA+0.15 mg/L 2,4-D+0.6 mg/L NAA.

关键词: 黄毛草莓; 愈伤组织; 正交试验; 植物激素
中图分类号: S324 **文献标志码:** A **文章编号:** 0258−7971(2020)02−0393−08

草莓(*Fragaria ananassa* Duch.)隶属于蔷薇科(Rosaceae)草莓属(*Fragaria* L.), 是多年生草本植物, 在园艺学上属于浆果类果树[1], 具有较高的经济价值和营养价值. 草莓在我国的栽培面积非常广泛, 仅次于同为浆果类的葡萄[2], 但相比于其他种类的水果, 草莓的品种相对单一. 此外, 由于多年种植会导致草莓品种种性退化、病毒病侵染严重、畸形果的比例增加和口感逐渐变差等情况的发生, 从而严重影响果农的经济收益以及草莓品种的优化[3].

黄毛草莓 (*Fragaria nilgerrensis* Schltdl) 为蔷薇科草莓属的多年生二倍体野生草莓, 是我国西南地区重要的野生白果草莓资源. 黄毛草莓独特的水蜜桃香味、白色浆果和较强的抗寒、抗旱、抗病性等特点, 使之在拓宽栽培草莓遗传背景方面具有很大的潜力[4].

草莓多以无性繁殖为主, 而长期无性繁殖极易导致病毒在草莓植株体内进行累积, 从而出现植株矮化、果实畸形、病斑出现、种性退化, 不利于推广优良品种[5]. 与传统自繁方式相比, 草莓组织培养技术不仅繁殖速度快、系数大, 而且可以获得脱毒苗, 保持品种优良特性[6]. 因此, 对于草莓组织培养技术的研究尤为重要, 而影响组培育苗成败的关键因素便是愈伤组织分化. 愈伤组织培养不仅是一种植物快繁的新手段, 同时也是植物改良, 种质保存和次生代谢产物研究的理想手段[7].

1978 年, 日本学者加古顺治最先报道了利用花药经过愈伤组织分化出不定芽形成脱毒草莓植株[3]. 王燕等以黄毛草莓匍匐茎茎尖为材料, 筛选出了适宜黄毛草莓丛生芽增殖快繁和离体生根的培养基[8]. 同时, 刘玲等以黄毛草莓叶片为材料, 筛选出了适宜黄毛草莓离体叶片不定芽再生的培养基[9]. 国内外对于草莓愈伤组织的研究多集中于章姬、红颜和法兰地等栽培种[10], 但关于野生黄毛草莓愈伤组织的研究尚未见报道. 本文通过单因素实验对 MS 培养基和 1/2MS 培养基进行筛选, 并利用 TDZ(噻苯隆)、6-BA(6-苄氨基腺嘌呤)、2,4-D(二氯苯氧乙酸)、NAA(萘乙酸)4 种激素之间的不同

收稿日期: 2019-08-21; 接受日期: 2019-11-15; 网络出版日期: 2020-02-11
基金项目: 国家自然科学基金地区项目(31760082); 云南省科技厅应用基础研究计划面上项目(2019FB-8).
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浓度组合来设计正交实验,以期筛选出黄毛草莓叶片和茎尖诱导愈伤组织诱导的最适培养条件.

1 材料和方法

1.1 材料 研究试验材料黄毛草莓实生苗于 2018 年采自昆明市呈贡区云南大学农学院培养室,愈伤组织诱导所用外植体采自黄毛草莓匍匐茎初代培养的组培苗.

1.2 方法

1.2.1 *愈伤组织诱导材料的获得与培养* 为了减少污染率和消毒对于外植体造成的损伤,本研究愈伤组织诱导材料都来自于无菌苗.

选取黄毛草莓匍匐茎茎尖作为外植体,先用 10% 的洗洁精浸泡 5 min,在流动的自来水下冲洗 30 min 后,用 75% 的酒精消毒 20 ~ 25 s;接着用无菌水清洗 1 次,0.1% 的升汞处理 5 ~ 7 min,无菌水冲洗 5 ~ 8 次,每次 1 min;最后用灭菌滤纸吸去茎尖上的水分,消毒后的材料待用.

待外植体在培养基内生长 40 d 后,选取生长健壮、长势一致的组培苗作为愈伤组织诱导材料.

1.2.2 *培养条件* 培养箱温度白天控制在 (25±2)℃,黑夜控制在 (20±2)℃,光照强度为 1 500 ~ 2 000 lx,白天光照时间 14 h,黑夜避光 10 h.

1.2.3 *培养基的筛选* 分别以 MS 培养基、1/2MS 培养基为基本培养基,添加 0.4 mg/L TDZ,0.2 mg/L 6-BA 筛选适合黄毛草莓叶片与茎尖诱导愈伤组织的基本培养基(表 1).

表 1 诱导黄毛草莓叶片与茎尖愈伤组织培养基

Tab. 1 Callus medium for leaf and shoot tip induction in *Fragaria nilgerrensis* mg/L

培养基	*ρ*(TDZ)	*ρ* (6-BA)
MS	0.4	0.2
1/2MS	0.4	0.2

1.2.4 *愈伤组织的诱导* 以正交实验因素水平表为依据,采用 $L_9(3^4)$正交表配制 9 种培养基(表 2).叶片剪成 0.8 ~ 1.2 cm 的方块状,在无菌环境下接种于培养基中,每种培养基接种 20 瓶,每瓶接种 5 ~ 6 个,重复 3 次;在解剖镜下切取 0.2 ~ 0.5 mm 的茎尖生长点,每瓶接种 4 ~ 5 个茎尖生长点,重复 3 次.两种材料的愈伤组织诱导都经过 7 d 的暗培养后转入正常光下培养 23 d,分别统计出愈伤组织诱导率并且观察愈伤组织的长势.

表 2 正交试验因素水平 $L_9(3^4)$

Tab. 2 Orthogonal test factor level $L_9(3^4)$ mg/L

水平	A *ρ*(TDZ)	B *ρ*(6-BA)	C *ρ*(2,4-D)	D *ρ*(NAA)
1	0.2	0.2	0.15	0.3
2	0.4	0.4	0.3	0.6
3	0.6	0.6	0.45	0.9

1.2.5 *愈伤组织诱导最优培养基的验证* 正交试验是一种均一性设计实验,无法包含所有处理组合,所以最优培养基可能并未显现出来.为验证正交试验结果显示的激素配比是否为最优组合,配制 20 瓶正交培养基与诱导愈伤组织长势最好的培养基进行比较,对比愈伤组织的长势以及诱导率.

1.2.6 *试验结果评定指标* 经过 30 d 的诱导培养后进行统计,相关结果利用 Excel 进行处理和分析.

愈伤组织诱导率(%)=(产生愈伤组织外植体数/接种外植体的总数)×100%

2 结果与分析

2.1 不同基本培养基对黄毛草莓叶片与茎尖诱导愈伤组织的影响 将生长健壮、长势一致的黄毛草莓组培苗叶片与茎尖生长点接种到以 MS 培养基与 1/2MS 培养基上.经过暗处理培养 7 d 后转入光照下培养,2 d 后大部分叶片开始卷曲,25 d 后叶片边缘开始出现一些黄色的松散颗粒状愈伤(图 1A),茎尖周围开始出现灰色的颗粒状愈伤(图 1B),暗处理和正常光照培养 30 d 后统计愈伤组织诱导率并进行记录.

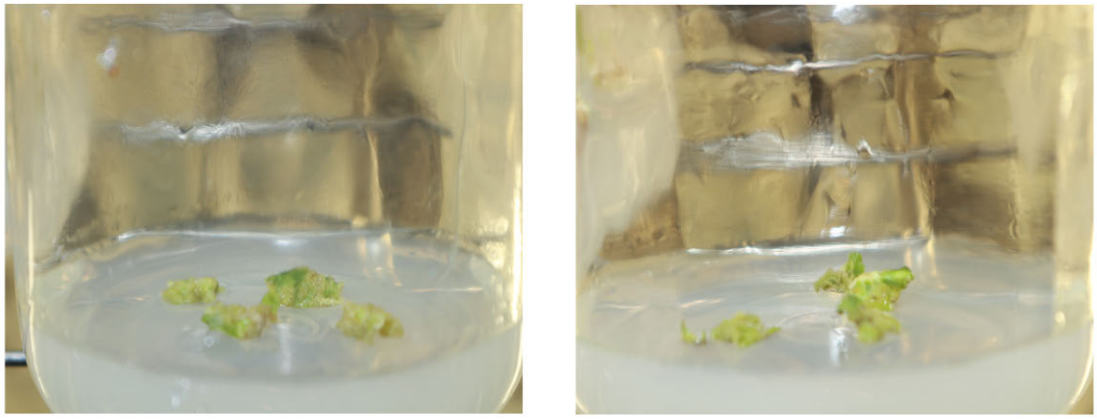
通过对表 3 的分析可知,在 MS 培养基上,叶片和茎尖愈伤组织发生率程度远高于 1/2MS 培养基,表明 MS 最适宜黄毛草莓无菌苗诱导愈伤组织的形成.

2.2 不同质量浓度配比的植物激素对黄毛草莓茎尖、叶片诱导愈伤组织的影响

2.2.1 *茎尖愈伤组织诱导* 在 MS 基本培养基中分别加入不同质量浓度的 TDZ、6-BA、2,4-D、NAA 植物激素,在暗培养后的 15 d,茎尖周围也会开始出现少量金黄色的松散状愈伤(图 2),再过 7 d 后愈伤成块,此时统计愈伤组织诱导率.

通过对表 4 的观察结果分析,极差 *R* 的值越大表示该因素的影响也越大,由此可知对黄毛草莓茎

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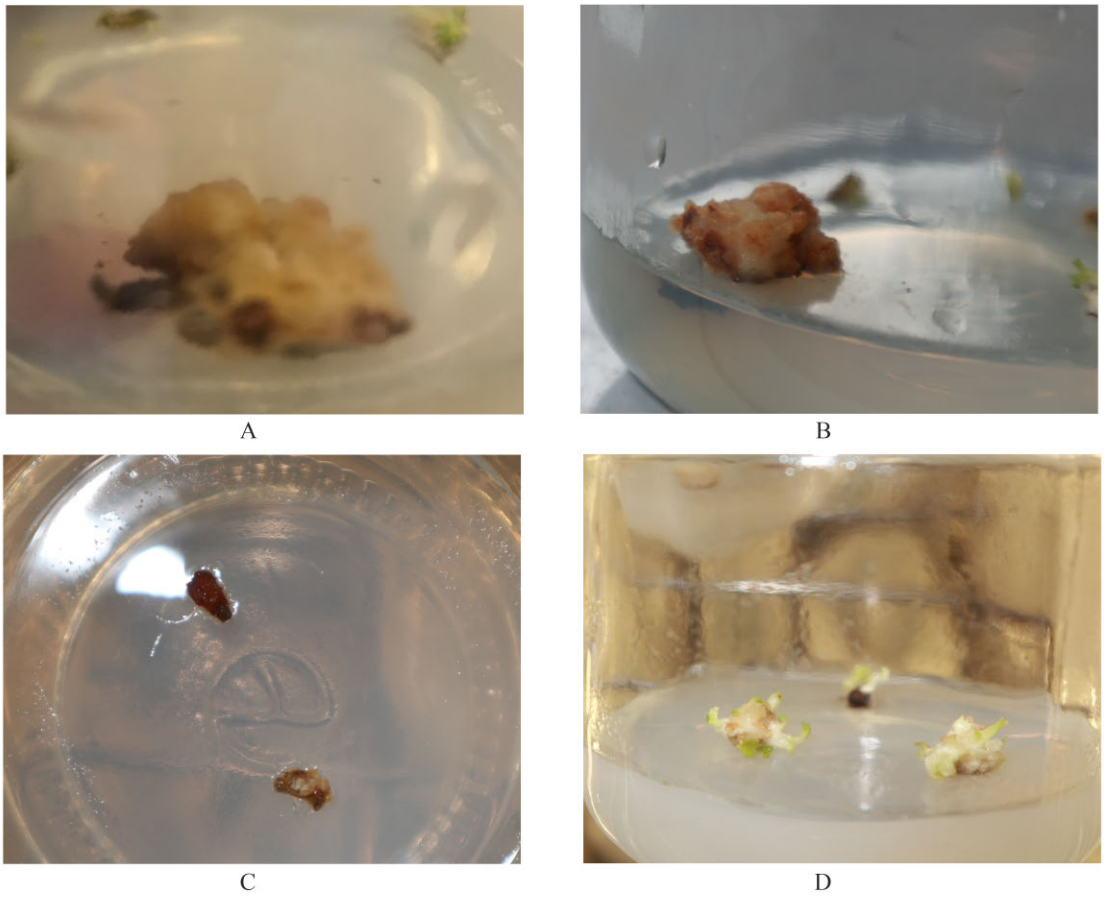


A　　B

A: MS 培养基诱导愈伤组织; B: 1/2 MS 培养基诱导愈伤组织.

图 1　不同培养基诱导黄毛草莓叶片愈伤组织情况(25 d)

Fig. 1　Callus induction from leaf on different medium (25 d)



A　　B

C　　D

A: 0.6 mg/L TDZ+0.2 mg/L 6-BA+0.45 mg/L 2,4-D+0.6 mg/L NAA; B: 0.4 mg/L TDZ+0.6 mg/L 6-BA+0.15 mg/L 2,4-D+0.6 mg/L NAA; C: 0.4 mg/L TDZ+0.4 mg/L 6-BA+0.45 mg/L 2,4-D+0.3 mg/L NAA; D: 0.2 mg/L TDZ+0.2 mg/L 6-BA+0.15 mg/L 2,4-D+0.3 mg/L NAA.

图 2　不同激素浓度配比下黄毛草莓茎尖诱导愈伤组织的情况(22 d)

Fig. 2　Callus induction from shoot-tips of *Fragaria nilgerrensis* under different hormone concentrations (22 d)

尖诱导愈伤组织的影响为: NAA>TDZ>6-BA>2,4-D, 说明萘乙酸(NAA)对于黄毛草莓茎尖的愈伤组织诱导率的影响最大. 表 4 显示当 NAA 浓度逐渐增大时, 愈伤组织诱导率呈现先增加后降低的变化趋势, 当 NAA 质量浓度为 0.6 mg/L 时愈伤组织诱导率最高. *K* 值愈大说明激素水平愈高, 结果显示

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表 3 不同基本培养基对黄毛草莓诱导愈伤组织的影响

无菌苗	培养基	ρ(TDZ)/(mg·L^{-1})	ρ(6-BA)/(mg·L^{-1})	接种数	出愈数	愈伤组织诱导率/%
叶片	MS	0.4	0.2	50	42	84
	1/2MS	0.4	0.2	50	10	20
茎尖	MS	0.4	0.2	46	31	67
	1/2MS	0.4	0.2	46	8	17

表 4 不同质量浓度配比的激素对茎尖愈伤组织诱导率的影响

Tab. 4 Effects of different concentrations of hormones on callus induction from shoot tips

试验编号	A ρ(TDZ)/(mg·L^{-1})	B ρ(6-BA)/(mg·L^{-1})	C ρ(2,4-D)/(mg·L^{-1})	D ρ(NAA)/(mg·L^{-1})	接种数	出愈数	愈伤组织诱导率/%
1	0.2	0.2	0.15	0.3	63	34	54
2	0.2	0.4	0.3	0.6	68	46	67.6
3	0.2	0.6	0.45	0.9	59	23	39
4	0.4	0.2	0.3	0.9	63	10	15.9
5	0.4	0.4	0.45	0.3	58	15	25.9
6	0.4	0.6	0.15	0.6	70	56	80
7	0.6	0.2	0.45	0.6	75	45	60
8	0.6	0.4	0.15	0.9	65	7	10.8
9	0.6	0.6	0.3	0.3	56	21	37.5
K1	53.533	43.3	48.267	39.133			
K2	40.6	34.767	40.333	69.2			
K3	36.1	52.167	41.633	38.3			
R	17.433	17.4	7.934	30.9			

TDZ 的均值 K1>K2>K3, 6-BA 的均值 K3>K1>K2,2,4-D 的均值 K1>K3>K2,NAA 的均值 K2>K1>K3, 故黄毛草莓茎尖愈伤组织诱导的最佳激素组合为 A1B3C1D2. 同时, 通过图 2 可以看出, 在不同激素(植物生长调节剂)配比的培养基下, 黄毛草莓茎尖诱导愈伤组织的过程中出现颜色由浅至深的现象, 并且愈伤组织的松软程度也不一样, 有的质地较硬, 有的出现褐化现象, 甚至有的愈伤组织在生长的过程中伴随着丛生芽的分化.

比较最优组合 A1B3C1D2 与正交试验中最优的 6 号培养基出愈率, 结果显示 A1B3C1D2 组合的愈伤组织诱导率达到了 90%, 略高于正交表中愈伤组织诱导率最高的 6 号培养基 85%(表 5). 而从愈伤组织的形态来看(图 3), 6 号培养基诱导的愈伤组织为乳白色, 且较为疏松, 而 A1B3C1D2 诱导的愈伤组织为浅绿色, 且较为密集, 长势明显更好. 这表明 A1B3C1D2 组合的培养基的确比 6 号培养基更适合黄毛草莓茎尖诱导愈伤组织的形成, 所以黄毛草莓茎尖诱导愈伤组织的最优培养基为: MS+0.2 mg/L TDZ+0.6 mg/L 6-BA+0.15 mg/L 2,4-D+0.6 mg/L NAA.

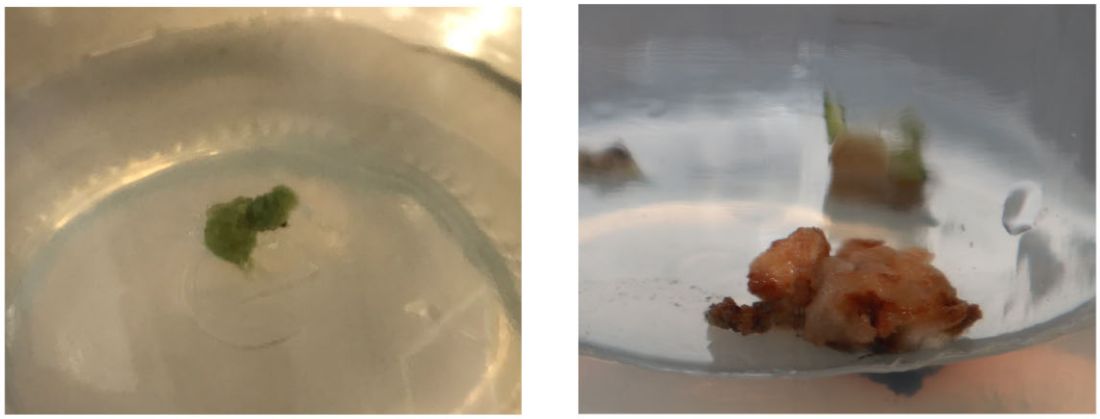
表 5 验证试验

Tab. 5 Validation tests

培养基	接种数	出愈数	愈伤组织诱导率/%
A1B3C1D2	20	18	90
6号	20	17	85

2.2.2 叶片愈伤组织诱导 在 MS 基本培养基中分别加入不同浓度的 TDZ、6-BA、2,4-D、NAA 植物激素, 暗培养 7 d 后叶片开始卷曲, 转入正常光照下处理 14 d 叶片上出现黄色的松散状愈伤(图 4), 再过 7 d 后愈伤布满叶片, 此时统计愈伤组织诱导率.

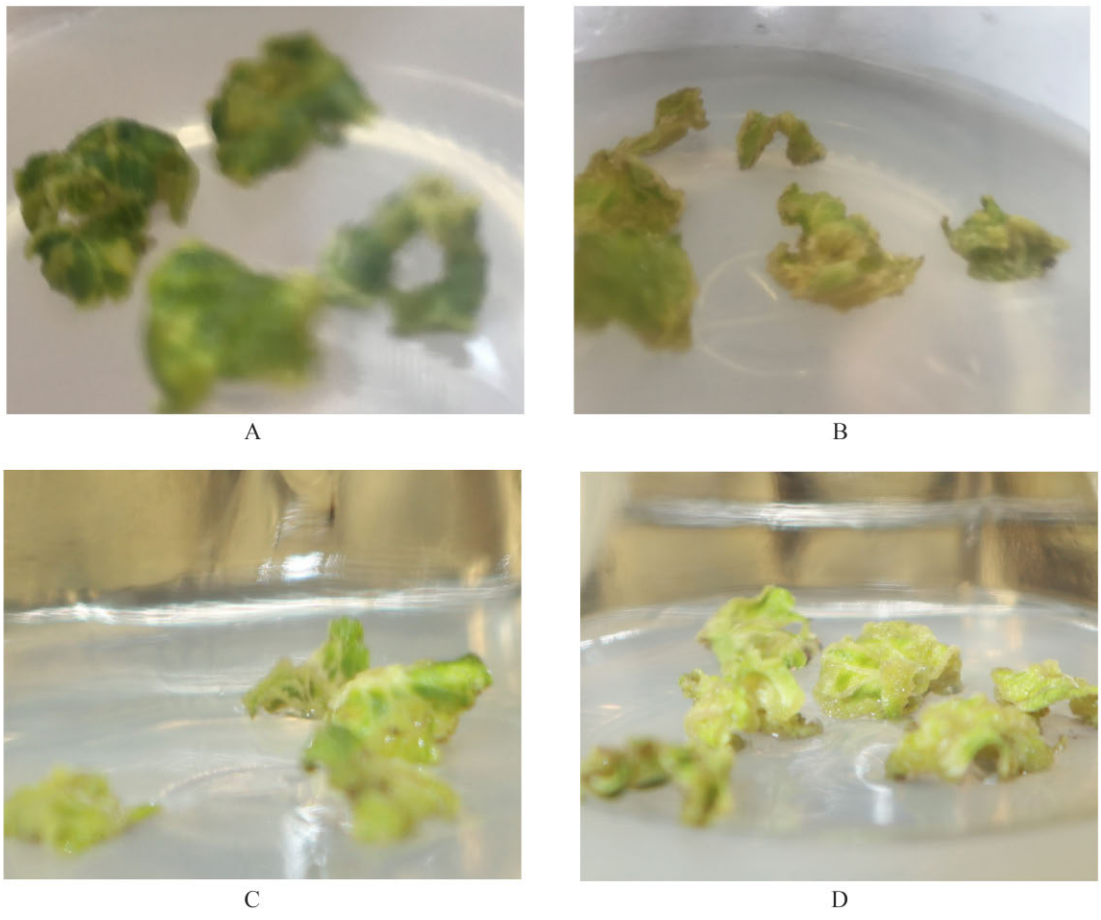
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A: A2B1C1D1 (MS+0.2 mg/L TDZ+0.6 mg/L 6-BA+0.15 mg/L 2,4-D+0.6 mg/L NAA); B: 6 号 (MS+0.4 mg/L TDZ+0.6 mg/L 6-BA+0.15 mg/L 2,4-D+0.6 mg/L NAA).

图 3　黄毛草莓茎尖愈伤组织诱导最优培养基与诱导率最高培养基生长情况比较(30 d)

Fig. 3　Comparison of shoot-tips callus growth between optimum medium and highest induction rate medium in *F. nilgerrensis* (30 d)



A: 0.6 mg/L TDZ+0.2 mg/L 6-BA+0.3 mg/L 2,4-D+0.6 mg/L NAA; B: 0.4 mg/L TDZ+0.2 mg/L 6-BA+0.45 mg/L 2,4-D+0.4 mg/L NAA; C: 0.4 mg/L TDZ+0.4 mg/L 6-BA+0.15 mg/L 2,4-D+0.6 mg/L NAA; D: 0.2 mg/L TDZ+0.4 mg/L 6-BA+0.3 mg/L 2,4-D+0.4 mg/L NAA.

图 4　不同激素浓度配比下黄毛草莓叶片诱导愈伤组织的情况(21 d)

Fig. 4　Callus induction from leaf of *Fragaria nilgerrensis* under different hormone concentrations (21 d)

通过对表 6 的观察结果分析, 极差 *R* 的值越大表示该因素的影响也越大, 因而对黄毛草莓叶片诱导愈伤组织的影响为: 2,4-D>TDZ>6-BA>NAA, 说明二氯苯氧乙酸(2,4-D)对于黄毛草莓叶片的愈伤组织诱导率的影响最大. 表 6 显示, 当 2,4-D 浓度越大时愈伤组织诱导率越低. *K* 值愈大就说明水平

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表 6 不同浓度配比的激素对愈伤组织诱导率的影响

Tab. 6 Effects of different concentrations of hormones on callus induction rate

试验编号	A ρ(TDZ)/(mg·L^{-1})	B ρ(6-BA)/(mg·L^{-1})	C ρ(2,4-D)/(mg·L^{-1})	D ρ(NAA)/(mg·L^{-1})	接种数	出愈数	愈伤组织诱导率/%
1	0.2	0.2	0.15	0.2	70	60	85.7
2	0.2	0.4	0.3	0.4	64	53	82.8
3	0.2	0.6	0.45	0.6	69	56	81.2
4	0.4	0.6	0.3	0.2	65	55	84.6
5	0.4	0.2	0.45	0.4	59	49	83.1
6	0.4	0.4	0.15	0.6	72	62	86.1
7	0.6	0.4	0.45	0.2	61	51	83.6
8	0.6	0.6	0.15	0.4	66	56	84.8
9	0.6	0.2	0.3	0.6	63	53	84.1
K1	83.233	84.633	85.533	84.3			
K2	84.6	83.567	83.833	84.167			
K3	84.167	83.8	82.633	83.533			
R	1.367	1.066	2.9	0.767			

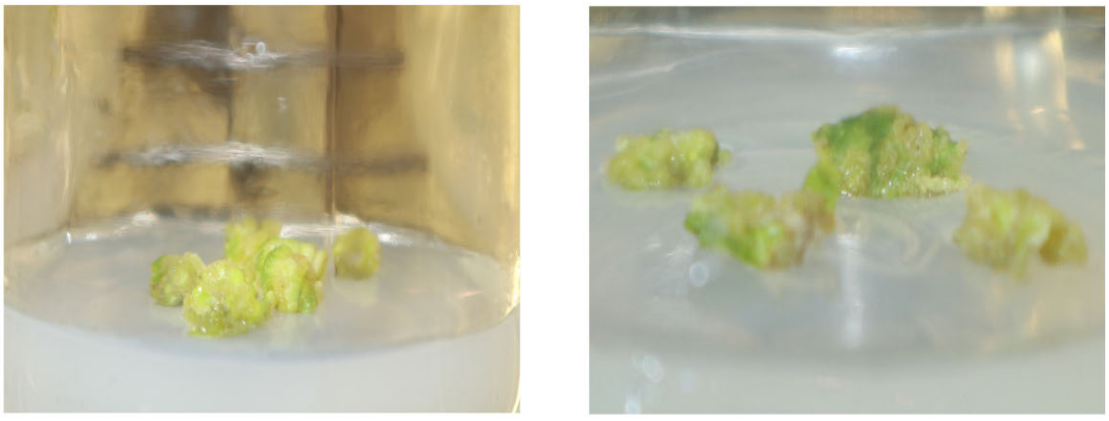
愈高, 由此可知 TDZ 的均值 K2>K3>K1, 6-BA 的均值 K1>K3>K2,2,4-D 的均值 K1>K2>K3,NAA 的均值 K1>K2>K3, 故黄毛草莓叶片愈伤组织诱导的最佳激素组合为 A2B1C1D1. 同时, 通过图 4 可以看出, 在不同激素配比的培养基下, 黄毛草莓叶片在诱导愈伤组织生长的过程中会出现由深绿至鲜绿色的现象, 并且愈伤组织的疏松程度是逐渐变得密集, 同时有的叶片仅仅出现卷曲而并未有明显愈伤生成的现象, 这说明激素配比的不同对生成愈伤组织的影响较大.

比较最优组合 A2B1C1D1 与正交试验中诱导率最高的 6 号培养基进行比较, 结果显示 A2B1C1D1 组合的愈伤组织诱导率达到了 95%, 高于正交表中

表 7 验证试验

Tab. 7 Validation tests

培养基	接种数	出愈数	愈伤组织诱导率/%
A2B1C1D1	20	19	95
6号	20	17	85



A B

A: A2B1C1D1 (MS+0.4 mg/L TDZ+0.2 mg/L 6-BA+0.15 mg/L 2,4-D+0.2 mg/L NAA); B: 6 号 (MS+0.4 mg/L TDZ+0.4 mg/L 6-BA+0.15 mg/L 2,4-D+0.6 mg/L NAA).

图 5 黄毛草莓叶片愈伤组织诱导最优培养基与诱导率最高培养基生长情况比较(30 d)

Fig. 5 Comparison of leave callus growth between optimum medium and highest induction rate medium in *F. nilgerrensis* (30 d)

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的 6 号培养基 85%(表 7). 且从愈伤组织的形态来看(图 5), 6 号培养基诱导的愈伤组织覆盖率较低, 叶片存在明显的原型状态, 而 A2B1C1D1 诱导的愈伤组织覆盖率明显较高, 且较为密集, 长势明显更好. 这表明 A2B1C1D1 组合的培养基的确比 6 号培养基更适合黄毛草莓叶片诱导愈伤组织的形成, 所以黄毛草莓叶片诱导愈伤组织的最优培养基为: MS+0.4 mg/L TDZ+0.2 mg/L 6-BA+0.15 mg/L 2,4-D+0.2 mg/L NAA.

3　讨论

我们的研究表明 MS 培养基和 1/2MS 培养基均能使黄毛草莓叶片和茎尖生长点诱导出愈伤组织, 但从实验结果可以得出 MS 培养基的效果明显强于 1/2MS 培养基. 这一结果与张爱丽等以章姬草莓茎段为材料研究最适愈伤培养基的基本培养基相符[10]. 结合王燕等的结果综合来看, 1/2MS 培养基更适于用来诱导草莓生根培养[8].

蔷薇科植物的愈伤组织研究结果表明, 不同外植体的愈伤组织诱导效果存在差异[11-12]. 对比黄毛草莓叶片与茎尖的愈伤组织诱导发现, 叶片更容易诱导出愈伤组织, 这一结果与宋金锋等所报道的晶瑶草莓叶片高诱导率相符[13], 可能是因为叶片中叶脉作为物质交换与运输的组织, 最早感受到生理生化环境的变化从而脱分化产生愈伤组织[14], 但愈伤组织团明显没有茎尖诱导生成的愈伤组织团密集, 长势也较茎尖愈伤组织差. 这一结果与戴小云等研究的核桃叶片愈伤质量没有叶柄愈伤质量好以及尤润等以华木莲叶片诱导愈伤的情况相符[15-16], 这可能是由于叶片存在脱分化的过程.

本试验筛选出黄毛草莓茎尖诱导愈伤组织的最适培养条件为: MS+0.2 mg/L TDZ+0.6 mg/L 6-BA+0.15 mg/L 2,4-D+0.6 mg/L NAA, 同时 NAA 对于黄毛草莓茎尖愈伤组织的诱导影响最大, 在 NAA 为 0.6 mg/L 时愈伤组织诱导率最高. 正如前人的研究表明, 单独使用 NAA 能促进植物愈伤组织中总酚、总黄酮的积累, 而适量结合 6-BA 能获得最高的活性物质含量[17]. 黄毛草莓部分愈伤组织在生长的过程中伴随着丛生芽的分化, 这可归因于 6-BA 具有促进细胞的分裂与分化同时提高出芽率的作用, 这与张爱丽等所报道的以章姬草莓茎段为材料可同步进行愈伤组织诱导和丛芽分化的结果相符[10].

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Callus induction of *Fragaria nilgerrensis*

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Abstract: In order to establish an efficient and rapid system of regeneration, the optimal medium for callus induction from leaves and stem tips in *Fragaria nilgerrensis* were screened. Compared with 1/2 MS medium, MS medium with 0.4 mg/L TDZ+0.2 mg/L 6-BA was more appropriate for callus induction. Then leaves and stem tips were used as explants to compare the different callus induction rate under four kinds of plant hormones with different concentration combinations, which were designed as orthogonal experiment of $L_9(34)$. The results showed that 2, 4-D has the greatest effect on callus induction of leaves in *F. nilgerrensis* and the optimal medium was MS +0.4 mg/L TDZ +0.2 mg/L 6-BA +0.15 mg/L 2, 4-D +0.2 mg/L NAA. While NAA has the greatest effect on callus induced from stem tips and the optimal medium was MS +0.2 mg/L TDZ +0.6 mg/L 6-BA +0.15 mg/L 2, 4-D+0.6 mg/L NAA.

Key words: *Fragaria nilgerrensis*; callus; orthogonal; plant hormones

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doi:10.3969/j.issn.1002-2481.2020.03.19

米勒魔芋休眠解除过程中激素及糖类变化

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摘 要:米勒魔芋是一种特殊价值较高的园艺作物,采收后的球茎具有较长休眠期。为探究米勒魔芋球茎休眠过程中主要内源激素及糖类物质变化情况,以米勒魔芋为材料,观察其芽点形态变化,并分析魔芋球茎从深度休眠期到萌芽期脱落酸(ABA)、生长素(IAA)以及糖类物质的含量变化。结果表明,ABA 在球茎深度休眠期含量较低,在萌动期前开始上升至最高值 213 ng/g,长芽后开始逐步下降;IAA 在开始长芽后,有比较大的增长,达到最大值 33.8 ng/g,进入萌芽期后球茎 IAA 含量回落至 28 ng/g;可溶性糖及可溶性蛋白含量随着贮藏时间的增加均呈现升高趋势,而球茎内淀粉逐渐转化,含量持续下降。ABA 对球茎休眠的维持具有重要作用,适量的 IAA 能促进球茎生根,淀粉可为魔芋球茎发芽提供营养物质及能量基础。

关键词:米勒魔芋;休眠;脱落酸;生长素;糖类物质
中图分类号:S632.3 **文献标识码**:A **文章编号**:1002-2481(2020)03-0358-06

Changes of Hormones and Carbohydrate during Dormancy to Dormancy-release of *Amorphophallus muelleri*

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Abstract: *Amorphophallus muelleri* is a kind of horticultural crop with high special value. The bulbs after harvest have a long dormant period. To explore the changes in the main endogenous hormones and biochemical content during the dormancy of konjac corms, this study used *Amorphophallus muelleri* as material to observe the morphological changes of buds, the changes of abscisic acid (ABA), auxin (IAA) and carbohydrate content in the period from deep dormancy to germination. The results showed that the ABA content was relatively low during the deep dormant period of the corm, and began to rise to a maximum value of 213 ng/g before the germination period, and gradually decreased after the long shoots. After growing, IAA had a relatively large increase, reaching a maximum at 33.8 ng/g. After the germination stage, the IAA content of the corm dropped back to 28 ng/g. The soluble sugar and soluble protein content increased with the raise storage period. The starch in the corm gradually transformed being continuously decreased. ABA had an important role in maintaining the dormancy of the corm. The appropriate amount of IAA could promote the rooting of the corm. The starch could provide the nutrients and the energy for the konjac corm to germinate.

Key words: *Amorphophallus muelleri*; dormancy; abscisic acid; auxin; carbohydrate

米勒魔芋(*Amorphophallus muelleri*)属天南星科(Araceae)魔芋属(*Amorphophallus*)多年生草本植物[1],起源于热带雨林,喜温暖湿润环境。我国的米勒魔芋主要生长在中缅边境一带境内,主要分布在云南省德宏州瑞丽弄岛、盈江县铜壁关的原始森林,以及思茅、临沧边境一带,另外在云南西双版纳、江城以及绿春的沟谷雨林中也有分布[2]。

米勒魔芋是珠芽类魔芋的一种[1],叶柄分叉处着生气生珠芽,与传统的花魔芋、白魔芋相比,既能利用球茎和实生种子繁殖,又能利用气生珠芽繁殖,具有产量高、抗病性强、耐高温高湿等特点[3]。米勒魔芋是一种开发潜能大、种植风险低、经济效益高的魔芋种质资源,能进行低成本生产,但与多种球宿根植物类似,具有休眠特性,一般在 15 ℃以下即进入休眠状态,停止生长[4]。近年来,国内学者对米勒魔芋以外的花魔芋、白魔芋的休眠及解除休眠过程的各类生理变化有了些许认识。王玉兰[5]、张禾[6]根据休眠过程中魔芋球茎内的生理生化反应特性,将球茎休眠划分成 4 个时期:休眠初期、深休眠期、芽萌动期以及芽伸长期。孙远明等[7]在研究花魔

收稿日期:2019-11-05
基金项目:云南几种主栽花卉新品种选育及绿色栽培技术研究与示范(部省合建 2018BSHJ0108)
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芋休眠特性时发现,花芽球茎处在 12 ℃以下时,休眠期一般会到次年 4 月，顶芽 ABA 含量到 3 月下旬才下降到 120 ng/g;孙远明等[8]、李川[9]利用提高越冬贮藏温度(如贮藏温度为 20～25 ℃)达到芽提前萌发的目的。孙远明等[10]在研究花魔芋球茎休眠与脱落酸和赤霉素的关系中也发现，外源添加 ABA 会影响球茎休眠,5 mg/L 以上浓度 ABA 处理解除休眠球茎,能明显抑制其顶芽的萌发。而根据相关文献,IAA 对萌发基本没有促进作用[11],但是能够促进生根。

为了适应长期的低温环境,米勒魔芋同其他魔芋种一样具有较长的休眠期，且萌芽时间参差不齐,米勒魔芋种植的产量、质量都不稳定。在生产过程中,魔芋球茎一般 3—4 月份才开始萌动,5 月份芽才出土[12]。长时间的休眠期在其他作物中鲜有出现,块茎的休眠期过长,因此,不能及时萌动而影响球茎的生长期和形成期，严重影响田间出苗整齐度,导致采收球茎产量高低不一;同时,较长的休眠期也增加了魔芋球茎贮藏成本以及储藏风险[13-14]。如何有效控制米勒魔芋生产用种休眠期,以降低种球储藏成本和储藏风险,延长田间生长期以提升产量,是米勒魔芋产业发展中迫切需要解决的关键科技问题。

本研究探索不同贮藏温度对米勒魔芋休眠解除的影响，分析休眠解除过程中内源 ABA、IAA 以及糖类含量变化,旨在能为米勒魔芋休眠的生理学研究提供数据支撑,同时也能为米勒魔芋种植产业的高产栽培管理提供参考,以期找到切实有效的外部手段提前打破休眠，促进米勒魔芋球茎萌发生长,在实际生产中提高种植产量及产品质量。

1　材料和方法

1.1　试验材料

供试米勒魔芋(*Amorphophallus muelleri*)休眠球茎由临沧滇晟农林发展有限公司提供。

1.2　试验方法

1.2.1　不同贮藏温度对米勒魔芋休眠解除的影响　本试验共设 4 个处理组,分别为:处理 A. 将球茎先置于超低温 9 ℃下预冷一个月，后转移至 25 ℃裸藏处理;处理 B.将球茎先置于正常低温 13 ℃下预冷一个月,后转移至 25 ℃裸藏处理;处理 C. 将球茎一直置于 25 ℃裸藏处理;处理 D(CK).将球茎于室内(昼温 17～25 ℃,夜温 5～12 ℃)裸藏处理。其中，处理 A、B、C 均为暗处理，贮藏室湿度为 70%。

从贮藏之日起,每日观察各组魔芋球茎的萌动情况。每隔 10 d 将叶面球茎取出,选取有代表性的芽生长状态的球茎,洗净表面后置于体式显微镜下观察并拍照，分别做整体形态观察及纵切解剖观察，选择合适的视野与倍数拍照跟踪记录芽萌发过程。

1.2.2　魔芋休眠解除过程中内源 ABA 及 IAA 含量的测定　选取大小一致的供试魔芋球茎置于室内裸藏。取深度休眠期前、中、后(XM-1、XM-2、XM-3),芽萌动期前、中、后(MD-1、MD-2、MD-3)及萌芽期前、中、后(MY-1、MY-2、MY-3)共 9 个不同时期的魔芋球茎切小块于 -80 ℃冰箱保存备用。依据郭磊等[15]的方法,采用 LC-MS 法测定魔芋球茎内源 ABA 及 IAA 含量。

1.2.3　魔芋休眠解除过程中糖类化合物的代谢　选取大小一致的供试魔芋球茎置于室内裸藏,从贮藏之日起,每隔 10 d 随机选择种球,将球茎切小块于 -80 ℃冰箱保存备用。

1.2.3.1　可溶性糖含量的测定　依据文献[16]的方法,在 MDA 测定中通过 450 nm 波长下的吸光度再乘以校正系数可间接得出可溶性糖含量。

1.2.3.2　淀粉含量测定　采用双波长法[17-19],通过测定支链淀粉和直链淀粉的含量来间接算出淀粉含量。

1.2.3.3　可溶性蛋白测定　采用考马斯亮蓝法,通过标准曲线求得对应蛋白浓度,经换算后得出样品中可溶性蛋白的含量。

1.3　数据处理

所有数据均通过 Excel 2010 进行计算处理,利用 SPSS 22.0 进行分析。

2　结果与分析

2.1　不同温度处理下米勒魔芋球茎芽萌动情况分析

由表 1 可知,经过 9 ℃的预冷处理能提前打破球茎休眠,15 d 时芽萌动率为 13%，但预冷时间不宜过长,否则易导致球茎冻害;13 ℃预冷处理及室温处理对球茎的生长无明显作用,15 d 时芽萌动率均为 0。25 ℃为米勒魔芋萌发最为适宜的贮藏温度,能够提前打破休眠,15 d 时芽萌动率达到 16%。试验证明,9 ℃预冷确实能促使珠芽魔芋提前萌发,但萌发率低于 25 ℃贮藏条件时的萌发率,25 ℃为珠芽魔芋萌发最为适宜的贮藏温度,能够提前打

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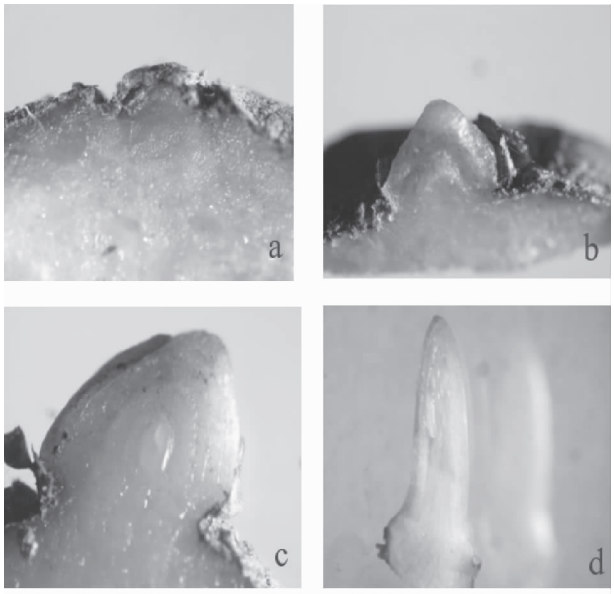
破休眠,促进球茎芽的生长,同时,25 ℃处理下 30 d 时球茎的萌发率为 39.5%,45 d 时球茎的萌发率能达到 95.0%,表示 25 ℃持续处理下能够较大程度的调节球茎发芽的时间,促进珠芽魔芋在田间出芽的整齐度。

表 1　不同温度处理球茎芽萌动情况分析

处理方法	芽萌动率 /%			
	15 d	30 d	45 d	60 d
A	13.0	26.0	52.0	100
B	0	27.0	43.0	100
C	16.0	39.5	95.0	100
D(CK)	0	31.0	65.5	100

2.2　米勒魔芋球茎顶芽发育情况分析

米勒魔芋球茎休眠时间较长,休眠期间,芽原基处有所膨大,但顶端分生组织活动很弱,叶原基不明显(图 1-a);球茎 3 月开始萌发,萌发时顶端分生组织进行平周分裂以及垂周分裂,向上产生叶原基细胞,并持续分裂,逐渐向上形成山峰状突起,最后形成叶柄轴(图 1-b);叶柄轴基部组织继续进行分裂,形成鳞片,上部裂叶原基继续分出裂叶轴和小裂片,进入生长阶段(图 1-c);当芽长超过 3 cm 时,叶柄轴上端小裂叶清晰可见,同时,叶轴基部开始有生根的结构(图 1-d)。



a为深度休眠期球茎;b为萌动期球茎,芽点处有小芽长出,但并未伸长;c为萌动期球茎,新芽开始伸长;d为萌芽期球茎,芽开始伸长,并开始有长根的现象

图1　米勒魔芋球茎芽萌发解剖(20×)

2.3　米勒魔芋从休眠期到萌芽期内源 ABA 及 IAA 含量的变化分析

2.3.1　米勒魔芋从休眠到萌发过程中激素 ABA 的变化　进入深度休眠期的米勒魔芋球茎中 ABA 的含量为 70 ng/g,且一直处于相对较低的水平,之后开始上升,直到开始长芽,在萌动期达到最高值 213 ng/g(图 2)。

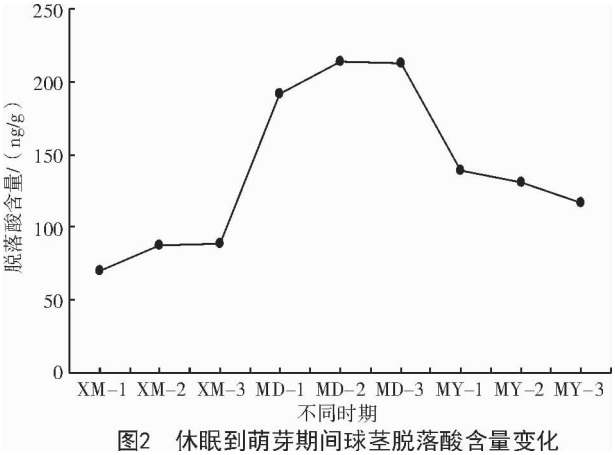


图2　休眠到萌芽期间球茎脱落酸含量变化

由表 2 可知,球茎从休眠到萌芽的整个过程,休眠期 ABA 含量与萌动期及萌芽期 ABA 的含量间均有显著性差异,而萌动期与萌芽期 ABA 的含量之间同样差异显著。

表 2　休眠到萌芽期间球茎 2 种内源激素含量分析　ng/g

不同时期	ABA 含量	IAA 含量
深度休眠期	82.20± 10.07c	26.57± 0.45b
萌动期	205.97± 12.11a	31.20± 2.25a
萌芽期	138.94± 54.99b	28.93± 2.38b

注:表中同列数据后不同小写字母表示差异显著(P<0.05)。

2.3.2　米勒魔芋从休眠到萌发过程中激素 IAA 的变化　IAA 能够促进生根,米勒魔芋球茎在解除休眠开始长芽的同时也伴随着根的生长,从图 3 可以看出,进入深度休眠期后,米勒魔芋球茎中 IAA 的含量为 27 ng/g,且一直小幅度下降,在进入萌动期,开始长芽后有比较大的增长,达到最大值 33.8 ng/g,进入萌芽期后球茎 IAA 含量回落至 28 ng/g,但仍高于休眠期。整体来看,米勒魔芋球茎打破休眠进入萌动期时 IAA 的含量与休眠期时 IAA 含量有显著性差异(表 2)。

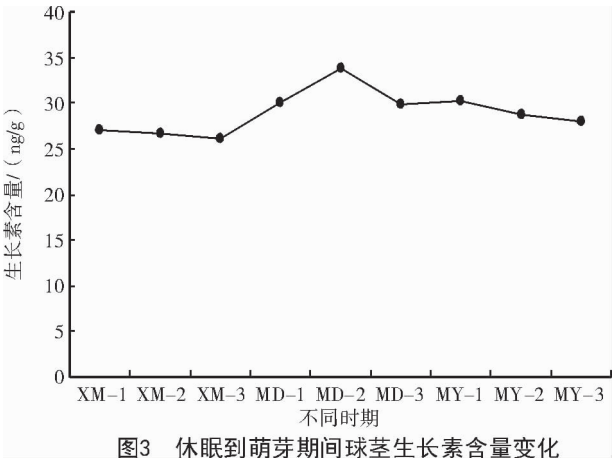


图3　休眠到萌芽期间球茎生长素含量变化

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2.4　米勒魔芋从休眠期到萌芽期糖类化合物含量的变化分析

2.4.1　米勒魔芋从休眠到萌发过程中可溶性糖含量的变化　从图 4 可以看出,从休眠到萌发过程中球茎内可溶性糖含量随着时间的加长呈先升高再下降后趋于平稳的趋势,休眠初期的可溶性糖含量为 0.41 mmol/g，在芽萌动前期，开始大幅上涨,在 30 d 达到最高含量(1.19 mmol/g),30 d 后开始大幅下降,40 d 时球茎已开始发芽，可溶性糖含量趋于稳定。

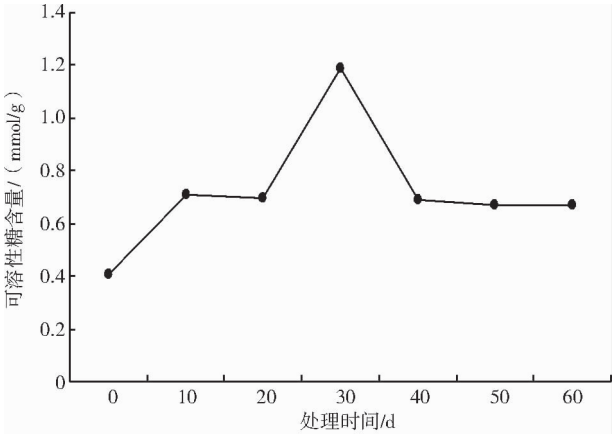


图4　休眠到萌芽期间球茎可溶性糖含量变化

2.4.2　米勒魔芋从休眠到萌发过程中淀粉含量的变化　由图 5 可知,魔芋球茎内淀粉含量总体都呈下降趋势。休眠初期球茎内的淀粉含量最高,可达 42.93%,后持续下降。淀粉在球茎内部为贮藏态的碳水化合物,供物质转化提供能量,随着贮藏的进行,淀粉逐渐转化,为芽的萌发提供能量,故而总体呈现下降趋势,在 50 d 时淀粉含量达到最低状态,含量为 10.13%。

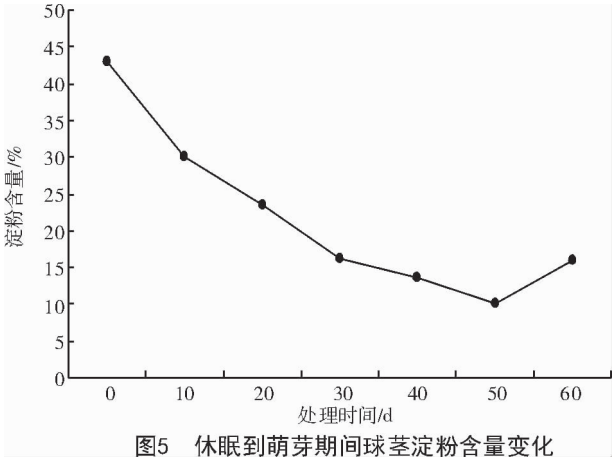


图5　休眠到萌芽期间球茎淀粉含量变化

2.4.3　米勒魔芋从休眠到萌发过程中可溶性蛋白含量的变化　从图 6 可以看出,贮藏初期可溶性蛋白含量最低,为 9.64 mg/g,在贮藏期 0～30 d 内,球茎内可溶性蛋白含量随着贮藏时间的加长持续增加,在 30 d 达到最高值,含量为 35.21 mg/g,后有所回落,但仍保持在 18.69 mg/g 的较高水平。

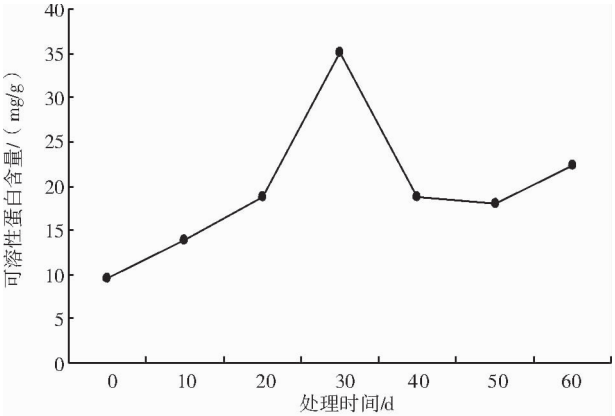


图6　休眠到萌芽期间球茎可溶性蛋白含量变化

3　结论与讨论

3.1　不同贮藏温度对米勒魔芋球茎休眠解除的影响

米勒魔芋喜温暖湿润,忌高温,对温度变化较为敏感,温度的高低能直接影响球茎的萌发及生长发育,赵琴等[20]的田间试验表明,随着温度的降低,魔芋种球的萌发率也会逐渐下降;孙远明等[10]研究也表明,魔芋球茎贮藏温度愈高,休眠期愈短,而低温可延长休眠期,温度愈低,效应愈显著,但魔芋的休眠属于生理性休眠,也需通过适当低温和光周期反应来调节[21]。本试验也证实,9 ℃预冷确实能促使米勒魔芋提前萌发，但萌发率低于 25 ℃贮藏条件时的萌发率,25 ℃为米勒魔芋萌发最为适宜的贮藏温度,能够提前打破休眠,促进球茎芽的生长,且在 25 ℃持续处理下能够较大程度的调节球茎发芽的时间,调整米勒魔芋在田间出芽的整齐度。

3.2　米勒魔芋球茎从休眠到萌芽内源激素含量的变化

脱落酸(ABA)能引起芽休眠以及叶片脱落,在种子休眠、萌发、气孔关闭、干旱、低温等非生物胁迫应答中起重要调控作用[22-24]。孙远明等[10]研究发现，花魔芋球茎的休眠及解除与 ABA 密切相关,ABA 具有维持休眠、抑制顶芽萌发和生长的作用,这在孙远航[23]及彭凤梅等[24]研究的白魔芋中也得到了印证。本试验中,在魔芋球茎收获后,ABA 含量下降到最低后开始上升,这与康朵兰[25]研究马铃薯块茎休眠期间生理生化变化时的研究结果一致。通过查阅文献发现,从休眠到萌发过程中,结合态 ABA 会逐渐转化为游离态 ABA[26],故在深度休眠后期,球茎在即将进入萌动期，开始长芽之前,ABA 含量

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在开始长芽前大幅上涨，在萌动期的芽中游离态 ABA 达到最高值，推断 ABA 对新芽的生长具有一定作用。现有大量的研究表明，ABA 和植物胚的形成密切相关[27-29]，具体 ABA 如何促进米勒魔芋球茎后期芽的生长，有待进一步研究。

生长素（IAA）参与植物体许多代谢动活，是一种能促进植物生长的激素，具有促进生根的作用[30-35]，苏小霞[36]则认为，IAA 还抑制植物生长，吲哚乙酸氧化酶活性在休眠解除过程中下降，导致生长素积累。在本研究中，米勒魔芋球茎在休眠期是 IAA 含量一直处于相对较低的水平，在萌动期却逐步升高，达到最高值 33.8 ng/g，应是吲哚乙酸氧化酶活性下降导致 IAA 积累。休眠解除后，球茎开始长根，IAA 含量有所回落，但仍高于休眠期，适宜浓度的 IAA 有助于根的生长。此外，有研究发现，IAA 含量受 GA 的上调[37]，但尚未得到证实，有待进一步的研究。

3.3 米勒魔芋从休眠期到萌芽期糖类化合物含量的变化

随着米勒魔芋球茎休眠的加深，球茎的各项生命活动均有减弱的趋势，糖类化合物代谢也逐渐减弱。贮藏初期可溶性糖含量处于相对较低的水平，而淀粉含量相对较高，随着贮藏时间的增加，可溶性糖含量有所增长，而球茎内淀粉含量持续下降。淀粉在球茎内部为贮藏态的碳水化合物，供物质转化提供能量，随着贮藏时间的增加，淀粉逐渐转化，为芽的萌发提供能量，故而整体呈现下降的趋势，这与高春英等[38]的研究结果一致。MILLER 等[39]也研究发现，鳞茎内多糖降解，直接增加了蔗糖含量，即增加了可溶性糖含量。淀粉在淀粉酶的作用下不断水解，使得球茎内可溶性糖含量增加，可溶性糖消耗为植物体的生长发育提供了物质与能量，淀粉及可溶性糖的变化与本试验的结果较为一致。

可溶性蛋白含量的增加能够促进休眠的解除，管毕财等[40]在研究低温打破龙牙百合休眠过程中可溶性蛋白变化时发现，一般情况下，可溶性蛋白的含量越高，鳞茎的休眠程度越低，所需的萌发时间也越短。本试验结果表明，可溶性蛋白含量变化在前 30 d 呈现增加的趋势，在 30 d 达到最高值（35.21 mg/g），证实了可溶性蛋白含量的增加能促进球茎的萌发。同时，可溶性蛋白是重要的贮能物质，在种子萌发时，可溶性蛋白在蛋白酶的作用下水解并连续不断的降解，产生大量游离的氨基酸运送至各个生长部位。夏宜平等[41]研究认为，可溶性糖含量出现变化的转折点可能与休眠解除有关，这与本试验测得可溶性蛋白在发芽时含量下降的结果一致，可溶性蛋白降解是为魔芋球茎萌发提供营养物质及能量基础。

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贝莱斯芽孢杆菌总RNA提取的Trizol改良法①

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摘 要 贝莱斯芽孢杆菌（*Bacillus velezensis*）是在农业、环境和发酵工业等领域具有重要应用潜力的细菌。但芽孢杆菌属细菌具有较厚的细胞壁且分泌蛋白多，常规方法难以抽提到高质量的总RNA。因此，建立和优化简便有效的贝莱斯芽孢杆菌总RNA提取方法，将为*B. velezensis*基因表达和功能研究提供重要的技术支撑。通过优化传统Trizol法，对菌体培养条件、菌体洗涤次数、液氮冷冻处理和溶菌酶裂解菌体方法进行改良，建立了一种简便有效的*B. velezensis*总RNA提取方法。

关键词 贝莱斯芽孢杆菌 ；总RNA提取 ；Trizol改良法
中图分类号 S432.1 **文献标识码** A **DOI：** 10.12008/j.issn.1009-2196.2020.07.009

A Modified Trizol Method for Total RNA Isolation from *Bacillusvelezensis*

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Abstract *Bacillus velezensis* is a Gram-positive bacteria, and it has prospects of application in agriculture, environment and fermentation industry. However, it is difficult to extract RNA with high quality and quantity from *Bacillus spp.*, because they have thick cell wall and secrete plenty of proteins that are easy to result in contamination. Therefore, a simple and effective method for total RNA isolation could provide technical support for the gene expression and gene function analysis of *B. velezensis*. A Trizol-based RNA extraction method for *B. velezensis* was optimized by modifying the bacterial cell growth conditions, washing times, liquid nitrogen freezing treatment, and lysozymatic break down of the bacterial cell wall, and a simple and effective method for total RNA isolation of *B. velezensis* was hence established.

Keywords *Bacillus velezensis* ; total RNA extraction ; modified Trizol method

贝莱斯芽孢杆菌（*Bacillus velezensis*）是芽孢杆菌属的一个新种，属于革兰氏阳性菌，存在于土壤、水体、动植物体内[1]。近年来，国内外研究人员相继分离到不同的*B. velezensis*菌株，发现其在促进植物生长、拮抗病原菌及食品发酵等方面发挥良好作用，且对环境友好、易于人工培养，在农业、食品、环保、工业和医药等领域具有重要的理论和应用研究价值[2-5]。获得高质量总RNA是进行RT-PCR、qRT-PCR、cDNA文库构建、Northern杂交、转录组和翻译组测序等研究的基础[6-8]。因此，高效、简便的*B. velezensis*总RNA提取方法对该菌的深入研究和利用至关重要。

目前常见的细菌总RNA提取方法有CTAB法、强变性剂法、SDS-Phenol法及热硼酸法等[7, 9]，这些方法各有优缺点，并根据菌体特性适用于不同细菌类型。有效的细胞裂解是提取高产量细菌总

① 基金项目：云南省科技厅科技计划项目（No.2019FD128），云南省教育厅科学研究基金（No.2018JS007）。
收稿日期：2020-03-22；编辑部E-mail：rdnk@163.com；责任编辑：龙娅丽；排版：龙娅丽。
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RNA的关键步骤。目前细菌细胞壁破碎的方法有酶解法、玻璃珠法和超声波法[10-11]。相对于真核生物，细菌的RNA含量少，半衰期短，易降解；内外环境以及操作体系无处不在的RNA酶（RNase）会导致细菌总RNA提取效果不理想[7]。此外，破碎的细胞内含物常与RNA形成难溶的物质，导致RNA难以分离。而国内关于高质量总RNA提取研究多集中于真核生物，尚缺乏高效、简便和经济的细菌高质量总RNA提取方法。

Trizol法能够快速高效分离不同的生物来源样品的总RNA，所提取的总RNA浓度和纯度高，不需要超速离心或多步酚—氯仿进行抽提[7]。然而，常规Trizol法不能够有效裂解细菌细胞壁，且对RNase的抑制效果并不理想，无法获得高质量的细菌总RNA[9]。因此，针对*B. velezensis*分泌蛋白量大且种类多、细胞壁厚等特点，本研究通过优化菌体培养条件、洗涤菌体次数、溶菌酶裂解细胞、液氮冷冻处理等方法，对传统Trizol法进行改良，获得了高质量的*B. velezensis*总RNA，为该菌的分子生物学研究提供了一种高效、简便和经济的总RNA提取技术。

1 材料与方法

1.1 材料

1.1.1 供试菌株

B. velezensis YQ1菌株由云南大学农学院植物病理研究室分离鉴定并于-80℃中保存。

1.1.2 主要试剂及仪器

Trizol试剂（Invitrogen，美国），0.1% DEPC水，溶菌酶（200 mg/mL）（Solarbio，北京），D 2 000 DNA Marker（天根，北京），Ribopure™Bacteria kit（Invitrogen，美国），台式高速冷冻离心机（Thermofisher，美国）等。

1.2 方法

1.2.1 常规Trizol法

（1）菌体培养及收集：将保存于-80℃的*B. velezensis* YQ1菌株接种于LB液体培养基中进行活化培养，30℃，180 r/min振荡培养16 h。①固体培养条件菌体培养与收集：用接种环蘸取上述活化菌液在LB固体培养基上划线，30℃恒温倒置培养24 h后，取50 mg菌体至1.5 mL离心管中。②液体培养条件菌体培养与收集培养：将1 mL上述活化菌液加入200 mL LB培养基中，30℃，180 r/min振荡培养，待OD_{600}值达到1.0，8 000 g，4℃离心5 min，弃上清后取50 mg菌体至1.5 mL离心管中。

（2）总RNA提取步骤：①往上述离心管加入0.75 mL Trizol试剂，移液器吸打混匀菌体，以最大转速涡旋振荡3～5 min；②室温孵育5～10 min。加入0.2 mL氯仿，上下颠倒混匀，室温孵育2～3 min；③4℃、12 000 g离心10 min；④吸取上清，加入等体积的异丙醇，上下颠倒混匀，置于20℃中冰冻10 min；⑤4℃、12 000 g离心10 min；弃上清，加1 mL 75%乙醇，温和振荡5～10 s；⑥4℃、7 500 g离心5 min；⑦弃上清，超级工作台中晾置5～10 min至乙醇完全挥发；⑧加入30～50 μL DEPC水溶解，获得的总RNA于-80℃冰箱保存。

1.2.2 Trizol改良法

（1）菌体培养条件优化：①固体培养条件下菌体培养时间优化：将活化菌液划线至LB固体培养基上，30℃倒置培养24、36和48 h，分别取50 mg菌体至1.5 mL离心管中。②液体培养条件下菌液OD_{600}值优化：1 mL活化的菌体加入200 mL LB培养基中，30℃振荡培养至菌液OD_{600}值为0.5、1.0、1.5和2.0时，8 000 g，4℃离心5 min，弃上清后取50 mg菌体至1.5 mL离心管中。

（2）菌体DEPC水处理：用25 mL 0.1% DEPC水洗涤菌体，洗涤次数分别设置为1、2、3、4次，充分混匀菌体后，4℃，8 000 g离心5 min，弃上清后取50 mg菌体至1.5 mL离心管中。

（3）溶菌酶处理：向最适DEPC水处理后的菌体中加1 mL DEPC水，混匀后分别加入20、30、40 μL的溶菌酶，37℃静置30 min，4℃、10 000 g离心1 min，收集菌体。

（4）液氮处理：将最适溶菌酶处理后的菌体置于1.5 mL离心管中，液氮冷冻2～3 min，未进行液氮处理的菌体设置为对照。加入Trizol试剂前将上述离心管取出后置于冰上融化。

（5）总RNA的提取：总RNA提取步骤同上述常规Trizol法总RNA提取步骤。

1.2.3 改良Trizol法与试剂盒法的比较

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Ribopure™Bacteria Kit 试剂盒总 RNA 提取方法如下：取 1 mL 菌液（OD_{600}为 1.0），将 250 μL 氧化锆珠滴入 0.5 mL 的螺旋盖中；离心收集样品，加入 350 μL RNA_{WTZ}，涡旋振荡均匀，将 RNA_{WTZ}中的细胞转移到 250 μL 的氧化锆珠，拧紧盖子，涡旋器中最大转速振荡 10 min，充分溶解；4℃，14 000 g 离心 5 min；溶菌产物转移到新的 1.5 mL 离心管，加入 0.2 倍体积的氯仿，混匀，室温孵育 10 min；4℃、14 000 g 离心 5 min；水相转移到新的 1.5 mL 离心管，加 0.5 倍体积的无水乙醇，完全混合，将滤器放入 2 mL 的收集管，将样品转移到滤器中；4℃、14 000 g 离心 1 min；弃滤筒，滤器转入新的滤筒，加 700 μL Wash Solution 1；4℃、14 000 g 离心 1 min；丢弃滤筒，过滤器放入收集管，加 500 μL Wash Solution 2/3；4℃、14 000 g 离心 1 min；弃滤筒，过滤器放入收集管，重复上述步骤一次；4℃、14 000 g 离心 1 min，转移到 2 mL 的收集管；将预热至 95℃的 25～50 μL 洗脱液加到滤池中心；4℃、14 000 g 离心 1 min；重复上述两步一次，获得的总 RNA 于-80℃冰箱保存。

1.2.4　总 RNA 质量及完整性检测

（1）1%琼脂糖凝胶电泳检测：取总 RNA 样品 4～5 μL，于 1%的琼脂糖凝胶电泳检测。200 V 恒压，16 min。凝胶成像仪上观察条带，并记录结果。

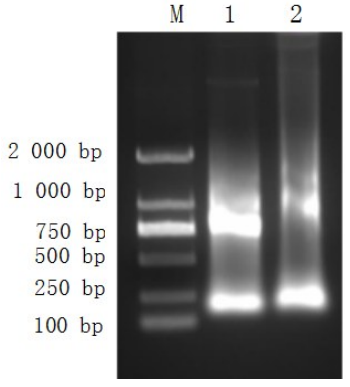
（2）RNA 纯度和浓度检测：取 2 μL 总 RNA 样品，用超微量紫外分光光度计（Thermofisher，美国）分别检测 A_{260}/A_{280}、A_{260}/A_{230}及浓度（ng/μL）。总 RNA 的 A_{260}/A_{280}值为 2.0 且 A_{260}/A_{230}值为 2.0～2.5 时为高纯度总 RNA。

（3）cDNA 的合成和扩增质量鉴定：利用 RT-PCR 法对 Trizol 改良法提取的总 RNA 进行反转录扩增质量鉴定，以常规法提取的总 RNA 作为对照。根据 Takara AMV（V 3.0）Reverse Transcriptase（Takara，大连）的说明进行 cDNA 第一链的合成和 RT-PCR 扩增。RT-PCR 扩增片段为 *B. velezensis* 16S rRNA 片段，所用引物为(F:5'-CTTCGGGTGTTA-CAAACTCTCG-3'；R：5'-TAAGCCAATCCCACAAATCTG-3')，利用 1%的琼脂糖凝胶电泳进行检测。

2　结果与分析

2.1　常规 Trizol 法提取

如图 1 所示，常规 Trizol 法提取的 *B. velezensis* 总 RNA 存在明显的降解，且 A_{260}/A_{280}和 A_{260}/A_{230}值均小于 2.0（表 1），说明有杂质污染。推测培养基残留，*B. velezensis* 培养过程中分泌物多，菌体细胞壁未完全破裂，造成常规 Trizol 法无法提取到该菌完整的总 RNA。



说明：M. D 2 000 DNA Marker；1. LB 固体培养基培养的 *B. velezensis* 总 RNA；2. LB 液体培养基培养的 *B. velezensis* 总 RNA。

图 1　常规 Trizol 法提取 *B. cvelezensis* 总 RNA

表 1　常规 Trizol 法提取 *Bacillus velezensis* 总 RNA 的浓度和质量

培养条件	浓度/（ng•μL^{-1}）	A_{260}/A_{280}	A_{260}/A_{230}
固体 LB 培养基	61.30	1.51	0.16
液体 LB 培养基	80.21	1.26	0.22

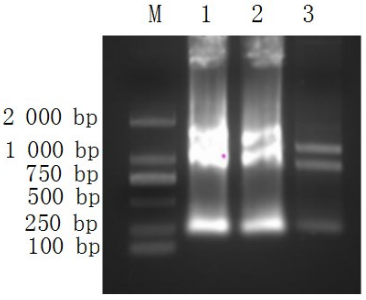
2.2　Trizol 改良法提取

2.2.1　*B. velezensis* 培养条件的优化

在进行菌体培养时，若 *B. velezensis* 生长对数期之前收集菌体，菌体量少；而进入对数生长期后，*B. velezensis* 分泌大量的胞外酶（包括 RNase），严重影响总 RNA 提取质量。为明确菌体培养环境及培养时间对 *B. velezensis* 总 RNA 提取的影响，分别取 LB 固体和液体培养基条件下不同培养时间的菌体进行总 RNA 提取。如图 2 和表 2 所示，在 LB 固体培养基条件下，24 h 的总 RNA 浓度和纯度相对较高，说明在 LB 固体培养基条件下，应尽量选取 24 h（对数生长早期）的菌体进行总 RNA 抽提。但 24、36、48 h 总 RNA 均有不同程度的降解，

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且A_{260}/A_{280}和A_{260}/A_{230}值均小于2.0（表2），说明有蛋白、酚类、多糖等杂质污染。



说明：M.D2 000 DNA Marker；1.24 h；2.36 h；3.48 h。

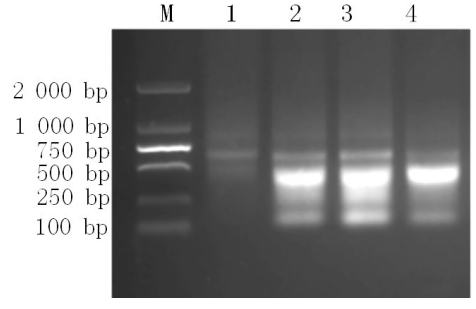
图2　LB固体培养基条件下不同培养时间对*B. velezensis*总RNA提取的影响

表2　LB固体培养基条件下不同培养时间*B. velezensis*总RNA的浓度和质量

时间/h	浓度/（ng•μL^{-1}）	A_{260}/A_{280}	A_{260}/A_{230}
24	361.30	1.53	1.16
36	214.00	1.65	0.65
48	69.20	1.72	1.06

如图3所示，在LB液体培养基条件下当菌体OD_{600}值为1.0和1.5时能够提取质量较高的总RNA，但存在降解，且A_{260}/A_{280}和A_{260}/A_{230}值均小于2.0（表3），说明有蛋白、酚类、多糖等杂质污染；而OD_{600}值为0.5和2.0时总RNA严重降解。说明在LB液体培养基条件下，菌体培养至OD_{600}值为1.0～1.5时是最佳的总RNA提取时间。

2.2.2　不同洗涤次数预处理对*B. velezensis*总RNA提取的影响



说明：M.D2 000 DNA Marker；1.OD_{600}=0.5；2.OD_{600}=1.0；3.OD_{600}=1.5；4.OD_{600}=2.0。

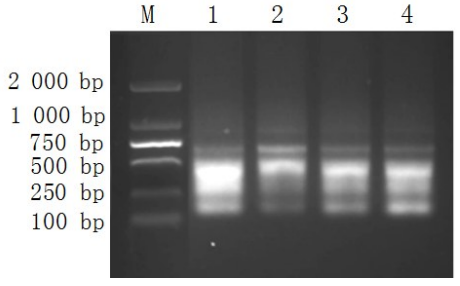
图3　LB液体培养基条件下*B. velezensis*菌体OD_{600}值对总RNA提取的影响

表3　LB液体培养基条件下不同*B. velezensis*菌体OD_{600}值的总RNA浓度和质量

OD_{600}值	浓度/（ng•μL^{-1}）	A_{260}/A_{280}	A_{260}/A_{230}
0.5	52.10	1.54	0.88
1.0	387.20	178	1.12
1.5	298.90	1.77	1.07
2.0	169.70	1.41	0.77

提取的影响

收集菌体时，残留的培养基和菌体分泌物等对总RNA提取存在严重影响，因此本研究采用DEPC水洗涤菌体的方法来减少杂质和RNase影响。为明确洗涤次数对减少杂质和菌体分泌物的效果，分别对LB液体培养基条件下收集的菌体（OD_{600}值为1.0）进行1、2、3、4次洗涤。如图4和表4所示，洗涤2次的*B. velezensis*总RNA质量较高，但均存在一定程度的降解。另外，DEPC水洗涤处理后，总RNA浓度较低，不利于后续进行RNA纯化处理。



说明：M.D2 000 DNA Marker；1.洗涤1次；2.洗涤2次；3.洗涤3次；4.洗涤4次。

图4　DEPC水洗涤处理对*B. velezensis*总RNA提取的影响

表4　DEPC水洗涤处理后*B. velezensis*总RNA的浓度和质量

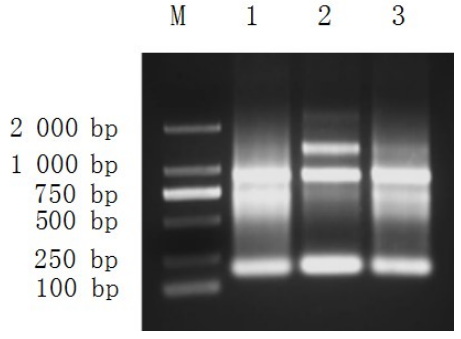
洗涤次数	浓度/（ng•μL^{-1}）	A_{260}/A_{280}	A_{260}/A_{230}
1	97.16	1.57	0.72
2	124.32	1.86	1.38
3	165.70	1.80	0.98
4	67.30	1.39	0.48

2.2.3　溶菌酶处理对*B. velezensis*总RNA提取的影响

为提高*B. velezensis*总RNA提取量，需完全裂解菌体细胞壁，释放核酸。本研究将上述DEPC

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水洗涤 2 次后的菌体，分别用 20 μL(20 mg)、30 μL(30 mg)、40 μL（40 mg）溶菌酶处理菌体。如图 5 和表 5 所示，溶菌酶处理后，总 RNA 浓度得到了明显提高，30 μL(30 mg）溶菌酶处理后提取的总 RNA 质量和浓度最高，但仍存在一定程度降解（表 5)。说明 30 μL(30 mg）溶菌酶能够完全裂解 *B. velezensis*（OD_{600}值为 1.0）细胞壁，保证高浓度的总 RNA 提取量。



说明：M. D 2 000 DNA Marker；1. 20 μL（20 mg）溶菌酶；2. 30 μL（30 mg）溶菌酶；3. 40 μL（40 mg）溶菌酶。

图 5　溶菌酶处理对 *B. velezensis* 总 RNA 提取的影响

表 5　溶菌酶处理后 *B. velezensis* 总 RNA 浓度和质量

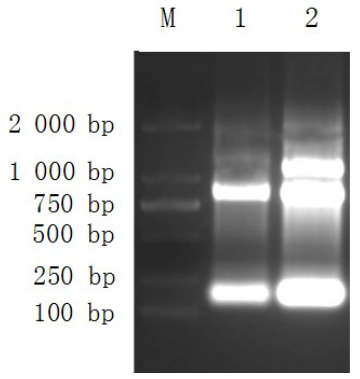
溶菌酶量/μL	浓度/(ng•μL^{-1})	A_{260}/A_{280}	A_{260}/A_{230}
20	1 412.88	1.82	0.85
30	1 821.08	1.98	1.69
40	1 421.32	1.90	1.01

2.2.4　液氮冷冻处理菌体对 *B. velezensis* 总 RNA 提取的影响

通过 DEPC 水洗涤和溶菌酶处理后，提取的总 RNA 仍存在降解情况，说明 *B. velezensis* 体内或提取过程中仍有 RNAase 的影响。为抑制 RNAase 活性，本研究对上述 30 μL（30 mg）溶菌酶处理的菌体进行液氮冷冻。如图 6 所示，提取菌体的总 RNA 可以清晰的看到 23S、16S 和 5S 条带。相较于未进行液氮处理的样品，液氮处理后提取的总 RNA 降解程度少，并且浓度和纯度高（表 6)，说明液氮处理能够有效降低 *B. velezensis* 内源性和提取过程中 RNAase 活性。

2.3　Trizol 改良法和试剂盒法比较

由以上结果确定了 *B. velezensis* 总 RNA



说明：M. D 2 000 DNA Marker；1. 未液氮冷冻处理；2. 液氮冷冻处理。

图 6　液氮冷冻处理对 *B. velezensis* 总 RNA 提取的影响

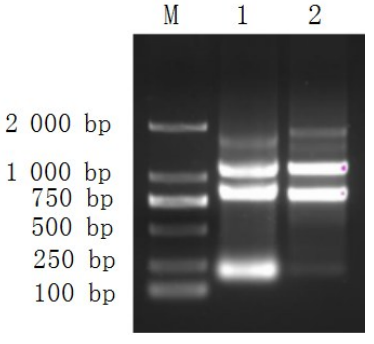
表 6　液氮冷冻处理提取 *B. velezensis* 总 RNA 的质量和浓度比较

处理	浓度/(ng•μL^{-1})	A_{260}/A_{280}	A_{260}/A_{230}
液氮冷冻	3 178.28	2.03	2.12
未液氮冷冻	1 832.40	1.93	1.74

Trizol 改良法。为比较其与试剂盒法提取的总 RNA 效果，选取革兰氏阳性菌总 RNA 提取试剂盒 Ribo-pure™ Bacteria Kit。如图 7 所示，2 种方法均能获得完整和高纯度的总 RNA，而 Trizol 法改良法提取的总 RNA 浓度更高，但相对于试剂盒法提取时间较长（表 7)。另外，试剂盒法提取的单个样本总 RNA 价格比 Trizol 改良法昂贵。因此 Trizol 改良法适用于 *B. velezensis* 大批量样本的总 RNA 提取，而试剂盒法适用于小批量样本的总 RNA 快速提取。

2.4　RT-PCR 扩增检测

以 Trizol 常规法和改良法所提取的总 RNA 为



说明：M. D 2 000 DNA Marker；1. 改良 Trizol 法；2. Ribopure™ Bacteria kit 法。

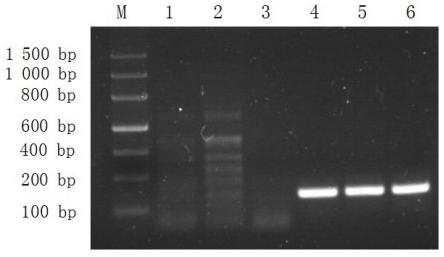
图 7　Trizol 改良法和试剂盒法提取的 *B. velezensis* 总 RNA 比较

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表7　Trizol改良法和试剂盒法提取的*B. velezensis*总RNA浓度和质量

方法	浓度/(ng•μL^{-1})	A_{260}/A_{280}	A_{260}/A_{230}	提取时间/h
改良Trizol法	3 824.20	2.02	2.05	2.0
试剂盒法	1 094.20	2.12	2.81	1.5

模板进行反转录合成cDNA第一链。随后以其为模板，利用*B. velezensis* 16S rRNA的荧光定量PCR引物进行PCR扩增。结果表明，Trizol常规法提取的总RNA无法扩增出16S rRNA片段，而Trizol改良法提取的总RNA能够扩增出明显的16S rRNA片段，且片段大小与预期相符（163 bp），说明Trizol改良法提取的总RNA能够满足RT-PCR要求。



说明：M. DNA Marker；1～3. Trizol常规法；4～5. Trizol改良法。

图8　Trizol常规法和改良法提取*B. velezensis*总RNA的RT-PCR检测结果

3　讨论与结论

*B. velezensis*不仅具有富含肽聚糖的细胞壁，还具有强大的胞外酶分泌系统，同时易产生内源性RNase，这些特点加大了其总RNA提取难度[10-11]。贝莱斯芽孢杆菌在对数生长早期，细胞分裂旺盛，胞内活动主要以细胞分裂相关为主，细胞壁积累较少，分泌物较少，易于破碎细胞并获得高质量的总RNA。由于常规Trizol法无法提取到*B. velezensis*完整的总RNA，因此，本研究比较了不同培养条件下，不同生长时期对*B. velezensis*总RNA提取的影响，结果表明，LB液体培养条件下的*B. velezensis*总RNA提取质量较好，且OD_{600}值为1.0～1.5是最佳提取时期。针对*B. velezensis*具有较强的胞外酶分泌系统特性，本研究通过DEPC水洗涤处理，在一定程度上减少了分泌物和残留培养基的影响，与枯草芽孢杆菌总RNA提取方法的研究结果相符[12]。本研究通过加入适量溶菌酶裂解菌体，提高了总RNA浓度，但该方法并不适用于粪肠球菌和金黄色葡萄球菌[13-14]，推测溶菌酶对不同革兰氏阳性菌的裂解效率存在差异。另外，本研究通过对菌体液氮冷冻处理后发现，液氮能够抑制菌体破裂后的RNAase活性，防止总RNA在提取过程中的降解[10]。本研究还比较了Trizol改良法与试剂盒提取*B. velezensis*总RNA浓度效果，结果表明Trizol改良法更加适合于大批量*B. velezensis*总RNA提取。

本研究提供了一种简便有效的*B. velezensis*总RNA提取方法，为满足*B. velezensis*相关分子生物学研究提供了重要的技术支撑。

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中国农业科技导报，2020，22(8)：178-186
Journal of Agricultural Science and Technology

云南热区咖啡种植地红壤细菌群落多样性分析

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摘　要：为了解云南热区咖啡种植地红壤细菌类群的生态分布情况，利用高通量测序技术分别对云南潞江坝(干热区)和芒市(湿热区)5个不同咖啡种植年限的红壤细菌多样性进行分析。16S rDNA测序质控后，获得五个咖啡种植地土样Clean Reads数平均为80 167，细菌物种数目平均为3 118。2个云南咖啡主产区红壤细菌群落划分为39个已知菌门，其中优势门为变形菌门、酸杆菌门、放线菌门、绿弯菌门、芽单胞菌门、拟杆菌门、浮霉菌门、疣微菌门和厚壁菌门。潞江坝与芒市咖啡种植地红壤的细菌群落组成、优势菌属、相对丰度、多样性指数均存在一定差异。其中，潞江坝咖啡种植5和8 a的红壤细菌物种数目和丰富度均高于芒市咖啡种植4和6 a的红壤，但与芒市咖啡种植20 a的红壤无显著差异。随咖啡种植年限的增加，潞江坝或芒市咖啡种植地红壤的细菌优势属均发生了一定变化，但细菌群落的丰富度和多样性无显著差异。该结果为云南热区咖啡种植红壤的细菌类群及其作用研究提供了参考信息，有助于云南咖啡种植的可持续发展。

关键词：咖啡；红壤；云南热区；细菌多样性；细菌群落结构
doi：10.13304/j.nykjdb.2019.0801
中图分类号：S154.3　　**文献标识码：**A　　**文章编号：**1008-0864(2020)08-0178-09

Diversity of Red Soil Bacterial Community in Coffee-growing Regions of Tropical Yunnan

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Abstract: For better understanding the distribution of red soil bacterial community in tropical coffee-growing regions of Yunnan, diversity of red soil bacterial community with different coffee cultivation years in Lujiangba (dry tropical region of Yunnan) and Mang city (humid tropical region of Yunnan) were investigated by a high-throughput sequencing. The clean reads with an average number of 80 167 and the observed bacterial species with an average number of 3 118 were detected from five soil samples by using 16S rDNA amplicon sequencing technique. A total of 39 bacterial phyla were detected, and Proteobacteria, Acidobacteria, Actinobacteria, Chloroflexi, Gemmatimonadetes, Bacteroidetes, Planctomycetes, Verrucomicrobia and Firmicutes were the most abundant phyla in all red soil bacterial communities. Bacterial community composition, abundance, diversity and dominant genera of red soil in Lujiangba and Mang city were apparently different. The species and richness of red soil bacterial community in coffee-growing region of Lujiangba planted for 5 and 8 a were more and higher than those in Mangshi for 4 and 6 a. But the species and richness of red soil bacterial community in coffee-growing region of Lujiangba had no significant difference with those in Mang city with 20 a cultivation. With increase of coffee cultivation years, the dominant genera

收稿日期：2019-09-26；**接受日期：**2020-02-13
基金项目：国家自然科学基金项目(41661071)；国家重点研发计划项目(2018YFD0201100)；云南省重大科技专项计划项目(2018ZG015)；云南省咖啡体系项目(2019KJTX009-1)；云南省教育厅科学研究基金项目(2019Y0030)。
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of red soil bacterial communities changed, but the bacterial community richness and diversity had no significant difference. These results provided insight into the function of red soil bacterial community in coffee-growing regions of Tropical Yunnan and would contribute to the sustainable development of Yunnan coffee.

Key words: coffee; red soil; tropical Yunnan; bacterial diversity; bacterial community structure

咖啡是全球主要饮料作物之一，具有重要的商业价值。云南热区具有咖啡种植的最佳海拔高度和气候条件，种植面积和产量均占全国咖啡的98%以上[1]。目前，云南咖啡产区主要集中在云南保山、德宏、临沧、普洱等地[2]。随着云南咖啡产业的快速发展，提高咖啡品质、解决栽培技术落后和加工滞后等问题对发展云南高原特色农业具有重要意义。

咖啡优良品质的形成与咖啡的生产量、施肥、耕作方式和自然环境的动态平衡息息相关[3-4]。土壤微生物是指示种植生态系统的重要指标，在种植土壤发育、有机质转化、生态系统平衡、土壤环境净化、生物修复等方面起着不可替代的作用，并且土壤微生物对植物生长和抗逆性具有促进作用。细菌是土壤微生物中数量最大的一个类群，可决定土壤微生物总量的分布、有机物的分解和养分的转化[5-7]。如落叶松人工防护林土壤微生物中细菌数量直接影响土壤微生物总量的变化趋势[8]；镉耐性促生细菌明显改善了镉污染土壤微生物环境，促进植物生长[9]。并且土壤中微生物生物量一定程度上代表该生态系统物质循环能力和支持植被生长发育的能力[10]。然而云南咖啡热区土壤研究主要集中在肥力评价，对土壤微生物的研究还较少[11-13]。

本研究以云南咖啡主产区保山市潞江坝(干热区)和德宏芒市(湿热区)不同年限的咖啡种植地红壤为研究对象，采用高通量测序技术分析其细菌群落结构、细菌种群丰度和多样性的变化，深入了解云南咖啡种植地红壤的细菌生态环境，为维持云南热区咖啡土壤质量和健康、培育优质的咖啡提供参考信息。

1　材料与方法

1.1　研究区域概况

云南热区是热带北缘与南亚热带地区的统称，热量条件≥10 ℃年积温6 000 ℃上，年平均气温>18 ℃，最冷月平均气温>10 ℃，年降雨量600~2 700 mm，干湿季分明，5—10 月为雨季，降雨量占全年的86%，11 月至翌年4 月为干季，降雨量仅占全年的14%，年日可照时数1 750~2 250 h。云南热区总面积8.1 万 km^2 左右，占云南省土地总面积的21%，主要分布在澜沧江、金沙江、怒江、红河、南盘江以及伊洛瓦底江的支流大盈江、龙江流域海拔1 400 m 以下的低热河谷。热区是云南自然地理系统的低热层，是云南巨大的山地系统的组成部分。热区湿润-半湿润区的土壤类型主要是砖红壤和赤红壤，半干旱区的土壤类型主要是燥红壤和褐红壤，pH 范围为4.1~6.5。

本研究区域位于云南咖啡主产地保山市潞江坝和德宏景颇族自治州芒市。德宏芒市属于湿润-半湿润区，保山潞江坝属于半干旱区。总的气候特点为光热充足，水分缺乏，干湿分明，太阳辐射较强。

1.2　样品采集

供试土壤采自云南省保山市隆阳区潞江坝和德宏傣族景颇族自治州芒市不同种植年限咖啡园。2017 年咖啡采摘结束后，按S 形取样法取10 个点作为1 个混合样，每块样地重复采集3 次，采集深度0~20 cm 耕层土壤。土样磨细过2 mm 筛后，保存于-80 ℃备用。供试土壤基本情况见表1。

1.3　土壤微生物总 DNA 提取

采用磁珠法土壤微生物基因组 DNA 提取试剂盒(天根生化科技有限公司)提取样本的微生物基因组 DNA。利用1%琼脂糖凝胶电泳和微量紫外分光光度计(Nanodrop 8 000，USA)检测 DNA 的纯度和浓度。随后取适量的 DNA 样品稀释至1 ng·μL^{-1}。

1.4　PCR 扩增及测序

利用引物515F/806R 扩增细菌16S rDNA 基因的V4 区域[14]。PCR 反应体系30 μL：2×Phusion Master Mix 15 μL，引物3 μL，gDNA 10 μL(5~10 ng)，H_2O 2 μL。PCR 反应程序：98 ℃预变性1 min；98 ℃ 10 s，50 ℃ 30 s，72 ℃ 30 s，循环30 次；72 ℃延伸5 min。PCR 产物质量和浓度通过2%琼脂糖凝胶电泳和微量紫外分光光度计进行检测。随后 PCR 产物等质量混样，充分混

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表1 云南咖啡种植红壤样品基本情况

Table 1 Summary of the red soil samples in coffee-growing regions of tropical Yunnan

样品编号 Soil code	位置 Location	产区 Production area	种植年限 Planting years/a
B5a	N 25°2′49″ E 98°50′5″	潞江坝 Lujiangba	5
B8a	N 25°1′56″ E 98°50′26″	潞江坝 Lujiangba	8
M2013	N 24°18′13″ E 98°32′49″	芒市 Mang city	4
M6a	N 24°21′46″ E 98°27′5″	芒市 Mang city	6
M20a	N 24°21′55″ E 98°25′27″	芒市 Mang city	20

匀后利用2%的琼脂糖胶电泳和GeneJET胶回收试剂盒(Thermo Scientific,USA)进行纯化回收。最后,样品送至北京诺禾致源科技股份有限公司进行土壤微生物高通量测序(基于IonS5TMXL测序平台)。

1.5 数据处理

利用Cutadapt(V1.9.1)[15]对reads进行低质量部分剪切和初步质控得到原始数据(raw reads),去除嵌合体[16-17]后最终获得Clean reads。利用Uparse软件(v7.0.1001)[18]对所有样品的全部Clean reads进行聚类,默认以97%的一致性(identity)将序列聚类成为OTUs(operational taxonomic units)。对OTUs代表序列进行物种注释和分类学信息,并分别在各分类水平(界、门、纲、目、科、属、种)统计各土样细菌群落组成[19-20]。对各土样数据进行均一化处理,最后进行α多样性和β多样性分析。α多样性指数组间差异分析和β多样性指数组间差异分析分别进行Tukey检验和wilcox检验。

2 结果与分析

2.1 测序质量分析

云南省保山市潞江坝和德宏州芒市不同咖啡种植年限的红壤细菌16S rDNA扩增子测序结果如表2所示,五个咖啡种植土样Clean reads数平

表2 云南热区咖啡种植地红壤细菌16S rDNA扩增子测序结果

Table 2 Sequencing quality of 16S rDNA of red soil samples in coffee-growing regions of tropical Yunnan

样品编号 Sample code	Raw reads	Clean reads	Base/nt	Avg Len/bp	Q20/%	GC/%	Effective/%
B5a.1	83 310	80 046	20 229 709	252	89.61	56.08	96.08
B5a.2	87 649	80 099	20 245 396	252	89.43	56.28	91.39
B5a.3	82 383	80 268	20 289 279	252	87.89	56.23	97.43
B8a.1	86 771	80 134	20 246 682	252	90.89	56.18	92.35
B8a.2	88 648	80 119	20 212 387	252	91.16	56.12	90.38
B8a.3	87 737	80 111	20 237 041	252	90.72	56.12	91.31
M2013.1	87 502	80 055	20 255 582	253	89.86	57.45	91.49
M2013.2	87 104	80 015	20 235 319	252	91.39	57.6	91.86
M2013.3	83 166	80 524	20 368 794	252	91.29	57.35	96.82
M6a.1	85 786	80 340	20 307 722	252	89.45	57.62	93.65
M6a.2	86 677	80 139	20 256 277	252	87.85	57.08	92.46
M6a.3	87 330	80 132	20 244 207	252	91.82	57.43	91.76
M20a.1	85 509	80 119	20 245 550	252	92.09	57.23	93.7
M20a.2	83 309	80 197	20 279 512	252	88.8	56.93	96.26
M20a.3	88 787	80 202	20 273 070	252	92.57	57.09	90.33

注:Raw reads是指过滤掉低质量碱基的序列;Clean reads是指过滤嵌合体后,最终用于后续分析的序列;Base是指最终Clean reads的碱基数目;Avg Len指Clean reads的平均长度;Q20(%)是指Clean reads中碱基质量值大于20(测序错误率小于1%)的碱基所占的百分比;GC(%)表示Clean reads中GC碱基的含量;Effective(%)表示Clean reads的数目与Raw reads数目的百分比。

Note: Raw reads are sequences that filter out low quality bases. Clean reads refer to the sequence that is ultimately used for subsequent analysis after filtering chimera; base refers to the number of bases of the final Clean reads; Avg Len indicates the average length of Clean reads; Q20(%) refers to the percentage of bases whose base mass value in Clean reads is greater than 20(sequencing error rate is less than 1%. GC(%) represents GC base content in Clean reads; Effective(%) represents the percentage of the number of Clean reads versus the number of Raw reads.

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均为 80 167，Clean reads 的平均长度为 252 bp，90%以上的碱基质量达到 Q20 水平，表明测序质量良好。

2.2　细菌群落组成和结构分析

2.2.1　不同咖啡种植区域红壤细菌群落结构特征　为研究云南热区咖啡种植地红壤细菌群落的多样性，对所有样品的 Clean reads 进行聚类和注释，五个咖啡种植地红壤细菌在界、门、纲、目、科、属、种分类水平下 OTUs 数目如表 3 所示。细菌分类学分析结果表明，五个咖啡种植地土样中共检测出细菌 39 门、109 纲、145 目、276 科、458 属。所有土样中优势细菌门(相对丰度>1%)为：变形菌门(Proteobacteria)、酸杆菌门(Acidobacteria)、放线菌门(Actinobacteria)、绿弯菌门(Chloroflexi)、芽单胞菌门(Gemmatimonadetes)、拟杆菌门(Bacteroidetes)、浮霉菌门(Planctomycetes)、疣微菌门(Verrucomicrobia)和厚壁菌门(Firmicutes)(表 4)。

表 3　云南热区咖啡种植地红壤细菌在各分类水平下的 OTUs 数目

Table 3　Number of OTUs of red soil samples in coffee-growing regions of tropical Yunnan at the different classification levels

样品名称 Sample name	界 Kingdom	门 Phylum	纲 Class	目 Order	科 Family	属 Genus	种 Species
B5a	66 443 b	65 838 a	64 475 a	59 706 ab	50 603 ab	33 256 a	5 094 a
B8a	67 663 ab	67 015 a	65 679 a	60 612 a	51 576 a	34 315 a	3 979 bc
M2013	68 667 a	66 002 a	65 243 a	54 517 c	47 579 c	23 398 d	4 002 b
M6a	66 616 ab	64 697 a	64 014 a	57 695 b	48 433 b	25 846 b	3 612 c
M20a	68 772 a	66 745 a	65 852 a	55 860 c	46 729 c	24 940 c	4 116 b

注：同一列不同字母表示差异具有统计学意义($P<0.05$)。

Note: Different letters in the same column indicate significant difference ($P<0.05$).

表 4　云南热区咖啡种植地红壤细菌群落相对丰度前 10 菌门的相对丰度

Table 4　Relative abundance of the top 10 phylum of red soil bacterial community in coffee-growing regions of tropical Yunnan

门 Phylum	B5a	B8a	M2013	M6a	M20a
变形菌门 Proteobacteria	36.31±0.52 d	37.72±0.34 d	22.31±0.31 a	24.49±0.28 b	27.89±0.99 c
酸杆菌门 Acidobacteria	13.84±0.30 a	13.83±0.51 a	20.16±1.00 b	20.90±0.46 b	19.46±0.75 b
放线菌门 Actinobacteria	19.99±0.58 b	16.74±0.49 a	16.28±0.60 a	20.20±0.84 b	17.48±0.30 a
绿弯菌门 Chloroflexi	2.88±0.20 a	2.91±0.18 a	19.39±0.25 d	16.83±1.32 c	13.46±0.27 b
芽单胞菌门 Gemmatimonadetes	6.84±0.17 c	7.29±0.32 c	3.72±0.16 b	2.84±0.23 a	2.71±0.18 a
拟杆菌门 Bacteroidetes	5.71±0.48 c	6.21±0.26 c	1.80±0.15 a	2.91±0.39 b	2.39±0.17 ab
浮霉菌门 Planctomycetes	3.05±0.09 a	3.66±0.10 ab	3.90±0.30 b	3.24±0.29 ab	4.85±0.14 c
疣微菌门 Verrucomicrobia	3.09±0.10 b	3.34±0.12 b	3.42±0.15 b	2.17±0.09 a	4.30±0.35 c
厚壁菌门 Firmicutes	2.81±0.33 b	2.57±0.24 b	1.17±0.12 a	1.16±0.24 a	1.19±0.16 a
装甲菌门 Armatimonadetes	0.45±0.02 a	0.50±0.02 a	1.66±0.12 c	0.49±0.03 a	0.96±0.03 b
其他菌门 Others	5.02±0.07 ab	5.24±0.22 b	6.20±0.09 c	4.77±0.07 a	5.32±0.13 b

注：同行不同字母表示差异具有统计学意义($P<0.05$)。

Note: Different letters in the same row indicate significant difference ($P<0.05$).

另外，5 个咖啡种植地土样的相对丰度前 35 的菌属中均存在氨氧化微生物(*Ammonia-oxidizing archaea*，AOA) Group I. 1 a-associated (*Candidatus Nitrosotalea*)，并且在潞江坝地区丰度较高(0.43%~0.67%)，说明氨氧化古菌(AOA)可能是云南热区咖啡种植地红壤的主要氨氧化微生物(图 1)。

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注: 不同颜色块代表相对物种丰度值,由蓝到红,相对丰度逐渐升高,只显示丰度最高的前 35 个属。

Note: Different color represents the relative species abundance value, and the relative abundance gradually increases from blue to red. Only top 35 genera with the highest abundance are shown.

图 1 云南热区咖啡种植地红壤在属水平上的细菌群落的物种丰度聚类

Fig.1 Clustering of bacterial community structures of red soil samples in coffee-growing regions of tropical Yunnan at level of genus

潞江坝与芒市咖啡种植区红壤细菌在群落组成和丰度上均存在明显差异(图 1 和表 4)。相对于芒市,潞江坝咖啡种植土样中的变形菌门(潞江坝平均为 37.02%,芒市平均为 24.90%)、芽单胞菌门(潞江坝平均为 7.06%,芒市为 3.09%)、拟杆菌门(潞江坝平均为 5.96%,芒市平均为 2.37%),以及厚壁菌门(潞江坝平均为 2.69%,芒市平均为 1.17%)相对丰度显著较高,而酸杆菌门

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(潞江坝平均为 13.83%,芒市平均为 20.17%)、绿弯菌门(潞江坝平均为 2.90%,芒市平均为 16.56%)和装甲菌门(潞江坝平均为 0.47%,芒市平均为 1.03%)相对丰度显著较低(表 4)。

另外,潞江坝与芒市咖啡种植地红壤细菌优势属(相对丰度>1%)也明显不同。B5a 细菌优势属共 9 个,分别为 *Mizugakiibacter*(8.54%)、*Rhizomicrobium*(2.34%)、*Gemmatimonas*(2.7%)、*Rhodococcus*(1.89%)、*Bryobacter*(1.67%)、*RB41*(1.40%)、*Nocardioides*(1.00%)、*Sphingomonas*(3.32%)、unidentified_*Mitochondria*(1.11%);B8a 细菌优势属共 8 个,分别为 *Mizugakiibacter*(8.97%)、*Rhizomicrobium*(3.12%)、*Gemmatimonas*(3.01%)、*Bryobacter*(1.71%)、*RB41*(1.30%)、*Sphingomonas*(2.94%)、unidentified_*Mitochondria*(1.51%)、*Granulicella*(1.07%)。而 M2013 细菌优势属共 11 个,分别为 *Blastococcus*(1.12%)、unidentified_*TK10*(2.25%)、*Gemmatimonas*(1.33%)、*Rhodanobacter*(1.14%)、*Bryobacter*(2.86%)、*Bradyrhizobium*(1.78%)、*Arthrobacter*(1.32%)、*Candidatus Solibacter*(2.37%)、*Sphingomonas*(1.26%)、*Acidothermus*(1.75%)、*Anaeromyxobacter*(1.24%);M6a 细菌优势属共 11 个,分别为 *Mizugakiibacter*(1.73%)、*Rhizomicrobium*(1.80%)、*Actinospica*(1.04%)、*Bryobacter*(3.18%)、*Bradyrhizobium*(1.73%)、*Burkholderia Paraburkholderia*(1.11%)、*Arthrobacter*(1.70%)、*Candidatus Solibacter*(2.13%)、*Pseudarthrobacter*(1.07%)、*Acidibacter*(1.33%)、*Acidothermus*(4.63%);M6a 细菌优势属共 8 个,分别为 *Rhizomicrobium*(2.01%)、*Bryobacter*(2.21%)、*Bradyrhizobium*(2.20%)、*Burkholderia Paraburkholderia*(2.56%)、*Candidatus Solibacter*(2.31%)、*Crossiella*(1.01%)、*Acidothermus*(2.95%)、*Variibacter*(1.24%)。

2.2.2　不同咖啡种植年限红壤细菌群落结构特征　随咖啡种植年限增加,潞江坝咖啡种植地土样中放线菌门的相对丰度减少了 3.25%;芒市咖啡种植地土样中变形菌门的相对丰度提高了 5.58%,而绿弯菌门和芽单胞菌门的相对丰度分别减少了 5.94% 和 1.01%;另外,放线菌门(20.20%)的相对丰度在 M6a 土样中丰度最高,而浮霉菌门(4.85%)和疣微菌门(4.30%)在 M20a 样品中丰度最高(表 4)。

2.2.3　云南热区咖啡种植地红壤细菌群落结构的主成分分析　为了解潞江坝和芒市不同咖啡种植年限的红壤细菌群落结构差异,对其进行了基于 OUT 水平的主成分分析(PCA),提取出最大程度反映样品间差异的两个主成分,贡献率分别为 28.34%和 15.96%。潞江坝和芒市的不同土样围绕 PC1 轴分开,潞江坝 B5a 和 B8a 分布在 PC1 的负轴,芒市 M2013、M6a 和 M20a 分布在 PC1 的正轴,说明云南干热区和湿热区的咖啡种植地红壤细菌群落结构差异明显(图 2)。另外,潞江坝 B5a 与 B8a 的细菌群落结构无明显差异,均分布在 PC2 负轴;而芒市 M2013 和 M6a 与 M20a 的细菌群落结构差异明显,M2013 和 M6a 分布在 PC2 正轴,而 M20a 分布在 PC2 负轴(图 2)。

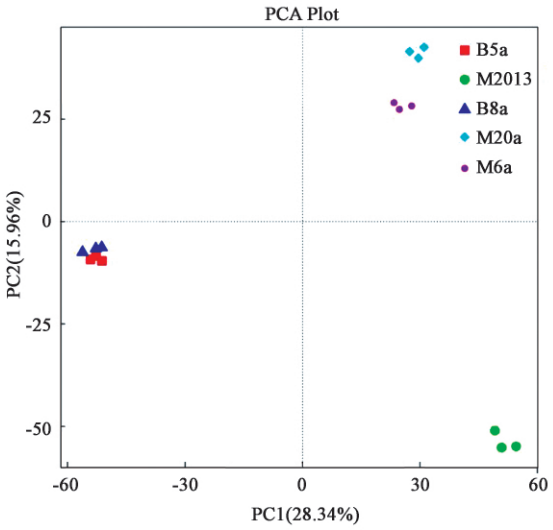


图 2　云南热区咖啡种植地红壤细菌群落 PCA 分析

Fig.2　Principal component analysis (PCA) of bacterial community composition of red soil samples in coffee-growing regions of tropical Yunnan

2.3　云南热区咖啡种植地红壤细菌群落的多样性分析

潞江坝 B5a 和 B8a 的细菌种类数目、丰富度(chao1 和 ACE 指数)均比芒市 M2013 和 M6a 多,但与 M20a 无显著差异,说明潞江坝咖啡种植 8 a 后和芒市 20 a 后红壤细菌种类数目和丰富度无明显差异,且在潞江坝咖啡种植 8 a 内和芒市咖啡种植 6 a 内并不能显著提高红壤细菌种类数目和丰富度(表 5)。潞江坝 B5a 与 B8a 的多样性指数 shannon 无显著差异,但与 M20a 差异显著;而

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芒市红壤细菌多样性指数 shannon 随着咖啡种植年限的增加而提高(表 5)。另外,潞江坝 B5a 与 B8a 的多样性指数 simpson 无显著差异,但与芒市 3 个咖啡种植地土样差异显著; 芒市红壤细菌多样性指数 simpson 随着咖啡种植年限的增加并无显著提高(表 5)。以上结果表明,咖啡种植年限增长对云南热区红壤细菌群落的丰富度和多样性无明显提高。

表 5 云南热区咖啡种植地红壤细菌群落的 α 多样性指数

Table 5 Bacterial diversity indexes of red soil samples in coffee-growing regions of tropical Yunnan

样品名称 Sample Name	种类 Observed Species	多样性 Diversity		丰富度 Richness		覆盖率 Goods coverage
		shannon	simpson	chao1	ACE	
B5a	3 303 b	9.401 ab	0.995 a	3 572.489 b	3 615.500 b	0.992 a
B8a	3 409 b	9.497 b	0.995 a	3 695.641 b	3 749.107 b	0.992 a
M2013	2 762 a	9.309 a	0.996 b	2 986.952 a	3 006.130 a	0.994 b
M6a	2 790 a	9.482 b	0.997 bc	3 023.702 a	3 057.376 a	0.993 b
M20a	3 329 b	9.876 c	0.997 c	3 602.652 b	3 628.620 b	0.992 a

注: 同一列不同字母表示差异具有统计学意义($P<0.05$)。

Note: Different letters in the same column indicate significant difference ($P<0.05$).

3 讨论

微生物类群数量是灵敏指示土壤环境质量变化的生物学指标之一,反映了土壤环境生态功能的演变规律[21]。云南保山市潞江坝和德宏芒市是云南主要咖啡种植区,其红壤优势细菌主要为变形菌门、酸杆菌门、放线菌门、绿弯菌门、芽单胞菌门、拟杆菌门、浮霉菌门、疣微菌门和厚壁菌门。本研究结果与稻田红壤和有机农田红壤的根际土壤细菌群落研究结果相似[22-24]。目前研究表明,氨氧化古菌(AOA)倾向于在酸性和低氮的寡营养环境中起作用,而红壤酸性强、有机质含量低、养分匮乏,因此,AOA 在红壤细菌中主导氨氧化作用[25-27]。本研究发现,在潞江坝和芒市咖啡种植地红壤中 AOA 丰度显著高于其他氨氧化细菌,可能是云南热区咖啡种植地红壤的关键氨氧化微生物。另外,2 个咖啡种植地的红壤中均发现有利于咖啡生长的优势菌属,如 *Sphingomonas*(降解化学杀虫剂)[28],*Blastococcus*(抵御不良环境、防治土壤病害)[29],*Arthrobacter*、*Acidothermus*、*Bryobacter* 和 *Candidatus Solibacter*(分解有机质,促进土壤碳循环)[30-33]。因此,研究云南热区咖啡种植红壤的细菌类群及其作用,将为保持云南咖啡生态系统平衡,促进云南咖啡产业的可持续发展提供重要参考。

咖啡种植年限低于 8 a,保山潞江坝相对于芒市咖啡种植地的红壤细菌种类多,并且细菌丰富度也较高,说明云南干热区相较于湿热区可能更有利于红壤细菌的生长。这可能与不同热区的海拔维度、气候条件、土壤含水量、土壤肥力、咖啡根系生物量、农业管理措施等因素密切相关。土壤微生物的生态系统复杂,并且时刻处于动态平衡的变化过程中,影响云南热区土壤微生物数量变化的主控因子还有待深入研究。

另外,不同咖啡种植年限下云南热区咖啡种植地红壤细菌在群落组成和丰度存在一定差异。随咖啡种植年限增加,潞江坝或芒市咖啡种植地红壤一些细菌优势属发生了变化,并且芒市咖啡种植地的红壤细菌群落组成相对潞江坝变化较大,但 2 个区域咖啡种植地红壤细菌群落的丰富度和多样性并无明显提高。目前不同种植年限下红壤微生物多样性研究还较少,有的研究发现土壤微生物数量和多样性随着种植年限的增加而增多[34-35],有的研究结果却不同[36-39]。这可能与土壤采样时间、采样深度、采样点肥力差异等有关,特别是与土壤的最高种植年限密切相关。最高种植年限不同,土壤的结构、通气性、水分状况、养分等理化性状均会发生变化,因而对土壤微生物多样性产生了重要影响。

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引用格式：何　迷，李小波，施继芳，等 . 低温对水稻光合特性的影响 [J]. 湖南农业科学，2020（8）：12-15.
DOI:10.16498/j.cnki.hnnykx.2020.008.004

低温对水稻光合特性的影响

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摘　要：光合作用在农作物生长发育过程中起到至关重要的作用，也是其产量高低的决定性因素，而低温冷害是影响水稻生长发育和产量的严重自然灾害之一，故前人就低温冷害对水稻光合特性影响展开了大量研究。综述了低温冷害对栽培稻和野生稻各生长发育时期包括光合速率、气孔导度、胞间 CO_2 浓度、蒸腾速率等光合特性指标的影响。同时从选育抗低温品种、采取合理使用外源物质及改变灌水层高度等有效栽培措施等方面提出了增强水稻抗低温能力的建议，对保证水稻在低温冷害时维持正常产量具有指导意义。

关键词：栽培稻；野生稻；低温；光合特性；综述
中图分类号：S511　　**文献标识码**：A　　**文章编号**：1006-060X（2020）08-0012-04

Effects of Low Temperature on Photosynthetic Characteristics of Rice

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Abstract：Photosynthesis not only plays an important role in the growth and development of crops, but also plays a decisive role in the yield. Low temperature and chilling injury is one of the serious natural disasters that affect the growth and yield of rice. Therefore, many studies have been carried out on the effects of low temperature and chilling injury on photosynthetic characteristics of rice. In order to provide theoretical guidance for maintaining normal yield of rice under chilling injury, the effects of chilling injury on photosynthetic characteristics of cultivated rice and wild rice at different growth stages, including photosynthetic rate, stomatal conductance, intercellular CO_2 concentration and transpiration rate, were reviewed. At the same time, the prospect was put forward from the aspects of breeding low temperature resistant varieties, rational use of exogenous substances and changing the height of irrigation layer, so as to improve the low temperature resistance of cultivated rice and wild rice, and ensure that they still have normal photosynthetic capacity under low temperature conditions, which has theoretical significance for ensuring the normal yield of rice under low temperature and cold injury.
Key words：cultivated rice; wild rice; low temperature; photosynthetic characteristics; review

水稻在我国粮食作物中种植面积最大、总产量最高 [1]，是典型的喜温喜光作物，其对温度条件敏感，在不同的生育时期均容易受到低温冷害的影响 [2-3]。低温对水稻生产的影响不仅是农业领域研究的重点，也一直是水稻气象研究的重点问题 [4-10]。我国水稻栽培历史悠久，稻谷总产量居世界总产量首位，遗传资源数量多且类型丰富，水稻杂种优势利用研究以及杂交稻的大面积种植居世界领先地位，对全球稻作科技的发展做出了重要贡献 [11]。在我国所有种植水稻的地区均发生过冷害，而且重大冷害发生频率 5 a 内就有一次，受灾严重年份水稻产量减少 50 亿 ~100 亿 kg[12]，对我国粮食安全产生重大影响。光合作用是作物生长发育和产量形成的生理基础，也是作物生产力高低的决定因素 [13]。而低温冷害对作物光合特性影响是直接导致水稻产量降低的主要原因，当水稻遇到低温冷害时，光合作用削弱的同时也降低了呼吸作用，改变膜脂过氧化与抗氧化系统，进而降低水稻抗寒能力，抑制水稻生长，最终导致产量降低 [14-17]。前人就低温冷害对水稻光合特性，包括生长发育时期光合速率、气孔导度、胞间 CO_2 浓度、蒸腾速率等光合特性指标的影响做了大量研究。栽培稻起源于野生稻，野生稻作为珍贵的种质资源，具有耐冷、耐旱、抗病、抗虫、抗贫瘠、高效营养、高产优质、药用等诸多优良基因，可以作为栽培稻改良和优良基因挖掘的重要资源 [18]。栽培稻在低温条件下光合特性也会受到影响 [19]。笔者就前人研究的低温对栽培稻和野生稻不同生育时期光合特性的影响进行综述，为水稻耐冷品种选育和低温环境条件下水稻栽培技术措施制定提供参考。

收稿日期：2020-01-15
基金项目：国家重点研发计划“七大农作物育种”重点专项（2017YFD0100201）；云南省科技计划项目青年项目（2018FD006）
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1　低温对水稻苗期光合特性影响

1.1　栽培稻苗期光合特性研究

苗期是水稻培育壮秧的关键时期，秧苗素质对产量的影响很大。如果水稻苗期遭遇低温冷害，必定会影响秧苗素质，降低产量 [20]。研究表明，在低温胁迫条件下，水稻幼苗的叶绿素含量、净光合速率、气孔

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导度、蒸腾速率均明显下降，叶绿素荧光参数有明显变化[21-27]。低温胁迫下，叶片呼吸强度降低，而叶片呼吸速率（r）与光合速率（po）关系密切，温度越低，po/r 的比值越大，说明呼吸作用降低越明显[28]，同时水稻叶片叶绿素的合成受到抑制或叶绿体功能紊乱，细胞膜透性增大，导致光合速率降低，气孔导度降低，最终导致产量下降。

1.2　野生稻苗期光合特性研究

徐孟亮等[29]研究表明，冷胁迫后，与典型籼稻、爪哇稻，典型粳稻 3 种不同类型的栽培稻相比，茶陵野生稻净光合速率和光系统Ⅱ光化学量子效率的变化小于典型籼稻和爪哇稻，大于典型粳稻，说明茶陵野生稻苗期耐冷性强于籼稻和爪哇稻，但弱于粳稻。黄芸香[30]研究中与中花 11（栽培稻对照）相比，干旱预处理 + 自然低温胁迫条件下，东乡野生稻、茶陵野生稻、柳江野生稻、海南野生稻叶绿素含量均高于对照；海南野生稻的最大光合速率、气孔导度、表观量子效率等气体交换参数以及叶绿素荧光参数均有高于对照的总趋势。陈志[31]研究表明，茶陵野生稻苗期耐冷性与其抗氧化系统冷胁迫后的适应性变化密切相关，对低温逆境响应迅速并具有较强的恢复能力。由此可见，与栽培稻相比，大多数野生稻均有较好的耐冷性。

2　低温对水稻幼穗期光合特性影响

2.1　栽培稻幼穗期光合特性研究

水稻的幼穗期是水稻生长发育关键的时期，若在该时期遭遇低温胁迫，将直接影响水稻的产量[32]。低温胁迫下，水稻幼穗期及其分蘖期叶片中叶绿素含量明显降低，光合速率、净光合速率、蒸腾速率明显呈下降趋势[33-38]。

2.2　野生稻幼穗期光合特性研究

易向军[39]研究表明，在幼穗期前的分蘖期，低温条件下，茶陵野生稻分蘖期叶片的净光合速率稍高于供试的栽培稻；其 PSⅡ最大的光化学量子效率（Fv/Fm）、光下 PSⅡ反应中心的激发能捕获效率（Fv'/Fm'）与非光化学淬灭系数（NPQ）均大于栽培稻，表现出了较强的光合作用能力；而邓志瑞等[40]研究表明，武育粳 8 号生长过程中，剑叶的叶绿素含量和光合速率都高于普通野生稻，这与其他相关研究有些许不同，可能与水稻品种类型、特性及其环境等因素有关。

3　低温对水稻孕穗期光合特性影响

3.1　栽培稻孕穗期光合特性研究

水稻的孕穗期是关系到水稻产量形成的关键时期[41]，相关研究结果表明，低温胁迫下，孕穗期栽培稻在叶片净光合速率、气孔导度及其蒸腾速率等方面均有明显的下降趋势[42-45]，植株最大光合速率，光饱和点及光能初始利用率随胁迫时间延长而下降、光化学淬灭系数，PS II 电子传递速率及 PS II 最大光能转化效率呈现先降后升趋势[46-47]。其原因可能是低温致使叶绿体的结构发生改变，表现在片层排列方向发生变化，基粒片层堆叠紧密，出现空泡甚至空洞[48]，因而叶绿素含量和光合速率下降，造成光合同化物减少，同时暗反应受到严重损害，对栽培稻的后期生长也有很大影响，从而降低产量；相关研究表明在低温持续 3 d 时，Ls（气孔限制值）逐渐升高，表明此时低温导致倒 2 叶光合速率降低的原因主要是气孔限制因素，而在低温持续 5 d 时，Ls 则逐渐降低，表明此时光合速率降低的原因是非气孔因素。这与朱红[49]抽穗后低温胁迫对水稻若干生理特性的影响研究中低温导致水稻叶片光合速率的下降的原因是非气孔因素作用的结果有些许出入。结果有待进一步研究验证。

3.2　野生稻孕穗期光合特性研究

刘凤霞等[50]研究结果表明，东乡野生稻孕穗开花期具有较强的耐冷性；杨志奇[51]研究结果也表明中国粳稻地方品种孕穗期耐冷性强且遗传具有多样性。由此可见，相比栽培稻而言，低温胁迫下，野生稻的光合特性会强于栽培稻，但这与野生稻及栽培稻的类型、生态环境等因素有关。

4　低温对水稻抽穗扬花期光合特性影响

4.1　栽培稻抽穗开花期光合特性研究

抽穗开花期若遭遇低温，不但影响水稻受精结实，还影响水稻叶片的光合作用和叶绿素的合成能力[52]。研究结果表明，低温胁迫下，抽穗开花期稻株叶片叶绿素含量、净光合速率、蒸腾速率、叶片气孔导度和胞间 CO_2 浓度均有不同程度的降低[53-55]，导致产量下降。

4.2　野生稻抽穗开花期光合特性研究

有研究指出普通野生稻耐冷性 > 粳稻耐冷性 > 籼稻耐冷性[56]；陈大洲[57]研究表明，东乡野生稻在抽穗开花期有较强耐冷性。低温导致水稻光合功能下降，最终导致减产，而野生稻具有较强的耐冷性，可见其光合功能下降的速率会低于栽培稻，与栽培稻相比还是具有较强优势的。

5　总　结

低温胁迫在栽培稻和野生稻的各个生长阶段都产生了影响，主要表现为对水稻叶片光合特性的影响，进而影响产量和品质。与栽培稻相比，大多野生稻具有较强的耐冷性，这与野生稻的生长环境及其适应性是密切相关的。

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6 展 望

综合来看野生稻在低温条件下，仍然能保持较高的光合能力，因此，挖掘野生稻有利于耐冷基因对栽培稻进行改良，提高栽培稻耐冷性，是培育耐低温栽培稻品种途径之一。当然影响水稻光合特性的因素不止低温冷害，还包括光照、水分、二氧化碳浓度、栽培方式以及水稻本身对各种环境条件下光合作用的恢复机制强弱等。水稻低温耐受性不仅受品种本身遗传特性影响，与低温条件栽培措施也密切相关，孙磊等[58-59]研究表明，低温条件下喷洒壳聚糖可以维持叶片较高的光化学效率，提高水稻抗低温胁迫的能力。杨军等[60]研究结果表明，降温期灌水和叶面喷施磷酸二氢钾，提高了早稻的叶绿素含量、光合速率和产量。陈海燕[61]研究表明，在低温胁迫条件下，合理使用硅制剂能增强水稻抗低温逆境能力，可见对于水稻光合能力也会有所提高。综上所述，在低温条件下，通过合理使用一些外源物质、改变灌水层高度等栽培措施也是提高水稻低温冷害后光合能力的重要途径。

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引用格式：程　卯，黄光福，李红平，等 . 一种两收模式下多年生稻产量潜力初探 [J]. 湖南农业科学，2020（10）：31-35.
DOI:10.16498/j.cnki.hnnykx.2020.010.009

一种两收模式下多年生稻产量潜力初探

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摘　要：以多年生稻品系 PR25 和再生稻品种渝粳优 5029（RR1）、甬优 1540（RR2）、佳辐占（RR3）、宜优 673（RR4）、内优 7075（RR5）为材料，采用单因素试验探讨多年生稻与再生稻产量、产量构成、生育期、SPAD、根系活力等性状的异同。结果表明：PR25 产量与再生稻相比，头季持平或者略低，第二季 PR25 产量为 471.10 kg/667m^2，显著高于所有再生稻品种，较 RR1、RR2、RR3、RR4 和 RR5 分别增产 48.22%、34.79%、60.95%、31.71%、45.58%；周年产量 PR25 为 1 031.63 kg/667m^2，亦显著大于再生稻品种，较 RR1、RR2、RR3、RR4 和 RR5 分别增产 5.82%、10.41%、47.00%、13.19% 和 42.66%。多年生稻 PR25 再生季的周年产量较再生稻高的主要原因是：多年生稻再生季的单位面积颖花数、有效穗数和结实率均高于再生稻的再生季；PR25 头季齐穗后剑叶 SPAD 衰减幅度较小，剑叶具有较强的光合能力，头季成熟期根系维持在较高活力水平且再生能力较强；多年生稻再生季营养生长期至营养生长与生殖生长并进期明显长于再生稻，进而全生育期较再生稻呈延长趋势，利于干物质积累。

关键词：多年生稻；再生稻；产量及其构成；产量潜力
中图分类号：S511　　**文献标识码**：A　　**文章编号**：1006-060X（2020）10-0031-05

Preliminary Study on Yield Potential of Perennial Rice Under Two-Harvest Model

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Abstract: A single factor experiment was conducted to investigate the differences of yield, yield components, growth period, SPAD and root activity between the perennial rice line PR25 and the ratooning rice varieties Yujingyou 5029 (RR1), Yongyou 1540 (RR2), Jiafuzhan (RR3), Yiyou 673 (RR4) and Neiyou 7075 (RR5). The results showed that: (1) the yield of PR 25 in the first season was approximate to or slightly lower than those of the ratooning rice varieties; in the second season, PR25 yielded 7 066.5 kg/hm^2, which was 48.22%, 34.79%, 60.95%, 31.71% and 45.58% higher than RR1, RR2, RR3, RR4 and RR5, respectively, reaching the significant levels; the annual yield of PR25 was 15 474.45 kg/hm^2, 5.82%, 10.41%, 47.00%, 13.19% and 42.66% higher than that of RR1 RR2, RR3, RR4 and RR5, respectively, reaching significant increases; (2) the main reason why the annual yield of PR25 in ratooning season was higher than that of ratooning rice was that the number of spikelets per unit area, effective panicles and seed setting rate of PR25 were higher than those of ratooning rice; after full heading, the flag leaf of PR25 had a strong photosynthetic capacity with a slight decrease of SPAD value; at the maturity period of the first season, PR25 maintained a high root activity and a strong regeneration ability; the period from vegetative growth to vegetative-reproductive growth of perennial rice was significantly longer than that of ratooning rice, furthermore, the whole growth period of perennial rice was longer than that of ratooning rice, which was beneficial to dry matter accumulation.

Key words: perennial rice; ratooning rice; yield and its composition; yield potential

再生稻是通过一定的栽培管理措施，使头季稻收割后稻桩上的休眠芽继续萌发生长成穗而收获的一季水稻。再生稻具有生育期短、日产量较高、省种省工节水、生产成本低和效益高等优点，是南方稻区种植一季稻热量有余而种植双季稻热量又不足的稻田提高复种指数、增加单位面积稻谷产量和经济收入的有效措施之一[1]。多年生稻为收割后，留下的稻桩安全越冬，第二季或第二年通过栽培手段使其再萌发成苗，形成新的稻株，如此周而复始，实现水稻一次种植、多（季）年收获，双季稻区可一年收 2 季，一季稻区一年收获 1 季，可以实现多年连续收获；种植多年生稻具有省种、省工等节本增效的优点[2]。Hu 等[3]在解析长雄野生稻地下茎无性繁殖特性遗传规律基础上，利用长雄野生稻无性繁殖特性成功培育出系列多年生稻品种（系），包括多年生稻 23（PR23）、PR25、PR107 等品种（系）。其中，PR23 在云南和老挝试种，表现出广泛的适应性和较强的多年生性[4]，并在 2018 年通过云南省审定，在云南不同生态区种植，产量在年份间基本稳定，与当地常规稻品种产量基本持平，米质受大米加工企业和广大消费者喜爱。多年生稻从

收稿日期：2020-05-10
基金项目：科技部“粮食丰产增效科技创新”专项（2016YFD0300508）
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第二季起，省去买种、育秧、犁田、耙田、移栽等环节，与再生稻相比可以更加节约生产成本，大幅度提高效益[5]，目前在云南适宜地区已经开始推广种植。而多年生稻品系 PR25，比 PR23 多年生性更强、米质更优。

研究表明，从头季稻收割后到再生稻成熟生育期仅 60 d 左右，与同期抽穗主季稻源库流相比，再生稻叶面积指数为主季稻的 1/8~1/5，穗粒数和产量为主季稻的 1/3 左右，但齐穗至齐穗后 10 d 再生稻光合速率较主季稻高 20%~70%，孕穗至成熟期再生稻净同化率是主季稻的 2.7~6.0 倍[6]。成熟期单茎鞘干质量大、茎鞘物质输出率高，有利于再生稻的高产[7]。徐富贤等[8]研究指出，头季稻齐穗到成熟叶片 SPAD 值衰减指数可作为鉴定再生力的指标。郑景生等[9]认为再生季稻再生分蘖的生育依赖于头季稻残留的根系，再生季稻穗数及产量与头季稻成熟期和再生季稻齐穗期根系活力呈极显著线性正相关。因此，头季稻成熟期根系伤流量可作为诊断再生力、筛选再生稻品种的重要指标。由此可知，再生稻头季成熟期物质积累、产量构成、剑叶 SPAD 值、根系伤流与再生季产量等再生指标密切相关。研究以多年生稻品系 PR25 和再生稻品种渝粳优 5029（RR1）、甬优 1540（RR2）、佳辐占（RR3）、宜优 673（RR4）、内优 7075（RR5）为材料，采用单因素试验探讨多年生稻与再生稻产量、产量构成、生育期、SPAD、根系活力等性状的异同，以期为多年生稻栽培生理基础研究和生产应用提供理论依据。

1 材料与方法

1.1 供试材料

供试材料为多年生粳稻品系 PR25 和再生稻品种渝粳优 5029（RR1）、甬优 1540（RR2）、佳辐占（RR3）、宜优 673（RR4）、内优 7075（RR5），共 6 个水稻品种（系）。

1.2 试验设计

试验于 2018 年 4—12 月在云南省西双版纳傣族自治州景洪市勐龙镇进行，试验田地处东经 100°39′47″，北纬 21°33′34″，海拔高 666 m。采用单因素随机区组设计，每个品种（系）设 3 个重复，共 18 个小区，小区面积 20 m^2，四周设保护行 4 行以上。

1.3 田间操作与管理

各品种（系）均于 2018 年 4 月 8 日播种，5 月 7 日移栽，株行距 20 cm × 25 cm，第一季收割后蓄留稻桩高度均为 5 cm。头季肥料纯氮用量为 12 kg/667m^2，氮、磷、钾施用比例为 2∶1∶2，其中，氮肥按基肥∶分蘖肥∶穗肥∶保根肥 =3∶3∶2∶2 施用，磷肥全部作基肥施，钾肥按基肥∶穗肥∶保根肥 =4∶4∶2 施用；保根肥为头季稻齐穗后 20 d 左右施入。第二季肥料的纯氮用量和氮、磷、钾比例与头季相同，其中，氮肥按基肥∶分蘖肥∶穗肥 =5∶3∶2 施用，磷肥全部作基肥施，钾肥按基肥∶穗肥 =1∶1 施用。水分管理：寸水活棵、浅水分蘖、够苗晒田，拔节抽穗期保持田间有水、干湿交替壮籽。移栽后 5~7 d 施用丙草胺、苄嘧磺隆等常规除草剂除草；在苗期到幼穗分化期做好稻飞虱、南方黑条矮缩病防治，分蘖盛期和破口期做好稻飞虱、稻纵卷叶螟、纹枯病、白叶枯病的防治。

1.4 测定项目与方法

1.4.1 *SPAD 值衰减率* 于头季稻齐穗后 10 d 和成熟期，用 SPAD 仪测定剑叶 SPAD 值，直到成熟收割为止。SPAD 衰减率 =（成熟期 SPAD− 齐穗后 10 d SPAD）/ 齐穗后 10 d SPAD。

1.4.2 *根系活力测定* 头季成熟期测定根系伤流量，每个小区测定 3 株。具体操作：第 1 天 19∶00，在离地面 10 cm 处割出伤口，用封口袋装脱脂棉连接在伤口处，次日早上 8∶30 收回，测定脱脂棉重量，计算单株单位时间伤流量。

1.4.3 *生育期记载* 记载头季播种期、移栽期、齐穗期、成熟期及收获期，再生季齐穗期和成熟期的具体时间。

1.4.4 *产量及产量构成* 头季每个小区取 12 株进行考种，测定结实率、千粒重、每穗粒数，每个小区数 30 株有效穗，计算理论产量，同时每个小区取 5 m^2 进行实割测产；再生季取 12 株进行考种，考察不同节位有效穗、结实率、千粒重，计算单株产量及不同节位对产量贡献率，同时每个小区取 5 m^2 进行实割测产。

1.4.5 *干物质积累* 于齐穗期和成熟期取样，每小区取 3 株，洗净泥沙，测定茎蘖数，然后去根，将样品分成茎、叶、穗 3 部分（成熟期将样品分成稻草、枝梗、实粒、秕粒 4 个部分），于 105℃杀青 20 min，然后在 80℃下烘 48 h 以上，冷却至恒温称干物重。

1.5 数据分析

数据采用 Excel 和 SPSS 23 软件进行整理分析。

2 结果与分析

2.1 多年生稻和再生稻产量及产量构成特点分析

如表 1 所示，头季产量为 RR1 > RR2 > PR25 > RR4 > RR3 > RR5，其中 RR1 产量最高为 657.07 kg/667m^2，RR2 次之为 584.83 kg/667m^2，PR25 排第三，其中 PR25 与 RR2 和 RR4 差异不显著，但显著（$P < 0.05$）高于 RR3 和 RR5；再生季产量 PR25 为 471.10 kg/667m^2，显著高于所有再生稻品种，较 RR1、RR2、RR3、RR4 和 RR5 分别增加 48.22%、34.80%、60.95%、31.71%、45.58%，周年产量 PR25 为 1 031.63 kg/667m^2，亦显著高于再生稻品种，较 RR1、RR2、RR3、RR4 和 RR5 分别增加 5.82%、10.41%、47.00%、13.19%、42.66%。

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再生稻品种头季产量和周年产量均以 RR1 最高，分别为 657.07 和 974.90 kg/667m^2，而再生季产量 RR4 最高，为 357.67 kg/667m^2。可以看出，PR25 产量与再生稻相比头季持平或者略低，但再生季和周年产量显著超过再生稻品种。

表 1　多年生稻和再生稻产量差异比较　（kg/667m^2）

品 种	头季产量	再生季产量	周年产量
PR25	560.50 ± 27.58 b	471.10 ± 20.68 a	1 031.63 ± 34.00 a
RR1	657.07 ± 49.14 a	317.83 ± 8.80 c	974.90 ± 49.23 b
RR2	584.83 ± 11.74 b	349.50 ± 8.89 b	934.33 ± 10.60 bc
RR3	409.10 ± 12.96 c	292.70 ± 0.72 d	701.78 ± 18.76 d
RR4	553.80 ± 20.30 b	357.67 ± 10.71 b	911.43 ± 18.65 c
RR5	399.57 ± 1.43 c	323.60 ± 10.12 c	723.16 ± 23.69 d

注：同列不同小写字母表示差异显著（P<0.05），下同（有季别的为同季比较）。

如表 2 所示，总的来看，再生季与头季比，颖花数、每穗粒数、千粒重有所降低，而有效穗数和结实率均有所增加。头季单位面积颖花数为 RR2 > RR4 > PR25 > RR1 > RR5 > RR3，每穗粒数为 RR2 > RR4 > RR1 > PR25 > RR5 > RR3，千粒重为 RR4 > RR3 > RR5 > RR1 > PR25 > RR2，有效穗数为 PR25 > RR1 > RR3 > RR5 > RR4 > RR2，结实率为 RR1 > RR3 > PR25 > RR2 > RR5 > RR4 >，头季 PR25 产量构成各因素与头季产量趋势基本一致。PR25 再生季单位面积颖花数、有效穗数和结实率均大于再生稻，其中颖花数较 RR1、RR2、RR3、RR4 和 RR5 分别增加 10.82%、0.68%、50.76%、35.00%、32.00%，其中除了与 RR2 差异未达显著水平，与其他品种差异均显著；有效穗数分别增加 51.49%、13.34%、6.08%、4.28%、5.52%，除与 RR2 差异显著之外，与其他再生稻差异均不显著；结实率分别增加 35.24%、27.57%、11.62%、17.55%、8.89%，每穗粒数和千粒重均排在第 3。从产量构成因素看，多年生稻再生季产量较再生稻高的主要原因是再生季单位面积颖花数、有效穗数和结实率高于再生稻。

表 2　多年生稻和再生稻产量构成分析

季 别	品 种	颖花数（×10^2 朵 /m^2）	每穗粒数（粒）	千粒重（g）	有效穗数（穗 /m^2）	结实率（%）
头 季	PR25	393 ± 16.86 b	145 ± 20.40 c	27.83 ± 0.67 d	273.50 ± 26.68 a	76.83 ± 0.46 b
	RR1	388 ± 31.99 b	147 ± 24.30 c	28.47 ± 0.64 cd	269.03 ± 56.73 a	89.37 ± 2.96 a
	RR2	546 ± 16.84 a	261 ± 12.71 a	23.43 ± 1.45 e	210.10 ± 16.68 b	68.67 ± 0.75 c
	RR3	257 ± 9.04 d	109 ± 1.00 d	30.33 ± 0.25 b	235.47 ± 7.52 ab	78.60 ± 1.02 b
	RR4	413 ± 1.43 b	182 ± 4.73 b	33.40 ± 0.02 a	226.80 ± 6.67 ab	60.27 ± 2.45 d
	RR5	325 ± 0.51 c	142 ± 10.50 c	29.47 ± 0.16 bc	230.10 ± 16.68 ab	62.70 ± 1.39 d
再生季	PR25	297 ± 15.54 a	88 ± 7.54 b	27.30 ± 0.69 bc	340.20 ± 46.69 a	87.73 ± 1.51 a
	RR1	268 ± 2.39 b	101 ± 3.49 a	25.47 ± 0.40 c	224.57 ± 10.19 b	64.87 ± 6.33 e
	RR2	295 ± 10.04 a	98 ± 3.22 a	25.90 ± 2.06 c	300.17 ± 20.01 a	68.77 ± 1.37 e
	RR3	197 ± 6.21 d	61 ± 1.22 d	28.60 ± 1.48 b	320.70 ± 19.19 a	78.60 ± 0.43 c
	RR4	220 ± 3.72 c	67 ± 0.36 cd	31.13 ± 0.32 a	326.23 ± 5.87 a	74.63 ± 0.53 d
	RR5	225 ± 3.40 c	70 ± 4.42 c	26.80 ± 1.37 bc	322.40 ± 19.25 a	80.57 ± 0.65 b

2.2　多年生稻和再生稻生育期差异分析

各品种再生季全生育期与头季相比均有所缩短，平均缩短 60.7 d，其中齐穗前生育期再生季较头季平均缩短 61.2 d，是造成全生育期缩短的主要原因（表 3）。头季齐穗期在 7 月 5—27 日，成熟期在 8 月 6 日—9 月 1 日，全生育期为 125~151 d，其中 RR5 最长，为 151 d，RR3 最短，为 125 d，各品种生殖生长期（齐穗后）差异不明显，为 31~36 d，PR25 和 RR5 最长，为 36 d；而营养生长期和营养生长与生殖生长并进期（播种至齐穗期）为 93~115 d，是造成全生育期差异的主要阶段。再生季各品种齐穗期为 9 月 6 日—11 月 10 日，成熟期为 10 月 6 日—12 月 15 日，各品种间相差较大；全生期为 PR25 最长为 110 d，较 RR1、RR2、RR3、RR4、RR5 分别延长 36、20、49、41、45 d，再生稻品种全生育期为 61~90 d，其中，PR25 的营养生长期及营养生长与生殖生长并进期（头季收获至再生季齐穗期）为 75 d，较 RR1、RR2、RR3、RR4、RR5 分别延长 34、23、44、41、44 d，生殖生长期（齐穗后）差异不明显。多年生稻和再生稻品种生育期差异主要在营养生长期，而生殖生长期差异不明显。多年生稻

表 3　多年生稻和再生稻生育期比较

季 别	品 种	齐穗期（月–日）	成熟期（月–日）	播种 / 收获—齐穗期（d）	齐穗—成熟（d）	全生育期（d）
头 季	PR25	07–22	08–27	110	36	146
	RR1	07–12	08–14	100	33	133
	RR2	07–24	08–14	102	31	133
	RR3	07–05	08–06	93	32	125
	RR4	07–23	08–26	111	34	145
	RR5	07–27	09–01	115	36	151
再生季	PR25	11–10	12–15	75	35	110
	RR1	09–24	10–27	41	33	74
	RR2	10–05	11–12	52	38	90
	RR3	09–06	10–06	31	30	61
	RR4	09–29	11–03	34	35	69
	RR5	10–02	11–05	31	34	65

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再生季营养生长期至营养生长与生殖生长并进期明显长于再生稻，进而全生育期较再生稻明显延长。

2.3　多年生稻和再生稻头季剑叶 SPAD 值及根系活力分析

如图 1 所示，头季各品种齐穗后 10 d 和成熟期 SPAD 值差异均不显著，其中，齐穗后 10 d PR25、RR1、RR2、RR3、RR4、RR5 的剑叶 SPAD 值分别为 38.02、39.06、39.84、39.09、36.77、39.07，成熟期为 RR2 > PR25 > RR5 > RR1 > RR3 > RR4；叶面积衰减率为 RR3 > RR1 > RR4 > RR5 > RR2 > PR25，其中 PR25 衰减率最低为 9.71%，较 RR3、RR1、RR4、RR5、RR2 分别减少 48.38%、43.50%、32.68%、28.34%、15.86%。灌浆结实期各品种 SPAD 差异不明显，但是后期 SPAD 衰减率多年生稻低于再生稻，说明后期多年生稻叶片早衰程度低于再生稻，剑叶光合持续时间较长。由图 2 可知，头季成熟期单株根系伤流量表现为 PR25 > RR2 > RR4 > RR5 > RR1 > RR3，其中 PR25 最大，为 47.9 mg/h，较 RR2、RR4、RR5、RR1、RR3 分别增加 5.8%、54.3%、92.2%、124.1%、213.3%，其中除了与 RR2 差异不显著外，与其他品种差异均达到显著水平。这说明多年生稻在头季成熟期仍能保持较高的根系活力。

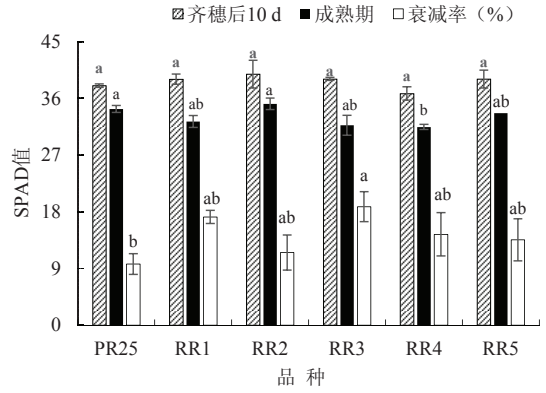


图 1　多年生稻和再生稻剑叶 SPAD 值

[图中不同小写字母表示品种间差异显著（$P < 0.05$），下同]

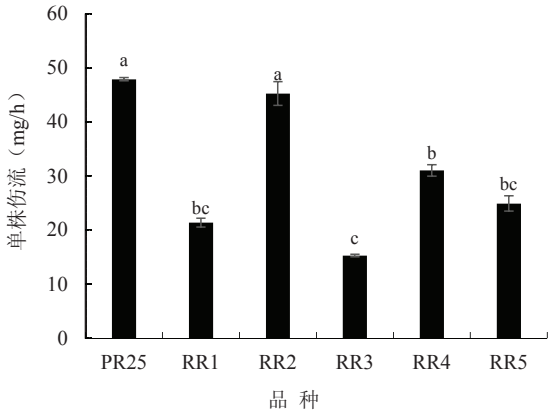


图 2　多年生稻和再生稻的根系伤流量

2.4　多年生稻和再生稻干物质积累分析

再生季与头季比较，齐穗期和成熟期干物质都明显减少（表 4），头季齐穗期干物质为 RR4 > RR5 > PR25 > RR2 > RR1 > RR3，其中，RR4 最大，为 1 265.4 g/m^2，RR3 最小，为 900.2 g/m^2，成熟期为 RR1 > PR25 > PR4 > RR2 > RR5 > RR3，其中，RR1 最大，为 1 825.0 g/m^2，RR3 最小，为 1 303.2 g/m^2。再生季齐穗期为 RR3 > RR4 > PR25 > RR1 > RR5 > RR2，成熟期（不含老稻桩）为 PR25 显著大于 RR1、RR2、RR3、RR4 和 RR5，分别增加 39.93%、28.16%、119.17%、26.14% 和 48.69%。再生季与头季相比，多年生稻干物质下降幅度低于再生稻，干物质积累量高于再生稻，是多年生稻产量高于再生稻重要原因之一。

表 4　多年生稻和再生稻干物质积累量分析

品 种	头 季（g/m^2）		再生季（g/m^2）		
	齐穗期	成熟期	齐穗期	成熟期（含老稻桩）	成熟期（不含老稻桩）
PR25	1 152.2 c	1 815.0 a	789.3 b	1 742.0 ab	1 486.2 a
RR1	942.3 e	1 825.0 a	757.3 bc	1 257.1 c	1 062.1 c
RR2	1 107.7 d	1 687.8 b	684.2 c	1 620.0 ab	1 159.6 b
RR3	900.2 f	1 303.2 d	982.9 a	1 519.8 bc	678.1 e
RR4	1 265.4 a	1 769.2 ab	868.0 ab	1 711.2 ab	1 178.2 b
RR5	1 226.5 b	1 585.3 c	727.0 c	1 847.6 a	999.5 d

3　结论与讨论

水稻产量由单位面积内有效穗数、每穗粒数、结实率和千粒重构成，这 4 个因素对再生稻产量的形成都具有重要作用，但作用程度存在明显差异 [10]。再生稻头季稻产量与每穗粒数的相关性最密切，其次为穗数，头季高产主要是在稳定穗数的基础上主攻大穗，形成巨大的库容量；再生季产量与单位面积穗数的相关性最密切，其次为每穗粒数，再生季高产应培育更多的穗数，形成巨大的库容量，进而获得更高产量 [11-18]。头季产量为 RR1 > RR2 > PR25 > RR4 > RR3 > RR5，其中 RR1 产量最高，为 657.07 kg/667m^2，RR2 次之，为 584.83 kg/667m^2，PR25 排第三位，从产量构成来看，头季单位面积颖花数为 RR2 > RR4 > PR25 > RR1 > RR5 > RR3，每穗粒数为 RR2 > RR4 > RR1 > PR25 > RR5 > RR3，应该说每穗粒数低于 RR2 和 RR1 是产量低于两者的主要原因。再生季产量为 PR25 为 471.10 kg/667m^2，显著高于所有再生稻品种，较 RR1、RR2、RR3、RR4 和 RR5 分别增加 48.22%、34.79%、60.95%、31.71% 和 45.58%，周年产量 PR25 为 1 031.63 kg/667m^2，亦显著高于再生稻品种，较 RR1、RR2、RR3、RR4 和 RR5 分别增加 5.82%、10.41%、47.00%、13.19% 和 42.66%，再生稻品种头季产量和周年产量均以 RR1 最高，分别为 657.07 和 974.90 kg/667m^2，而再生季产量 RR4 最高，为 357.67 kg/667m^2。再生季 PR25 单位面积颖花数、有效穗数和结实率均大于再生稻，其中，

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颖花数较 RR1、RR2、RR3、RR4 和 RR5 分别增加 10.82%、0.68%、50.76%、35.00% 和 32.00%（除了与 RR2 差异未达显著水平外，与其他品种差异均显著）；有效穗数分别增加 51.49%、13.34%、6.08%、4.28% 和 5.52%（除与 RR2 差异显著之外，与其他再生稻差异均不显著）；结实率分别显著增加 35.24%、27.57%、11.62%、17.55% 和 8.89%，且差异均达显著水平。从产量构成因素看，多年生稻再生季产量较再生稻高的主要原因是再生季单位面积颖花数、有效穗数和结实率均高于再生稻。

目前，对再生稻生育期研究主要围绕留稻桩高度展开。李品汉 [19]、马静 [20] 等研究表明，不论是抛秧还是手插，随着留桩高度的增加，有效积温减少，生育期相应缩短。严斧等 [21] 的研究指出，留桩 35 cm 的抽穗最早，成熟也最早，抽穗最迟的是留桩 50 cm 的处理。此研究中多年生稻与再生稻均采用低留稻桩 5 cm，对于多年生稻而言，低留桩是可行的，而再生稻还是应留稻桩 25 cm。PR25 再生季全生育期最长，为 110 d，较 RR1、RR2、RR3、RR4、RR5 分别延长 36、20、49、41、45 d，再生稻品种全生育期为 61~90 d，其中，PR25 的营养生长期和营养生长与生殖生长并进期（头季收获至再生季齐穗期）为 75 d，较 RR1、RR2、RR3、RR4、RR5 分别延长 34、23、44、41、44 d，生殖生长期（齐穗后）差异不明显。多年生稻和再生稻品种生育期差异主要在营养生长期，而生殖生长期差异不明显。造成生育期差异的原因是品种间差异，还是由于不同留稻桩高度引起的，有待进一步研究。徐富贤 [8] 等研究指出，头季稻齐穗到成熟叶片 SPAD 值衰减指数可作为鉴定再生力的指标。其中 PR25 衰减率最低，为 9.71%，较 RR3、RR1、RR4、RR5 和 RR2 分别减少 48.38%、43.50%、32.68%、28.34% 和 15.86%。灌浆结实期各品种 SPAD 差异不明显，但是后期 SPAD 衰减率多年生稻低于再生稻，说明后期多年生稻叶片早衰程度低于再生稻，剑叶光合持续时间较长。郑景生等 [9] 认为再生季稻再生分蘖的生育依赖于头季稻残留的根系，再生季稻穗数及产量与头季稻成熟期和再生季稻齐穗期根系活力呈极显著线性正相关，因此，头季稻成熟期根系伤流量可作为诊断再生力、筛选再生稻品种的重要指标。头季成熟期单株根系伤流量表现为 PR25 > RR2 > RR4 > RR5 > RR1 > RR3，其中 PR25 最大，为 47.9 mg/h，较 RR2、RR4、RR5、RR1 和 RR3 分别增加 5.8%、54.3%、92.2%、124.1% 和 213.3%，多年生稻根系活力在头季成熟期维持在较高水平。此研究中多年生稻与再生稻比较产量等再生指标都表现出优势，主要原因是多年生稻 PR25 头季齐穗后剑叶 SPAD 衰减幅度较小，剑叶具有较强的光合能力，头季成熟期根系维持在较高活力水平，再生能力较强；再生季多年生稻营养生长期至营养生长与生殖生长并进期明显长于再生稻，进而全生育期较再生稻明显延长，利于干物质积累，从而充分发挥多年生稻产量潜力。PR25 在“一种两收”模式下具有较高的产量潜力。

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中国油料作物学报
Chinese Journal of Oil Crop Sciences
2020,42(5):796-806
doi:10.19802/j.issn.1007-9084.2019265

不同群体中大豆脂肪酸组分QTL定位研究

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摘要:以低亚麻酸材料FA10为父本,分别与中豆32和中豆43杂交得到两个F_2分离群体。采用气相色谱法对两个群体进行脂肪酸含量测定,利用SSR分子标记进行群体基因型分析,开展不同群体中大豆脂肪酸组分QTL定位研究。采用区间作图法,在两个群体分别定位到39个和18个与脂肪酸组分相关的QTL。将定位的QTL与前人报道的脂肪酸组分QTL进行比较发现,有40个QTL与前人报道的QTL具有相同或相近的标记或标记区间;比较本研究两个群体的定位结果发现,4对与亚麻酸含量相关的QTL既与前人定位结果一致,又在两个群体中被重复检测到。这些稳定检测到的脂肪酸组分QTL对于大豆脂肪酸的分子标记辅助选择具有一定的指导意义。

关键词:大豆;脂肪酸;遗传背景;QTL定位
中图分类号:S565.1　　**文献标识码:**A　　**文章编号:**1007-9084(2020)05-0796-11

QTL mapping of fatty acids in different F_2 populations in soybean

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Abstract: Soybean is one of the most important oil crops in China and around the world. Soybean oil contains mainly five fatty acids, and the relative composition of saturated and unsaturated fatty acids determines the quality and use of soybean oil. In this study, a low linolenic line, FA10, was used as a common male parent to cross with Zhongdou 32 and Zhongdou 43 to construct two F_2 segregating populations. Gas Chromatography (GC) method was used to determine the concentrations of fatty acids and SSR markers were used to analyze the genotype for the individuals in the two populations. The objective of this study was to identify repeatable QTLs associated with fatty acids in different genetic backgrounds in soybean. Using Interval Mapping (IM) method, a total of 39 and 18 QTLs for fatty acids were detected in the two populations, respectively. Compared with the QTLs reported in previous studies, 40 QTLs showed same or close markers or marker intervals. Comparing the results from the two populations in this study, four pairs of QTLs for linolenic showed high consistency in marker intervals and confirmed the results in previous studies. All these stable QTLs for fatty acids might provide useful information for the improvement of fatty acids in soybean.

Key words: soybean; fatty acids; genetic background; QTL mapping

大豆是世界上重要的油料作物之一,其产量占世界油料作物产量的60%[1],提供了全球大约1/3的植物油用量[2]。大豆油中饱和脂肪酸和不饱和脂肪酸的含量及组成比例直接影响了大豆油的品质和用途[3]。富含不饱和脂肪酸的大豆油主要用作食用植物油,其不饱和脂肪酸除了可以满足人体营养必需外,还可以起到降低胆固醇的作用,对高血压及心脑血管疾病也有辅助治疗作用[4]。但食用大豆油中含有多个不饱和双键的脂肪酸组分(亚油酸和亚麻酸),在贮藏过程中容易被氧化而产生酸败,从而影响到食用油的风味和稳定性[5]。通过育种途径提高大豆脂肪酸中油酸(只含一个双键)含量的同时,

收稿日期:2019-11-10
基金项目:国家自然科学基金(31560396,31860385);云南省科技计划面上项目(2018FB061)
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降低亚油酸和亚麻酸的含量,是提高大豆油品质最为安全和有效的方法,因而也是大豆品质育种的主要育种目标之一。

大豆脂肪酸含量是受多基因控制的数量性状,易受环境因素影响[6]。利用分子标记辅助选择育种,可以大大提高大豆脂肪酸遗传改良的选择效率。目前,国内外已有大量学者对大豆脂肪酸含量进行了 QTL 定位研究[7~11]。大豆重要数据库网站 Soybase(http://soybase. org/)上已公布与棕榈酸、硬脂酸、油酸、亚油酸和亚麻酸性状相关的 QTL 分别有 41、32、43、44 和 68 个。Li 等[12]以 Luheidou2 和 Nanhuizao 杂交产生的 200 个重组自交系为材料,对大豆脂肪酸组分进行 QTL 定位研究,共定位到 26 个与脂肪酸含量相关的稳定 QTL,表型贡献率为 0. 40%~37. 0%;Leite 等[13]以 Lineage 69 和 Tucunaré 杂交产生的 244 个 F_2 为材料,对大豆油分含量进行 QTL 定位与分析,在 K 和 H 连锁群上检测到 2 个与含油量相关的 QTL,表型变异解释率为 7. 80%~46. 75%;Xie 等[14]利用合丰 25×东农 L-5 构建的重组自交系群体为材料,定位到与亚麻酸相关 QTL 6 个,与亚油酸相关 QTL 4 个,与油酸相关 QTL 4 个,与棕榈酸相关 QTL 4 个,与硬脂酸相关 QTL 1 个,检测到的 QTL 在不同环境下的平均遗传贡献率为 2. 53% ~ 37. 30%;Teng 等[15]以东农 46×半野生系 L-100 构建的 129 个重组自交系为材料,在 10 个环境下进行大豆油分 QTL 定位研究,共检测到 7 个 QTL 与大豆油分相关,在不同环境下的表型贡献率为 2. 24%~17. 54%;Ning 等[16]以 Dongnong47×Jiyu89 和 Dongnong47×Zaoshu18 的回交群体后代为材料,对两个群体在不同环境下进行大豆油分 QTL 定位分析,两群体分别定位到 9 个和 7 个相关 QTL,在两群体中重复检测到 3 个 QTL,大多数定位 QTL 表型解释率低于 10%;南金平[17]以中黄 13×迟黄豆和中黄 13×泰兴牛毛黄乙的回交群体后代为材料,对两群体的脂肪和脂肪酸组分含量进行 QTL 定位,两群体分别定位到 56 个和 71 个相关的 QTL,其中与油酸相关的标记区间 Satt256-Satt615 在两个群体均被定位到。

虽然目前国内外已经报道了大量与大豆脂肪酸相关的 QTL,但是能在不同环境或不同遗传背景中被重复检测到的 QTL 较少[18~20]。本研究利用低亚麻酸材料 FA10 为共同父本,分别与中豆 32 和中豆 43 杂交,构建了两个 F_2 分离群体。以此分离群体为材料,采用 SSR 分子标记,开展不同遗传背景下的大豆脂肪酸 QTL 定位研究,以期获得能在不同遗传背景下被重复检测到的脂肪酸 QTL,研究结果将对大豆脂肪酸性状的分子标记辅助选择具有一定的指导意义。

1 材料和方法

1.1 材料与田间试验

2016 年夏以低亚麻酸材料 FA10 为共同父本,分别与中豆 32 和中豆 43 杂交,获得杂交 F_1 种子。FA10 亚麻酸含量极低,平均含量约为 3%;中豆 32 和中豆 43 分别为晚熟夏大豆和早中熟夏大豆品种,两者脂肪酸组分含量与普通常规品种相当(表 1)。2016 年冬在云南大学农学院西双版纳试验基地加代,收获得到两个 F_2 群体,其中群体 1(Pop1,中豆 32×FA10,下同)包含 188 个单株,群体 2(Pop2,中豆 43×FA10,下同)包含 77 个单株。

2017 年夏季将两个供试群体种植于云南大学农学院富民试验基地。条播,行长 2. 00 m,行距 0. 50 m,株距 0. 10 m,一般大田管理。成熟后分单株收获,脱粒晒干后用于脂肪酸品质分析。

1.2 大豆脂肪酸提取及含量检测

取若干粒干燥的大豆种子,借助电动微型粉碎机将其充分研磨,依据 Christie 所描述的甲酯化提取法提取大豆脂肪酸[21]。采用 Thermo Fisher TRACE 1310 气相色谱分析仪,用气相色谱法内标法检测脂肪酸含量;色谱柱为 Thermo Fisher RT-Wax(30 m×0. 25 mm×0. 25 μm);进样量:1 μL,采用分流进样方式,分流比:25:1,恒流:2 mL/min,吹扫流量:5 mL/min;进样口温度为 250℃;载气为氮气:40 mL/min;氢气:35 mL/min;空气:350 mL/min;程序升温方式为起始温度 90℃,以 20℃/min 的速率升温至 160℃,保持 1 min,以 2℃/min 的速率升温至 220℃,保持 2 min。检测器为火焰离子检测器(FID),温度为 250℃。每个样品重复检测 3 次,取平均值作为其脂肪酸组分含量。

1.3 大豆 DNA 提取与 SSR 基因型分析

田间采集苗期大豆幼嫩叶片,用锡箔纸包裹后置于液氮中。随后转移至-80℃冰箱中存储,以备提取 DNA。按照改进的 CTAB 方法提取 DNA[22]。将提取的 DNA 溶解于 ddH_2O 中,使用 Thermo Fisher Nano Drop2000 检测 DNA 质量和浓度,使用时统一稀释浓度为 50 ng/μL。

参照“大豆公众图谱”Soybase(https://soybase.org)网站上发表的 SSR 标记信息,在 20 个连锁群上每 5~10 cM 均匀选取 SSR 标记,共筛选了 1005 个

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SSR标记。根据网上公布的引物序列,交由上海生物工程服务有限公司合成SSR引物。PCR反应体系和扩增条件按照Soybase上提供的方法进行。

利用1005对引物分别对两个群体亲本进行SSR基因型分析,筛选出在亲本间具有多态性的标记。再利用筛选出来的多态性标记分别对两个群体的单株进行基因型分析,将与母本相同的带型记作“A”,与父本一样的带型记作“B”,杂合的带型记作“H”,缺失记为“X”。

1.4 表型及QTL分析

采用Microsoft Excel 2010软件对两个F_2群体亲本和后代单株脂肪酸组分进行最小值、最大值、平均值、标准差和变异系数的基本统计量分析,采用SPSS 19软件进行脂肪酸组分间的相关性分析。

采用IciMapping软件构建遗传图谱。结合脂肪酸含量表型数据,采用IciMapping软件中的区间作图法(IM),LOD阈值设置为2.5,分别对两个群体进行与脂肪酸相关的QTL定位分析。

2 结果与分析

2.1 Pop1和Pop2群体脂肪酸含量表型分析

对两个群体亲本及后代单株的脂肪酸含量进行基本统计量分析,结果如表1所示。两个群体的亲本在脂肪酸组分含量上均存在差异:共同父本FA10的硬脂酸和油酸含量均高于两群体的母本中豆32和中豆43,而棕榈酸和亚麻酸含量均低于中豆32和中豆43;其中FA10的油酸含量与中豆43差异较大,与中豆32差异较小;FA10的亚油酸含量高于中豆32,但低于中豆43。两个群体的母本中豆32和中豆43在脂肪酸含量上也存在差异:中豆32油酸含量较高,为43.67%,中豆43亚油酸含量较高,为58.45%;中豆32的棕榈酸、油酸、亚麻酸含量均高于中豆43,其他组分含量均低于中豆43。对两个群体的后代个体进行脂肪酸含量分析表明,两个F_2群体的脂肪酸含量均存在较大变异(表1),其中油酸变异幅度差异较大,Pop1群体中变异系数为13.91%,Pop2群体中变异系数为22.02%;其中亚麻酸含量变异系数较大,均在21%以上,其中Pop1群体高达28.02%。

对两个群体脂肪酸含量分布进行分析表明,两个群体的各脂肪酸含量基本呈正态分布(图1),表明该数据可以用于QTL定位分析。

对两个F_2分离群体的棕榈酸、硬脂酸、油酸、亚油酸和亚麻酸含量进行相关性分析可知(表2):两群体内各脂肪酸含量相关性的趋势相同;其中棕榈酸与亚油酸、亚麻酸呈显著正相关,与油酸呈显著负相关;硬脂酸与亚油酸、亚麻酸呈显著正相关,与油酸呈显著负相关。

2.2 遗传图谱构建

利用合成的1005对SSR引物对两个F_2分离群体的亲本进行多态性筛选,Pop1群体获得清晰的多态性标记153个,Pop2群体获得清晰的多态性标记116个。利用多态性标记分别对两个群体的后代进行SSR分析,获得两个分离群体的基因型数据。剔除个体缺失严重的标记,利用IciMapping软件对两个群体分别构建遗传图谱,其中Pop1构建的遗传图

表1 两个F_2群体亲本和后代单株脂肪酸含量(%)的基本统计

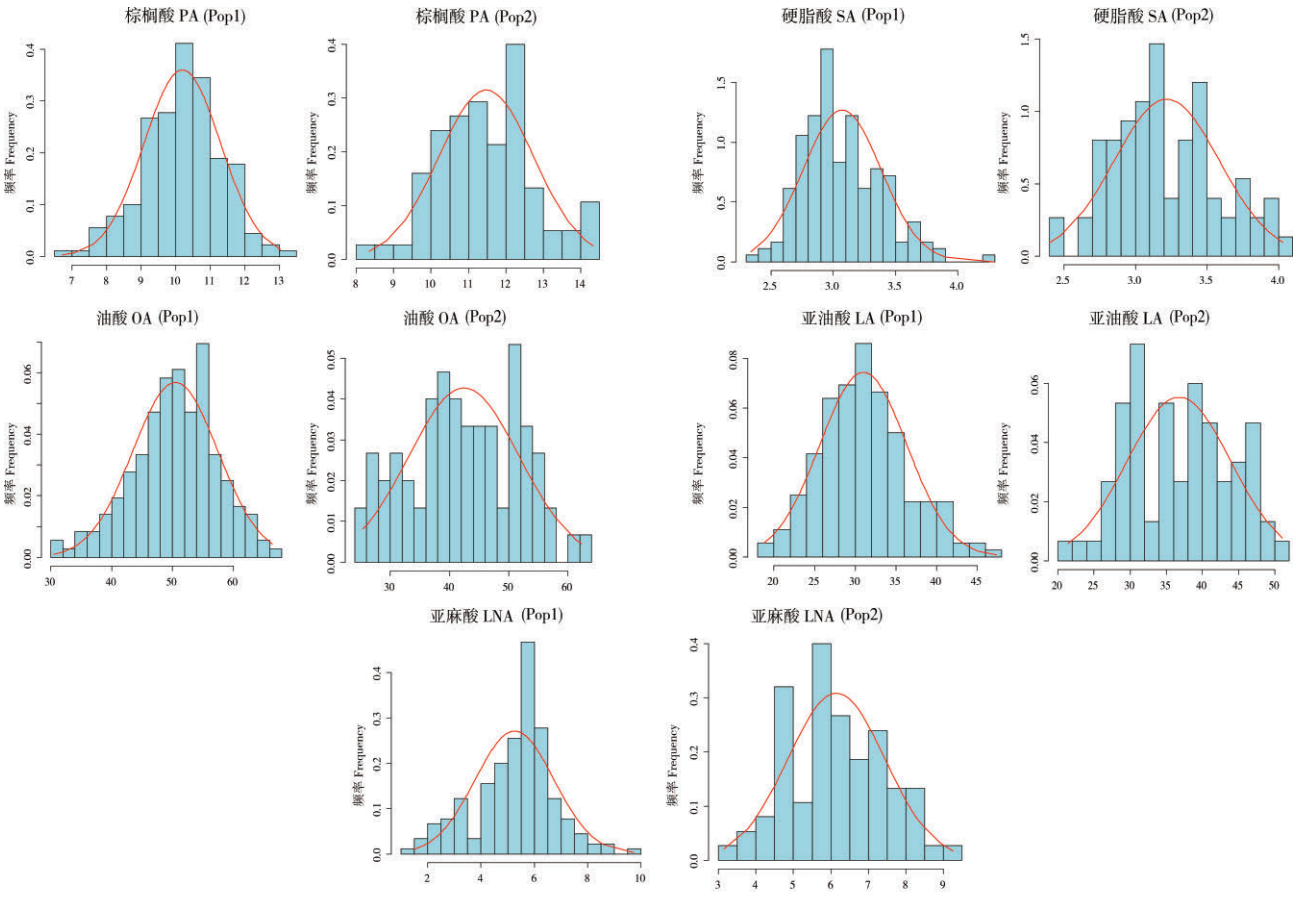
Table 1 Descriptive statistics of fatty acids (%) for parents and F_2 individuals in F_2

性状 Trait	群体 Population	亲本 Parent		群体 Population						
		父本 FA10	母本 Female	最小值 Minimum	最大值 Maximum	平均值 Mean	标准差 SD	变异系数 CV/%	偏度 Skewness	峰度 Kurtosis
棕榈酸	Pop1	9.05	11.15	6.72	13.03	10.20	1.11	10.87	−0.35	0.31
PA	Pop2		11.08	8.34	14.33	11.47	1.27	11.04	0.13	−0.06
硬脂酸	Pop1	3.40	2.95	2.34	4.29	3.07	0.31	10.24	0.63	0.63
SA	Pop2		3.34	2.40	4.03	3.22	0.38	11.43	0.27	−0.40
油酸	Pop1	48.12	43.67	30.45	66.39	50.49	7.02	13.91	−0.39	0.08
OA	Pop2		18.94	25.40	62.22	42.42	9.34	22.02	−0.03	−0.81
亚油酸	Pop1	36.39	34.00	18.76	47.32	30.99	5.36	17.29	0.43	0.05
LA	Pop2		58.45	21.46	51.02	36.74	7.19	19.57	0.05	−0.94
亚麻酸	Pop1	3.04	8.23	1.48	9.73	5.26	1.47	28.02	−0.34	0.32
LNA	Pop2		8.19	3.15	9.26	6.15	1.29	21.03	0.15	−0.46

注:Pop1代表中豆32×FA10群体,Pop2代表中豆43×FA10群体

Note: Pop1 represents Zhongdou 32 × FA10 population; Pop2 represents Zhongdou 43×FA10 population; PA: Palmitic acid; SA: Stearic acid; OA: Oleic acid; LA: Linoleic acid; LNA: Linolenic acid

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注:PA:棕榈酸;SA:硬脂酸;OA:油酸;LA:亚油酸;LNA:亚麻酸

Note: PA: Palmitic acid; SA: Stearic acid; OA: Oleic acid; LA: Linoleic acid; LNA: Linolenic acid

图 1　Pop1 和 Pop2 群体脂肪酸含量(%)表型频率分布图

Fig. 1　Frequency distribution of phenotype for fatty acids (%) in Pop1 and Pop2 populations

表 2　两个 F_2 群体脂肪酸含量的相关分析

Table 2　Correlation analysis of fatty acids in F_2 populations

	棕榈酸(PA)	硬脂酸(SA)	油酸(OA)	亚油酸(LA)	亚麻酸(LNA)
棕榈酸(PA)	1	0.041	−0.898**	0.850**	0.595**
硬脂酸(SA)	0.545**	1	−0.660**	0.593**	0.533**
油酸(OA)	−0.814**	−0.533**	1	−0.986**	−0.673**
亚油酸(LA)	0.674**	0.466**	−0.958**	1	0.563**
亚麻酸(LNA)	0.561**	0.224**	−0.555**	0.324**	1

注:对角线下方为 Pop1 群体各脂肪酸之间的相关系数,上方为 Pop2 群体各脂肪酸之间的相关系数;**: 表示在 $P<0.01$ 水平上显著相关

Note: PA: Palmitic acid; SA: Stearic acid; OA: Oleic acid; LA: Linoleic acid; LNA: Linolenic acid; Numbers below diagonal represent the correlation coefficients among fatty acids in Pop1, and numbers above diagonal represent the correlation coefficients among fatty acids in Pop2; **: Significant correlation was found at $P < 0.01$

谱包含 140 个标记,Pop2 构建的遗传图谱包含 116 个标记。Pop1 和 Pop2 构建的遗传图谱平均间距分别为 18.03 cM 和 21.76 cM,均涵盖大豆的 20 个连锁群(图 2、图 3)。

2.3　大豆脂肪酸 QTL 分析

利用 IciMapping 软件中的区间作图法对 Pop1 和 Pop2 群体的脂肪酸含量进行 QTL 定位分析,两群体共定位到 57 个与脂肪酸含量相关的 QTL(表 3、表 4)。Pop1 和 Pop2 群体分别定位到 39 个和 18 个 QTL,分布在除 A2、E、I 和 M 以外的 16 个连锁群上(图 2、图 3)。

检测到与棕榈酸相关的 QTL 有 11 个,其中 Pop1 群体检测到 9 个 QTL 分别位于 A1、B2、D1a、D1b 和 L 等 8 个连锁群上,表型解释率为 2.36%~7.96%,

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表3 Pop1群体定位到的大豆脂肪酸组分相关QTL

Table 3 QTLs for fatty acids detected in Pop1

性状 Trait	连锁群 LG	位点 Position/cM	标记区间[a] Marker	置信区间 CI	LOD	表型解释率 PVE/%	加性效应 Add	显型效应 Dom	参考文献 Reference
棕榈酸	A1	116	Satt225- Satt200	95.5 - 121.5	2.64	2.64	-0.28	-0.64	
PA	A1	309	Sat_344- Satt545	299.5 - 323.5	4.25	3.87	0.21	-0.90	
	B2	0	Satt687- *Satt070*	0 - 25.5	4.46	2.36	0.25	-0.78	Reinprecht 等[23]
	D1a	41	Sat_106- Satt184	19.5 - 74.5	2.68	7.96	-0.75	0.71	
	D1b	227	Staga002- *Satt579*	219.5 - 234.5	7.75	7.81	1.14	0.43	Panthee 等[24]
	D2	119	*Satt397- Satt543*	105.5 - 132.5	3.15	5.08	0.84	0.74	Xie 等[14]
	F	396	*Satt663- Sat_262*	380.5 - 403.0	3.23	3.23	0.22	-0.74	Wang 等[25]
	K	61	BARCSOYSSR_09_1530- *Satt178*	58.5 - 63.5	16.40	7.54	1.00	-0.25	Wang 等[25]; Xie 等[14]
	L	185	Satt182- Satt588	174.5 - 198.5	2.81	7.87	0.70	0.87	
硬脂酸	B1	13	Satt484-BARCSOYSSR_11_0106	1.5 - 23.5	3.30	11.12	-0.02	0.47	
SA	C2	152	BARCSOYSSR_06_0217-*Sat_402*	141.5 - 165.5	3.81	6.13	-0.23	-0.26	Li 等[26]; Fan 等[27]
	F	59	Satt554- *AW756935*	52.5 - 68.5	2.68	2.19	-0.11	-0.11	Reinprecht 等[23]
油酸	A1	98	*Satt225-Satt200*	81.5 - 121.5	2.97	6.21	2.98	4.00	Diers 等[28]
OA	B1	103	BARCSOYSSR_11_0106- BRCSOYSSR_11_1342	82.5 - 109.5	2.66	7.37	3.16	8.46	
	B2	0	*Satt687- Satt070*	0 - 7.5	2.52	1.82	-1.38	3.57	Diers 等[28]; Kim 等[29]
	K	60	Sat_352- BARCSOYSSR_09_1530	56.5 - 67.5	4.65	3.50	-3.90	0.50	
亚油酸	A1	89	*Satt225- Satt200*	75.5 - 105.5	2.91	5.05	-2.42	-4.15	Diers 等[28]; Bachlava 等[30]
LA	B1	103	BARCSOYSSR_11_0106- BRCSOYSSR_11_1342	86.5 - 109.5	2.75	4.04	-2.73	-6.16	
亚麻酸	A1	141	Satt200- Sat_271	131.5 - 148.5	7.32	3.34	-1.03	1.06	
LNA	A1	305	*Sat_344-* Satt545	299.5 - 311.5	14.57	3.89	1.03	-1.12	Bachlava 等[30]
	B1	119	*BARCSOYSSR_11_0106- BRCSOYSSR_11_1342*	110.5 - 137.5	4.89	1.11	0.93	0.66	Li 等[26]
	B2	0	*Satt687- Satt070*	0 - 12.5	7.25	1.43	0.29	-1.42	Spencer 等[7]; Reinprecht 等[23]; Bachlava 等[30]; Xie 等[14]; Ha 等[31]
	C1	6	BARCSOYSSR_04_1294-*Satt646*	0 - 19.5	3.04	1.10	0.28	0.96	Li 等[26]
	C2	8	*Satt457- Sat_130*	0 - 29.5	2.52	1.32	0.33	1.20	Fan 等[27]
	C2	166	Sat_402- *Satt277*	155.5 - 176.5	3.21	0.67	-0.37	0.49	Fan 等[27]
	D1a	56	*Sat_106-* Satt184	38.5 - 77.5	3.49	4.68	-1.46	0.83	苗兴芬等[32]
	D1b	194	***Sat_096- Staga002***	193.5 - 194.5	5.79	4.45	-0.27	-2.83	Bachlava 等[30]; Li 等[26]; Xie 等[14]
	D1b	216	***Staga002- Satt579***	214.5 - 219.5	24.41	4.69	1.25	-1.34	Bachlava 等[30]; Xie 等[14]
	D2	114	***Satt397- Satt543***	98.5 - 130.5	6.08	2.84	1.35	0.84	朱明月[33]
	D2	507	Sctt008- Sat_296	497.5 - 513.5	3.26	1.77	-0.12	-1.42	
	F	229	BARCSOYSSR_13_0309- Satt510	208.5 - 242.5	4.27	3.96	0.07	-2.28	
	F	403	Satt663- *Sat_262*	382.5 - 403.0	3.28	0.69	0.24	-0.73	Hyten 等[34]; Akond 等[10]
	H	17	***Sat_180- Satt142***	9.5 - 23.5	4.07	3.36	1.03	1.37	Zheng 等[11]; Ha 等[31]
	J	84	*Satt674- Sat_224*	73.5 - 92.5	2.84	4.21	0.95	2.11	Li 等[26]
	J	164	*Sct_065- Satt380*	138.5 - 174.5	2.95	0.66	-0.58	0.40	Akond 等[10]
	K	60	Sat_352- BARCSOYSSR_09_1530	58.5 - 60.5	19.83	3.53	1.11	-1.04	
	L	168	*Satt182-* Satt588	149.5 - 182.5	2.58	1.86	0.77	0.74	Kim 等[29]; Bachlava 等[30]
	N	66	BARCSOYSSR_03_1424- *BARCSOYSSR_03_0499*	53.5 - 82.5	8.19	3.38	1.54	0.81	Ha 等[31]; Fan 等[27]
	O	211	BARCSOYSSR_10_1087- *Satt420*	201.5 - 220.5	5.68	4.48	-1.07	1.91	Kim 等[29]; Li 等[26]

注:PA:棕榈酸;SA:硬脂酸;OA:油酸;LA:亚油酸;LNA:亚麻酸;a:标记区间用红色加粗字表示的,表明该QTL在本试验中的两个群体中被重复检测到;标记区间用斜体字表示的,表明该QTL与前人报道过的结果一致;标记区间用斜体、加粗字表示的,表明该QTL在本试验的两个群体及前人的报道中被重复检测到

Note: PA: Palmitic acid; SA: Stearic acid; OA: Oleic acid; LA: Linoleic acid; LNA: Linolenic acid; a: Marker intervals showed in red bold indicate the QTLs were detected in two populations in this study; marker intervals showed in italics indicate the QTLs were reported in previous studies; marker intervals showed in bold and italics indicate the QTLs were detected in two populations in this study and in previous studies;

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表 4 Pop2 群体定位到的大豆脂肪酸组分相关 QTL

Table 4 QTLs for fatty acids detected in Pop2

性状 Trait	连锁群 LG	位点 Position /cM	标记区间[a] Marker	置信区间 CI	LOD	PVE /%	Add	Dom	参考文献 Reference
棕榈酸	D1b	4	*Sat_139–Sat_096*	0 – 22.5	2.61	15.14	0.66	0.34	Reinprecht 等[23]; Panthee 等[24]
PA	H	69	Satt142 – *Sat_401*	56.5 – 77.5	5.84	25.58	−0.10	−1.43	南金平等[35]
硬脂酸	B2	147	*Sat_355* – BARCSOYSSR_14_1479	118.5 – 163.5	3.37	7.49	−0.26	−0.04	Panthee 等[24]; Li 等[26]
SA	C2	59	Satt681 – *Satt227*	42.5 – 79.5	2.62	4.22	−0.19	0.23	Li 等[26]; Fan 等[27]
	D1b	4	Sat_139 – Sat_096	0 – 23.5	2.91	5.79	0.19	0.15	
	G	190	*Satt309 – Sat_143*	178.5 – 200.5	3.06	19.03	0.14	0.57	Reinprecht 等[23]
	H	70	*Sat_401* – Sctt009	55.5 – 86.5	2.86	4.65	−0.06	−0.30	Fan 等[27]
油酸	C1	40	Sat_367 – Satt338	34.5 – 45.5	3.58	23.11	−11.65	−0.26	
OA	D1b	7	*Sat_139* – Sat_096	0 – 24.5	2.86	7.53	−4.84	−6.01	Hyten 等[34]
	H	60	Satt142 – Sat_401	51.5 – 76.5	5.09	14.21	1.72	13.18	
亚油酸	C1	39	Sat_367 – Satt338	32.5 – 49.5	3.18	12.81	8.56	0.30	
LA	C1	112	Satt338 – Satt646	109.5 – 124.5	2.54	10.71	−1.63	12.65	
	H	58	*Satt142 – Sat_401*	49.5 – 74.5	4.13	8.53	−1.28	−10.40	Fan 等[27]
亚麻酸	C1	41	*Sat_367 – Satt338*	29.5 – 55.5	2.94	16.05	1.35	−0.33	Li 等[26]
LNA	D1b	14	***Sat_139 – Sat_096***	0 – 27.5	3.74	14.95	0.43	1.82	Bachlava 等[30]; Li 等[26]; Xie 等[14]
	D1b	220	***Satt703 – Satt579***	206.5 – 234.5	6.07	18.30	1.43	−0.13	Bachlava 等[30]; Li 等[26]
	D2	42	***Satt310 – Satt543***	30.5 – 49.5	3.00	7.08	0.39	1.13	朱明月[33]
	H	66	***Satt142 – Sat_401***	55.5 – 81.5	5.19	9.80	−0.38	−1.48	Zheng 等[11]; Ha 等[31]

注:PA:棕榈酸;SA:硬脂酸;OA:油酸;LA:亚油酸;LNA:亚麻酸;a:标记区间用红色加粗字表示的,表明该 QTL 在本试验中的两个群体中被重复检测到;标记区间用斜体字表示的,表明该 QTL 与前人报道过的结果一致;标记区间用斜体、加粗字表示的,表明该 QTL 在本试验的两个群体及前人的报道中被重复检测到;PVE:表型解释率;Add:加性效应;Dom:显性效应

Note: PA: Palmitic acid; SA: Stearic acid; OA: Oleic acid; LA: Linoleic acid; LNA: Linolenic acid; a: Marker intervals shown in red bold indicate QTLs were detected in two populations in this study; marker intervals shown in italics indicate QTLs were reported in previous studies; marker intervals showed in bold and italics indicate the QTLs were detected in two populations in this study and in previous studies

LOD 值为 2. 64~16. 40;Pop2 群体检测到 2 个 QTL 位于 D1b 和 H 连锁群,表型解释率分别为 15. 14% 和 25. 58%,LOD 值为 15. 14 和 5. 84。

检测到与硬脂酸相关的 QTL 有 8 个,其中 Pop1 群体检测到 3 个 QTL,分别位于 B1、C2 和 F 连锁群,表型解释率分别为 11. 12%、6. 13% 和 2. 19%,LOD 值分别为 3. 30、3. 81 和 2. 68;Pop2 群体检测到 5 个 QTL 分别位于 B2、C2、D1b、G 和 H 连锁群,表型解释率为 4. 22%~19. 03%,LOD 值为 2. 62~3. 37,其中位于标记区间 Satt309–Sat_143 的 QTL 效应值较大,表型解释率为 19. 03%。

检测到与油酸相关的 QTL 有 7 个,其中 Pop1 群体检测到 4 个 QTL 分别位于 A1、B1、B2 和 K 连锁群,表型解释率为 6. 21%、7. 37%、1. 82% 和 3. 50%,LOD 值分别为 2. 97、2. 66、2. 52 和 4. 65;Pop2 群体检测到 3 个 QTL 分别位于 C1、D1b 和 K 连锁群,表型解释率分别为 23. 11%、7. 53%、14. 21% 和 12. 81%,LOD 值分别为 3. 58、2. 86 和 5. 09,其中位于标记区间 Sat_367–Satt338 的 QTL 解释了 23. 11% 的表型变异。

检测到与亚油酸相关的 QTL 有 5 个,其中 Pop1 群体检测到 2 个 QTL 位于 A1 和 B1 连锁群,表型解释率分别为 5. 05% 和 4. 04%,LOD 值为 2. 91 和 2. 75;Pop2 群体检测到 3 个 QTL 位于 C1 和 H 连锁群,表型解释率为 12. 81%、10. 71% 和 8. 53%,LOD 值为 3. 18、2. 54 和 4. 13。

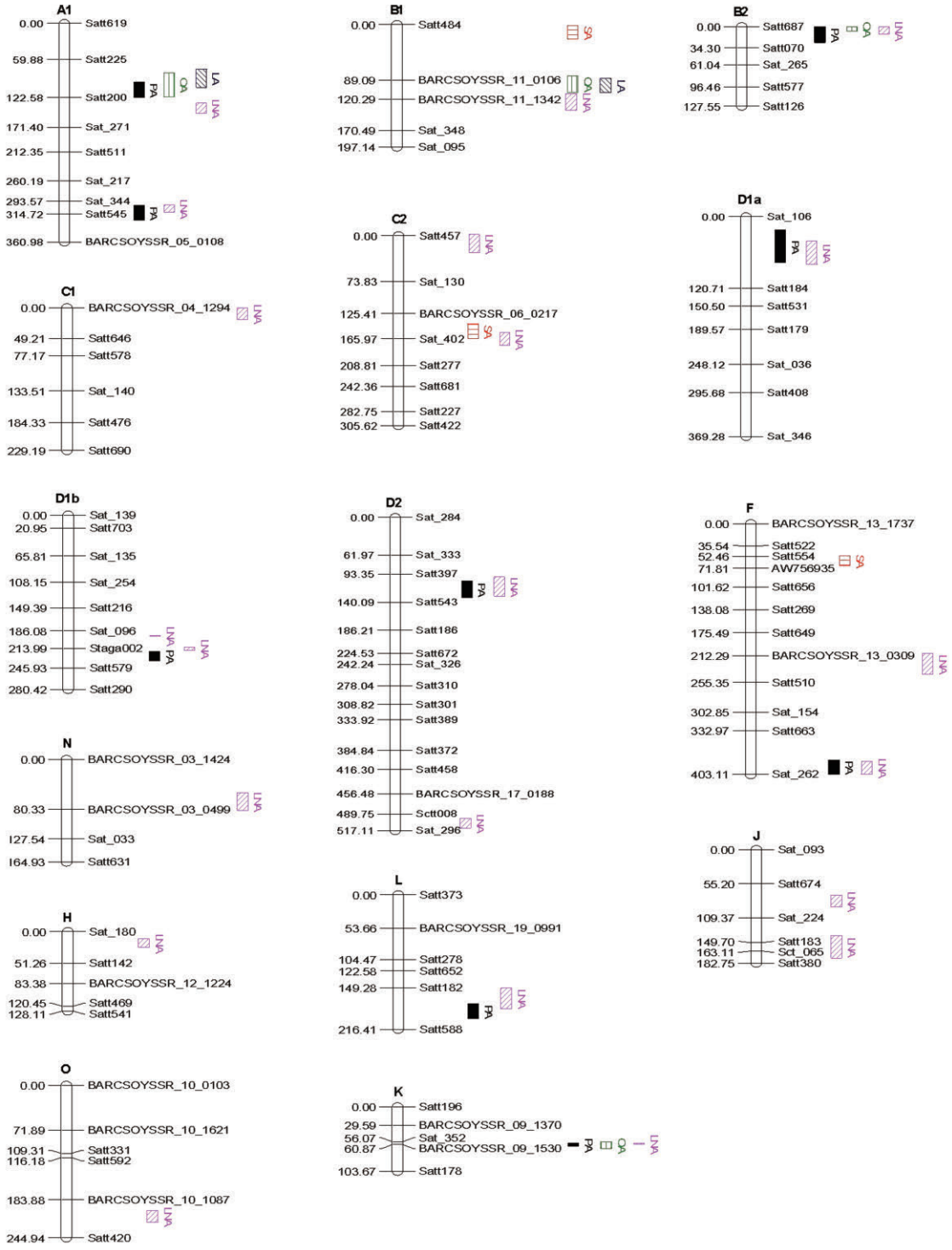
检测到与亚麻酸相关的 QTL 有 26 个,其中 Pop1 群体检测到 21 个 QTL 分别位于 A1、B1、B2、C1 和 C2 等 15 个连锁群,表型解释率为 0. 66%~4. 69%,LOD 值为 2. 52~24. 41;Pop2 群体检测到 5 个 QTL 分别位于 C1、D1b、D2 和 H 连锁群,表型解释率分别为 0. 66%~18. 30%,LOD 值为 2. 94~6. 07,其中位于标记区间 Sat_139–Sat_096 的 QTL 表型解释率最高,为 18. 30%。

2. 4 Pop1 和 Pop2 群体 QTL 分析结果比较

对两个群体定位到的脂肪酸组分 QTL 进行比较发现,能在两个群体中被重复检测到的 QTL 有 4 对,均与亚麻酸含量相关,分别位于 D1b、D2 和 H 连

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锁群上(表3和表4,标记区间红色加粗表示)。其中D1b连锁群上有2对QTL,标记区间分别为Sat_096-Staga002 与 Sat_139-Sat_096、Staga002-Satt579 与Satt703-Satt579,表型解释率分别为4.45%、14.95%、4.69%和18.30%,LOD值分别为5.79、3.74、24.41和6.07;D2连锁群上有1对QTL,为Satt397-Satt543与Satt310-Satt543,表型解释率为2.84%和7.08%,LOD值为6.08和3.00;H连锁群上有1对QTL,为Sat_180-Satt142与Satt142-Sat_401,表型解释率为3.36%和9.80%,LOD值为4.07和5.19。



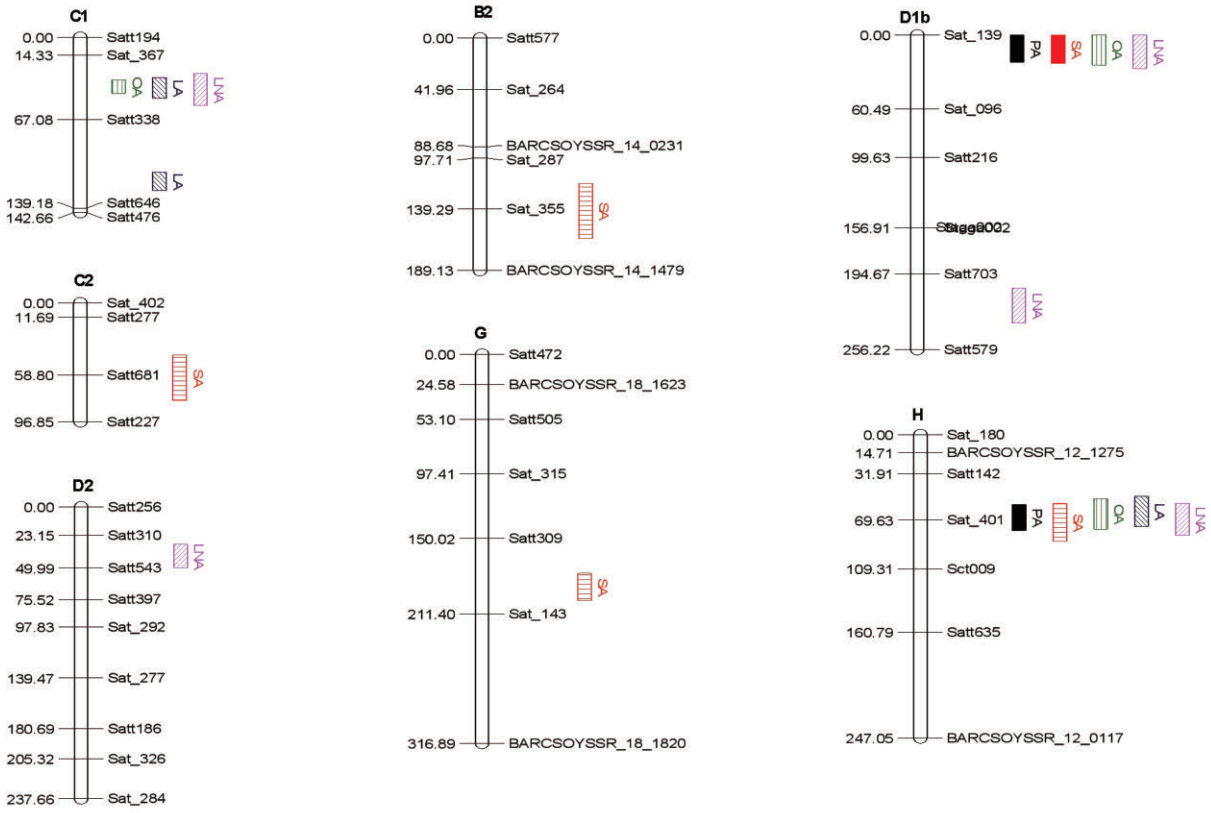
注:PA:棕榈酸;SA:硬脂酸;OA:油酸;LA:亚油酸;LNA:亚麻酸

Note: PA: Palmitic acid; SA: Stearic acid; OA: Oleic acid; LA: Linoleic acid; LNA: Linolenic acid

图2 Pop1群体中定位的脂肪酸组分QTL

Fig. 2 Map locations of QTLs for fatty acids detected in Pop1

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注:PA:棕榈酸;SA:硬脂酸;OA:油酸;LA:亚油酸;LNA:亚麻酸

Note: PA: Palmitic acid; SA: Stearic acid; OA: Oleic acid; LA: Linoleic acid; LNA: Linolenic acid

图 3 Pop2 群体中定位的脂肪酸组分 QTL

Fig. 3 Map locations of QTLs for fatty acids detected in Pop2

3 讨论

在植物中,脂肪酸的合成主要发生在质体上[36],大多数的不饱和脂肪酸都是在饱和脂肪酸的基础上,经过脂肪酸脱氢酶催化而成,如油酸在△12-脂肪酸脱氢酶作用下合成亚油酸,再在△15-脂肪酸脱氢酶催化而合成亚麻酸[37]。已有研究表明,脂肪酸各组分间存在显著相关性:亚油酸与亚麻酸呈极显著正相关,油酸与亚油酸、亚麻酸呈极显著负相关[38-42],棕榈酸与亚油酸、亚麻酸呈极显著正相关[33],油酸与硬脂酸、棕榈酸呈极显著负相关[43]。本研究中,5 种脂肪酸组分的相关性趋势在两个群体相同:棕榈酸与亚油酸、亚麻酸呈显著正相关,与油酸呈显著负相关;硬脂酸与亚油酸、亚麻酸呈显著正相关,与油酸呈显著负相关,该结果与已有报道结论相同。对两个群体中定位的各脂肪酸组分 QTL 进行分析表明,一些标记或标记区间同时与多种脂肪酸组分 QTL 相关:比如在 Pop1 群体中,A1 连锁群上的标记 Satt200 同时与棕榈酸、油酸、亚油酸和亚麻酸相关,B1 连锁群上的标记 BARCSOYSSR_11_0106 同时与油酸、亚油酸和亚麻酸相关,B2 连锁群上的标记区间 Satt687-Satt070 同时与棕榈酸、油酸和亚麻酸相关;在 Pop2 群体中,C1 连锁群上的标记区间 Sat_367-Satt338 同时与油酸、亚油酸和亚麻酸相关,D1b 连锁群上的标记区间 Sat_139-Sat_096 同时与棕榈酸、硬脂酸、油酸和亚麻酸相关,H 连锁群上的标记区间 Satt142-Sat_401 同时与 5 种脂肪酸相关等。这些脂肪酸组分 QTL 的重叠现象,可能是因为基因的一因多效,也可能是脂肪酸组分 QTL 的紧密连锁。因此在进行大豆脂肪酸的遗传改良时,应注意不同组分之间的相关关系,使之达到符合育种目标的最佳平衡。

由于受遗传背景或环境的影响,相同性状在不同群体或不同环境下的定位结果可能不同[44,45]。在开展数量性状的分子标记辅助选择时,那些能在不同遗传背景、不同环境或不同研究中被重复检测到的 QTL 可能具有更高的利用价值[46]。本研究以低亚麻酸材料 FA10 为共同父本分别与中豆 32 和中豆 43 杂交构建了两个 F_2 分离群体,采用 SSR 分子标记,在两个群体中分别定位到 39 个和 18 个与脂肪酸含量相关的 QTL。将定位到的脂肪酸组分 QTL 与前人报

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道的QTL进行对比分析,结果表明:两群体共有40个QTL与前人报道的位点可能相同,其中Pop1群体中5个与棕榈酸相关、2个与硬脂酸相关、2个与油酸相关、1个亚麻酸相关和17个与亚麻酸相关的QTL,以及Pop2群体中2个与棕榈酸相关、4个与硬脂酸相关、1个与油酸相关、1个亚油酸相关和5个与亚麻酸相关的QTL与前人报道的脂肪酸相关QTL具有相同或相近(<10 cM)的标记或标记区间(表3和表4)。这40个QTL中,效应值在15%以上的有5个,分别与棕榈酸、硬脂酸和亚麻酸相关(表3和表4),且在前人的研究中多次被定位到[23, 24, 26, 30, 35],表明这5个QTL可能是能在不同遗传背景和环境中稳定遗传的脂肪酸QTL。本研究定位的另外17个与脂肪酸组分相关的QTL和前人研究没有相同或相近的标记或标记区间,可能是新的脂肪酸QTL,其中1个与油酸相关的QTL效应值较大,解释了23.11%的遗传变异,另外16个QTL的效应值较小,表型解释率低于15%。将两个群体定位到的脂肪酸组分QTL进行比较发现,两群体重复检测到4对QTL,分别位于D1b、D2和H连锁群,均与亚麻酸含量相关(表3和表4,图2和图3)。与已报道的脂肪酸相关QTL进行比较,结果表明:本研究在D1b连锁群上定位到的标记区间与Bachlava等[30]、Li等[26]、Xie等[14]和Wang等[25]的定位结果相近,其中标记区间Satt703-Satt579的表型解释率较高,为18.30%,有可能是一个与亚麻酸相关的稳定QTL区间;在D2连锁群上,本研究定位的标记区间与朱明月[33]有相同的标记区间,但表型解释率较低;在H连锁群上,检测的标记区间与Ha等[31]和Zheng等[11]的定位结果相近,但表型解释率较低,分别为3.36%和9.80%。

本研究两个群体共定位到57个与脂肪酸相关的QTL,但能在两个群体重复检测到的QTL较少,仅有4对(表3和表4),均与亚麻酸含量有关。可能原因有:1、本研究所用的两个群体均是F_2群体,无法进行多年多点的田间试验,导致两群体脂肪酸含量表型鉴定结果一致性较差;2、F_2群体的表型值基于单株表现,无法使用群体来代表其表型值,结果可能存在较大的误差而影响两个群体结果的一致性;3、Pop2群体较小,仅有77个单株,导致该群体检测QTL的能力弱,同时导致每个QTL的遗传解释率偏高。虽然本研究定位的QTL中仅有4对能在两个群体中被重复检测到,但是有超过70%的QTL与前人的研究结果保持一致(表3和表4),表明本研究定位的绝大部分QTL可重复性好。这些能在两个群体中或不同研究中被重复检测到的QTL是稳定的脂肪酸相关QTL,可用于大豆脂肪酸改良的分子标记辅助选择。

4 结论

利用低亚麻酸材料FA10为共同父本分别与中豆32和中豆43杂交构建了两个F_2分离群体,采用SSR分子标记,在两个群体中分别定位到39个和18个与脂肪酸含量相关的QTL。其中40个QTL与前人的结果具有相同或相近的标记位点与区间,4对与亚麻酸含量相关的QTL既与前人研究结果一致,又在两个群体中被重复检测到。这些稳定的脂肪酸相关QTL可用于大豆脂肪酸改良的分子标记辅助选择。

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 DOI:10.3969/j.issn.1006-8082.2021.01.003 中国稻米 2021,27(1):9-13

多年生稻稻瘟病抗性评价

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摘 要:利用长雄野生稻(*Oryza longistaminata*)地下茎无性繁殖特性培育多年生稻(Perennial Rice, PR)已经成功并开始示范推广。多年生稻表现出一定的稻瘟病抗性,但其所具有的稻瘟病抗性来源尚不清楚。本研究通过田间病情调查、接种鉴定以及抗性基因检测等3种方法,对育成的多年生稻23(PR23)、云大24(PR24)、云大25(PR25)、云大101(PR101)、云大107(PR107)、父本长雄野生稻、母本RD23、(RD23/长雄野生稻)F_1进行稻瘟病抗性评价。结果表明,父本长雄野生稻、(RD23/长雄野生稻)F_1代及5个多年生稻品种(系)表现为高抗稻瘟病,而母本RD23表现高感稻瘟病,推测PR23、PR24、PR25、PR101和PR107这5个多年生稻品种(系)的稻瘟病抗性可能来源于长雄野生稻。其中,PR23、PR25稻瘟病抗性基因可能来自于长雄野生稻的*Pi5*基因和*Pita-2*位点,PR24稻瘟病抗性基因可能是来自长雄野生稻的*Pita-2*位点,PR107稻瘟病抗性基因可能来自于长雄野生稻的*Pi5*基因和*Pish*位点;PR101中未检测到本文中涉及到的基因或位点,推测其稻瘟病抗性来自长雄野生稻内未知的稻瘟病抗性基因。本研究结果将为多年生稻稻瘟病抗病育种、品种布局、植保技术制定等提供一定参考。

关键词:多年生稻;稻瘟病;抗性评价;长雄野生稻

中图分类号:S435.111.4+1;S511 **文献标识码:**A **文章编号:**1006-8082(2021)01-0009-05

稻瘟病是由稻梨孢菌(*Magnaporthe oryzae*)引起的水稻真菌病害之一,具有发病急、破坏力强、发病地区广泛、发病时期及发病部位类型多、造成经济损失严重等病害特征[1]。利用抗病基因改良品种是目前防治该病害最有效的措施,至今已定位了100多个稻瘟病抗性主效基因,并克隆了30多个稻瘟病抗性主效基因[2]。利用已开发的功能分子标记,可准确地检测不同水稻品种中存在的稻瘟病抗性基因[3]。

利用长雄野生稻地下茎无性繁殖特性培育的多年生稻已在生产上推广应用[4-6]。多年生稻不仅能实现免耕、减缓耕作带来的水土流失等问题,同时还能减少人力和农资投入[7]。在多年生稻试验示范和推广过程中发现,其对稻瘟病具有一定抗性,但具体的抗性水平和抗病基因还未明晰。虽然已发现长雄野生稻第12号染色体上具有一定稻瘟病广谱抗性基因*Pi57*[8],但该基因是否导入多年生稻品种(系)并引起相应抗性反应还未知。因此,本研究对5个多年生稻品种(系)PR23、PR24、PR25、PR101、PR107,及其父本长雄野生稻、母本RD23及(RD23/*O. longistaminata*)F_1进行田间病情调查、接种抗性鉴定,以及分析已知的多个R基因在多年生稻品种(系)中有无,初步明确多年生稻对稻瘟病的抗性情况,为多年生稻的稻瘟病抗病育种和生产布局提供参考。

1 材料与方法

1.1 试验材料

以多年生稻品种(系)PR23、PR24、PR25、PR101、PR107、长雄野生稻(多年生稻父本)、泰国优质籼稻RD23(多年生稻母本)、(RD23/*O. longistaminata*)F_1及本地感稻瘟病品种丽江新团黑谷(LTH)为供试品种。

供试稻瘟病菌为强致病力CH091C菌株,由云南省农业科学院农业环境资源研究所保存和提供。

1.2 田间病情调查

2019年6月,在云南省西双版纳州多年生稻示范推广田块进行多年生稻品种(系)的叶瘟田间病情情况调查。采用五点取样法,各品种(系)取样数量为100株,参照国际水稻研究所叶瘟抗性评价分级标准[9]进行病级分级,按照病情指数=∑(各级病株数×该病级值)/(调查总株数×最高级值)×100进行病情指数计算。病情指数为0表示高抗(HR),0~5%表示抗(R),5.1%~15%表示中抗(MR),15.1%~25%为中感(MS),25.1%~50%为感(S),>50%为高感(HS)。水稻苗瘟、叶瘟抗性

收稿日期:2020-11-23

基金项目:云南省重大科技专项"多年生稻种质创新及应用研究"(2019ZG013)

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表 1 水稻苗瘟叶瘟抗性分级标准(IRRI)

病级	受害情况	抗性水平
0	叶片无病斑	高抗(HR)
1	病斑为针头大小褐点	抗(R)
2	褐点稍大	抗(R)
3	圆形至椭圆形的灰色病斑,边缘褐色,病斑直径 1~2 mm	中抗(MR)
4	梭形病斑,长 1~2 cm,通常局限于两条叶脉间,受害面积不超过叶面积的 2%	中抗(MR)
5	梭形病斑,受害面积不超过叶面积的 10%	中感(MS)
6	梭形病斑,受害面积不超过叶面积的 11%~25%	中感(MS)
7	梭形病斑,受害面积不超过叶面积的 26%~50%	感(S)
8	梭形病斑,受害面积不超过叶面积的 51%~75%	感(S)
9	全叶枯死	高感(HS)

表 2 稻瘟病抗性基因检测的引物序列及相关信息

目的基因	前引物序列	后引物序列	目标片段/bp		退火温度	文献
			抗病(R)	感病(S)	/℃	
Pizt	ATGTGGATGCTGTGTTAT	TAGTTTGCTGCTCAATAAGTA	176		55	[10]
Pi2	CAGCGATGGTATGAGCACAA	CGTTCCTATACTGCCACATCG	450	282	53	[11]
Pi9	GCTGTGCTCCAAATGAGGAT	GCGATCTCACATCCTTTGCT	291		55	
Pikh	CCCAACATTGGTAGTAGTGC	TCCTTCATACGCAACAATCT	258	401	51	[12]
Pi5	ATAGATCATGCGCCCTCTTG	TCATACCCCATTCGGTCATT	206	307	57	[11]
Pik	GGATAGCAGAAGAACTTGAGACTA	CATGTCTTTCAACATAAGAAGTTCTC	140		55	
	GGATAGCAGAAGAACTTGAGACTG	CATGTCTTTCAACATAAGAAGTTCTC		140		
Pikp	GGTGTTTGGGAACCTGAACCCT	TTTCTGTTCGTCGGATGCTC	158		55	
	GGTGTTTGGGAACCTGAACCCTG	TTTCTGTTCGTCGGATGCTC		158		
Pi1	GTGCTGCTGTGGCTAGTTTG	AGTCCCCGCTCAATTTTTCT	460		58	[13]
Pikm-1	CAGTAGCTGTGTCTCAGAACTATG	AAGGTACCTCTTTTCGGCCAG	290	323	56	[14]
Pikm-2	TGAGCTCAAGGCAAGAGTTGAGGA	TGTTCCAGCAACTCGATGAG	174	213	58	[14]
**Pita-2*	TTGAGAGCGTTTTTAGGATG	TCGGTTTACTTGGTTACTCG	169		55	
Piz	GCATTTTTAGCTATGAATCTGGAT	TGTAGAATGAGGTGAGTTATTAACA	200		55	
**Pish*	CCACTTTCAGCTACTACCAG	CCACTTTCAGCTACTACCAG	136		55	[15]
**Pi7*	CCACTTTCAGCTACTACCAG	GAGCCTTCTGGTTTCTGCTATGC	184		55	[15]
**Pi20*	GAGATGGCCCCCTCCGTGATGG	TGCCCTCAATCGGCCACACCTC	255		55	[16]
**Pi57*	GTATTACGCTCGATAGCGGC	GTATCCTTTCTCGCAATCGC	148		55	[8]
	GTATTACGCTCGATAGCGGC	GTATCCTTTCTCGCAATCGC	169		55	

* 为 SSR 连锁分子标记。

分级标准见表 1。

1.3 苗瘟抗性水平评价

以 LTH 为感病对照，利用 5%次氯酸钠对 RD23、PR23、PR24、PR25、PR101、PR107 种子以及长雄野生稻地下茎尖组织进行消毒后催芽，每个材料在播种盘(规格为 30 cm×10 cm)中播 2 行,每行 10 株,共 20 株,待长至 3 叶 1 心时接种稻瘟病菌。稻瘟病菌 CH091C 菌株于燕麦培养基(燕麦 10.0 g、琼脂 4.0 g、蔗糖 5.0 g,加水至 300 mL,120℃高温高压灭菌)上进行活化培养,培养 7 d 后将菌丝洗净放置于光照培养箱中,产孢培养 2 d 后配置浓度约 1×10^5 个/mL 孢子悬浮液。用喷壶均匀喷洒于水稻叶片上，放置于培养箱中 25℃保湿培养 24 h。接种 7 d 后,参照国际水稻研究所苗瘟抗性鉴定分级标准进行抗性水平鉴定。

1.4 稻瘟病抗性基因检测

利用已知的多个稻瘟病抗性基因功能标记和紧密连锁 SSR 标记对多年生稻材料进行 PCR 扩增（表 2),扩增产物利用 1.0%(目标产物>500bp）或 2.0%的琼脂糖凝胶和 8%聚丙烯酰胺凝胶电泳进行检测（目标产物<500 bp)。

2 结果与分析

2.1 稻瘟病田间病情调查结果

通过调查多年生稻示范推广田的稻瘟病发病情况,发现多年生稻品种(系)和父本长雄野生稻无明显叶瘟症状,呈现较强的稻瘟病抗性,PR23、PR24、PR25、

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表 3 多年生稻品系(种)田间病情调查和苗瘟抗性评价结果

材料名称	田间叶瘟病级	田间病情指数/%	田间叶瘟抗性评价	苗瘟抗性水平评价
长雄野生稻	0 级	0	HR	R
RD23	3~8 级	63.33	HS	S
F_1	0~1 级	0.74	R	–
PR23	0~1 级	1.44	R	R
PR24	0~2 级	1.47	R	R
PR25	0~2 级	1.56	R	R
PR101	0~1 级	1.11	R	R
PR107	0~2 级	1.33	R	R
LTH	–	–	–	S

表 4 10 个 R 基因在多年生稻品系(种)及亲本中的分布检测

R 基因	长野	RD23	F_1	PR23	PR24	PR25	PR101	PR107	Yes/No	单基因系	CH091C 鉴定反应
Pik	+	–	–	–	–	–	–	–	N	KRBLK KA	R
Pikp	–	–	–	–	–	–	–	–	N	KRBLKP-K60	R
Pi5	+	–	–	+	–	+	–	+	Y	IRBL5-M	R
Pi2	–	–	–	–	–	–	–	–	N	IRBLZ5-CA	R
Pizt	+	+	+	+	+	+	+	+	N	IRBLZT-T	R
Pi1	–	+	+	+	+	+	–	–	N	IRBL1-CL	R
Pi54	+	+	+	–	+	–	+	–	N	KRBLKH-K3	R
Pi9	+	+	+	–	–	–	+	–	N	IRBL9-W	R
Pikm	+	–	–	–	–	–	–	–	N	IRBLKM-TS	R
Piz	–	+	+	+	+	+	+	+	N	IRBLZ FU	R

“+”携带 R 基因;“–”不携带 R 基因。

表 5 5 个 R 基因在多年生稻及其亲本中的多态性检测

R 基因	长野	RD23	F_1	PR23	PR24	PR25	PR101	PR107	Yes/No	单基因系	CH091C 鉴定反应
Pish	R	S	H	S	S	S	S	R	Y	IRBLSH-S	R
Pi7	S	R	H	S	S	S	S	S	N	IRBL7-M	R
Pita-2	R	S	H	R	R	R	S	S	Y	IRBLTA2-PI	R
Pi20	S	S	S	S	S	S	S	S	N	IRBL20-IR24	R
Pi57	R	S	S	S	S	S	S	S	N	–	R

R,扩增目的条带;S,扩增非目的条带;H,扩增分别来自 2 个亲本的目的带和非目的带。

PR101、PR107、长雄野生稻、F_1 病情指数分别为 1.44%、1.67%、1.56%、1.11%、1.33%、0、0.74%(表 3)。而母本 RD23 具有典型稻瘟病斑,调查病株最轻病级达 3 级(叶片出现椭圆灰白病斑),大部分病株病级分布于 5~7 级(受害面积 10%~50%),最高病级可达 8 级(叶部受害面积为 51%~75%),病情指数为 63.33%(表 3)。

2.2 苗瘟抗性水平评价

接种强致病力稻瘟病菌 CH091C 菌株 7 d 后,PR23、PR24、PR25、PR101、PR107 和长雄野生稻仅有针尖大小病斑,表现为抗病。而感病对照丽江新团黑谷(LTH)和多年生稻品种(系)母本 RD23 均具有典型叶瘟症状,表现为感病(表 3)。多年生稻品种(系)苗期接种稻瘟病菌进行稻瘟病抗性水平鉴定与田间病情调查结果较为一致,说明多年生稻品系(种)具有较强的抗瘟性,同时该特性可能来源于父本长雄野生稻。

2.3 稻瘟病抗性基因检测

利用功能标记检测 10 个已知的 R 基因在多年生稻中的分布,在 PR23 和 PR25 中均检测到抗性基因 *Pizt*、*Pi5*、*Pi1*、*Piz*,但只有 *Pi5* 在长雄野生稻以及 PR23、PR25 中检测得到,且在 RD23 中缺失,所以 PR23、PR25 所携带的 *Pi5* 基因可能来源于长雄野生稻(表 4)。

PR107 携带 *Pizt*、*Piz*、*Pi5* 稻瘟病抗病基因,仅 *Pi5* 在长雄野生稻检测到,而 RD23 中缺失,因此来源于长雄野生稻的 *Pi5* 可能为 PR107 的稻瘟病抗病基因(表 5)。

利用与 *Pish*、*Pita-2*、*Pi7*、*Pi20*、*Pi57*(*t*)基因分别连锁的 SSR 标记 RM212(*Pish*)、RM6905(*Pita-2*)、RM27384/RM27386(*Pi7*)、OSR32(*Pi20*)、RM5364/RM7102(*Pi57*(*t*))对 5 个多年生稻品系(种)进行基因型评价,发

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现 PR23、PR24、PR25 中携带来自长雄野生稻的 *Pita-2* 位点，而 PR107 中携带来自长雄野生稻的 *Pish* 位点（表 5）。推测 PR23、PR24、PR25 的稻瘟病抗性基因可能来源于长雄野生稻的 *Pita-2* 位点，而 PR107 的稻瘟病抗性基因可能来源于长雄野生稻的 *Pish* 位点。

因此，PR23 和 PR25 的稻瘟病抗性基因可能来源于长雄野生稻的 *Pi5* 基因和 *Pita-2* 位点，PR24 稻瘟病抗性基因可能来源于长雄野生稻的 *Pita-2* 位点，PR107 稻瘟病抗性基因可能来源于长雄野生稻的 *Pi5* 基因和 *Pish* 位点，本文涉及到的稻瘟病抗性基因或位点 PR101 中均不存在，推测其稻瘟病抗性来自长雄野生稻未知的稻瘟病抗性基因。

3 结论与讨论

稻瘟病是危害最严重的水稻病害之一，培育和推广抗病品种是目前最科学的防治对策。由于稻瘟病菌变异频率较高，大部分抗病品种的抗性会随着应用时间的增长而逐渐降低直至消失[17]。因此，需要不断挖掘、鉴定新的抗病品种进行推广或作为抗源应用于未来的稻瘟病抗性育种中。本文针对具有抗稻瘟病潜力的 5 个主栽多年生稻品种（系）的抗性水平进行了初步探究。在田间病情调查中，这 5 个多年生稻品种（系）的病情指数均未达到感病系数，抗性鉴定中，这 5 个多年生稻品种（系）对接种稻瘟病菌为完全免疫反应。因此，可以认为这些多年生稻抗稻瘟病。此次多年生稻田间抗性的评价是针对其自然状态下的发病情况来评定，反应了多年生稻在田间的自然抗性的差异，今后可以进一步建立病圃对多年生稻的抗病水平进行更精准的评定。除此之外，对多年生稻抗性水平的全面解析还需进一步利用更多的优势生理小种对其抗谱进行测定，并结合田间鉴定。

多年生稻亲本的田间病情调查结果和抗性鉴定均显示，长雄野生稻为抗性品种，RD23 为感病品种，多年生稻的稻瘟病抗性可能来源于父本长雄野生稻。且有前人报道，基于长雄野生稻转录组中发现大量导致栽培稻感稻瘟病的基因在长雄野生稻中没有检测到相应的基因信号，长雄野生稻可能是稻瘟病抗性基因库[18]。本文利用功能性分子标记和紧密连锁 SSR 标记对多个稻瘟病抗性基因在多年生稻品种（系）的分布及遗传背景进行分析，发现 3 个抗性基因位点 *Pish*、*Pita-2*、*Pi5* 可能为多年生稻中来源于长雄野生稻的抗性基因。另外，本研究中还发现部分多年生稻的稻瘟病抗性可能来源于长雄野生稻中其余已知基因或未知基因，这些未知基因很可能是长雄野生稻中未被挖掘的稻瘟病抗性新基因，需进行更深入的研究。

无论是田间自然发病、接种鉴定，还是鉴定部分已知的稻瘟病抗性基因或位点，均表明已经育成的多年生稻 23、云大 24、云大 25、云大 101、云大 107 具有较好的稻瘟病抗性，这对多年生稻的示范推广、品种布局、植保技术等示范推广具有实际指导意义。

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Evaluation of Rice Blast Resistance in Perennial Rice

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Abstract: Perennial rice has been developed successful by utilizing the rhizome trait of the wild rice species, *Oryza longistaminata* and demonstrated widely, exhibiting broad-spectrum resistance to rice blast. To understand the reason of resistance to blast of perennial rice, disease investigation in famer's field, inoculation identification and molecular detection of rice blast resistant genes were carried out among nine accessions including five perennial rice varieties (lines) as Perennial Rice 23(PR23), Yunda24 (PR24), Yunda25(PR25), Yunda101 (PR101), Yunda107 (PR107), male parent *O. longistaminata*, female parent RD23, F_1(RD23/O. *longistaminata*) and Lijiangxintuanheigu(LTH). The results showed that all the five perennial rice varieties(lines), their male parent *O. longistaminata* and the original F_1 (RD23/*O. longistaminata*) had high resistance to rice blast, while their female parent RD23 showed high susceptibility, which indicated that the rice blast resistance gene of these five perennial rice lines may originated from *O. longistaminata*. The resistance of PR23 and PR25 might owing to the *Pi5* gene and *Pita-2* locus from *O. longistaminata*, and the resistance of PR24 might due to the *pita-2* locus from *O. longistaminata*, while that of PR107 might inherited from *O. longistaminata* with the *Pi5* gene and *Pish* locus. However, none of the known rice blast resistance genes or loci mentioned in this study were harbored by PR101, speculated that the unknown genes from *O. longistaminata* inherited by PR101 archived its blast resistance. The results of this study provided a basis and strategy for blast disease resistance breeding, perennial rice varieties distribution and plant protection technology decision in future.

Key words: perennial rice; rice blast; resistance evaluation; *Oryza longistaminata*

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Analysis of Characteristics of Temporal and Spatial Variation of Rice Production in the World

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Abstract: The average annual growth rate of rice area, yield per unit area and total yield per unit area increased by 0.67%, 1.65% and 2.33%, respectively, according to the analysis of global rice production from 1961 to 2018. Rice is cultivated in the continents of Asia, Africa, America, Europe and Oceania. Among the rice planting areas in the world, Asia accounts for 87.9%, Africa accounts for 7.7%, America accounts for 4.0%, and Europe and Oceania account for less than 0.5%. Compared with the 1960S, the global rice planting area and the distribution of total rice production in the near 10 years have changed. The harvest area of rice in Africa has increased, and the harvest area of rice in Asia has decreased. Global rice yield growth factor analysis showed that 60% to 70% of the total yield growth was contributed from the increase of yield per unit area. The contribution factors of total rice production growth varied greatly among different continents.

Key words: rice; yield; growth rate; temporal and spatial distribution; world

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马铃薯番茄共生新个体培育技术

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摘　要：嫁接作为一项农业生产技术，已在农业、经济作物和园艺植物中广泛使用。在农业育苗和生产中，嫁接是一种提高抗性、解决连作障碍、增产增收的重要技术教学手段，对作物产量、品质、抗性等均有重要影响。本文主要介绍和采用 2 种方式进行紫色马铃薯与樱桃番茄嫁接。通过嫁接技术将 2 种茄科作物融合到单株植物上，培育了马铃薯—番茄共生作物，实现地上部分长番茄，地下部分结马铃薯的效果。试验提高了作物品质、增加了抗性并增加了空间利用率，为其它作物的嫁接提供理论依据。

关键词：番茄；马铃薯；嫁接；共生作物
中图分类号：S339. 4+5　　文献标识码：A　　DOI：10. 19754/j. nyyjs. 20210115023

随着经济社会的发展和消费结构的升级，中国居民消费呈现出一种多元化、个性化的发展趋势，高效化、立体化栽培逐渐成为农业的主要发展目标。实施绿色特色农业促进项目，可以发挥区域比较优势，促进特色化发展，形成差异化竞争局面。马铃薯和番茄在中国均具有十分重要的地位。本文以立体化种植的角度为导向，在嫁接培育的方法上取得了进展，通过以马铃薯为砧木，番茄为接穗嫁接融合试验，培育了马铃薯—番茄共生作物。

1　材料与方法

1.1　试验材料

试验选用的接穗是红色樱桃番茄，果实形状为圆形，果形周正，果肉厚、耐裂，含糖量高，口味较好，风味独特，且对土壤适应性强，产量高，耐热耐湿性强。砧木是云南地方紫色马铃薯品种，幼苗直立，生长势强；芽眼浅，块茎大小中等，长椭圆形；抗性强，花青素含量高，薯肉为紫红色，商品性好，口感香面。栽培基质是砂质红壤、腐殖土按 1∶3 比例混合均匀使用，基肥以农家肥为主，配合氮磷钾复合肥、辛硫磷 1∶800 倍液均匀喷洒拌匀。嫁接过程中需要嫁接刀、嫁接膜、嫁接夹、嫁接器、加仑盆等。

1.2　试验方法

试验于 2019 年 11 月—2020 年 8 月在云南大学试验温室进行。试验使用了传统嫁接和嫁接器嫁接 2 种方法。砧木和接穗采用了马铃薯—番茄嫁接，并以马铃薯—马铃薯嫁接、番茄—番茄嫁接为对照。同时，在 1 月、2 月、3 月不同月份进行嫁接，因不同时期光照、温度、水分等农业气象条件不同，比较哪一时期的农业气象条件更利于嫁接成活率提高。嫁接试验时，每种方法 20 个重复，共 360 次嫁接试验。

1.2.1　前期准备

1.2.1.1　1 月嫁接

2019 年 11 月下旬，将农家肥与土壤混拌，加入基肥，充分拌匀，为樱桃番茄种子播种做准备。12 月中旬，再次准备基质，为马铃薯播种做好准备（先播种番茄是因为番茄的生长周期更长，便于嫁接时 2 种作物生长情况一致，提高嫁接成活率）。加仑盆土深位置在满盆的 3/5 左右，为后期培土留够空间。

1.2.1.2　2 月嫁接

2019 年 12 月下旬，将农家肥与土壤混拌，加入基肥，充分拌匀，为樱桃番茄种子播种做准备。2020 年 1 月中旬，再次准备基质，为马铃薯播种做好准备。

1.2.1.3　3 月嫁接

收稿日期：2020-12-11
基金项目：云南省教育厅科学研究基金项目“马铃薯番茄嫁接关键技术及应用研究”（项目编号：2019J0030）
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2020 年 1 月下旬，将农家肥与土壤混拌，加入基肥，充分拌匀，为樱桃番茄种子播种做准备。2 月中旬再次准备基质，为马铃薯播种做好准备。

1.2.2 番茄种子处理

准备好的樱桃番茄种子用 15% 次氯酸钠消毒 10min，用常温清水清洗干净后，再用 30～32℃清水不断搅动，浸种 4h，种子充分吸水后，白天控制在 28℃、夜间控制在 10℃催芽。26h 后露白，清水漂洗，均匀播种于加仑盆内，浇水。6～7d 后出苗。

1.2.3 马铃薯种薯处理

用 0.2%～0.3%高锰酸钾溶液喷洒在种薯上，喷洒要均匀彻底，直至种薯被淋湿完全进行消毒，喷湿后密闭保存 1.5h，放于室内通风处晾干。移至室温 15～20℃的温暖室内，铺上稻草或松叶把优选的马铃薯种薯放于上面，再用稻草或松叶覆盖进行遮光催芽，待马铃薯芽长至 0.5～0.8cm 时，播种于加仑盆，浇水。16d 出苗。

1.3 嫁接时间

1 月 16 日、2 月 15 日和 3 月 16 日，马铃薯长到 10～12cm，番茄嫁接苗株高达 8～10cm 或 6～8 片叶时进行嫁接。

1.4 嫁接方法

1.4.1 传统嫁接

在 12～15℃温度条件下，取嫁接苗顶端茎节 5～6cm，茎端削成刀面平滑的楔形（使用嫁接刀前，先用 75%的酒精擦拭浸泡几分钟，以免嫁接刀中带有细菌，使植株感病），用嫁接刀从马铃薯距离地面 8～9cm 处切断，在切口处向下切 1cm 左右，将嫁接苗插入切口，用嫁接膜包住，再使用嫁接夹固定。取过尖的番茄仍可做定植苗使用。

1.4.2 嫁接器嫁接

在同样的情况下，使用嫁接器对番茄和马铃薯进行嫁接。用嫁接器将番茄和马铃薯切开，以马铃薯为砧木，番茄为接穗，将 2 种作物在切口处用嫁接膜固定住，再用嫁接夹夹住。

1.5 嫁接后管理

在温度 12～15℃、75%～80%的空气湿度条件下，进行嫁接口愈合，白天温度保持在 25～28℃，夜间温度 22～23℃，嫁接后前 3d 完全遮光，切口结合过程用清水进行喷雾，使空气湿度接近 80%～85%状态，之后逐渐增强光照，降低湿度。嫁接 10d 后转入正常管理，再过 10d 统计嫁接成活率。

1.5.1 水分管理

春末夏初，嫁接苗成活后，在加仑盆土深 3/5 以上苗周围覆土，起到保水作用和防止倒伏。嫁接苗生长期间，特别是在始花期和薯块膨大期要注意灌水及保持土壤的湿润。嫁接苗成活到薯块快速膨大、樱桃番茄开始结果时期，是嫁接植株合成和积累营养的重要时期，对水分的要求逐渐加大。若块茎膨大期和樱桃番茄开始挂果期间缺水，会发生早衰情况，出现叶子发黄蔫萎，导致降低产量。这一时期要及时浇水，避免马铃薯因干旱导致早衰现象，影响产量。

1.5.2 追肥

在嫁接苗成活后结合灌水进行第 1 次复合肥溶液追肥。根据苗情，生长旺期进行第 2 次追施，同时可用磷酸二氢钾进行叶面喷施，能够增加植株对钾的吸收和提高抗性。灌溉与施肥在时间、数量和方式上合理配合，可以促进作物根系粗壮扎实，提高作物品质和产量。

1.5.3 病虫防治

气温回升后，要注意蚜虫、菜青虫、红蜘蛛的防治，以防病毒病传播。可选用 20%的金满枝可湿性粉剂 1200 倍液，或 43%爱卡螨可湿性粉剂 1500 倍液喷雾防治，喷时使叶片湿润充分。对疫病、病毒病进行防治，选用 25%辛硫磷乳油 1000 倍液，70%代森锰锌可湿性粉剂 600 倍液喷施。后续喷药次数视病情控制情况而定，尽量减少施肥量和用药量。后期如果病情反复，继续再喷 1 次。

2 结果与分析

2.1 嫁接成活率

嫁接成活率 = 嫁接成活的株数/总嫁接株数×100%

嫁接成活率如图 1 所示。

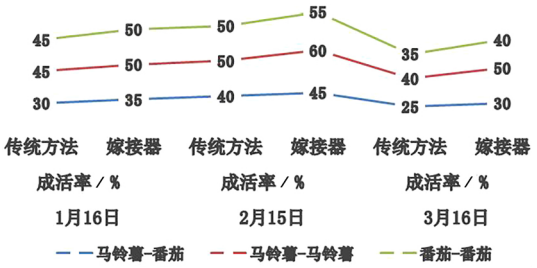


图 1 马铃薯—番茄嫁接成活率

嫁接试验中，1 月气温偏低，不利于嫁接成活；3 月气候虽适于植株生长，但春天干燥，水分散失快，不利于嫁接成活。2 月气温、水分等气候条件适宜，有利于切口水分的保持，减少水分散失，嫁接成活率与 1 月、3 月相比较高。

2.2 抗病虫能力

植株发病率 =（染病株数/调查总株数）×100%

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发病率如表 1 所示。

表 1　马铃薯—番茄嫁接植株发病率

砧木—接穗	发病率				
	菜青虫	红蜘蛛	疫病	TY 病毒	病毒病
马铃薯—番茄	偏低	偏低	偏低	中等	偏低
马铃薯—马铃薯	偏高	中等	偏高	中等	中等
番茄—番茄	中等	偏高	无	偏高	偏高

马铃薯—番茄嫁接、马铃薯—马铃薯嫁接、番茄—番茄嫁接中，植株的抗病虫害能力有所不同。马铃薯—马铃薯植株上主要病虫害为菜青虫和疫病，番茄—番茄植株上主要病虫害是红蜘蛛、TY 病毒和病毒病。与马铃薯—马铃薯植株和番茄—番茄植株相比，马铃薯—番茄共生植株的抗病虫害能力较强。对感病植株交叉喷施辛硫磷和代森锰锌后，有效防治了植株的病虫害，有的植株甚至不再感病，但少部分植株因抗性较弱，在感病后直接死亡。

2.3　影响嫁接成活率原因分析

2.3.1　嫁接时间

由于嫁接成活率会受到砧木和接穗成熟度和水分含量的影响，所以要选择适宜的时间来进行嫁接。每年早春进行，2 月份为宜。马铃薯和番茄长到 6～8 片真叶时为宜。成熟度不够或苗长得过大，特别砧木、接穗木质化程度高、中空和水分含量低都不利于成活。

2.3.2　砧木和接穗的亲和力

接穗的形成层和砧木的形成层对齐，经嫁接后愈合生长的能力会增强。嫁接时切口两面削成楔形，尽量保持平滑，把接穗和砧木靠齐，让二者维管束紧密结合。在马铃薯—番茄嫁接中，嫁接器嫁接成活率 35%比传统嫁接方法成活率 30%高的原因，是因为使用嫁接器，砧木和接穗的削面平整、光滑，而且嫁接器容易操作，减少了切口暴露时间，水分散失少。同属植物亲和力高，易于成活。

2.3.3　农业气象因子

2.3.3.1　光照

适度的光照能促进光合作用和同化产物的生成，加快伤口愈合，但不宜是直射光和强光，因为直射光和强光加快水分蒸发，嫁接后应采用遮阳网遮光 3d，以促进新生组织生长。

2.3.3.2　温度

选择适宜温度嫁接，15～20℃温度范围内，过高过低的温度都不利于伤口愈合和成活。

2.3.3.3　水分

应保持一定的湿度条件，过小接穗水分供应不足，会影响伤口愈合；如果湿度过大，会影响砧木的呼吸作用，伤口容易腐烂，不利于伤口愈合。

3　结论与讨论

经试验和总结，可以通过以下途径提高嫁接成活率：保证砧木和接穗的成熟度和质量；掌握技术关键要领，尽量保证削面光滑平整，尽量缩短嫁接操作时间，砧木和接穗形成层对形成层，保持嫁接口贴合充分紧密；加强嫁接后的管理。改善嫁接空间范围的农业气象小环境，提供适宜的气象因子影响条件。嫁接技术是蔬菜种植生产中克服连作障碍、提高植株抗性逆性、获得丰产的一种有效途径，通过其能实现远缘杂交，产生新的、更广泛的变异，在马铃薯、番茄共生作物繁殖、改良中发挥关键作用。嫁接技术在新型农业如观光农业中的地位越来越高。马铃薯—番茄嫁接可用透明玻璃器皿种植，以地上部分长番茄、地下部分长马铃薯的形式用于观光展览，也为其它植物嫁接用于观光农业提供了一个思路。

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中国农学通报 2021,37(1):61-66
Chinese Agricultural Science Bulletin

苹果SSR-PCR反应体系的建立与优化

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摘 要:为建立苹果属植物SSR-PCR反应优化体系,采用$L_{16}(4^5)$正交实验和退火温度梯度实验,分析反应体系中使用的模板、引物、Mg^{2+}、dNTPs以及Top Taq酶浓度对扩增产物的影响,并对这5个因素进行4个水平不同浓度梯度的筛选和优化。结果表明,引物浓度对SSR-PCR反应体系的影响最大,通过综合分析最终确定SSR-PCR的25 μL最佳反应体系为:30~60 ng DNA模板,1.2 μL正反向引物(5 μmol/L),0.5 μL dNTPs(2.5 mmol/L),0.5 μL Top Taq酶(2.5 U/μL)以及2.5 μL 10× Top Taq buffer(含Mg^{2+})。利用优化后的反应体系对蔷薇科苹果属的21种植物材料进行检测,实验结果表明扩增产物均在100~200 bp左右,可扩增出2~3个有效等位基因。通过优化反应体系中的各影响因素,建立了最佳的SSR-PCR反应体系,可为苹果属植物不同种群的遗传变异研究及亲缘关系的分子鉴定提供前期研究基础。

关键词:苹果;SSR-PCR最佳反应体系;正交设计试验;种质资源;单因素试验
中图分类号:S661.1 **文献标志码:**B **论文编号:**casb20200100068

Establishment and Optimization of SSR-PCR Reaction System of Apple Plants

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Abstract: To establish an optimal SSR-PCR system for apple plants, the $L_{16}(4^5)$ orthogonal test and the annealing temperature gradient experiment were used to analyze the effect of template concentration, primer concentration, Mg^{2+}concentration, dNTPs concentration and Top Taq enzyme concentration in the SSR reaction system on the amplification products. Additionally, the above five factors were screened and optimized at 4 different levels. The results showed that the primer concentration factor had the greatest impact on the SSR-PCR reaction system. Though analysis, the optimal reaction system of 25 μL for SSR-PCR was finally determined to be as follows: 30-60 ng DNA template, 1.2 μL forward and reverse primer (5 μmol/L), 0.5 μL dNTPs (2.5 mmol/L), 0.5 μL Top Taq enzyme (2.5 U/μL) and 2.5 μL 10×Top Taq buffer (containing Mg^{2+}). Finally, optimized reaction system was used for PCR amplification using 21 different apple plants, and the amplification products were about 100-200 bp displaying 2-3 alleles. The optimal SSR-PCR reaction system can provide a preliminary basis for the genetic variation research and molecular identification of genetic relationships in different populations of apple plants.

Keywords: apple; SSR-PCR optimal reaction system; orthogonal design experiment; germplasm resource; single factor test

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基金项目:云南省科技计划"云南特色水果品质提升与优势品牌创建"(2019ZG002);国家自然科学基金"苹果砧木平邑甜茶PcG蛋白MhMSI1调控单性生殖的分子机理"(31660566)。

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收稿日期:2020-01-20,**修回日期:**2020-03-24。

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0 引言

苹果是蔷薇科苹果亚科苹果属(*Malus*)植物，为多年生落叶乔木，是国内主要的落叶果树之一，苹果产业在农业产业结构调整和农民增收中具有重要地位[1]。国内的主产区主要分布在四川、河北、河南、辽宁、山东、山西、陕西、甘肃和新疆等地[2]，不同区域内苹果的品种和栽培生产方式差异较为明显，形成了有地域特色的品种优势，如新疆‘冰糖心富士’、山东‘红富士’、山西‘秦冠’、河北‘王林’等[3]。中国具有悠久的苹果栽培历史，是世界上苹果种植面积最大、总产量最高的国家[4]，但良种苗木繁育体系不健全是影响苹果产量的重要原因之一[5]。中国已经成为世界上最大的苹果生产国和消费国[6]，但自主培育具有地域特色的主栽品种较少，野生和地方特优种质资源的创新与利用日益突出，因此应充分挖掘利用国内资源，扩大优异基因来源，为选育具有自主知识产权的品种提供依据。

国内苹果种质资源丰富、品种类型多样，包括大量的野生种、半野生种及栽培品种，在果树栽培、生产和育种上具有较高的经济价值[7-9]。目前鉴定植物资源的方法主要有文献考证、野外考察、标本采集、品种的分类学鉴定以及DNA分子标记等方法[10]。而DNA分子标记是近年来使用最为广泛的一种遗传标记形式，也是继形态学标记、生化标记和细胞标记之后的一种较为理想的标记方法，同时也是一种鉴定种质资源较为有用的技术。它是以蛋白质、核酸分子的突变为基础，通过检测生物个体在基因或者基因型上所产生的变异来反映生物个体之间的差异[11]。DNA分子标记包含限制性片段长度多态性技术(RFLP)、序列标签位点技术(STS)、随机扩增多态性DNA技术(RAPD)、扩增片段长度多态性技术(AFLP)、简单序列重复技术(SSR)及单核苷酸多态性技术(SNP)等[12]，其中SSR分子标记应用较为广泛。SSR分子标记即微卫星DNA，其序列串联重复，并且主要分布于植物基因组的编码区和非编码区中，在分类水平上具有很好的保守性，并且具有突变速率快、多态性高、重复性好、能区分纯合子和杂合子等特点，是一种理想的分子标记[13]。苹果SSR标记主要基于二核苷酸重复序列进行开发[14]，SSR标记通过基因组扫描技术(GSA)及谱系基因分型技术可以很好地覆盖苹果基因组[15]，前者主要用于绘制基因的抗性基因[16]，后者主要用于等位基因的挖掘以及对性状关联的评估[17]。由于不同物种的最佳SSR-PCR反应体系不同，有必要对苹果属植物的SSR-PCR反应体系进行优化确定。

笔者以21种苹果属植物作为实验材料，采用L_{16}正交实验对模板浓度、引物浓度、Mg^{2+}浓度、dNTPs浓度以及Top Taq酶浓度等5个因素进行优化，并通过退火温度梯度实验对SSR-PCR扩增体系进行优化改良，旨在为利用SSR分子标记鉴定蔷薇科苹果属植物种质资源提供实验技术支撑。

1 材料与方法

1.1 材料

实验于2018—2019年在云南省云南大学农学院进行，所有实验均重复3次。实验所采用的21种苹果属材料均采自云南省昭通市水果推广站实验基地，详见表1。利用随机抽取的‘粉红女士’品种的DNA作为SSR-PCR反应优化体系模板。引物设计参照Liebhard等[18]的研究，由擎科生物工程股份有限公司合成，详见表2。PCR反应所用的dNTPs、Top Taq酶、DNA marker，10×Top Taq buffer (Mg^{2+})以及6× DNA上样缓冲液购自云南金邦(普麦)生物科技有限公司(AP151-11)。

表1 21种苹果属植物

编号	品种	编号	品种	编号	品种
1	德国红星	8	金帅	15	北斗
2	华艳	9	美国8号	16	新红王将
3	金世纪	10	中秋王	17	红蛇
4	红元帅	11	密脆	18	国光
5	乔纳金	12	假红肉	19	华美
6	中原	13	华丹	20	皮诺娃
7	烟富3	14	七月红	21	粉红女士

1.2 仪器设备

高速离心机(HC-2518)，供试PCR仪(Eppendorf AG 22331)，琼脂糖凝胶电泳仪(DYCP-31DN)，聚丙烯酰胺凝胶电泳仪(MGV-202-33)，切胶仪(天根生物科技有限公司)，超微量分光光度计(tGem Spectrophotometer Plus)。

表2 引物序列

引物名称	F(5’-3’)	R(3’-5’)
CH03d11	ACCCCACAGAAACCTTCTCC	CAACTGCAAGAATCGCAGAG
CH01h02[1]	AGAGCTTCGAGCTTCGTTTG	ATCTTTTGGTGCTCCCACAC

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1.3 实验方法

1.3.1 DNA提取及质量检测

(1)DNA提取。方法参照Easy plant Genomic DNA kit(云科生物技术有限公司,code#E11101)。21种苹果属材料分别取0.1 g的嫩叶组织,液氮研磨后,参照DNA提取试剂盒说明书提取,提取的DNA用65℃预热的100 μL ddH_2O溶解,-20℃保存。

(2)浓度检测。通过超微量分光光度计检测DNA浓度。1 μL ddH_2O进行调零,检测1 μL DNA待测液的浓度及OD_{260}/OD_{280}值,取*OD*值于1.8~2.0范围内的DNA用于后续实验。

1.3.2 SSR-PCR反应体系正交设计实验 随机挑选'粉红女士'作为优化体系的DNA模板,CH01h02[1]作为引物,对表3中的引物浓度、Top Taq buffer(Mg^{2+})、dNTPs、Top Taq酶以及引物浓度等5个因素的4个水平进行正交实验,$L_{16}(4^5)$16个组合体系正交表结果如表4。反应体系总体积为25 μL,实验3次重复。

1.3.3 PCR扩增与检测

(1)PCR扩增程序。94℃预变性5 min,94℃变性30 s,49~58℃退火30 s,72℃延伸30 s,共35个循环,72℃后延伸10 min,PCR产物4℃保存。取1 μL PCR扩增产物加入0.2 μL DNA上样缓冲液,分别用1.2%的琼脂糖凝胶电泳及8%的聚丙烯酰胺凝胶电泳进行检测。

(2)产物检测。根据琼脂糖凝胶电泳的条带清晰度以及条带的数量判断模板浓度、引物、Mg^{2+}、dNTPs以及Top Taq酶浓度是否适合反应体系;根据聚丙烯酰胺凝胶电泳条带的清晰度来筛选引物。

1.3.4 最适SSR-PCR反应温度的筛选 参照何正文等[19]的实验方法,对聚丙烯酰胺凝胶电泳(PAGE)条带进行判断和筛选,其中特异性强、弥散度弱、条带清晰的条带视为正确扩增条带。根据扩增正确条带数判断SSR-PCR反应最适组合。

表3 SSR-PCR反应体系因素及水平

水平	因素				
	模板DNA浓度/(ng/μL)	引物浓度/(μmol/μL)	Top Taq buffer(Mg^{2+})/(μmol/μL)	dNTPs/(μmol/μL)	Top Taq/(U/μL)
1	2.25	1.50	2.25	2.50	2.50
2	2.25	3.00	3.00	2.50	2.50
3	2.50	2.75	2.50	2.25	2.25
4	3.00	3.00	2.25	2.50	2.00

表4 苹果属植物SSR-PCR反应的正交实验设计[$L_{16}(4^5)$]

组合编号	模板DNA浓度/(ng/μL)	引物浓度/(μmol/μL)	Top Taq buffer(Mg^{2+})/(μmol/μL)	dNTPs/(μmol/μL)	Top Taq/(U/μL)
1	1.2	0.1	1	0.05	0.25
2	1.2	0.2	2	0.10	0.50
3	1.2	0.3	3	0.15	0.75
4	1.2	0.4	4	0.20	1.00
5	1.6	0.1	1	0.05	0.25
6	1.6	0.2	2	0.10	0.50
7	1.6	0.3	3	0.15	0.75
8	1.6	0.4	4	0.20	1.00
9	2.0	0.1	1	0.05	0.25
10	2.0	0.2	2	0.10	0.50
11	2.0	0.3	3	0.15	0.75
12	2.0	0.4	4	0.20	1.00
13	2.4	0.1	1	0.05	0.25
14	2.4	0.2	2	0.10	0.50
15	2.4	0.3	3	0.15	0.75
16	2.4	0.4	4	0.20	1.00

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运用最佳的SSR-PCR反应体系进行PCR实验，设置PCR反应程序不变，将退火温度设置49、52、55、58℃ 4个温度梯度进行实验（根据引物的退火温度设定），寻找最适退火温度。

2 结果与分析

2.1 蔷薇科苹果属品种DNA提取及浓度测定

对21个品种的DNA进行提取，琼脂糖凝胶电泳检测发现，所有样品的谱带清晰明亮，无明显杂质，DNA完整性较好（图1）。随后通过DNA浓度和纯度的测定，筛选出OD_{260}/OD_{280}值在1.8~2.0之间DNA样品（表5）。

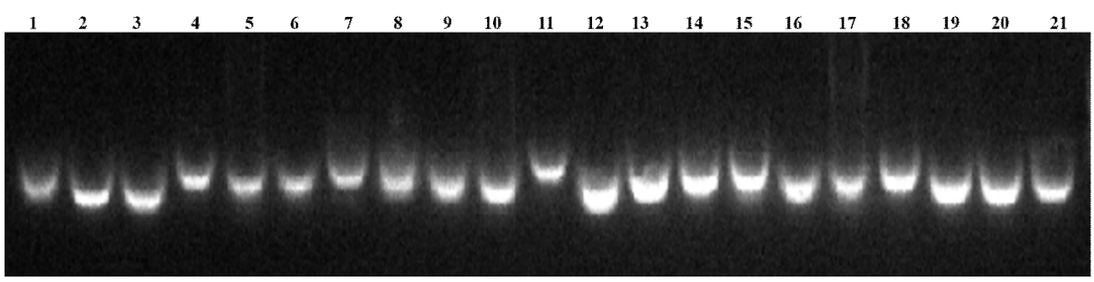
2.2 苹果属植物SSR-PCR反应体系的优化

随机选取CH01h02[1]作为引物（表2），根据表3中16个不同浓度梯度水平的组合进行PCR扩增，将反应产物进行聚丙烯酰胺凝胶电泳实验(PAGE)。由SSR-PCR正交实验结果可以看出，不同组合的扩增结果差异明显，组合6、11、14下扩增出的条带数量多且条带清晰明亮。经过多次重复实验发现组合11、14所扩增出的条带清晰度较高，实验结果较为稳定（图2）。经过多次实验证明，组合14可作为最优体系，即30~60 ng DNA模板、1.2 μL正反向引物(5 μmol/L)、0.5 μL dNTPs (2.5 mmol/L)、0.5 μL Top Taq酶(2.5 U/μL)以及2.5 μL 10×Top Taq buffer（含Mg^{2+}），用ddH_2O补至25 μL。

为进一步证明初步结论的准确性，对正交实验的各因素及水平进行分析（表6），其中*K*值代表在同一水

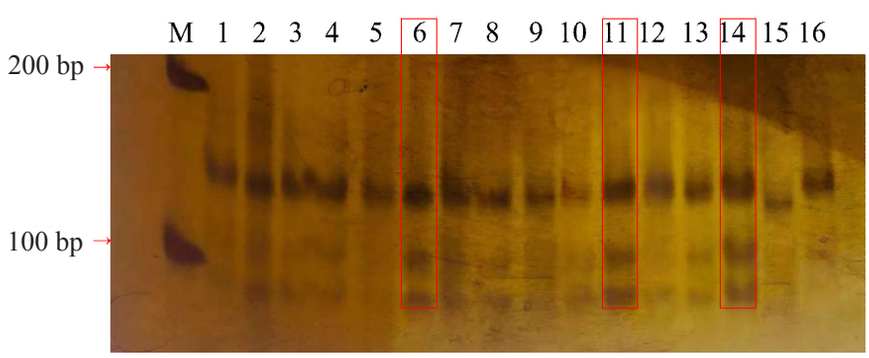
表5 21种苹果属植物品种的DNA浓度及*OD*值测定结果

编号	品种	浓度/(ng/μL)	OD_{260}/OD_{280}
1	德国红星	966.10	1.93
2	华艳	619.60	1.89
3	金世纪	1124.3	2.00
4	红元帅	494.90	2.05
5	乔纳金	1170.3	2,01
6	中原	104.90	1.90
7	烟富3	721.70	1.94
8	金帅	1809.1	1.82
9	美国8号	530.60	2.01
10	中秋王	2853.7	2.07
11	密脆	518.40	1.96
12	假红肉	835.80	1.81
13	华丹	446.40	2.00
14	七月红	974.00	2.02
15	北斗	260.40	2.10
16	新红王将	650.20	1.85
17	红蛇	466.50	1.91
18	国光	275.90	1.91
19	华美	547.30	1.89
20	皮诺娃	496.50	1.96
21	粉红女士	817.20	1.93



1~21表示不同苹果品种（顺序同表1）

图1 蔷薇科苹果属DNA质量检测电泳结果



M为DL700 DNA Marker，1~16分别指表3中1~16个不同组合

图2 正交设计SSR-PCR反应体系扩增（聚丙烯酰胺凝胶电泳检测）结果

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平下所产生的扩增条带的平均值，*R*值能够反映某因素的极差，*R*值越大影响越显著。通过对模板DNA浓度、引物浓度、Mg^{2+}浓度、dNTPs浓度、Top Taq酶浓度5个因素在4个水平内的综合分析发现，对结果影响从大到小依次为引物浓度、Top Taq buffer(Mg^{2+})、模板DNA浓度、Top Taq酶及dNTPs。经验证，上述实验中所获得的SSR-PCR反应体系为适合本实验的最适体系。

2.3 最适退火温度的筛选

引物的退火温度对PCR结果的影响较大，为减少误差，在以上反应体系的基础上对引物的退火温度进行了筛选。结果发现，当退火温度为52℃时，PCR扩增出的条带数目清晰（图3）。

2.4 最佳反应体系的稳定性检验

为了检测SSR-PCR反应的稳定性，随机选用另外一对引物CH03d11进行验证。以21种蔷薇科苹果属品种DNA为模板，利用优化后的SSR-PCR反应体系进行扩增，结果发现，21个品种的扩增结果条带清晰，无拖带并且大部分都具有2个或者3个等位基因，大小在100~200 bp左右（图4），说明该扩增体系比较稳定，可应用于蔷薇科苹果属植物SSR扩增反应。

3 结论与讨论

SSR是基于特异性引物进行PCR的分子标记技术[20]，凡是影响PCR扩增效果的因素，如Mg^{2+}、DNA聚合酶、引物、模板DNA、dNTPs等，均会影响到SSR-

表6 SSR-PCR反应体系的正交实验直观分析

项目	扩增条带数				
	模板DNA浓度	引物浓度	Top Taq buffer(Mg^{2+})	dNTPs	Top Taq
K_1	2.25	1.50	2.25	2.50	2.50
K_2	2.25	3.00	3.00	2.50	2.50
K_3	2.50	2.75	2.50	2.25	2.25
K_4	3.00	3.00	2.25	2.50	2.00
R	0.75	1.50	0.75	0.25	0.50

注：K_1~K_4为同因素下的同一水平的实验均值，*R*为极差。

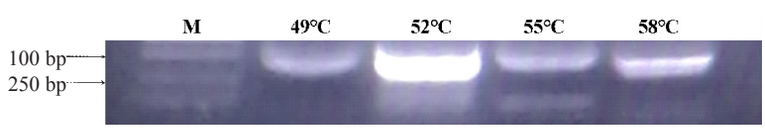
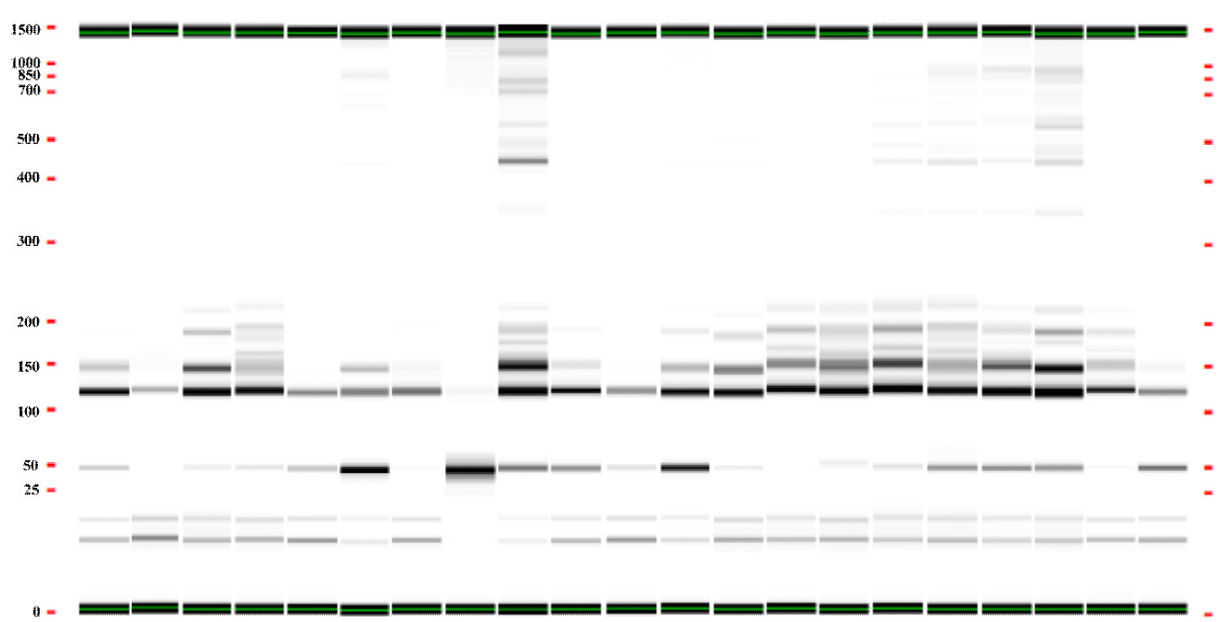


图3 SSR-PCR反应体系最佳退火温度



M为DL2000 DNA Marker，1~20表示不同苹果品种（顺序同表1）

图4 SSR-PCR最佳反应体系检验结果

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PCR的结果。不同物种的SSR-PCR分析报道虽多，但大多是采取单因素梯度实验，过程复杂且不能兼顾各因素之间的相互影响[21-22]。本试验采用正交设计实验能够分析不同因素之间的交互作用[23]，同时也克服了单因素实验规模巨大、顾此失彼的问题[24]，此种方法效率更高、稳定性更好，能够较为全面地找出各因素之间的规律[25]。因而本试验以此来获得最优反应体系，减少SSR技术的误差。

本试验研究前期参考Liebhard等[18]的研究，共选取了108对引物，通过琼脂糖凝胶电泳试验筛选出32对引物，本试验随机挑选了其中的2对引物进行后续的体系优化。研究发现，琼脂糖凝胶电泳的条带极易发生弥散拖尾现象，当模板浓度过低时产物容易弥散；模板浓度过高时引物与dNTPs过早耗尽，结果不稳定；引物浓度过高时会产生非特异性产物，极易形成引物二聚体[26-27]。PCR产物拖尾主要是由于退火温度过低，当退火温度较低时容易出现条带拖尾。通过正交设计实验发现，在SSR-PCR反应过程中，引物浓度的影响最为明显，但模板浓度、dNTPs、Mg^{2+}以及退火温度也起到了较为关键的作用，需根据实验的具体要求灵活安排以提高结果的准确性，将人为因素降低到最小。在整个实验过程中，确定每个引物的最适退火温度实验过程比较繁琐，本研究只列举了引物CH03d11的退火温度。当采用多个引物同时扩增时，反应程序可采用降落PCR方法[28]，降落PCR能够有效降低各组分浓度及退火温度引起的非特异性产物产生，大大缩短实验时间。通过本实验，最终确定适用于蔷薇科苹果属植物SSR-PCR的最适反应体系为：30~60 ng DNA模板，1.2 μL正反向引物(5 μmol/L)，0.5 μL dNTPs(2.5 mmol/L)，0.5 μL Top Taq酶(2.5 U/μL)以及2.5 μL 10×Top Taq buffer(含Mg^{2+})，用ddH_2O补至25 μL。该体系经过验证可用于后续蔷薇科苹果属植物变异遗传稳定及分子身份证的构建研究。

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 DOI:10.3969/j.issn.1006-8082.2021.02.013 中国稻米 2021,27(2):63-67

多年生稻白叶枯病抗性评价

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摘 要:利用长雄野生稻地下茎无性繁殖特性培育多年生稻已经成功,并在生产上进行了示范推广。为明确多年生稻品种(系)对白叶枯病的抗性表现,通过田间病情调查、抗性水平鉴定和水稻白叶枯病抗性基因检测 3 种方法,对多年生稻品种(系)多年生稻 23(简称 PR23,下同)、云大 24(PR24)、云大 25(PR25)、云大 101(PR101)、云大 107(PR107)及其父本长雄野生稻、母本 RD23 和 F_1(RD23/长雄野生稻)的白叶枯病抗性进行评价。结果表明,长雄野生稻高抗白叶枯病;尽管 PR23、PR24、PR25、PR107 携带白叶枯病抗性基因 *Xa1*、*Xa4*、*Xa23*、*xa25* 的等位基因,但在田间自然发病条件下均易感白叶枯病,说明这几个抗性基因对这 4 个多年生稻品种(系)不起抗病作用;而 PR101 在田间自然发病条件下表现为抗白叶枯病,并含有白叶枯病抗性等位基因 *xa25*、*Xa27*,说明这 2 个基因可能是 PR101 抗白叶枯病的基因。本研究结果为明确多年生稻对白叶枯病菌的抗病反应,以及抗白叶枯病育种和多年生稻生产布局提供了一定依据。

关键词:多年生稻;白叶枯病;抗性评价

中图分类号:S435.111.4+7;S511 **文献标识码:**A **文章编号:**1006-8082(2021)02-0063-05

多年生稻是指种植一次可以连续收获多年(季)的水稻,即从第 2 年(季)起不再需要买种、育秧、犁田耙田、栽秧等生产环节,大大减少了劳动力投入和减轻了劳动强度,是一项轻简化的稻作生产技术。该项技术包括了多年生稻品种及配套的耕作栽培技术[1-2]。近年来,云南大学已成功利用长雄野生稻(*Oryza longistaminata*)地下茎无性繁殖特性培育出多年生稻品种[1-3]。其中,多年生稻 23(PR23)已通过云南省审定(审定编号:滇审稻 2018033 号),云大 24(PR24)、云大 25(PR25)、云大 101(PR101)、云大 107(PR107)等品系也开始试验试种。通过前期在云南省内多年多点试验结果来看,尽管多年生稻品种(系)的父本长雄野生稻携带有水稻白叶枯病抗性基因 *Xa21*[4-5],但多年生稻品种(系)在各地的白叶枯抗性表现不一。多年生稻品种(系)是否具备白叶枯病抗性并携带相关抗病基因并不十分清楚,导致在多年生稻抗白叶枯病育种和生产应用上指导性不强。

因此,本研究通过田间病情调查、人工接种抗性水平鉴定和抗性基因检测 3 种方法,对多年生稻品种(系)PR23、PR24、PR25、PR101、PR107 及其亲本长雄野生稻、RD23 和 F_1(RD23/长雄野生稻)进行白叶枯病抗性鉴定,以明确多年生稻品种(系)的白叶枯病抗性水平,为今后多年生稻遗传育种、生产防控,以及产业布局提供一定科学依据。

1 材料与方法

1.1 试验材料

以 PR23、PR24、PR25、PR101、PR107、长雄野生稻(多年生稻的父本)、RD23(多年生稻的母本)、F_1(RD23/长雄野生稻)共计 8 个材料进行白叶枯病抗性鉴定。其中,长雄野生稻来源于非洲尼日尔,RD23 是泰国优质籼稻,杂交后通过幼胚挽救技术获得了 F_1 植株,经过多世代的自交,选育出 PR23、PR24、PR25、PR101、PR107 等多年生稻品种(系)。

1.2 试验地点

试验材料于 2019 年种植于西双版纳州多年生稻育种基地,海拔为 580 m,年平均气温 22.6 ℃,年降水量 1 200 mm。西双版纳州气候高温高湿,水稻生产易感染白叶枯病。田间调查在西双版纳傣族自治州示范推广田块进行。

1.3 田间管理

接种用品种(系)7 月 15 日播种,8 月 5 日移栽大田,每个品种栽 4 行,移栽行株距为 20 cm×20 cm,单苗移栽,每行 10 株。示范田早稻播种期为 1 月 15 日,移栽期为 3 月 1 日,晚稻播种期和移栽期与接种用品种

收稿日期:2020-12-23

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表 1 水稻白叶枯病分级和评价标准

病级	抗性反应	标准评价
0	剪口处无病斑	高抗(HR)
1	剪口处有小病斑,长度不超过 2 cm	抗(R)
3	病斑下延伸 2 cm 以上,不超过叶长的 1/4	中抗(MR)
5	病斑占叶面积 1/4 以上至 1/2	中感(MS)
7	病斑长度占叶面积 1/2 以上至 3/4	感(S)
9	病斑长度超过叶片的 3/4	高感(HS)

表 2 PCR 鉴定所用引物序列

抗病基因	正向引物(5'-3')	反向引物(5'-3')	片段大小/bp		退火温度/℃	参考文献
			抗病	感病		
Xa1	ACTGCCCTCTTGCACACGCCATTGG	CCGGTACATCAGTATTGTCCATCGG	552		58	[11]
Xa3/Xa26	ATGGCTTGACCTAAGTGGA	TGTTCTGAGTGCAGGAGTG	1 100		55	[11]
Xa4	ATCGATCGATCTTCACGAGG	TGCTATAAAAGGCATTCGGG	157		61	[12]
xa5	CCGGAGCTCGCCATTCAAGTTCTTG	TGCTCTTGACTTGGTTCTCC	145	170	53	[13]
Xa10	CATTAGCACAGCCGAAACTC	ATGAAACGAACGGTCAAACA	540		59	[14]
xa13	AGCTCCAGCTCTCCAAATG	GGCCATGGCTCAGTGTTTAT	1 000	280	57	[15]
Xa21	CGATCGGTATAACAGCAAAAC	ATAGCAACTGATTGCTTGG	1 400	1 300	58	[16]
Xa23	TAAGTTCTACATCGACCCCA	CACATGAAGAGCTGGAAAGG	760		58	[17]
xa25	TTCTGTTCCTGTGGCTTTG	TGGATCACTCGCTTCTGCA	930	860	55	[18]
Xa27	TAGTGTCTAAATACAGGGACT	GAGTACTTTGCTCTGATGCTC	149	174	54	[19]

一致,移栽行株距为 20 cm×20 cm,每丛栽 1~2 苗。接种田和示范田田间水肥管理参照当地大面积水稻生产田。

1.4 白叶枯病抗性鉴定

1.4.1 田间病情调查

6 月、10 月对西双版纳州试验田及示范推广田块的 PR23、PR24、PR25、PR101、PR107,以及长雄野生稻、RD23 和 F_1(RD23/长雄野生稻)进行早稻和晚稻的田间白叶枯病病情调查,采用五点取样调查法,病级评价参照已有标准[6](表 1)。

1.4.2 田间接种鉴定

选取 9 个不同致病力的水稻白叶枯病生理小种用于抗性水平鉴定,即 YP1、YP2、YP3、YP4、YP5、YP6、YP7、YP8、YP9 菌株(由云南农业大学植物病理研究室分离鉴定并提供)。其中,YP1 为弱致病力菌株,YP6 为强致病力菌株。病原菌分别于 PSA 培养基(马铃薯 200 g,葡萄糖或蔗糖 20 g,琼脂 15~20 g,蒸馏水 1 000 mL)上 30℃培养 72 h 后,配制 3×10^8 CFU/mL 菌液备用。选取生长时期一致的多年生稻叶片,于 15:00、温度为 28℃~30℃时,采用剪叶法接种[7],即剪去叶尖 1~3 cm 进行伤口接种。每个菌株接种 4 个植株,每个植株接种 3~5 个叶片(剑叶和上 3 叶)。接种 21 d 后调查白叶枯病发病情况。

1.4.3 抗性基因检测

1.4.3.1 DNA 制备 取 0.1 g PR23、PR24、PR25、PR101、PR107、长雄野生稻、RD23、F_1(RD23/长雄野生稻)的幼嫩叶片,CTAB 法[8]提取其基因组 DNA,置于-20℃冰箱保存备用。

1.4.3.2 PCR 鉴定 基于已报道和克隆的 10 个抗白叶枯病基因,包括 7 个显性抗病基因(*Xa1*、*Xa3*、*Xa4*、*Xa10*、*Xa21*、*Xa23*、*Xa27*)和 3 个隐性抗病基因(*xa5*、*xa13*、*xa25*)。利用已发表的功能标记或分子标记进行 PCR 扩增(表 2),利用聚丙烯酰胺凝胶电泳[9]和 1%~2%的琼脂糖凝胶电泳[10]对 PCR 产物进行检测。

2 结果与分析

2.1 田间病情调查

从表 3 可见,长雄野生稻、F_1、PR101 在早、晚稻全生育期对白叶枯病均表现出中抗以上抗性水平;RD23 在早稻全生育期表现出中抗以上抗性水平,在晚稻的分蘖期和孕穗期表现出中感和感(表 3);5 个多年生稻品种(系)均不同程度感白叶枯病,其中,PR107 感病最严重,在早、晚稻苗期、分蘖期和孕穗期均表现出感病。

2.2 抗性水平评价

从供试菌种看,YP6 菌株致病力最强,除了长雄野生稻外,能使多年生稻品种(系)以及 RD23 和 F_1 均感病;YP1 菌株致病力相对最弱,8 个材料均对其表现出抗性(表 4)。

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表 3 多年生稻白叶枯病田间病情调查结果

季节	时期	长雄野生稻	RD23	F_1	PR23	PR24	PR25	PR101	PR107
早稻	苗期	HR	R	R	R	R	R	R	MS
	分蘖期	R	R	R	MS	R	MS	R	MS
	孕穗期	R	MR	MR	MS	MS	MS	R	S
晚稻	苗期	HR	R	R	R	R	R	R	MS
	分蘖期	R	MS	MR	MS	MS	MS	R	S
	孕穗期	R	S	MR	S	S	S	MR	HS

表 4 多年生稻品种(系)及其亲本和 F_1 田间病情调查和抗性水平评价结果

供试菌种	长雄野生稻	RD23	F_1	PR23	PR24	PR25	PR101	PR107
田间自然发病	R	S	MR	S	S	S	MR	HS
YP1	R	R	R	MR	MR	R	MR	MR
YP2	R	R	MR	MR	MR	MR	R	S
YP3	R	MR	MR	MR	MR	MR	MR	MS
YP4	HR	MR	R	MR	R	MR	R	S
YP5	R	MS	R	MR	MR	MR	MR	MS
YP6	MR	MS	MS	S	MS	S	MS	S
YP7	R	R	R	MS	MS	MS	R	S
YP8	R	R	MR	MS	MS	MS	MR	MS
YP9	MR	MS	MS	S	MS	MS	MR	S

表 5 白叶枯病抗性基因在多年生稻品种(系)中的分子检测结果

分子标记	长雄野生稻	RD23	F_1	PR23	PR24	PR25	PR101	PR107
Xa1	+	+	+	+	+	+	+	+
Xa3/Xa26	−	−	−	−	−	−	−	−
Xa4	+	+	+	+	+	+	+	+
xa5	−	−	−	−	−	−	−	−
Xa10	−	−	−	−	−	−	−	−
xa13	−	−	−	−	−	−	−	−
Xa21	+	−	−	−	−	−	−	−
Xa23	+	−	+	+	−	+	−	−
xa25	−	−	+	−	−	−	+	−
Xa27	+	−	+	−	−	−	+	−

“+”表示携带检测基因;“−”表示不携带检测基因。

从多年生稻品种(系)看,长雄野生稻对 9 个供试菌种均表现出抗病;PR101 对 YP6 菌株表现为中感,但对其他 8 个菌株均表现为中抗以上;PR23、PR24 和 PR25 对 9 个菌株具有不同的抗性表现,对 YP6、YP7、YP8、YP9 菌株的感病程度与田间病情调查结果一致;PR107 对除 YP1 外的 8 个菌株均表现出感病(表 4)。

不同致病力生理小种的抗性水平鉴定结果虽然与田间调查结果存在一定差异,但感抗病情况基本吻合,说明该抗性水平评价结果可以直接指导田间白叶枯病害防治。

2.3 抗病基因检测

检测结果(表 5)发现,长雄野生稻、RD23、F_1、PR23、PR24、PR25、PR101 和 PR107 具有 *Xa1*、*Xa4*、*Xa23* 和 *xa25* 等位基因,说明这些材料具有潜在的 *Xa1*、*Xa4*、*Xa23*、*xa25* 抗性基因。田间病情调查结果(表 4)表明,RD23、PR23、PR24 和 PR25、PR107 均感白叶枯病,而长雄野生稻、F_1 和 PR101 均抗白叶枯病,说明 RD23、PR23、PR24、PR25、PR107 携带的为感病等位基因,长雄野生稻、F_1、PR101 可能携带部分相关基因的抗病等位基因。

Xa3/Xa26 基因的功能标记检测显示,在长雄野生稻、RD23、F_1、PR23、PR25、PR101 和 PR107 都未扩增出基因条带,推测长雄野生稻、RD23、F_1、PR23、PR25、PR101 和 PR107 中可能不携带 *Xa3/Xa26* 基因。*Xa21* 基因的功能标记检测显示,只有长雄野生稻中扩增出抗病基因带型,而在 RD23、F_1、PR23、PR25、PR101 和 PR107 扩增出感病基因带型,PR24 中未扩增出条带,推测 *Xa21* 基因虽然是来源于长雄野生稻的白叶枯病抗性基因,但是没有遗传到其衍生的多年生稻品种(系)中。白叶枯病抗性基因 *xa25* 在 RD23、PR23、

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PR24、PR25 和 PR107 中为感病基因型（860 bp),且 RD23、PR23、PR24、PR25 和 PR107 均感白叶枯病,而在 F_1 和 PR101 中为抗病基因型(约 930 bp),推测 *xa25* 可能在 F_1 和 PR101 的抗白叶枯病中起到了积极作用。*Xa27* 基因功能标记检测表明，在长雄野生稻、F_1 和 PR101 中扩增出了抗病基因带型,PR107 中未扩增出条带，在 RD23、PR23、PR24、PR25 扩增出感病基因带型，这与长雄野生稻、F_1、PR101 在田间病情调查结果相符,说明 PR101 可能携带 *Xa27* 抗病基因,起到主要抗病作用。

PR23、PR24、PR25 和 PR107 携带已知的白叶枯病抗性基因 *Xa1*、*Xa4*、*Xa23*、*xa25* 的等位基因,但均感病,说明这几个抗病等位基因在这些材料中为感病基因型;虽然长雄野生稻携带已知白叶枯病抗性基因 *Xa1*、*Xa4*、*xa25*、*Xa21*、*Xa23*、*Xa27*，但从其 F_1 及衍生的 5 个多年生稻品种（系）的田间病情调查结果和携带基因看,*Xa27* 和 *xa25* 基因可能是由长雄野生稻遗传给 PR101 并赋予其白叶枯病抗性的主要原因。

因此,PR101 抗白叶枯病是由于其可能携带来自长雄野生稻的 *Xa27*、*xa25* 基因，而 PR23、PR24、PR25 和 PR107 这 4 个多年生稻品种(系)不具备本研究中用到的 10 个抗白叶枯病基因。

3　结论和讨论

白叶枯病在籼稻和粳稻中发病情况具有一定差异,一般籼稻重于粳稻,晚稻重于早稻[20]。根据对不同致病力白叶枯病菌生理小种抗性水平鉴定发现,PR101 对大部分白叶枯病生理小种表现出抗性，并与田间生产条件下表现一致;PR23、PR24 和 PR25 仅对部分白叶枯病生理小种具有抗性，需要进一步进行白叶枯病抗性遗传改良;PR107 对大部分白叶枯病生理小种均感病,是制约其推广应用的主要限制因素,急需进行白叶枯病的抗性改良。

多年生稻 PR23、PR24、PR25、PR101 和 PR107 中均不含 *xa5*、*Xa3/Xa26*、*xa13*、*Xa10*、*Xa21* 抗病等位基因。PR23、PR24、PR25 和 PR107 携带白叶枯病抗性等位基因 *Xa1*、*Xa4*、*Xa23*、*xa25*，但根据田间病情调查发现这几个品系均感病，说明 *Xa1*、*Xa4*、*Xa23*、*xa25* 抗性等位基因在 PR23、PR24、PR25、PR107 中不起抗病作用；在长雄野生稻中检测到携带有 *Xa1*、*Xa4*、*Xa21*、*Xa23*、*xa25* 和 *Xa27* 等白叶枯病抗性等位基因,但从其 F_1 及衍生的 5 个多年生稻品种(系)的田间病情调查结果和携带基因看,*xa25*、*Xa27* 基因可能是通过长雄野生稻遗传到 PR101 的白叶枯病抗性等位基因。

xa25 是一个隐性的抗白叶枯病主效基因，与其对应的显性基因是 *Xa25*。*xa25* 的供体品种是明恢 63,对白叶枯菌生理小种 PXO339 表现为专化性抗性[21]。由于 *xa25* 的启动子突变,导致 PthXo2 无法被识别,则其表达便不受诱导,表现为抗性[22-23]。*xa25* 的表达受显性等位基因 *Xa25* 的调控。王石平等[24]通过转基因技术将 *xa25* 的部分 DNA 片段导入到水稻品种中,抑制其表达从而增强水稻品种的抗性。所以猜想 PR101 可能是由于抑制了 *xa25* 的表达而表现为抗白叶枯病。

已有报道,白叶枯病抗性等位基因 *Xa27* 来自小粒野生稻(*O. minuta*)[7,25],而来源于长雄野生稻的 *Xa27* 等位基因也可能是白叶枯病的抗性等位基因。WU 等[26]发现,*Xa27* 的表达是通过增加叶片维管束次生细胞壁厚度来抵御病原菌的侵染,推测 PR101 能抵抗白叶枯病可能是通过增加维管束次生细胞壁的厚度来抵御侵害。

多年生稻 PR101 对白叶枯病表现出良好抗性,可作为改良水稻抗白叶枯病的基因库。深入研究多年生稻 PR101 对白叶枯病的抗性反应,有助于水稻白叶枯病抗病育种研究，拓宽水稻抗白叶枯病育种的材料基础;而在利用 PR23、PR24、PR25 和 PR107 进行稻作生产时,需要注意及时有效的进行白叶枯病防治。

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Identification of Bacterial Blight Resistance in Perennial Rice

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Abstract: Perennial rice (PR), using vegetative propagation via Rhizome as perenniality donor from *Oryza longistaminata*, has been bred successfully and demonstrated widely. In order to clarify the resistance performance of perennial rice varieties (lines) to bacterial leaf blight, the perennial rice varieties (lines) PR23, PR24, PR25, PR101, PR107, and their male *Oryza longistaminata*, female parents RD23 and F_1 (RD23/ *Oryza longistaminata*) were used as the materials to evaluate their resistance to bacterial blight by three methods: field disease investigation, resistance level identification and rice bacterial blight resistance gene detection. The results showed that, *O. longistaminata* presents high resistance to bacterial blight. Although PR23, PR24, PR25, PR107 carry the alleles of bacterial blight resistance genes *Xa1*, *Xa4*, *Xa23*, *xa25*, but they are all susceptible to bacterial blight in the field. It shows that these resistance genes do not play a role in disease resistance in these four perennial rice varieties (lines). PR101 shows resistance to bacterial blight under natural disease conditions in the field, and contains the bacterial blight resistance alleles *xa25*, *Xa27*, indicating that these two genes may be PR101 resistance genes to bacterial blight. The results provided a basis and strategy for both bacterial blight resistance breeding and application of perennial rice in future.

Key words: perennial rice; bacterial blight; resistance evaluation

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植物科学学报 2021，39(4)：407～414
Plant Science Journal http://www.plantscience.cn

DOI: 10.11913/PSJ.2095-0837.2021.40407
李蓉，眭梦洁，王其刚，余春霞，杜光辉，晏慧君. 月季品种‘绿萼’*RcAG* 基因启动子克隆及分析[J]. 植物科学学报，2021，39(4)：407-414
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月季品种‘绿萼’*RcAG* 基因启动子克隆及分析

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摘 要： 月季品种‘绿萼’(*Rosa chinensis* ‘Viridiflora’)是中国古老月季最宝贵资源之一，其花瓣、雄蕊及雌蕊均萼片化。本研究以花器官正常发育的月季品种‘月月粉’(*R. chinensis* ‘Old Blush’)为对照，利用实时荧光定量 PCR 技术对 *RcAG* 基因在‘绿萼’花器官中的表达情况进行分析，阐明 *RcAG* 基因在‘绿萼’花器官发育中的作用。结果显示，*RcAG* 基因在‘绿萼’中的表达明显下调。进一步克隆‘绿萼’和‘月月粉’的 *RcAG* 基因启动子，序列分析结果表明两个启动子均含有 TATA、TATC-box 和 MBS 等顺式作用元件，但在‘绿萼’*RcAG* 基因启动子中发现了光响应元件 MRE 和昼夜节律调控元件 Circadian，而光响应元件 TCT-motif 只在‘月月粉’*RcAG* 基因启动子中被发现。同时，本研究采用重亚硫酸盐测序技术分析了‘绿萼’和‘月月粉’*RcAG* 基因启动子甲基化的情况，发现‘绿萼’*RcAG* 基因启动子区域中有 4 个 CpG 位点均发生甲基化，其甲基化程度远高于‘月月粉’。研究结果表明 *RcAG* 基因在‘绿萼’花器官中的下调表达可能与其启动子的顺式作用元件及甲基化修饰相关。

关键词： 月季品种‘绿萼’；花器官发育；荧光定量 PCR；启动子克隆；甲基化分析

中图分类号： Q943.2 **文献标识码：** A **文章编号：** 2095-0837(2021)04-0407-08

Cloning and analysis of *RcAG* promoter in *Rosa chinensis* ‘Viridiflora’

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Abstract: *Rosa chinensis* ‘Viridiflora’ is a valued Chinese garden rose cultivar and represents an important rose germplasm resource，in which the petals，stamens，and pistils are converted into sepal-like organs (i.e.，sepalody). Expression of the *RcAG* gene in flower tissues of the *R. chinensis* ‘Viridiflora’ and ‘Old Blush’ varieties was analyzed by real-time quantitative polymerase chain reaction (PCR). Results showed that the expression level of *RcAG* was down-regulated in *R. chinensis* ‘Viridiflora’. To explore the reason for its down-regulation，the promoter sequences of *RcAG* were cloned in ‘Viridiflora’ and ‘Old Blush’，respectively. Results showed that the two promoter sequences contained TATA-box，TATC-box，and MBS cis-elements. Notably，MRE and Circadian were specifically found in the *RcAG* promoter in ‘Viridiflora’，while the TCT motif was only found in ‘Old Blush’. Furthermore，double sulfite sequencing technology was used to analyze promoter methylation. Results showed that all four CpG sites in the promoter region of ‘Viridiflora’ were methylated，and the

收稿日期： 2020-12-11，**修回日期：** 2021-04-23。
基金项目： 国家自然科学基金项目(32060693，31660579)。
This work was supported by grants from the National Natural Science Foundation of China (32060693，31660579).
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degree of methylation was much higher than that of 'Old Blush'. Our findings suggest that the down-regulation of the *RcAG* gene in 'Viridiflora' may be related to its promoter cis-elements and methylation.

Key words: *Rosa chinensis* 'Viridiflora'; Flower organ development; Real-time quantitative PCR; Promoter cloning; Methylation analysis

月季(*Rosa chinensis*)被称为"花中皇后",是中国十大传统名花,也是世界上重要的观赏植物[1]。同时,因其具有美丽的花型、艳丽的花色及宜人的花香而位居世界四大鲜切花之首。现代月季类型多样,品种众多。其中,花色、花型奇特的月季品种更为人们喜爱[2,3]。'绿萼'(*R. chinensis* 'Viridiflora')是中国古老月季宝贵的资源之一,其花朵形态十分奇特,表现为花瓣、雄蕊和雌蕊均转变为萼片状,在欧美各国深受人们欢迎[4]。

研究表明,植物的开花过程受到一系列花发育基因的调节和控制,花型的改变可以通过控制花器官发育相关基因的表达来实现[5]。目前,解析花器官发育机理的ABCDE模型已被广泛接受,*A*(*AP1*、*AP2*)、*B*(*PI*、*AP3*)、*C*(*AG*)、*D*(*STK*、*SHP1*、*SHP2*)、*E*(*SEP 1-4*)5种类型的花器官特性基因由于具有共同的保守区域被命名为MADS-box类同源异型基因,其编码的转录因子在观赏植物,特别是显花植物的花器官分化等方面有着重要的调控作用[6,7]。'绿萼'作为植物花器官发育同源异型突变体[8],一直受到广大研究者的重视。Chmelnitsky等[9]在2003年通过Northern杂交技术分别分析了花器官正常和畸形的月季花中*MASAKO C1*的同源基因*RhAG*在花发育各个阶段的表达量,发现在正常花型的月季花芽分化的各个阶段均有明显表达,而在畸形花的月季花芽分化早期均不表达,从而推测月季花器官萼片化可能由*RhAG*的异常表达导致。

目前,已有多种观赏植物*AG*同源基因被克隆,如百合(*Lilium*)[10]、蜡梅(*Chimonanthus praecox* (L.) Link)[11]、牡丹(*Paeonia suffruticosa* Andr.)[12]、山茶(*Camellia japonica* L.)[13]、兰科植物[14,15]等,尽管这些基因在序列上有差异,但功能相近[16]。本研究前期克隆得到的*RcAG*基因在月季品种'月月粉'('Old Blush')中全长931 bp,在'绿萼'中全长1086 bp,经NCBI序列比对,二者显示出极高的同源性;同时,与其他蔷薇属植物中的*AG*基因也有较高的同源性,说明该基因在进化过程中可能存在一定的保守性。

启动子是重要的顺式作用元件,它与多种反式作用因子及RNA聚合酶的相互作用是启动子调控基因转录的实质[17,18],在植物基因表达调控中发挥重要作用。DNA甲基化是表观遗传修饰主要的机制之一,植物DNA甲基化模式的改变通常对其生长发育造成影响,甲基化程度过高或不足都有可能导致植物形态异常[19]。启动子甲基化通过影响转录因子和顺式作用元件的结合,从而抑制转录的发生[20]。

本研究分析了*RcAG*基因在两个月季品种'绿萼'及'月月粉'幼蕾期的表达;同时克隆'月月粉'和'绿萼'*RcAG*基因启动子序列,通过分析顺式作用元件及启动子的甲基化情况进一步明确*RcAG*基因的功能,进而揭示*RcAG*基因在'绿萼'花器官发育中的作用及其花器官萼片化的发育机制,以期为深入研究植物花器官萼片化发育的分子机制提供参考。

1 材料与方法

1.1 实验材料

中国古老月季'绿萼'及'月月粉'种植于云南省农业科学院花卉研究所雨树村基地。于幼蕾期取嫩叶、萼片、花瓣、雄蕊、雌蕊,经液氮快速冷冻后于-80℃超低温冰箱保存、备用(图1)。

1.2 实验方法

1.2.1 *RcAG*基因在'绿萼'及'月月粉'花器官不同部位的表达分析

本研究基于*RcAG*基因的全长cDNA序列,设计RT-qPCR引物(表1)。利用试剂盒分别提取'绿萼'及'月月粉'的嫩叶、萼片、花瓣、雄蕊、雌蕊的总RNA并合成cDNA,以*RcGAPDH*序列为内参,'月月粉'和'绿萼'各部位的cDNA为模板进行RT-qPCR反应,反应体系及程序参照眭梦洁等[21]的方法。

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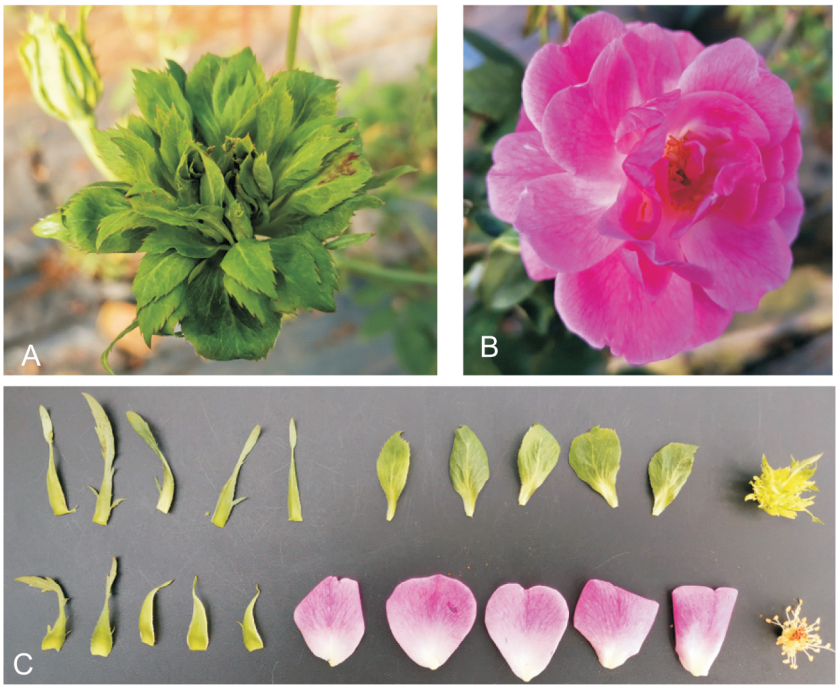


图 1　中国古老月季品种 '绿萼' (A) 和 '月月粉' (B) 及其花器官分解图(C)
Fig. 1　*Rosa chinensis* 'Viridiflora' (A) , *R. chinensis* 'Old Blush' (B) , and dissected flower organs (C)

表 1　引物信息
Table 1　PCR primer information

引物名称 Primer	引物序列 (5′-3′) Primer sequence (5′-3′)
RT-qPCR-F	AGTCACATTCTGCAAGCGTC
RT-qPCR-R	GCATATTCATAGAGGCGCCC
RcAG promoter-F	AGGACCGTGTTGGGTTTGTT
RcAG promoter-R	CATTGGAGTGGTCGCTAGGC
RcAG promoter methylation-F	TAGGTTTTTGTTTTTATTTTTGTTT
RcAG promoter methylation-R	ACCAAATTCCACAACTACCTAACC

1. 2. 2　'绿萼' 及 '月月粉' 中 *RcAG* 基因启动子的克隆

基于 Saint-Oyant 等[22] 发布的 '月月粉' 全基因组序列(https: //iris.angers.inra.fr/obh/) ，使用 NCBI(https: //www.ncbi.nlm.nih.gov/) 网站提供的引物设计工具 Primer-BLAST 设计 *RcAG* 基因启动子引物。PCR 反应程序为: 94℃预变性 4 min; 94℃变性 30 s，60℃退火 20 s，72℃延伸 90 s，40 个循环; 72℃ 保持 7 min，4℃保存。对目的片段进行胶回收，转入大肠杆菌感受态细胞 DH5α 中培养。提取质粒，阳性克隆鉴定后送测序。

1. 2. 3　*RcAG* 基因启动子的序列及甲基化分析

得到 *RcAG* 基因启动子序列后，使用 DNAMAN 软件进行序列拼接，并使用 PlantCARE 软件对获得的启动子序列进行顺式作用元件分析。使用 CpG Plot/CpG Report/Isochore(http: //www.ebi.ac.uk/emboss/cpgplot/index.html) 和 CpG finder (http: //www.softberry.com/berry.phtml? topic = cpgfinder&group = programs&subgroup = promoter) 等在线软件预测 '月月粉' 和 '绿萼' *RcAG* 基因启动子的 CpG 岛，根据预测到的 CpG 岛设计引物(表 1) 。使用 MethPrimer(http: //www.urogene.org/methprimer/index1.html) 在线工具设计甲基化检测引物(表 1) 。使用 DNA 重亚硫酸盐转化试剂盒对 '绿萼' 、 '月月粉' 基因组 DNA 进行重亚硫酸盐转化，之后参照试剂盒方法进行 PCR 扩增反应。对目的片段进行胶回收、转化，阳性克隆鉴定后送测序。使用 DNAMAN 软件对测序结果进行序列拼接和比对，使用在线软件 QUMA (http: //quma.cdb.riken.jb/) 对两种月季的甲基化情况进行分析。

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2 结果与分析

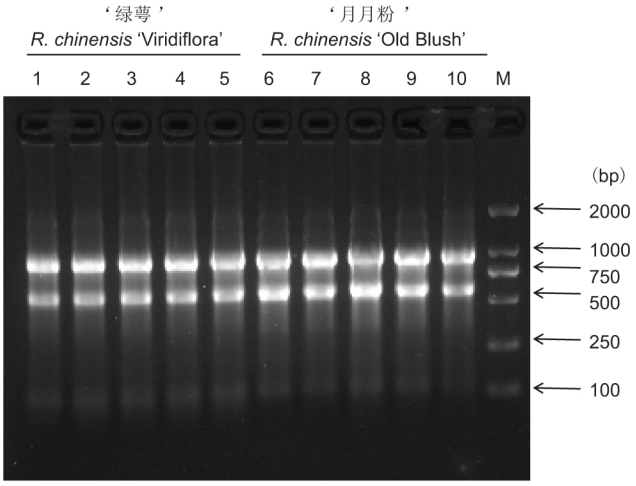
2.1 *RcAG* 基因在‘绿萼’及‘月月粉’花器官不同部位的表达分析

本研究以花器官正常发育的‘月月粉’为对照，分别提取‘绿萼’和‘月月粉’的嫩叶、萼片、花瓣、雄蕊、雌蕊5个不同部位的总RNA。对提取的RNA进行电泳检测(图2)，结果显示28S和18S条带清晰明亮，RNA完整性较好；所有RNA样品$OD_{260/280}$均在1.88～2.01，纯度较好，可以满足后续实验要求。

RT-qPCR结果表明，*RcAG*基因在‘绿萼’雄蕊和雌蕊中几乎不表达，而在花器官正常的‘月月粉’雄蕊和雌蕊中有较高的表达水平(图3)。

2.2 *RcAG* 基因启动子克隆及序列分析

本研究利用设计的*RcAG*基因启动子克隆特异引物，克隆获得‘月月粉’和‘绿萼’中*RcAG*基因ATG上游约1500 bp的片段(图4)，分别命名为pAG-OB和pAG-V。*RcAG*基因启动子顺式作用元件分析结果显示，两个启动子均含有顺式作用调节元件A-box；光响应元件GATA-motif、Box 4、G-box、ATC-motif；参与干旱诱导的MYB结合位点MBS，防御和应激元件TC-rich；水杨酸反应元件TCA-element；赤霉素响应元件P-box、TATC-box；厌氧诱导元件ARE；生长素反应元件AuxRR-core以及MeJA反应元件CGTCA-motif。此外，发现pAG-OB中存在光响应元件TCT-motif，而pAG-V中存在光响应元件MRE和昼夜节律调控元件Circadian(表2)。



M：DL 2000 DNA marker；1和6：嫩叶；2和7：萼片；3和8：花瓣；4和9：雄蕊；5和10：雌蕊。下同。
1，6：Tender leaf；2，7：Sepal；3，8：Petal；4，9：Stamen；5，10：Pistil. Same below.

图2 凝胶电泳检测结果
Fig. 2 Detection results of RNA gel electrophoresis

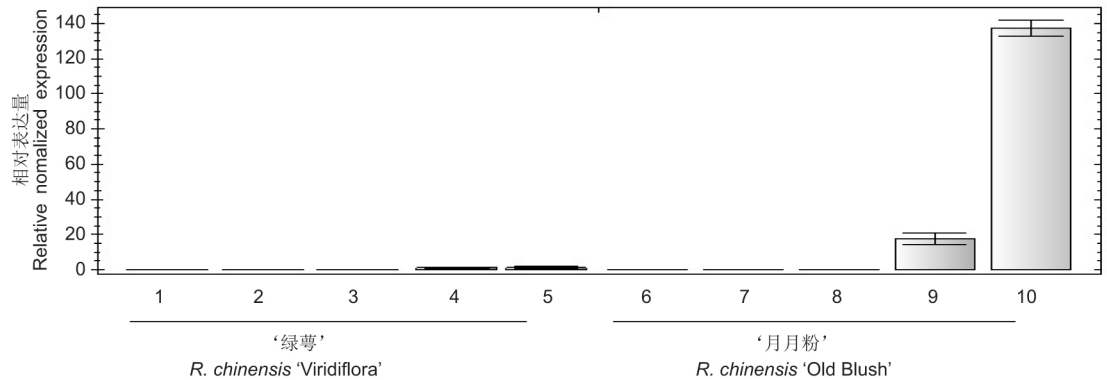
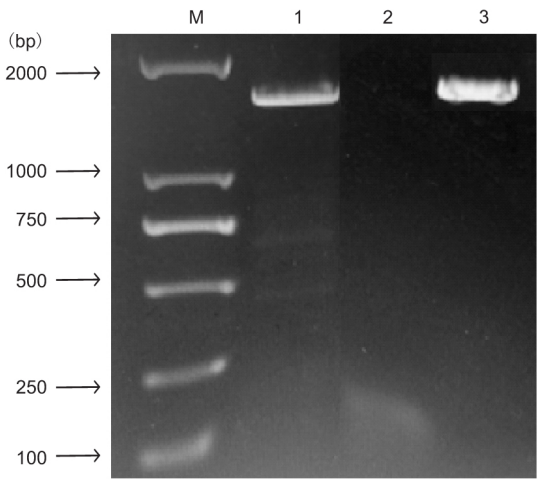


图3 *RcAG*基因在‘绿萼’和‘月月粉’不同组织部位中的表达
Fig. 3 Expression level of *RcAG* gene in different tissues of ‘Viridiflora’ and ‘Old Blush’

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t ‘月月粉’; 2: 阴性对照; 3 ‘绿萼’。
t ‘Old Blush’; 2: Negative control; 3 ‘Viridiflora’.

图 4　*RcAG* 基因启动子的 PCR 扩增产物
Fig. 4　Amplification products of *RcAG* promoter

2.3　*RcAG* 基因启动子甲基化分析

本研究对测序结果进行序列拼接，从引物位置截取亚硫酸氢盐处理后的 DNA 序列，发现 *RcAG* 基因启动子甲基化引物克隆得到的序列与原序列长度相同，‘绿萼’序列长度为 462 bp，‘月月粉’为 454 bp。两者的启动子甲基化程度分析结果见表 3。

CpG 位点甲基化情况分析结果表明，‘绿萼’启动子区域甲基化程度极高，有 4 个甲基化 CpG 位点分别在序列的 26、60、123、251 位，占全部 CpG 位点的 80%；而‘月月粉’序列中有 9 个 CpG 位点位于序列的 26、50、54、117、169、212、240、245、367 位，启动子甲基化程度却偏低，只有位于第 1、第 4、第 6 的 3 个 CpG 位点被甲基化，甲基化比例为 33%，远低于‘绿萼’中的比例(表 3、图 5)。

表 2　‘绿萼’和‘月月粉’*RcAG* 基因启动子中的顺式作用元件
Table 2　Cis-acting elements of *RcAG* promoter in ‘Viridiflora’ and ‘Old Blush’

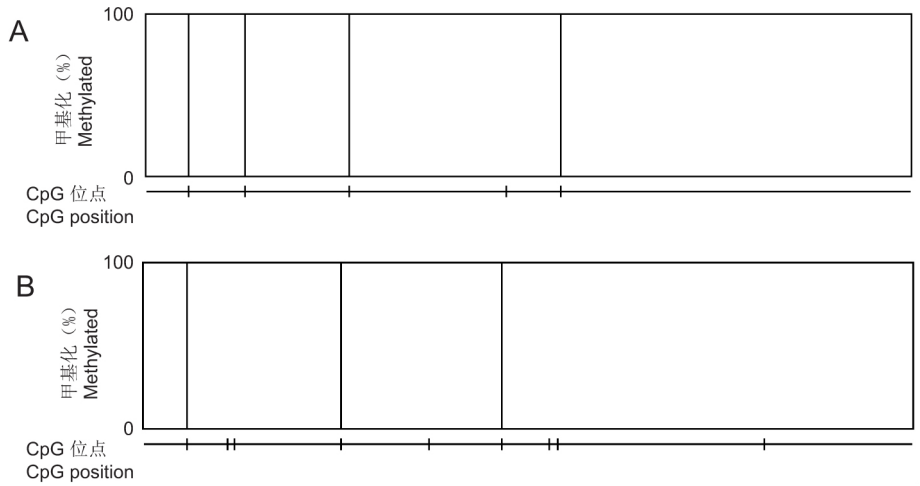
植物材料 Material	顺式元件 Cis-element	功能 Function
	AuxRR-core	参与生长素反应的顺式作用调控元件
	A-box	顺式作用调节元件
	Box 4	光响应元件属于保守 DNA 模块
	TATC-box	赤霉素反应的顺式作用元件
	ATC-motif	光响应元件属于保守 DNA 模块
‘月月粉’ ‘Old Blush’	CGTCA-motif	参与 MeJA 反应的顺式作用元件
	G-box	光反应中的顺式作用调节元件
‘绿萼’ ‘Viridiflora’	GATA-motif	光响应元件的一部分
	MBS	参与干旱诱导的 MYB 结合位点
	TC-rich repeats	参与防御和应激反应的顺式作用元件
	TCA-element	参与水杨酸反应的顺式作用元件
	P-box	赤霉素响应元件
	ARE	厌氧诱导的顺式作用调控元件
‘月月粉’ ‘Old Blush’	TCT-motif	光响应元件的一部分
‘绿萼’ ‘Viridiflora’	MRE	参与光反应的 MYB 结合位点
	Circadian	参与昼夜节律控制的顺式作用调控元件

表 3　‘绿萼’和‘月月粉’*RcAG* 基因启动子亚硫酸氢盐序列信息
Table 3　Bisulfite sequence information of *RcAG* promoter in ‘Viridiflora’ and ‘Old Blush’

植物材料 Material	比对长度 Alignment length	甲基化-CpG (%) Me-CpG (%)	未转换的(%转换) Unconverted (% converted)	甲基化模式 Methylation pattern
‘绿萼’ ‘Viridiflora’	0 (0) /462 (100.0)	4 (80.0)	0/59 (100.0)	●●●○●
‘月月粉’ ‘Old Blush’	7 (0) /454 (98.5)	3 (33.3)	0/58 (100.0)	●○○●○●○○○

注: ●表示该位点甲基化程度为 100%; ○表示该位点甲基化程度为 0%。
Notes: ● indicates methylation degree of site is 100%; ○ indicates methylation degree of site is 0%.

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A ‘绿萼’; B ‘月月粉’。
A ‘Viridiflora’; B ‘Old Blush’.

图 5 *RcAG* 基因启动子 CpG 甲基化对比
Fig. 5 Comparative analysis of CpG methylation in *RcAG* promoter

3 讨论

对于开花植物来说，花器官的发育是植物发育过程中的重要阶段。近年来，花发育模型从经典 ABC 模型[23]逐步发展完善为 ABCDE 模型[24]，对植物花器官发育的研究起到指导作用。中国古老月季‘绿萼’是研究花器官发育机理的宝贵材料，其花器官发育萼片化的现象一直受到广大育种工作者的关注。ABCDE 模型中 5 类基因的表达与植物花器官的形态变化密不可分，其中，C 类基因的沉默可能导致花瓣、雄蕊、雌蕊等器官转变为萼片化[25-27]。Chmelnitsky 等[9]发现 *RhAG* 基因在正常月季花的花芽分化的各个阶段均有明显表达，而在畸形月季花的花芽分化早期均不表达。本课题组前期的研究也确认 *RcAG* 基因的显著下调表达在‘绿萼’花器官萼片化过程中起决定性作用[28]。本研究以花器官正常发育的‘月月粉’为对照，利用实时荧光定量 PCR 方法验证了 *RcAG* 基因在‘绿萼’花器官中的表达，与前人研究结果一致[28]。

本研究克隆得到两个月季品种中的 *RcAG* 基因启动子，顺式作用元件分析结果表明在启动子序列中两者均存在多个典型的 TATA 和 CAAT 框; TATA 框没有方向性，可以指导起始前复合物的形成，决定转录起始位点[29]，若其中发生碱基互换很可能导致最小启动子失活[30]。CAAT 框是启动子中常见的顺式作用元件，对基因转录有较强的激活作用，无方向和距离特异性，与顺式作用元件有着协同作用[31]。此外，在两种月季的 *RcAG* 基因启动子中，均含有 GATA-motif、Box 4、G-box、ATC-motif 等多个光响应元件，还有赤霉素响应元件(P-box、TATC-box) 、生长素反应元件(AuxRR-core) 、MBS、ARE 和 TC-rich 元件等，说明 *RcAG* 基因的表达可能受到光照[32]、植物生长调节剂、空气含氧量和干旱等外界刺激的影响。陈仲等[33]研究发现光响应元件 TCT-motif 与植物开花过程密切相关，而赵玉飞[34]研究发现 MRE 也属于光响应元件。但在本研究中，TCT-motif 在只在‘月月粉’*RcAG* 基因启动子中出现，而 MRE 只在‘绿萼’中出现，昼夜节律调控元件 Circadian 也为‘绿萼’特有[34]，说明不同的启动子顺式作用元件和昼夜节律调控可能与‘绿萼’的萼片化发育有关。

Dubois 等[35]对 *RhAG* 等 6 个候选基因在重瓣月季和单瓣月季中的表达进行分析，发现 *RhAG* 基因在重瓣月季和单瓣月季中的表达有差异; 进一步利用原位杂交技术证实了 *RhAG* 基因的差异表达，但尚不明确其原因。Jones[36]研究发现基因的表达受到抑制可能与启动子区域甲基化相关。本研究通过重亚硫酸盐测序技术对‘绿萼’和‘月月粉’*RcAG* 基因启动子甲基化程度进行分析，结果表明‘绿萼’中 *RcAG* 基因启动子甲基化程度明显高于‘月月粉’，说明‘绿萼’中

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RcAG 基因表达下调可能受到启动子甲基化的影响。

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作物学报 ACTA AGRONOMICA SINICA 2022, 48(2): 380–395 http://zwxb.chinacrops.org/
ISSN 0496-3490; CN 11-1809/S; CODEN TSHPA9 E-mail: zwxb301@caas.cn

DOI: 10.3724/SP.J.1006.2022.04273

棉籽油分和 3 种主要脂肪酸含量 QTL 分析

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摘 要: 本研究对棉籽油分、棕榈酸、油酸和亚油酸含量进行了不同遗传体系的 QTL 分析, 为相关性状挖掘出更多有用的基因信息。分别于 2017 年和 2018 年, 利用陆地棉亲本 HS46 (P_1)和 MARCABUCAG8US-1-88 (P_2)所构建的 188 个重组近交系分别与双亲杂交构建 F_1 群体 BC (P_1)和 BC (P_2)。基于这些回交群体种子, 采用专为种子性状设计的母体和胚核基因组 QTL 定位的混合线性遗传模型及 QTL Network-CL-2.0-Seed 软件, 对棉籽油分、棕榈酸、油酸和亚油酸含量进行 QTL 定位分析。共检测到 7 个控制棉籽油分含量、3 个控制棕榈酸含量、2 个控制油酸含量和 3 个控制亚油酸含量的 QTL, 均具有显著或极显著的源自母体和胚 2 个核基因组的加性主效应, 其中有 7 个 QTL 的表型变异贡献率大于 10%。研究结果可为这些性状的分子标记辅助选择育种提供更为可靠的参考, 为这些性状的分子遗传机制研究提供理论基础。

关键词: 棉花; QTL 定位; 油分含量; 脂肪酸含量; 胚核基因组; 母体核基因组

QTLs analysis of oil and three main fatty acid contents in cottonseeds

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Abstract: In this study, in order to uncover more useful gene information for these traits, the analysis of QTLs controlling the contents of oil, palmitic acid, oleic acid, and linoleic acid in cottonseeds was conducted in different genetic systems. Based on a set of 188 recombinant inbred lines (RILs) derived from an intra-specific cross between two upland germplasms HS46 (P_1) and MARCABUCAG8US-1-88 (P_2), double backcross populations BC (P_1) and BC (P_2) were constructed in 2017 and 2018, respectively. BC (P_1) and BC (P_2) populations were obtained through backcrossing each of the two parents (HS46 and MARCABUCAG8US-1-88) with the 188 RILs, respectively. The cottonseeds from these backcross populations were used for QTLs analysis of oil, palmitic acid, oleic acid, and linoleic acid contents. To map QTLs for these traits, the mixed linear genetic model and the QTL Network-CL-2.0-Seed software, which were developed especially for seed traits, were applied. In total, seven QTLs for the oil content, three for the palmitic acid content, two for the oleic acid content, and three for the linoleic acid content were detected. Significant additive effects of QTLs from maternal plants and embryos were detected for all the QTLs. Seven QTLs, each of which explained more than 10% of total phenotypic variation, were found. The detected QTLs in the present study may give us better understanding of the molecular inheritance for these four traits, and provide more reliable information for molecular marker assisted breeding.

Keywords: cotton; quantitative trait loci (QTL); oil content; fatty acid; embryo nuclear genome; maternal nuclear genome

棉籽是棉花的重要副产品, 是重要的油料资源。棉籽的棉仁中含油量高约 30%~40% [1], 油分中大约含有 26%棕榈酸(C_{16-0})、2%硬脂酸(C_{18-0})、15%油酸(C_{18-1})、55%亚油酸(C_{18-2})等多种脂肪酸[2]。其中, 不饱和脂肪酸油酸和亚油酸对人类心脑血管健康非常有益, 且其含量高。棉籽油的品质好, 已成为重要的

本研究由国家自然科学基金项目(31760404)和云南大学农学学科建设项目(C176210302)资助。
This study was supported by the National Natural Science Foundation of China (31760404) and the Agricultural Discipline Construction Project of Yunnan University (C176210302).

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Received (收稿日期): 2020-12-14; Accepted (接受日期): 2021-07-12; Published online (网络出版日期): 2021-08-10.
URL: https://kns.cnki.net/kcms/detail/11.1809.S.20210810.1018.004.html

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食用油之一。同时, 棉籽油还可加工成生物燃料, 也已成为重要生物能源之一[3]。因此, 根据各种用途的需要, 棉籽含油量的提高与脂肪酸组分含量的改良是棉花育种的重要目标之一。棉籽含油量及其组分构成属于复杂的数量性状。已有的研究表明棉籽油分变异幅度较大, 为 15%~33% [4]。在不同的研究中, 油分遗传规律的研究结果有所不同。Singh 等[5]、季道藩等[6]、Wu 等[7]认为油分含量主要由基因的加性和显性效应控制。Dani 等[8]发现油分主要受制于母体效应, 但在他所研究的杂交组合中, 也有一些杂交组合的油分表现主要受胚加性效应影响, 同时具有显著的胚显性效应。王国印等[9]、Yuan 等[10]、秦利等[11]则认为棉籽油分含量主要由基因加性效应控制。这些差异与材料的遗传背景有关。Lukonge 等[12]认为不同类型的棉籽在油酸、肉豆蔻酸、亚油酸、亚麻酸等脂肪酸组分上也存在一定变异。Dowd 等[13]研究 35 种基因型棉籽在多年份多地点试验下的脂肪酸组分差异中发现, 棕榈酸、油酸及亚油酸含量变异主要来自基因型与环境的互作效应, 且油酸、棕榈酸含量与亚油酸含量呈现显著的负相关性。这些结果说明棉籽脂肪酸组分表现受多种因素的影响。随着分子技术的发展, 油分及棉籽油组分的遗传研究进入到分子水平。溶血磷酸酯酰转移酶(lysophosphatidic acid acyltransferase, LPAT)是植物油脂合成途径中的关键酶。Zou 等[14]将酵母的 *LPAT* 基因转入拟南芥, 其种子含油量提高了 8%~48%; 丁检等[15]在棉花研究中, 也确定了 8 个 LPAT 家族基因, 发现其中的 *LPAT6* 和 *LPAT8* 在种子中高表达, 说明 LPAT 也在棉籽的油脂合成过程中发挥了重要的作用。刘丽等[16]克隆了棉花的酮脂酰 ACP 还原酶基因 *GhKAR* 和烯脂酰 ACP 还原酶基因 *GhENR*, 通过转基因研究, 发现其转基因株系后代种子含油量提高 10.2%~14.1%, 不饱和脂肪酸含量提高 4.04%~6.02%。张军[17]将芝麻、蓖麻、油葵和花生的二酰甘油酰基转移酶基因 *DGAT1* 转入棉花, 种子含油量提高 6.37%~7.41%。Chapman 等[18]利用 1 个抑制棉花去饱和酶基因 *FAD2* 的表达载体, 获得了具有高油酸棉籽的棉花转基因系, 其油酸含量提高到 21%~30%, 有的株系甚至提高到 47%。Liu 等[19]构建了棉籽的 2 种关键酶基因 *ghSAD-1* 及 *ghFAD2-1* 的 RNAi 载体, 转入棉花 Coker 315, 结果发现转基因系的硬脂酸含量由 2%~3%提高到 40%, 油酸含量由 15%增加到 77%。Liu 等[20]将来自 Coker 201 的转录因子基因 *GhWRI1* 转入陆地棉 Sumian 20, 发现转基因后代棉籽油分含量显著提高, 从 25.4%增加到 31.22%。由此可见, 棉籽油分相关基因无论是油分合成途径中关键酶基因, 还是油分调控基因, 这些基因的挖掘和利用对实现棉籽品质改良均有着重要意义。

数量性状 QTL 定位技术是发掘基因的重要手段。近年来, 很多学者对棉籽油分含量进行了 QTL 定位分析。Song 等[21]利用陆地棉标准系 TM-1 和海岛棉 Hai 7124 发展而来的回交群体 BC_1S_1 研究了与棉仁油分含量相关的 QTL, 在 D 亚组 8 号染色体上确定了 1 个与油分含量相关的 QTL。Yu 等[22]利用来自陆地棉和海岛棉的回交群体在 4 个环境中共检测到 17 个与油分含量相关的 QTL, 其中有 1 个 QTL 在各环境下都能被检测到。刘小芳等[23]以 196 个陆地棉重组近交系检测到 1 个稳定的油分含量 QTL 位于 16 号染色体上, 解释了 8.19%~8.85%的表型变异。Liu 等[24]利用陆地棉 Yumian1 和 T586 的重组近交系构建了高密度遗传图谱, 确定了 15 个油分含量 QTL, 解释了 2.0%~39.8%的表型变异, 但只有 7 号和 11 号染色体上的 2 个 QTL 比较稳定。另外, 还分别检测到了 8 个亚油酸 QTL、10 个油酸 QTL、13 个棕榈酸 QTL。刘欣欣[25]通过关联分析发现陆地棉 A05 染色体上有 1 个油分基因 *GhA05KAS1*, 解释 14.23%的表型变异。

有关棉籽油分及其组分含量的 QTL 研究越来越很多[26-28], 但主要集中在种子核基因组上。由于棉花种子生长在母体植株上, 棉籽油分等品质性状除了受种子自身基因组的影响外, 还会受到母体植株核基因组的影响。因此, 本研究采用了专为棉籽性状建立的, 且涉及胚和母体核基因组 QTL 定位的混合线性遗传模型及相应软件 QTL Network-CL-2.0-Seed 对棉籽油分含量及 3 种主要脂肪酸即棕榈酸、油酸和亚油酸含量进行 QTL 定位, 并分析控制棉籽这些性状相关 QTL 的胚加性主效应、胚显性主效应、母体加性主效应以及相应的环境互作效应, 了解这些 QTL 在棉花胚和母体植株核基因组上的表达情况, 从而为这些品质性状的分子标记辅助选择育种提供更为详细的遗传信息。

1　材料与方法

1.1　试验材料

本试验中所用的重组自交系(recombinant inbred lines, RIL)群体衍生于 HS46 和 MARCABUCAG8US-1-88 (后文简称 MAR), 共有 188 个重组近交系。母

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本 HS46 为高产、优质的陆地棉品种，父本 MARCABUCAG8US-1-88 为陆地棉种质材料。两亲本的棉籽油分、蛋白质含量等品质性状存在较大差异[11]。1991 年在美国密西西比州进行杂交，获得 F_1。1992 年，从同一 F_1 植株上获得 100 个 F_2，之后各世代均按单粒传(single seed descent, SSD)方式进行自交选择。直至第 8 代家系相对稳定，从 94 个 F_2 单株的 F_8 家系中，每个家系随机选取 2 行，形成 188 个重组近交系。这些近交系之间在植株形态、纤维和棉籽成分方面都表现出显著差异[11]。

1.2 田间试验

2017 年 4 月和 2018 年 4 月，将双亲 HS46 和 MARCABUCAG8US-1-88 (MAR)以及 188 个重组近交系分别种植在云南开远和元谋。采用完全随机区组设计，小区长 7 m，单行种植，行距及株距分别为 0.8 m 和 0.3 m, 2 次重复。在 2017 年和 2018 年的盛花期，利用重组自交系(RILs)群体作为母本分别与 2 个亲本进行回交(即 RILs×HS46 和 RILs×MAR)，依次获得回交群体 BC (P_1)和 BC (P_2)。田间管理同大田生产。收获期收取亲本自交种子、188 个重组近交系种子及回交群体的杂交种子，用于棉籽油分及脂肪酸含量的 QTL 定位分析。

1.3 性状测定

先从2个亲本HS46、MAR和2个回交群体中筛选成熟饱满的种子，然后分别随机取样，每一个样品约为200粒棉籽。用浓硫酸脱绒、晒干、去壳，获得棉仁。棉仁在烘箱里38℃烘40 h后，用旋风磨粉机磨成棉仁粉。所有样品在密闭容器中进行水分平衡48 h以上，棉粉水分含量约为7%。利用FOSS 5000型近红外光谱分析仪(FOSS NIRSystems, Inc, USA)扫描收集光谱(波长1100~2498 nm)，采用Quampah等[29]构建的棉仁油分及脂肪酸定标模型及其分析方法获得棉仁中油分、棕榈酸、油酸及亚油酸含量数据。在这4个性状的定标模型中，油分含量(RPD=11.495)和亚油酸含量(RPD=5.026)的近红外仪测定值与化学方法测定值表现高度一致性；棕榈酸(RPD=1.914)和油酸含量(RPD=1.999)的2种方法测定值一致性稍差一些，但由于其RPD>1.5，因此仍适用于基因定位和育种材料的选择。

1.4 遗传图谱

基于孔广超[30]构建的遗传图谱进行定位研究。该遗传图谱是以本研究材料中 RIL 群体为作图群体进行构建的图谱，包括 388 个标记，归入 30 个连锁群。其中 15 个连锁群定位于 14 条染色体上(7 个属于棉花 A 亚基因组，7 个属于 D 亚基因组)；另有 15 个连锁群尚未确定在相应染色体上，据其长度依次递减命名为 LG1~LG15。分子标记间隔平均为 5 cM。本研究根据该图谱中的标记，从棉花基因组数据库(https://www.cottongen.org/)中选取 500 个包括 SSR、RAPD、AFLP 等分子标记(附表 1)，对两亲本及双向高代回交群体进行筛选，采用 JoinMap 3.0 构建连锁图谱，对原图谱中的分子标记分布进行验证。

1.5 QTL 定位分析

利用SPSS 16.0软件对2017年和2018年试验材料的棉籽油分、棕榈酸、油酸及亚油酸含量进行基本统计分析。采用可以分析棉籽性状的胚和母体植株核基因组遗传主效应和环境互作效应，专门针对复杂种子性状进行基因定位的遗传模型[31]及软件QTL Network-CL-2.0-Seed[32]，对2个环境条件下棉籽油分及3个重要脂肪酸含量进行胚和母体植株2套基因组的QTL定位。QTL命名原则根据McCouch等[33]提出的命名方法，即*q*+性状名+染色体或连锁群序号+QTL数目序号。

2 结果与分析

2.1 棉籽油分及 3 种脂肪酸含量在亲本、BC (P_1)和 BC (P_2)回交群体中的表现

双亲和回交群体 BC (P_1)、BC (P_2)的棉籽油分及 3 个脂肪酸含量值列于表 1。结果表明，这些性状在两亲本之间的差异除了 2017 年的油酸含量和 2018 年的亚油酸含量外，均存在显著性。油分含量在 BC (P_2)群体的表现要明显高于 BC (P_1)群体，表明亲本 MARCABUCAG8US-1-88 的遗传背景更有利于棉籽油分含量的提高。同时还发现同一群体的各性状在 2017 年和 2018 年的表型值差别较大，说明这些性状易受环境影响。

本试验涉及的 4 个性状在 2 年的回交群体中表现出广泛的连续变异现象，都超出了双亲均值(图 1)。并且，这些性状在群体中分布参数值偏度和峰度的绝对值都小于 1，呈近似正态分布，说明这些性状都是受多基因控制的数量性状，则可以使用这 2 个亲本的后代群体对这些性状进行基因定位。这 4 个性状在不同环境中的差异表现表明 2 个种植环境差别较大，环境互作效应分析和基因定位更有效。

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表 1　亲本和回交群体的棉籽油分及 3 种脂肪酸含量表现

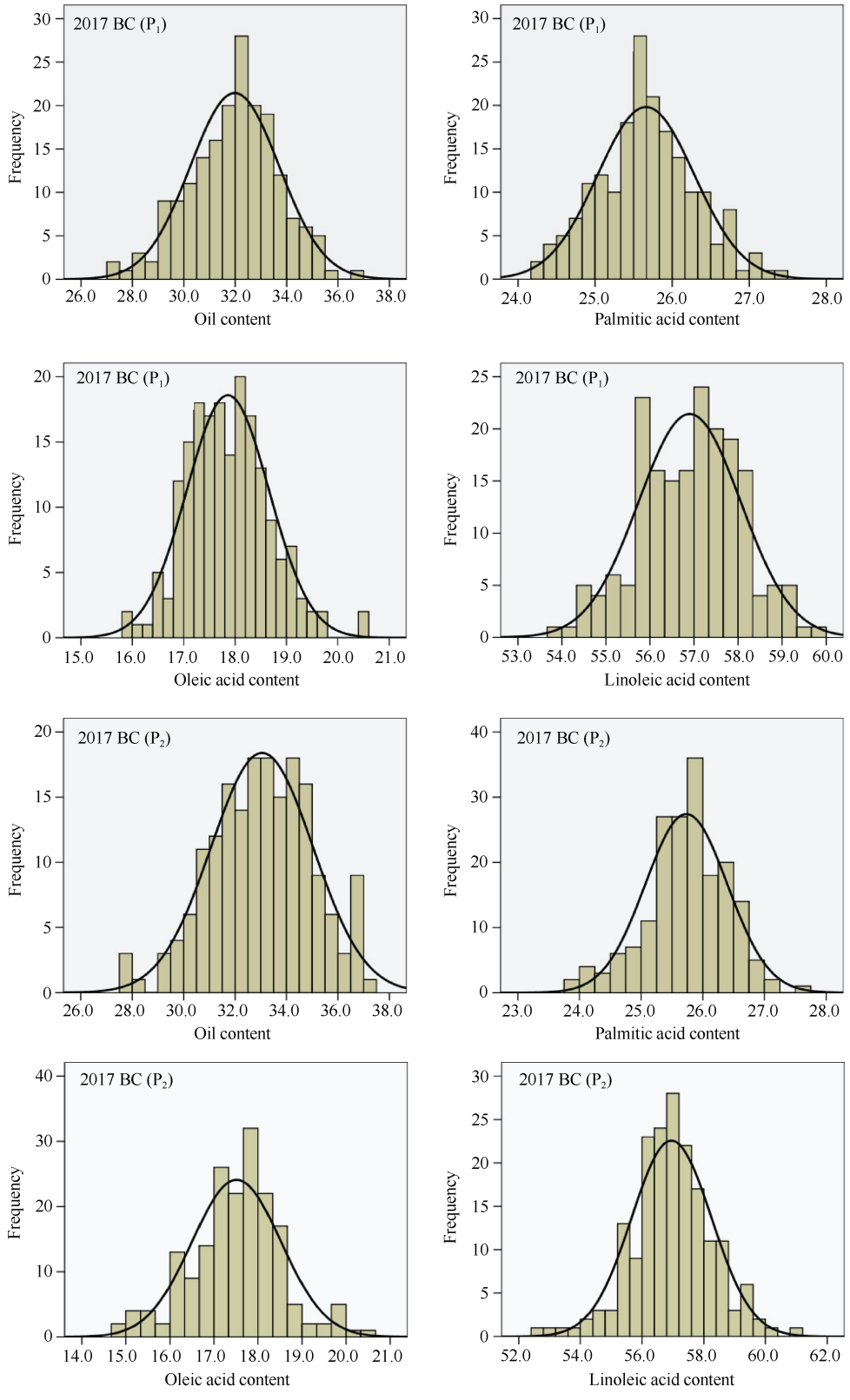
Table 1　Statistical analysis of cottonseed oil and three fatty acid contents in parents and two backcross populations for two years

年份 Year	性状 Trait	回交群体 BC (P_1)						回交群体 BC (P_2)						亲本 Parents	
		平均值 Mean	标准差 SD	最小值 Min.	最大值 Max.	偏度 Skew.	峰度 Kurt.	平均值 Mean	标准差 SD	最小值 Min.	最大值 Max.	偏度 Skew.	峰度 Kurt.	HS46	MAR
2017	油分含量 Oil content	32.28	1.85	25.26	38.06	−0.23	0.12	33.05	1.98	27.59	37.30	−0.18	−0.17	31.01 a	33.49 b
	棕榈酸含量 Palmitic acid content	25.66	0.63	24.19	27.40	0.13	−0.07	25.73	0.67	23.82	27.75	−0.38	0.56	25.49 a	25.94 b
	油酸含量 Oleic acid content	17.86	0.80	15.84	20.53	0.34	0.34	17.51	1.01	14.88	20.34	−0.11	0.54	17.31	17.01
	亚油酸含量 Linoleic acid content	56.90	1.16	53.81	59.79	−0.13	−0.31	56.85	1.29	52.80	60.85	−0.14	0.88	57.55 a	56.81 b
2018	油分含量 Oil content	32.86	0.92	29.58	35.08	−0.80	0.75	33.58	0.97	30.94	36.82	−0.22	0.29	29.70 A	32.69 B
	棕榈酸含量 Palmitic acid content	24.72	0.57	23.13	26.45	0.17	0.36	25.16	0.51	23.26	27.17	−0.37	0.09	24.63 A	25.22 B
	油酸含量 Oleic acid content	17.38	0.68	15.63	19.15	0.10	−0.07	17.02	0.59	15.24	18.85	−0.01	0.33	16.60 a	16.21 b
	亚油酸含量 Linoleic acid content	58.47	0.97	55.76	61.14	−0.05	0.21	58.06	0.70	56.16	59.80	−0.03	0.12	59.06	58.45

同一行的 a 和 b、A 和 B 分别表示同一年份同一性状在 0.05 或 0.01 水平差异显著。

In the same row, a and b, A and B represent the significant difference in the same trait of parents in the same year at the 0.05 and 0.01 probability levels, respectively; SD: standard deviation; Skew.: skewness; Kurt.: kurtosis.

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(图 1)

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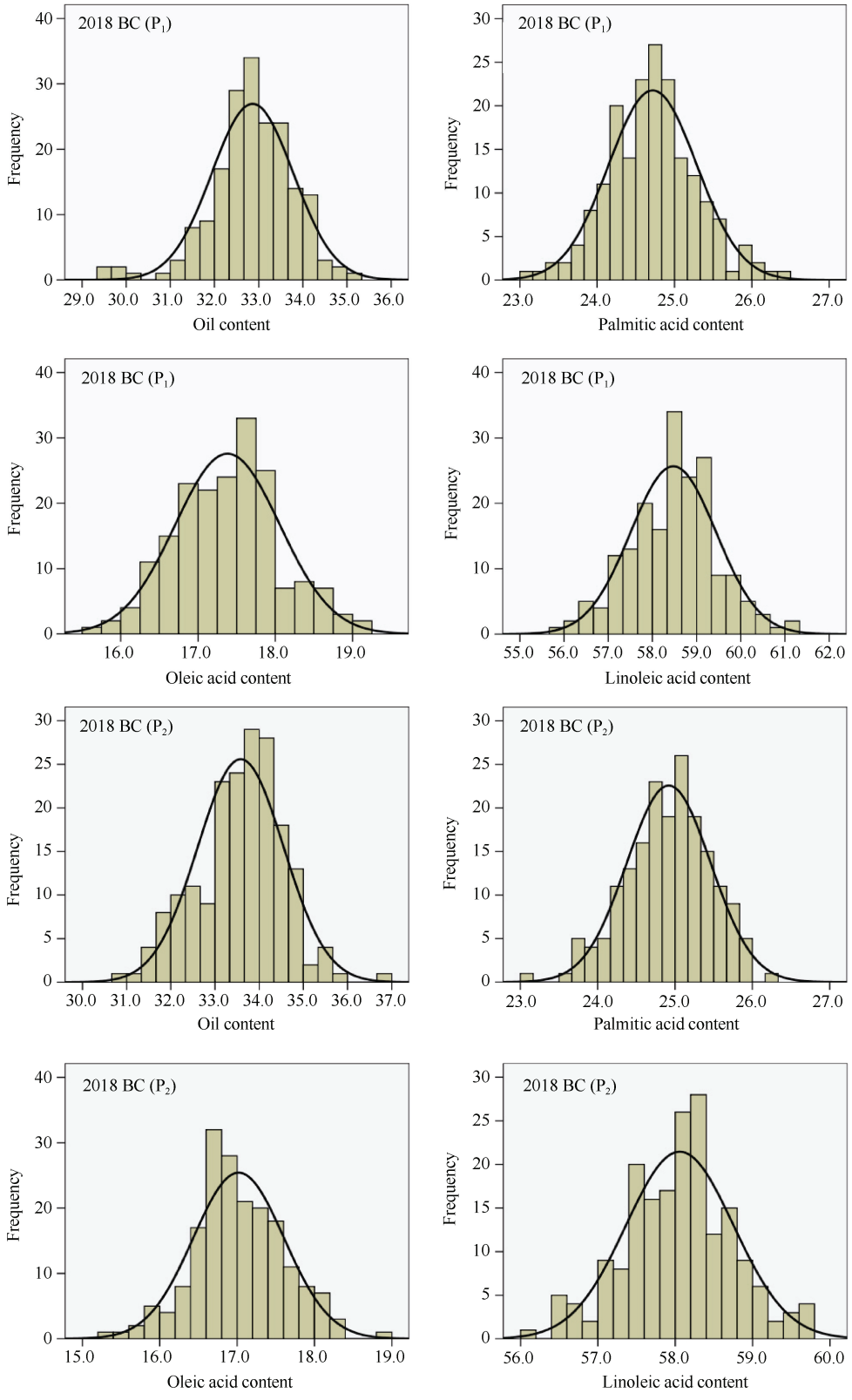


图 1　棉籽油分、棕榈酸、油酸和亚油酸含量分别在 BC (P_1)和 BC (P_2)群体中分布(2017 年和 2018 年)

Fig. 1　Frequency distribution of oil, palmitic acid, oleic acid, and linoleic acid contents in cottonseeds of BC (P_1) and BC (P_2) in 2017 and 2018

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2.2 4 个性状的表型相关性

由表 2 可知, 油分、棕榈酸、油酸及亚油酸含量之间存在显著相关性。油分含量与棕榈酸含量存在显著正相关性, 又分别与油酸和亚油酸含量存在极显著的负相关性。棕榈酸含量与油酸含量存在显著正相关性, 与亚油酸含量存在极显著负相关; 油酸与亚油酸含量存在极显著负相关。表明油分含量的提高与棕榈酸含量增加有关; 减少亚油酸含量, 有利于提高油酸含量, 也促进棕榈酸含量的提高。

2.3 QTL 定位分析

通过验证, 基于本研究材料中的 RIL 群体所构建的遗传图谱仍适用于 BC (P_1)和 BC (P_2)的定位研究。本试验中检测到与棉籽油分、棕榈酸、油酸及亚油酸含量有关的 QTL 分别有 7、3、2 和 3 个(表 3 和图 2)。这些 QTL 同时具有显著或极显著的胚加性效应和母体加性效应, 说明这些位点的基因表达同时受到胚和母体核基因组的影响, 而且这些遗传效应能稳定遗传。其中有 8 个 QTL 的胚显性主效应也达到了显著或极显著水平, 带有这些位点基因的后代容易产生杂种优势。另外, 有 9 个 QTL 是被检测出了显著的环境互作效应, 表明这些位点基因的表达容易受环境的影响。在所有检测到的 QTL 中, 其中有 7 个 QTL 的表型贡献率大于 10% (表 4), 而且同时具有较大的遗传效应(表 3); 有 9 个 QTL 以加性主效应为主。还有多个控制不同性状的 QTL 共定位在同一位点或同一连锁群的相邻区域, 如: *qC*$_{16\text{-}0}$*-15-1* 和 *qC*$_{18\text{-}2}$*-15-1* 均被定位在 D 亚组 15 号染色体的 BNL1579 与 BNL4082 标记区间。在 18 号染色体(D05)和第 6 连锁群上不同性状的 QTL 呈集中分布趋势, 说明这些染色体与油分相关性状关系比较密切。同时, 也表明这 4 个性状在分子水平上可能具有相关性。

2.3.1 油分含量 共检测到 7 个控制油分含量的 QTL (表 3), 共解释了 61.8%的表型变异。其中 *qOil-3-1* 定位在 3 号染色体(即 A03)上; *qOil-18-1*、*qOil-18-2* 和 *qOil-18-3* 定位在 18 号染色体(D05)上; *qOil-LG3-1*、*qOil-LG7-1* 和 *qOil-LG8-1* 分别位于第 3、7 和 8 连锁群上, 其染色体位置尚未确定。这些 QTL 均同时具有显著的胚加性和母体加性主效应, 说明这些位点的基因在胚和母体 2 套核基因组中可以同时表达, 并通过加性效应控制油分含量。有 3 个 QTL (*qOil-18-3*、*qOil-LG7-1* 和 *qOil-LG8-1*)具有明显的环境互作效应, 说明这些 QTL 的表达容易受到环境条件变化的影响。其余 4 个 QTL 在 2 个不同年份及环境下均未检测到环境互作效应, 说明这些 QTL 在不同环境下的表达较为稳定。*qOil-LG7-1* 表型贡献率最高, 达 27.5% (表 4); 虽然它具有显著的胚和母体加性主效应, 但其胚加性与环境互作效应的表型贡献率比重大, 说明该 QTL 的基因仅在特定环境下对油分含量影响很大。*qOil-18-1* 被定位在 18 号染色体(D05)上的 JESPR204 (c18)和 CIR099 (c18)的分子标记区间, 具有最大的胚加性和母体加性主效应, 解释 12.0%的表型变异, 其主要由胚加性和母体加性效应的遗传贡献率组成, 而且并未检测到环境互作效应, 表明这是一个稳定的重要 QTL。*qOil-3-1* 和 *qOil-LG3-1* 也都具有较大的胚加性主效应和母体加性主效应。尽管其加性主效应的表型贡献率较低(分别为 2.50%和 5.00%), 但这 2 个位点没有检测到显著的环境互作效应, 说明这 2 个 QTL 对油分改良仍然很重要。

2.3.2 棕榈酸含量 共检测到 3 个与棕榈酸含量有关的 QTL, 只有 *qC*$_{16\text{-}0}$*-15-1* 定位在 15 号染色体(D02)上(表 3)。单个 QTL 解释的表型变异范围为 1.5%~36.5% (表 4)。3 个 QTL 均具有显著的胚和母体加性主效应, 说明这些位点的基因在胚和母体核基因组上均可表达。有 2 个 QTL 没有检测到环境互作效应, 说明这 2 个位点的基因表达受环境影响较小。*qC*$_{16\text{-}0}$*-LG3-1* 具有最大的表型贡献率, 可解释

表 2 棉籽 4 个品质性状的相关性分析

Table 2 Correlation analysis among four quality traits of cottonseeds

性状 Trait	油分 Oil content	棕榈酸 Palmitic acid content	油酸 Oleic acid content	亚油酸 Linoleic acid content
油分 Oil content	1.00000			
棕榈酸 Palmitic acid content	0.45145**	1.00000		
油酸 Oleic acid content	–0.14629**	0.15922**	1.00000	
亚油酸 Linoleic acid content	–0.13874**	–0.64037**	–0.73753**	1.00000

*和**分别表示在 0.05、0.01 水平显著相关。

* and ** mean significant correlation at the 0.05 and 0.01 probability levels, respectively.

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表 3　棉籽油分及 3 种脂肪酸的 QTL 定位及其遗传效应

Table 3　Genetic effects and detection of the QTLs for oil content and three fatty acid contents of cottonseed across environments

性状 Trait	QTL	连锁群 Chr./LG	标记区间 Marker interval	位置 Position (cM)	范围 Range (cM)	a^e	d^e	a^m	a^eE1	a^eE2	d^eE1	d^eE2	a^mE1	a^mE2
油分含量 Oil content	*qOil-3-1*	Chr.3	MUCS127(c3)–NAU808	4.8	2.0–7.3	9.49**	0.05	−9.32**	−0.02	−0.05	0.11	−0.10	0.02	0.06
	qOil-18-1	Chr.18	JESPR204(c18)–CIR099(18)	0.0	0.0–1.0	−21.10**	−0.10	20.88**	0.09	0.00	−0.00	0.00	−0.09	−0.00
	qOil-18-2	Chr.18	TM0409a–NAU1001	14.8	13.9–16.8	−5.02**	−0.23**	5.24**	−0.07	−0.04	−0.07	0.07	0.07	0.03
	qOil-18-3	Chr.18	NAU5152b–NAU3053a	76.7	72.3–79.7	−0.32**	0.11*	0.50**	−0.12*	−0.01	0.08	−0.08	0.12*	0.01
	qOil-LG3-1	lg3	NAU5146c–RAPD-I10-620	51.4	48.6–58.4	13.45**	−0.01	−13.72**	0.07	0.08	0.00	−0.00	−0.07	−0.08
	qOil-LG7-1	lg7	NAU983–TMB1791	0.0	0.0–2.0	0.81**	−0.28**	−0.90**	−0.00	0.22**	−0.22*	0.21**	−0.00	−0.22**
	qOil-LG8-1	lg8	NAU4919b*–NAU4960d	31.7	30.7–34.5	1.84**	0.05	−1.48**	−0.16*	0.00	0.07	−0.09	0.16*	−0.01
棕榈酸含量 Palmitic acid content	$qC_{16\text{-}0}$*-15-1*	Chr.15	BNL1579–BNL4082(c15)	25.1	23.1–27.0	5.72**	−0.06*	−5.66**	−0.02	−0.00	0.00	−0.00	0.02	0.00
	$qC_{16\text{-}0}$*-LG3-1*	lg3	NAU5146c–RAPD-I10-620	56.4	50.6–62.4	−3.88**	0.04	3.74**	0.11**	−0.13**	−0.00	0.00	−0.11**	0.13**
	$qC_{16\text{-}0}$*-LG6-1*	lg6	NAU4852–NAU1247	40.5	39.2–42.5	−2.02**	−0.04*	2.10**	−0.01	−0.00	0.05	−0.05	0.02	0.00
油酸含量 Oleic acid content	$qC_{18\text{-}1}$*-18-1*	Chr.18	NAU5152a–NAU1232	44.6	40.6–47.6	−1.44**	0.01	1.28**	0.21**	−0.20**	0.00	−0.00	−0.21**	0.20**
	$qC_{18\text{-}1}$*-LG6-1*	lg6	NAU456–NAU5328-170	19.9	17.9–21.9	1.74**	−0.09**	−1.83**	0.10**	−0.02	−0.10*	0.09*	−0.10**	0.02
亚油酸含量 Linoleic acid content	$qC_{18\text{-}2}$*-6-1*	Chr.6	NAU932*–NAU2566	81.4	75.4–91.0	10.47**	0.01	−10.60**	0.04	0.00	0.11*	−0.11*	−0.04	−0.00
	$qC_{18\text{-}2}$*-15-1*	Chr.15	BNL1579–BNL4082(c15)	25.1	23.1–27.0	−4.83**	0.08*	4.71**	−0.27**	0.29**	−0.00	0.00	0.26**	−0.28**
	$qC_{18\text{-}2}$*-LG6-1*	lg6	NAU5328-170–RAPD-G17-120	23.0	22.0–24.0	−5.45**	−0.09*	5.64**	−0.00	−0.05	0.15**	−0.15**	0.00	0.04

位置指 QTL 在染色体或连锁群上离第一个分子标记的距离; 范围指 95%置信区间的 QTL 定位范围。a^e: 胚加性主效应; d^e: 胚显性主效应; a^m: 母体加性主效应; a^eE1: 环境 1 与胚加性互作效应; a^eE2: 环境 2 与胚加性互作效应; d^eE1: 环境 1 与胚显性互作效应; d^eE2: 环境 2 与胚显性互作效应; a^mE1: 环境 1 与母体加性互作效应; a^mE2: 环境 2 与母体加性互作效应; 环境 1 指开远种植环境, 环境 2 指元谋种植环境。*和**分别表示遗传效应的检测效果在 0.05 和 0.01 的显著水平。分子标记中的*表示偏分离标记。

Chr./LG represents a particular linkage group (lg) or chromosome (Chr.); position represents the distance of F peak value from the top marker on linkage group for QTLs; range: the 95% confidence interval for the QTL (Unit: CM). a^e: embryo additive main effect; d^e: embryo dominance main effect; a^m: maternal additive main effect; a^eE1 and a^eE2: embryo additive interaction effects in environment 1 and environment 2; d^eE1 and d^eE2: embryo dominance interaction effects in environment 1 and environment 2; a^mE1 and a^mE2: maternal additive interaction effects in environment 1 and environment 2, respectively. *, ** mean significance for the detection of genetic effects at the 0.05 and 0.01 probability levels, respectively. The segregation distorted markers were indicated with "*" following the marker name.

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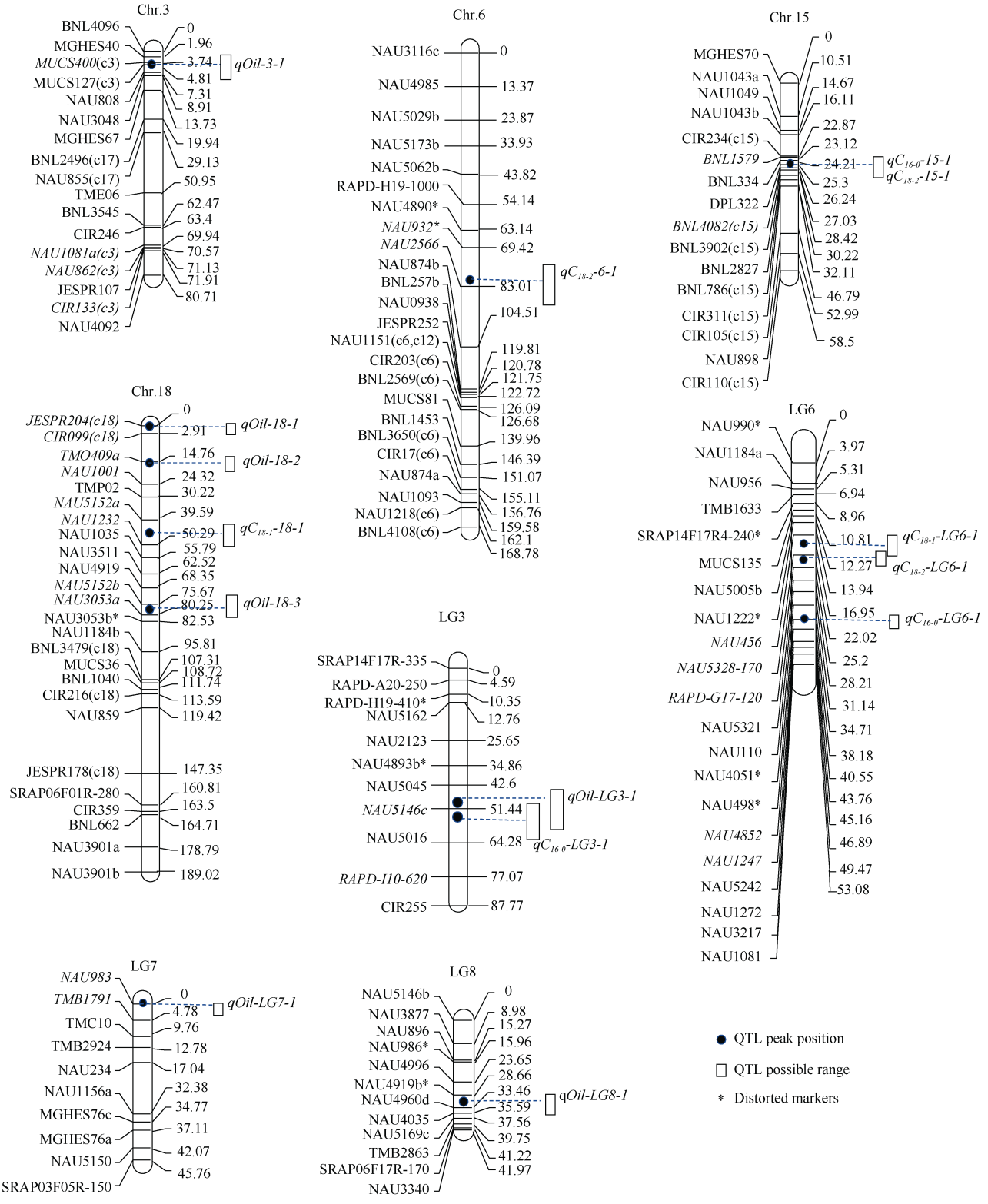


图 2 棉籽油分及 3 种脂肪酸含量 QTL 在胚和母体植株核基因组上的位置

Fig. 2 QTLs mapping of embryo and maternal plant nuclear genomes for oil and three fatty acids' content of cottonseeds

36.5%的表型变异，它具有显著的胚和母体加性主效应及其环境互作效应，没有显著的胚显性主效应及其环境互作效应，这说明该QTL主要具有加性效应。在3个控制棕榈酸含量的QTL中，*qC16-0-15-1*具有最大的胚和母体加性主效应，没有检测到环境互作效应，而且能解释11.4%的表型变异，均来自加性主

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效应, 表明该QTL对棕榈酸含量的改良具有重要意义。总体来看, 控制棕榈酸含量的这3个QTL的母体加性和胚加性主效应值及其表型贡献率明显。虽然有2个检测到了显著的显性效应, 但表型贡献率过低, 这些说明棕榈酸含量主要受基因加性效应影响。另外, 环境因素对棕榈酸含量的影响也不可忽视。

2.3.3 油酸含量　本研究中检测到 2 个与油酸含量有关的 QTL (表 3), 其中 *qC*$_{18\text{-}1}$*-18-1* 定位在第 18 染色体(D05)上, 另 1 个定位在第 6 连锁群上。这 2 个 QTL 同时具有显著的胚和母体加性主效应, 并且方向相反, 说明他们在胚和母体核基因组中的表达有所不同。另外, 这 2 个 QTL 均被检测到显著的基因-环境互作效应, 说明环境条件对亚油酸含量具有重要的影响。存在于第 6 连锁群上的 NAU456 和 NAU5328-170 之间的 *qC*$_{18\text{-}1}$*-LG6-1* 具有较大的胚和母体加性主效应, 总表型贡献率为 9.4% (表 4), 其中 7%来自加性主效应, 说明该 QTL 的表达对油酸含量的表现很重要。该位点来自 HS46 亲本的等位基因通过胚加性主效应能增加 1.74%的油酸含量, 而来自 MARCABUCAG8US-1-88 的等位基因通过母体加性主效应能增加 1.83%的油酸含量。此外, 还发现该 QTL 具有显著的胚显性主效应、胚显性与 2 种环境的互作效应、胚加性和母体加性与开远种植环境的互作效应, 此表明该 QTL 在特定环境及遗传背景中的基因表达对油酸含量的影响不可忽视。*qC*$_{18\text{-}1}$*-18-1* 具有较高的遗传贡献率, 解释 20.9%的表型变异; 尽管其胚和母体加性的遗传贡献率较低, 但这两种遗传效应值相对其他各种效应较大。另外, 该 QTL 只具有显著的加性主效应及其环境互作效应, 说明该位点基因主要是通过加性效应来控制油酸含量, 而且在适宜环境下, 该位点对油酸含量的遗传改良仍然很重要。

2.3.4 亚油酸含量　本试验共检测到3个与亚油酸含量有关的QTL即*qC*$_{18\text{-}2}$*-6-1*、*qC*$_{18\text{-}2}$*-15-1*和*qC*$_{18\text{-}2}$*-LG6-1*, 分别定位在6号染色体(A06)、15号染色体(D02)和第6连锁群上(表3), 解释5.1%~37.3%的表型变异(表4)。这3个QTL均具有显著的胚和母体加性主效应, 表明这些QTL在胚和母体核基因组上的表达对亚油酸含量都具有重要的影响。另外, 3个QTL都具有明显的环境互作效应, 表明这些QTL的表达易受环境条件影响。*qC*$_{18\text{-}2}$*-15-1*具有37.3%的遗传贡献率, 它位于15号染色体上的分子标记BNL1579和

表 4　棉籽油分及 3 种脂肪酸的 QTL 遗传贡献率

Table 4　Contribution rates of QTLs for oil and three fatty acid contents in cottonseed kernel

性状 Trait	QTL	R^2 (a^m)	R^2 (a^e)	R^2 (d^e)	R^2 (a^mE)	R^2 (a^eE)	R^2 (d^eE)	R^2 (T)
油分含量	*qOil-3-1*	1.1	1.4	0.0	0.0	0.0	0.0	2.5
Oil content	*qOil-18-1*	5.3	6.7	0.0	0.0	0.0	0.0	12.0
	qOil-18-2	0.3	0.4	0.0	0.0	0.0	0.0	0.7
	qOil-18-3	0.0	0.0	0.0	5.4	0.0	0.0	5.4
	qOil-LG3-1	2.3	2.7	0.0	0.0	0.0	0.0	5.0
	qOil-LG7-1	0.0	0.0	0.0	0.0	21.3	6.2	27.5
	qOil-LG8-1	0.0	0.1	0.0	8.6	0.0	0.0	8.7
棕榈酸含量	*qC*$_{16\text{-}0}$*-15-1*	5.0	6.4	0.0	0.0	0.0	0.0	11.4
Palmitic acid content	*qC*$_{16\text{-}0}$*-LG3-1*	2.2	3.0	0.0	11.6	19.6	0.0	36.5
	qC$_{16\text{-}0}$*-LG6-1*	0.7	0.8	0.0	0.0	0.0	0.0	1.5
油酸含量	*qC*$_{18\text{-}1}$*-18-1*	1.4	2.5	0.0	8.0	8.8	0.0	20.9
Oleic acid content	*qC*$_{18\text{-}1}$*-LG6-1*	3.3	3.7	0.0	1.8	0.0	0.6	9.4
亚油酸含量	*qC*$_{18\text{-}2}$*-6-1*	5.5	6.7	0.0	0.0	0.0	0.9	13.1
Linoleic acid content	*qC*$_{18\text{-}2}$*-15-1*	1.1	1.4	0.0	14.2	20.6	0.0	37.3
	qC$_{18\text{-}2}$*-LG6-1*	1.6	1.8	0.0	0.0	0.0	1.7	5.1

R^2(a^m): 母体加性主效应的贡献率; R^2(a^e): 胚加性主效应的贡献率; R^2(d^e): 胚显性主效应的贡献率; R^2(a^eE): 胚加性与环境互作的贡献率; R^2(d^eE): 胚显性与环境互作的贡献率; R^2(a^mE): 母体加性与环境互作的贡献率; R^2(T): 单个 QTL 的总贡献率。

R^2(a^e): the phenotypic variations explained by the a^e; R^2(d^e): the phenotypic variations explained by the d^e; R^2(a^m): the phenotypic variations explained by am; R^2(a^eE): the phenotypic variations explained by embryo additive interaction effects in environment; R^2(d^eE): the phenotypic variations explained by embryo dominance interaction effect in environment; R^2(a^mE): the phenotypic variations explained by maternal additive interaction effect in environment; R^2(T): the phenotypic variations explained by individual QTL.

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BNL4082 (c15)之间, 不仅同时具有显著的胚加性、胚显性和母体加性主效应, 还具有显著的胚和母体加性与环境的互作效应。该 QTL 的加性效应及其环境互作效应占大部分的遗传效应, 这表明该位点主要是以基因的加性效应控制着亚油酸含量的表现。存在于 6 号染色体上的 NAU932*和 NAU2566 之间的 *qC*$_{18\text{-}2}$*-6-1* 具有最大的胚和母体加性主效应, 来自 HS46 的等位基因所产生的胚加性主效应能使亚油酸含量增加 10.47%, 来自 MARCABUCAG8US-1-88 的等位基因所产生的母体加性主效应也能使亚油酸含量增加 10.6%。该 QTL 解释的表型变异率为 13.1%, 其中胚和母体加性效应的表型贡献分别为 5.5%和 6.7%。表明该 QTL 是控制亚油酸含量的 1 个重要位点。

3 讨论

3.1 棉籽油分含量等 4 个品质性状 QTL 在胚和母体核基因组上的表达

本研究涉及的棉籽油分及3种主要脂肪酸含量是棉籽品质育种的重要目标。由于棉籽品质性状不同于一般的农艺性状, 属于棉花子代的性状, 其发育受多套遗传体系中的基因影响[34]。因此, 对这些性状的基因定位时, 有必要考虑多个遗传体系的影响。本研究所用的定位遗传模型是专为双子叶植物种子品质性状QTL定位而建立, 能同时分析胚和母体2套核基因组上QTL位点。基于胚和母体核基因组2套遗传体系进行QTL定位, 这种方法将QTL的母体影响因素区分出来, 这样更有利于QTL的育种利用。本研究结果发现, 4个棉籽品质性状QTL均表现出在胚和母体核基因组2套遗传体中同时表达的现象, 这对加深理解棉籽品质性状的分子遗传特性具有重要意义, 同时, 在这些棉籽品质性状的分子改良方案制定时更加有针对性。

数量性状的表型一般容易受环境条件变化的影响, 相同的分离群体在不同的环境条件下, 检测的QTL的数目、效应可能不完全一致。因此, 多环境鉴定是提高QTL定位结果的精确性的重要因素。在不同环境下都能检测到的QTL具有更好的可靠性[35], 针对具有显著遗传效应的QTL进行分子标记辅助选择会有更好的育种效果[36-37]。另外, 定位群体也是影响QTL精确性的一个重要因素, 永久性的高代定位群体能使定位结果更加精确[31]。因此, 为获得更准确的定位结果, 本研究基于重组近交系的双向回交群体, 在2个环境中对棉籽油分等4个重要品质性状进行了多环境跨遗传体系的QTL分析。目前最理想的定位群体是F_2群体, 其分离广泛, 遗传信息丰富, 但重复性较差; DH及RIL群体重复性好, 但遗传信息不及F_2群体丰富。而本研究中的回交群体, 母本为RIL群体, 父本分别为纯合的轮回亲本HS46和MARCABUCAG8US-1-88, 因此, 它既有F_2群体丰富多样的遗传信息, 又能像RIL一样, 具有很好的重复性。利用这样的群体进行QTL定位, 其结果更精确可靠[32]。本研究共检测到15个QTL, 这些结果能为这些性状的改良提供更为全面、可靠的遗传信息。尤其是遗传贡献率大于10%的QTL, 如定位18号染色体(D05)上的位于记JESPR204 (c18)和CIR099 (c18)区间的*qOil-18-1*, 其表型贡献率为12.0%, 也未检测到环境互作效应, 而且位点还具有最大的胚加性和母体加性主效应, 说明这是一个稳定的重要QTL。因此, 类似这样的QTL在其相应性状的分子改良中可以发挥更为重要的作用。

目前, 关于棉籽油分及脂肪酸的QTL定位研究的报道逐渐增加。但因定位群体、定位软件及材料种植的环境不同, 研究结果之间有着较大的差异, 这是QTL研究的特点。正因为这个特性, 整合不同研究结果, 才能发现更多的共同QTL, 共同QTL可能更稳定、更有利用价值。本研究定位结果跟有些学者的研究结果差异较大, 只有少数QTL相同。如与Liu等[38]的研究结果比较, 仅在15号染色体上共同定位到一个棕榈酸含量QTL和在18号染色体上定位到1个油分含量QTL和1个油酸含量QTL; Yu等[22]采用陆地棉与海岛棉的杂交发展而来的回交群体(BIL), 在4个环境下检测到17油分含量相关的QTL分布在12个染色体上, 但仅在3号染色体上共同定位到一个油分含量QTL; Wang等[39]的研究结果中发现有30个油分QTL, 只有3号(A亚组)和18号(D亚组5号染色体)染色体上与本研究结果相同, 这可能是因为试验材料、定位方法及环境的差异而导致, 也可能与本研究的一些定位结果还未确定染色体的位置有关。但在不同研究结果中, 同一性状差异QTL为发掘控制该性状所有基因奠定基础。本研究结果也跟有些研究结果有很多一致性, 如秦利[11]采用本研究相同的重组近交系群体, 在种子基因组上定位出3个与油分相关的QTL, 其中位于18号染色体和第3连锁群上的*qOC18-2*和*qOC-LG3-1*在本研究中也能检测到。Liu等[24]采用连锁不平衡关联图谱对陆地棉棉

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籽油分进行了定位, 在10条染色体上共发现有15个油分QTL, 其中3号(A亚组)和18号(D亚组5号染色体)染色体的QTL与本研究一致。综合来看, 发现3号和18号染色体与油分相关性状关系密切, 在遗传改良时值得重点考虑。

另外, 本研究发现, *qC*$_{16\text{-}0}$*-15-1*和*qC*$_{18\text{-}2}$*-15-1*共定位在15号染色体上的同一位置。在18号染色体和第3、4、6、7连锁群上不同性状的QTL呈集中分布, 说明这些区域与油分相关性状密切。这个结果从分子层次上, 也体现了这4个性状相关性。这为棉籽油分及3个脂肪酸含量的遗传改良提供了更科学的指导依据。

4　结论

本研究利用陆地棉的特殊回交群体, 采用专为双子叶植物的种子性状设计的跨遗传体系的QTL定位模型及软件, 对棉籽油分和 3 种重要的脂肪酸含量进行定位分析, 共检测到了 15 个 QTL。所有 QTL 均检测到了来自胚和母体核基因组的遗传效应, 说明种子性状受 2 套核基因组的影响, 对这些 QTL 利用时, 母体植株基因所产生的影响不可忽视。本研究发现了 7 个油分含量 QTL, 共解释了 61.8%的表型变异。其中, *qOil-3-1* 和 *qOil-18-1* 是 2 个重要的 QTL; 在 3 个与棕榈酸含量有关的 QTL 中, *qC*$_{16\text{-}0}$*-15-1* 对棕榈酸含量最重要; 共检测到 2 个油酸含量 QTL, 其中, *qC*$_{18\text{-}1}$*-18-1* 能解释 20.9%的表型变异, 主要通过加性效应来控制油酸含量, 是一个控制油酸含量的重要位点; 共检测到 3 个重要的亚油酸含量 QTL, 尤其是 *qC*$_{18\text{-}2}$*-6-1*。这些位点既为这些性状的分子标记辅助选择育种提供更加可靠的参考, 又为其分子遗传机制的解释奠定更为扎实的基础。此外, 还发现在 15 号、18 号染色体和第 3、4、6、7 连锁群上的 QTL 呈集中分布现象, 说明这些染色体与油分相关性状关系密切, 在这些性状的遗传改良时可以重点考虑。

致　谢: 感谢浙江大学祝水金教授在本论文研究过程中给予了大量的支持。本研究中所用的 RIL 群体由祝教授提供, 并且祝教授还为回交群体的棉籽 4 个性状的测定提供了免费测试平台。

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附表 1　棉籽 4 个性状 QTL 位点相关分子标记信息

Table S1　Information from molecular genetic marker related to QTL for four traits of cottonseeds

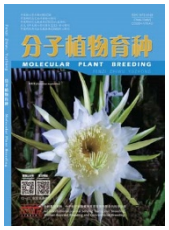
染色体/连锁群 Chr./Linkage group	分子标记 Molecular marker	引物 Primer (5′−3′)	标记类型 Marker type	标记引物来源 Source of marker primer	标记来源 Source of marker	重复基序 Repeat motif
Chr3	MUCS127(c3)	F: TTTGTACTCATGTTCCCGCC; R: GCATGCTTGAAGAAGGTTCC	SSR	Park et al.[40]	*G. arboreum* EST	TCTT(2)CTT(4)
Chr3	NAU808	F: GATGGCTACCTCCCTTTGTA; R: CGTAAGGAAGCCTAGCAAAA	SSR	Han et al. [41-42]	*G. hirsutum* EST	none
Chr18	JESPR204(c18)	F: CTCCAGGTTCAATGGTCTG; R: GCCATGTTGGACAAGTAGTC	SSR	Reddy et al.[43]	*G. hirsutum* genome	(CTT)20
Chr18	CIR099(18)	F: ATGATTCAAGTCGCGT; R: TTCAAGGCTGAGTCAAA	SSR	Nguyen et al.[44]	*G. hirsutum* genome	(GT)8
Chr18	TMO409a	F: CAGAGGACGAAGGTAGCAG; R: TGGTGGGTTTCACTTTCACA	SSR	USDA-ARS	*G. hirsutum* genome	(CA)10+(GA)5
Chr18	NAU1001	F: TAAAGGAAGAAAACCCACCA; R: TTTCTCTTCAACAGCAGCAG	SSR	Han et al.[41-42]	*G. hirsutum* EST	(ACA)7
Chr18	NAU5152b	F: TCCTTTCTACCCATGCCTAC; R: GTCACGAGAAGCAGAGGACT	SSR	Han et al.[41-42]	*G. hirsutum* EST	(tct)9
Chr18	NAU3053a	F: TTGGAGGTGTCAGATCCTCT; R: AAGATCCATGACCCAGAAAA	SSR	Han et al.[41-42]	*G. hirsutum* EST	(at)13
LG3	NAU5146c	F: ATCTCCATTCCCTCTCCTCT; R: ATAAACGCAACGGATAAAGG	SSR	Han et al.[41-42]	*G. hirsutum* EST	(ttta)5
LG3	RAPD-I10-620	GTCCACACGG	RAPD	购于上海赛百盛生物技术公司 The marker primer purchased from SBS Genetech Co., Ltd	*G. hirsutum* genome	None
LG7	NAU983	F: GAAGGTCATGTTTTGCATTG; R: AGCCATCAAATGACCAGTCT	SSR	Han et al.[41-42]	*G. hirsutum* EST	(CAGCAT)3
LG7	TMB1791	F: GAGATCGTTTATCATTCCCACA; R: TCTACCGGCCTCGTAAGCTA	SSR	Yu et al.[45]	*G. hirsutum* genome	(GA)15+(CA)8
LG8	NAU4919b*	F: ATCTAATGGGGGAGGAAGAG; R: CTCCTTGAAACCCTTTGAGA	SSR	Han et al.[41-42]	*G. hirsutum* EST	(cca)6

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(附表 1)

染色体/连锁群 Chr./Linkage group	分子标记 Molecular marker	引物 Primer (5′–3′)	标记类型 Marker type	标记引物来源 Source of marker primer	标记来源 Source of marker	重复基序 Repeat motif
LG8	NAU4960d	F: CGGCCTACCTCTTCCTCT; R: TTAACGACAATTCCATCGTG	SSR	Han et al.[41-42]	*G. hirsutum* EST	(tcc)6
Chr15	BNL4082(c15)	F:GTAAAATGAAATAAAATAAAAGGAGAGA; R: TTCAACACCGCCAAACATAA	SSR	Brookhaven National Laboratory, NY, USA	*G. hirsutum* genome	(GA)24
LG3	NAU4852	F: ATCTCAAGGCCCAAATTACA; R: CCTAAATTGTCAAGCGAGGT	SSR	Han et al.[41-42]	*G. hirsutum* EST	(cat)6
LG3	NAU1247	F: AATTGGCTTCCAAGAGACTG; R: CACCATCATCATCAAACTCG	SSR	Han et al.[41-42]	*G. hirsutum* EST	(AAG)8
Chr18	NAU5152a	F: TCCTTTCTACCCATGCCTAC; R: GTCACGAGAAGCAGAGGACT	SSR	Han et al.[41-42]	*G. hirsutum* EST	(tct)9
Chr18	NAU1232	F: GTGGAATGTGGTGATGAGAA; R: CAATCCCACCCCATATCTA	SSR	Han et al.[41-42]	*G. hirsutum* EST	(TGTA)5
LG6	NAU456	F: GAAGGAGGGCGAAAAACATA; R: TTGGAGATTACGCGACCTTC	SSR	Han et al.[41-42]	*G. hirsutum* EST	(CAC)8
LG6	NAU5328-170	F: AATGAGAGTGGCGGCTATAC; R: CTTATAGCTCTGGGCAGCAT	SSR	Han et al.[41-42]	*G. hirsutum* EST	(aatgca)3
Chr6	NAU932*	F: ACAAAGCAAATATGGGTTCC; R: CCCCCTTAAAATGGAAGAAG	SSR	Han et al.[41-42]	*G. hirsutum* EST	(GGTGAA)3
Chr6	NAU2566	F: CAAAATCCACCCCCTCCT; R: CCCTTCCCAGGAAAGTTTAT	SSR	Han et al.[41-42]	*G. hirsutum* EST	(aata)4
Chr15	BNL1579	F: CATGAGAACTTCATGCATATTCA; R: ATTTAAGTGCCTCCGTCCCT	SSR	Brookhaven National Laboratory, NY, USA	*G. hirsutum* genome	(AG)17
LG6	RAPD-G17-120	GACGGATCAG	RAPD	购于上海赛百盛生物技术公司 The marker primer purchased from SBS Genetech Co., Ltd	*G. hirsutum* genome	None

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分子植物育种
Molecular Plant Breeding
ISSN 1672-416X,CN 46-1068/S

《分子植物育种》网络首发论文

题目：抗稻瘟病基因特异分子标记开发及在云南粳稻中的应用

作者：周伍民，董丽英，刘树芳，刘沛，张先闻，李迅东，黄立钰，杨勤忠

网络首发日期：2021-09-29

引用格式：周伍民，董丽英，刘树芳，刘沛，张先闻，李迅东，黄立钰，杨勤忠．抗稻瘟病基因特异分子标记开发及在云南粳稻中的应用[J/OL]．分子植物育种．https://kns.cnki.net/kcms/detail/46.1068.s.20210928.0151.008.html



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网络首发时间：2021-09-29 12:04:38
网络首发地址：https://kns.cnki.net/kcms/detail/46.1068.s.20210928.0151.008.html

研究报告

Research Report

抗稻瘟病基因特异分子标记开发及在云南粳稻中的应用

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摘　要　为明确云南省水稻主栽粳稻品种的抗性遗传背景，本研究根据 *Pi2*、*Pi9*、*Piz-t*、*Pi50/Pigm*、*Pita-2* 和 *Pi5* 等 6 个已克隆的抗性基因序列开发基因特异性分子标记，并结合 *Pia*、*Pib*、*Pii*、*Pid3*、*Pid4* 和 *Pi54* 等 6 个已发表的抗性基因特异标记，对 1980-2020 年云南省培育的 163 个粳稻品种/系进行抗性基因的分子鉴定。结果表明，本研究开发的 6 个基因的特异分子标记能特异性地检测品种中是否含有靶标基因；利用 12 个基因的特异分子标记对云南 163 个粳稻品种/系检测发现，163 个品种中能检测到的抗性基因数量为 0-5 个，其中携带 5 个抗性基因的品种为‘楚粳 48’和‘楚粳 54’，并发现 3 个品种未检测到本研究中的抗性基因。在 12 个检测的基因中，品种中携带频率最高的基因为 *Pi54* (67.48%)；其次是 *Pib* 和 *Pii*，频率为 58.89% 和 42.33%；而 *Pita-2*、*Piz-t*、*Pid3*、*Pia* 和 *Pid4* 分布较少，频率为 12.26%、9.20%、7.97%、7.97%和 2.45%；*Pi2*、*Pi9*、*Pi50/Pigm* 以及 *Pi5* 在这些粳稻品种/系中均未检测到。基于 12 个抗性基因特异分子标记对云南近 40 年来选育的粳稻品种/系的抗性基因检测结果表明，云南粳稻品种中持有的抗性基因数量有限，需引入新的有效抗源以拓宽云南粳稻品种的抗性遗传基础，提高水稻品种的抗性水平。本研究开发的 6 个广谱稻瘟病抗性基因特异分子标记可用于品种中抗性基因的鉴定及分子标记辅助选择育种，以提高抗性品种的筛选效率，可为抗病品种的选育及解决水稻生产上稻瘟病抗性弱的实际问题提供理论依据。

关键词　水稻；稻瘟病；抗性基因；分子标记

基金项目：本研究由云南省基础研究计划(202001AS070006)、国家自然科学基金(31860524)和云南省科技人才与平台计划(202105AG070001)共同资助

Development of Blast Resistant Gene-Specific Molecular Markers and Their Application in Improved *Japonica* Rice Cultivars of Yunnan

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Abstract To clarify genetic basis for resistance to rice blast of these cultivars/lines, gene-specific molecular markers were developed, respectively, based on the sequences of six blast resistant genes, including *Pi2*, *Pi9*, *Piz-t*, *Pi50/Pigm*, *Pita-2* and *Pi5*, and then the genotypes of blast resistant genes in a total of 163 japonica rice varieties/lines cultivated from 1980 to 2020 in Yunnan Province were detected using the six molecular markers and other six published molecular markers specific to resistance genes *Pia*, *Pib*, *Pii*, *Pid3*, *Pid4*, and *Pi54*. The results showed that the six molecular markers developed in this study could specifically detect whether the cultivars/lines carry the target genes and the surveyed cultivars/lines carried 0 to 5 resistance genes Among the twelve rice blast resistant genes detected. Among Among them, the cultivars Chujing 48 and Chujing 54 possessed 5 resistant genes, and three cultivars did not harbor blast resistant genesdetected. Among the 12 resistance genes tested in this study, the highest frequency gene in all surveyed cultivars was *Pi54* (67.48%), which followed by *Pib* (58.89%) and *Pii* (42.33%). The carried frequency of *Pita-2*, *Piz-t*, *Pid3*, *Pia*, and *Pid4* was 12.26%, 9.20%, 7.97%, 7.97%, and 2.45%, respectively. Interestingly, *Pi2*, *Pi9*, *Pi50/Pigm*, and *Pi5* were not detected in all of these cultivars/lines. Rice blast resistance genes carrying in improved *japonica* rice cultivars/lines bred during the past 40 years in Yunnan has been identified with 12 gene-specific molecular markers, and the data revealed that

the limited blast resistance genes were detected in these *japonica* rice cultivars/lines. Therefore, new resistant resources need to be introduced to broaden the resistant genetic basis, so as to improve the resistant ability of *japonica* rice cultivars/lines to rice blast in Yunnan. This study has developed six gene-specific molecular markers, which could be used to determine if the varieties/cultivars carry these genes, and further used in marker-assisted selection for disease-resistant rice breeding to improve the selection efficiency of blast resistance genes, and could provide a theoretical basis for breeding disease-resistant varieties and solving the practical problems of weak rice blast resistance in rice production.

Keywords *Oryza sativa*; Rice blast; Resistance gene; Molecular marker

水稻(*Oryza sativa* L.)是世界上最重要的粮食作物之一，全球一半的人口以稻米为主食(Khush, 2005)，由稻瘟病菌 *Magnaporthe oryzae*(无性态为 *Pyricularia oryzae*)引起的稻瘟病是水稻生产中危害最为严重的病害之一(Couch and Kohn., 2002)。生产实践证明，合理利用抗性品种是控制稻瘟病最经济、有效和环境友好的措施(Sharma et al., 2012; Savary et al., 2019)，而抗性品种的培育离不开有效抗性基因的合理利用。传统鉴定水稻品种中抗性基因的方法主要是根据一套特定致病性的稻瘟病菌株对品种的抗、感表型鉴定，进而对品种持有的抗性基因进行推定，存在工作量大、菌株数量有限和接种环境影响等问题(Fang et al., 2018; 兰波等, 2019)。随着抗性基因不断被鉴定及分子定位，与抗性基因紧密连锁的分子标记被不断开发并用于分子标记辅助选择育种，且部分标记被用于品种中是否持有特定抗性基因的分析(Hayashi et al., 2004; 李进斌等, 2012)。但鉴定品种中是否存在目标抗性基因时，紧密连锁标记会受到遗传背景、连锁不平衡和目的基因成簇等因素的影响。而抗性基因的成功克隆，提供了直接用于基因特异性分子标记开发的 DNA 序列，基因特异分子标记的应用实现了目标基因的直接鉴定，解决了前述传统方法及紧密连锁标记应用中存在的问题，提高了抗性基因鉴定的准确性(Liu et al., 2012)。通过基因特异分子标记明确已育成品种中抗性基因的构成，可为品种布局和新品种培育提供理论依据。

通过广泛的遗传分析，迄今已从稻种中已鉴定、定位了 100 多个稻瘟病抗性基因，其中 28 个主效抗

性基因已被克隆(Kalia and Rathour., 2019)。这些基因中，除 *Pid2*、*pi21*、*Ptr/Pita-2* 是编码特殊结构的蛋白外，其余的均编码植物中具有核苷酸结合位点-亮氨酸重复结构(Nucleotide-binding site, Leucine-rich repeats, NBS-LRR)的抗病蛋白(Zhang et al., 2019)。*Pib* 基因是第一个被克隆的稻瘟病抗性基因(Wang et al., 1999; 杨勤忠等, 2009)，随后 Fjellstrom 等(2004)通过比较 *Pib* 基因与感病品种中等位基因序列差异，开发出显性特异分子标记 Pibdom，此标记可以准确检测品种中是否存在抗性基因 *Pib*。之后根据 *Pid3* (Shang et al., 2009)、*Pia* (Okuyama et al., 2011)、*Pi54* (Ramkumar et al., 2011)和 *Pii* (Takagi et al., 2013)等基因序列设计的 InDel、CAPS 或显性标记等基因特异分子标记相继被开发并广泛用于品种中抗性基因的检测分析。潘争艳等(2019)用 9 个抗性基因特异引物对辽宁省已审定的 260 份粳稻品种进行检测分析，结果发现抗性基因 *Pikh* 检出率最高，所有参试品种均未检测到 *Pi9* 基因，仅在一个品种中检测到 *Pi40* 基因；王小秋等(2020)利用 14 个抗性基因的特异性标记对江苏省育成的 195 个粳稻新品种/系进行检测分析，结果发现 *Pib*、*Pita* 和 *Pikh* 基因在供试品种中的分布频率较高，均在 45%以上，所有检测品种中均不含有 *Pigm* 基因。此外，水稻中具有广谱抗稻瘟病特性的基因 *Pi2* (Zhou et al., 2006)、*Pi9* (Qu et al., 2006)、*Piz-t* (Zhou et al., 2006)、*Pi50/Pigm* (Su et al., 2015; Deng et al., 2017)、*Pita-2* (Zhao et al., 2018)和 *Pi5* (Lee et al., 2009)等先后被成功克隆，为根据基因序列开发特异标记，研究水稻品种中广谱抗性基因的构成，指导分子标记辅助选择育种及品种的合理布局提供依据。

水稻作为云南省重要的粮食作物之一，常年种植面积约 10^6 hm^2 (蒋志农, 1995, 云南科技出版社, pp.1-3)，且云南地处中国西南低纬高原，既是稻种遗传资源多样化中心，又是稻瘟病菌的起源地(杨忠义等, 2009; Saleh et al., 2014)。稻瘟病菌群体的构成极为复杂，诸多单一的抗性基因在生产上抗性表现不佳，育成品种抗性弱的现象突出(涂敏等, 2011; 董丽英等, 2018)。利用云南稻瘟病菌单胞菌株对抗性单基因系研究表明，*Pi2*、*Pi5*、*Pi9* 等基因在云南具有较好的利用价值(董丽英等, 2012)。本研究拟根据 *Pi2*、*Pi9*、*Piz-t*、*Pi50/Pigm*、*Pita-2* 和 *Pi5* 等 6 个已克隆的广谱抗稻瘟病基因序列开发基因特异性分子标记，为水稻品种中抗性基因分子鉴定和分子标记辅助选择育种提供技术支持；并结合 *Pia*、*Pib* 和 *Pii* 等 6 个抗性基因的特异

标记，对云南省 1980-2020 年间培育的 163 个粳稻品种/系进行抗性基因分子鉴定，以期解析这些粳稻品种所携带的抗性基因种类及数量，明确其抗性遗传背景，为抗病品种的合理布局以及抗病新品种选育中如何拓宽抗性遗传基础提供科学依据。

1 结果与分析

1.1 基因特异性分子标记设计及检测

根据 *Pi2*、*Pi9*、*Piz-t*、*Pi50/Pigm* 基因及其旁系同源基因序列比较结果，在基因序列特有碱基位置上定点设计引物，分别设计 *Pi2*、*Pi9*、*Piz-t* 这 3 个基因的显性特异标记，经 PCR 扩增检测发现这 3 个显性标记能特异扩增靶标基因(图 1)，扩增的片段长度与预期相符(表 1)。*Pi50/Pigm* 基因特异标记设计则通过该位点存在的同源序列进行比较后，设计出可用 *Ava* II 进行 PCR 产物酶切后区别 *Pi50/Pigm* 与不含抗性基因的 CAPS 标记(图 2)，扩增出 335 bp 的产物用 *Ava* II 酶切消化后电泳，产生长度为 81 bp 和 254 bp 两条片段的个体不持有 *Pi50/Pigm* 抗病基因，而 335 bp 的 PCR 产物(不含有 *Ava* II 酶切位点)不能被酶切的品种持有 *Pi50*/*Pigm* 抗病基因(图 3A)；但当用其它品种 Arang、Lainokkha、Siboras 和 Terungdaun 等进行扩检测时，发现 *Ava* II 并不能酶切产物，测序分析发现，这些品种携带有另外一个以 Arang 品种的片段为代表的单倍型 New 1，与 *Pi50/Pigm* 序列相比，其含有一个 *Sty* I 的酶切位点，使 PCR 产物被酶切成 286 bp 和 49 bp 的片段，而梅 4 号中扩增出来的同源基因 *pi50*-NBS-2 可被 *Sty* I 酶切成 108 bp 和 227 bp 的片段，同源基因 *pigm*-R8 可被切成 108、178 和 49 bp 的片段(图 2)，因而在谷梅 4 号扩增产物存在 5 条带，分子量最大的一条未被酶切开的为 *Pi50/Pigm* 基因的条带(图 3B)，综上所述，因 *Pi50/Pigm* 基因不持有 *Ava* II 和 *Sty* I 酶切位点，可根据 PCR 产物酶切后的结果判定品种是否持有 *Pi50/Pigm* 基因。利用 *Pii* 基因特异引物检测时，发现 PCR 产物酶切后不能将 *Pii* 和其等位基因 *Pi5* 区别开(图 4A)，分析基因序列发现 2 个基因在靶标序列上均有相同的酶切位点，进一步根据 *Pii* 和 *Pi5* 基因序列的比较，基于 *Pi5* 基因序列具有 4 bp 的插入，而在此位置两端设计特异引物，经检测可有效将两个等位基因 *Pi5* 和 *Pii* 区别开(图 4B)。*Pita-2* 基因特异标记为共显性标记，持有 *Pita-2* 基因的品种 PCR 扩增产物缺失 12 bp (图 4C)。以上结果表明，本研究开发的 6 个基因特异引物可用于检测水稻品种中是否持有特定的稻瘟病抗性基因。

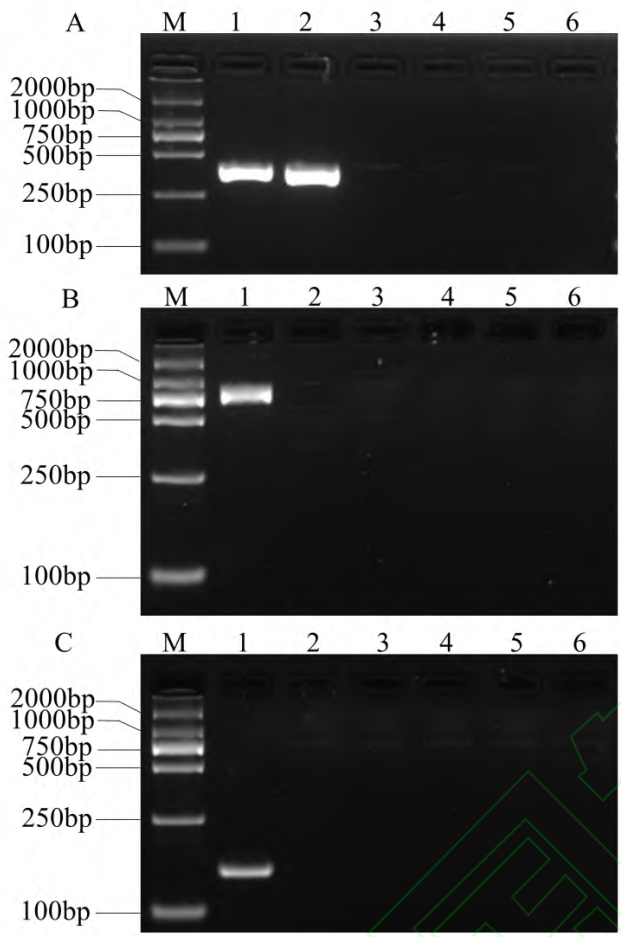


图 1 3 个稻瘟病抗性基因特异分子标记检测
注: M: DL2000 DNA Marker; A: *Pi2* 基因; 1~6: IRBLZ5-CA (*Pi2*), IRBLZ5-CA® (*Pi2*), IRBLZT-T (*Piz-t*), IRBL9-W (*Pi9*), 谷梅 4 号(*Pi50/Pigm*)和 LTH; B: *Pi9* 基因; 1~6: IRBL9-W (*Pi9*), IRBLZ5-CA (*Pi2*), IRBLZ5-CA® (*Pi2*), IRBLZT-T (*Piz-t*), 谷梅 4 号(*Pi50/Pigm*)和 LTH; C: *Piz-t* 基因; 1~6: IRBLZT-T (*Piz-t*), IRBLZ5-CA (*Pi2*), IRBLZ5-CA® (*Pi2*), IRBL9-W (*Pi9*), 谷梅 4 号(*Pi50/Pigm*)和 LTH

Figure 1 Detection of three specific molecular markers of rice blast resistance genes
Note: M: DL2000 DNA Marker; A: *Pi2* gene; 1~6: IRBLZ5-CA (*Pi2*), IRBLZ5-CA® (*Pi2*), IRBLZT-T (*Piz-t*), IRBL9-W (*Pi9*), Gumei 4 (*Pi50*/*Pigm*) and LTH; B: *Pi9* gene; 1~6: IRBL9-W (*Pi9*), IRBLZ5-CA (*Pi2*), IRBLZ5-CA® (*Pi2*), IRBLZT-T (*Piz-t*), Gumei 4 (*Pi50*/*Pigm*) and LTH; C: *Piz-t* gene; 1~6: IRBLZT-T (*Piz-t*), IRBLZ5-CA (*Pi2*), IRBLZ5-CA® (*Pi2*), IRBL9-W (*Pi9*), Gumei 4 (*Pi50*/*Pigm*) and LTH

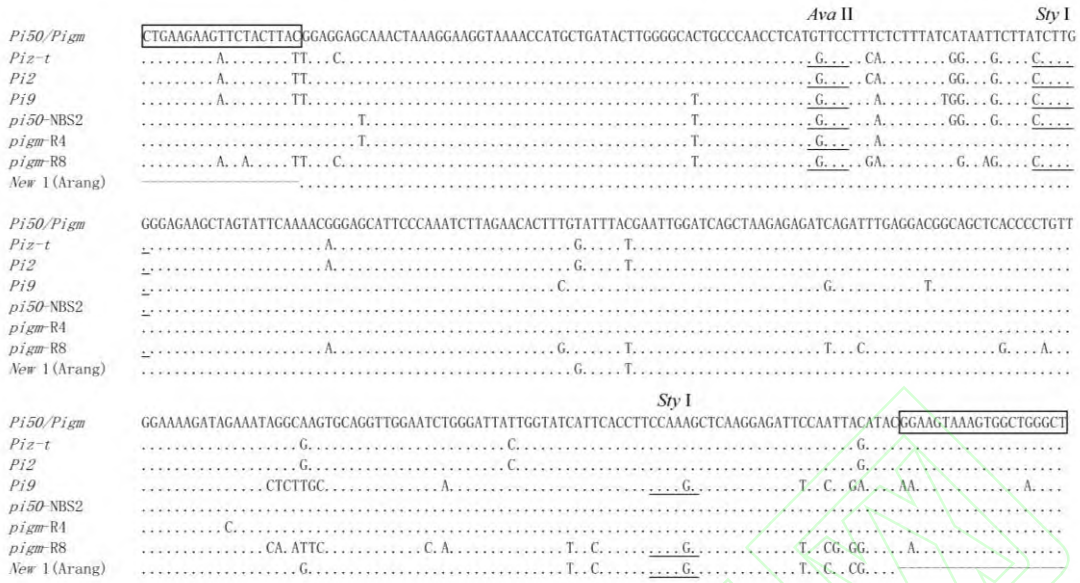


图 2 *Pi50*/*Pigm* 位点同源基因序列比较分析
注: 方框内的 DNA 序列为 PCR 扩增 *Pi50*/*Pigm* 基因片段的正向及反向引物序列; 点表示碱基与 *Pi50/Pigm* 相同, 短线位置表示新同源基因的引物结合的位置; 下划线的碱基为限制性内切酶 *Ava* II 和 *Sty* I 的酶切位点
Figure 2 Multiple alignment of the DNA sequences of *Pi50/Pigm*, *Pi2*, *Pi9*, *Piz-t* and their homologs
Note: DNA sequences of Forward and reverse primers used for amplification of *Pi50/Pigm* homologous fragments are boxed respectively; Dots represent nucleotides identical to Pi50/Pigm, and dashes represent the positions of forward and reverse primers; The underlined nucleotides represent the restriction sites for both *Ava* II (GGWCC) and *Sty* I (CCWWGG)

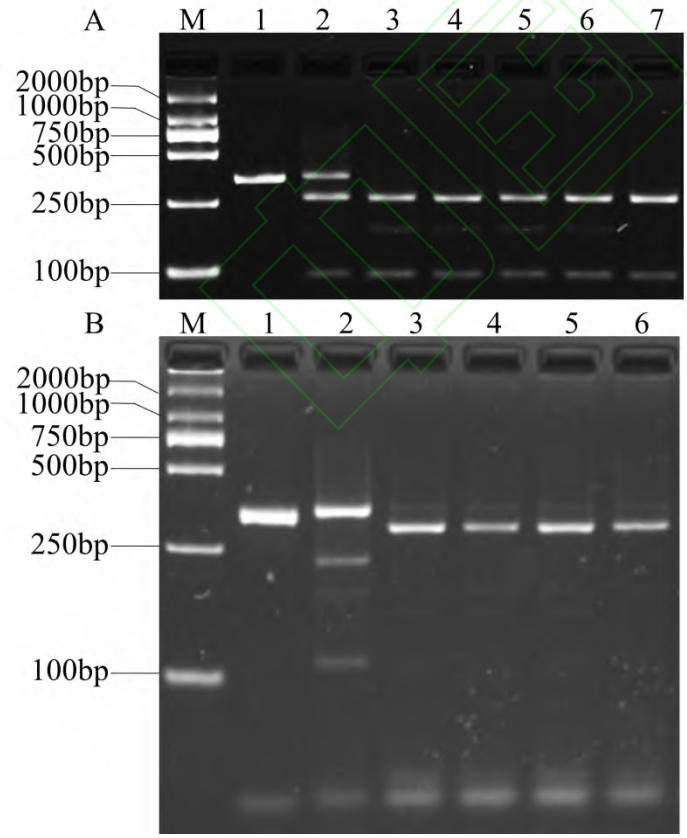


图 3 *Pi50/Pigm* 特异性分子标记检测
注: M: DL2000 DNA Marker; A: *Ava* II 酶切检测; 1: 未酶切的谷梅 4 号扩增片段; 2~7: 谷梅 4 号(*Pi50/Pigm*), IRBLZ5-CA (*Pi2*), IRBLZ5-CA® (*Pi2*), IRBLZT-T (*Piz-t*), IRBL9-W (*Pi9*)和 LTH; B: *Sty* I 酶切检测; 1: 未酶切的谷梅 4 号扩增片段; 2~6: 谷梅 4 号(*Pi50/Pigm*), Arang, Lainokkha, Siboras 和 Terungdaun.

Figure 3 Detection of *Pi50/Pigm* specific molecular markers
Note: M: DL2000 DNA Marker; A: Enzyme digestion of *Ava* II; 1: Undigested Guimei 4;2~7: Gumei 4 (*Pi50/Pigm*), IRBLZ5-CA (*Pi2*), IRBLZ5-CA® (*Pi2*), IRBLZT-T(*Piz-t*), IRBL9-W (*Pi9*) and LTH; B: Enzyme digestion of *Sty* I; 1: Undigested Guimei 4; 2~6: Gumei 4 (*Pi50/Pigm*), Arang, Lainokkha, Siboras and Terungdaun.

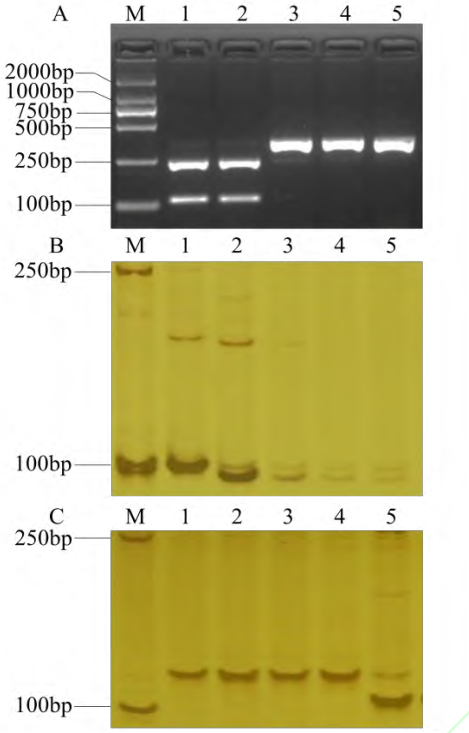


图 4 *Pi5* 和 *Pita-2* 特异性分子标记检测
注: M: DL2000 DNA Marker; A: *Pii* 分子标记扩增产物 *Pvu* Ⅱ酶切后结果; B: *Pi5* 特异性分子标记扩增检测结果; C: *Pita-2* 特异性分子标记扩增检测结果; 1~5: IRBL5-M (*Pi5*), IRBLI-F5 (*Pii*), LTH, IRBLTA-K1 (*Pita*)和 IRBLTA2-PI (*Pita-2*)
Figure 4 Detection of *Pi5*and *Pita-2* specific molecular markers
Note: M: DL2000 DNA Marker; A: The result of enzyme digestion of *Pvu* Ⅱ amplified product with *Pii* molecular marker; B: The result of *Pi5* specific molecular marker amplification; C: The result of *Pita-2* specific molecular marker amplification; 1~5: IRBL5-M (*Pi5*), IRBLI-F5 (*Pii*), LTH, IRBLTA-K1 (*Pita*) and IRBLTA2-PI (*Pita-2*)

1.2 云南省粳稻品种/系中稻瘟病抗性基因检测

利用本研究开发的 6 个抗性基因(*Pita-2*, *Pi2*, *Pi9*, *Piz-t*, *Pi50/Pigm*, *Pi5*)和已报道的 6 个抗性基因(*Pia*, *Pib*, *Pid3*, *Pid4*, *Pi54*, *Pii*)共计 12 个基因的特异引物对 163 份粳稻品种/系进行检测，结果发现云南粳稻育成品种中含有的抗性基因数为 0-5 个 (表 1)，其中‘楚粳 48 号’和‘楚粳 53 号’中检测到 5 个抗性基因，为检测到抗性基因数量最多的品种；在 7 个品种中检测到含 4 个抗性基因；含有 3 个、2 个和 1 个抗性基因的品种分别占供试品种的 22.08%、48.46%和 22.08%；在这些品种中，有 3 个品种(‘楚粳 9 号’、‘楚粳 22 号’和‘西南 175’)未检测到本研究中的 12 个抗性基因。

表 1 粳稻品种中稻瘟病抗性基因分子检测
Table 1 Molecular detection of resistance genes in improved *japonica* cultivars

序号 NO.	品种名称 Name cultivar	审定时间 Registration date	抗性基因 Resistance gene												总计 Total
			Pita-2	*Pia*	*Pib*	*Pid3*	*Pid4*	*Pi54*	*Pi2*	*Piz-t*	*Pi9*	*Pi50/Pigm*	*Pii*	*Pi5*	
1	楚粳 4 号 Chujing 4	1985	-	+	-	-	-	+	-	-	-	-	-	-	2
2	楚粳 5 号 Chujing 5	1986	-	-	-	-	-	+	-	-	-	-	-	-	1

3	楚粳 6 号 Chujing 6	1990	-	-	-	-	-	+	-	-	-	-	-	-	1
4	楚粳 7 号 Chujing 7	1991	-	-	-	-	-	+	-	-	-	-	-	-	1
5	楚粳 8 号 Chujing 8	1990	-	-	-	-	-	+	-	-	-	-	-	-	1
6	楚粳 9 号 Chujing 9	-	-	-	-	-	-	-	-	-	-	-	-	-	0
7	楚粳 13 号 Chujing 13	-	+	-	-	-	-	-	-	-	-	-	-	-	1
8	楚粳 14 号 Chujing 14	1995	+	-	-	-	-	-	-	-	-	-	-	-	1
9	楚粳 15 号 Chujing 15	-	-	-	-	-	-	+	-	-	-	-	-	-	1
10	楚粳 17 号 Chujing 17	1997	+	-	-	-	-	-	-	-	-	-	-	-	1
11	楚粳 18 号 Chujing 18	-	-	-	-	-	-	+	-	-	-	-	-	-	1
12	楚粳 19 号 Chujing 19	-	+	-	-	-	-	+	-	-	-	-	-	-	2
13	楚粳 21 号 Chujing 21	-	-	-	-	+	-	+	-	+	-	-	-	-	3
14	楚粳 22 号 Chujing 22	1999	-	-	-	-	-	-	-	-	-	-	-	-	0
15	楚粳 23 号 Chujing 23	1999	+	-	+	-	-	-	-	-	-	-	+	-	3
16	楚粳 25 号 Chujing 25	2002	+	-	-	-	-	+	-	-	-	-	+	-	3
17	楚粳 26 号 Chujing 26	2005	-	-	-	-	-	-	-	-	-	-	+	-	1
18	楚粳 27 号 Chujing 27	2005	-	-	-	+	-	-	-	+	-	-	-	-	2
19	楚粳 28 号 Chujing 28	2007	-	-	+	-	-	+	-	-	-	-	-	-	2
20	楚粳 29 号 Chujing 29	2007	-	+	-	-	-	-	-	-	-	-	-	-	1
21	楚粳 30 号 Chujing 30	2007	-	-	+	-	-	+	-	-	-	-	-	-	2
22	楚粳 31 号 Chujing 31	2010	-	-	-	-	-	+	-	-	-	-	-	-	1
23	楚粳 32 号 Chujing 32	2011	-	-	-	-	-	+	-	-	-	-	-	-	1
24	楚粳 34 号 Chujing 34	-	-	-	+	-	-	-	-	-	-	-	+	-	2
25	楚粳 35 号 Chujing 35	-	-	-	+	-	-	+	-	-	-	-	-	-	2
26	楚粳 36 号 Chujing 36	-	-	-	+	-	-	-	-	-	-	-	+	-	2
27	楚粳 37 号 Chujing 37	2014	-	-	+	-	-	+	-	-	-	-	+	-	3
28	楚粳 38 号 Chujing 38	2014	-	-	+	-	-	+	-	-	-	-	-	-	2
29	楚粳 40 号 Chujing 40	2015	-	+	+	-	-	+	-	-	-	-	-	-	3
30	楚粳 41 号 Chujing 41	2016	-	-	+	+	-	+	-	+	-	-	-	-	4
31	楚粳 42 号 Chujing 42	2016	-	-	-	+	-	-	-	+	-	-	-	-	2
32	楚粳 45 号 Chujing 45	2017	-	+	+	-	-	+	-	-	-	-	-	-	3
33	楚粳 48 号 Chujing 48	2019	-	+	+	+	-	+	-	+	-	-	-	-	5

34	楚粳 53 号 Chujing 53	-	-	-	+	+	-	+	-	+	-	-	+	-	5
35	楚粳 54 号 Chujing 54	-	-	-	-	+	-	-	-	+	-	-	-	-	2
36	轰杂 135 Hongza 135	1989	-	-	-	-	-	+	-	-	-	-	+	-	2
37	云稻 1 号 Yundao 1	2005	-	-	+	-	-	+	-	-	-	-	-	-	2
38	滇粳优 5 Dianjingyou 5	2005	-	-	+	-	-	+	-	-	-	-	-	-	2
39	滇系 4 号 Dianxi 4	2001	-	-	+	-	-	+	-	-	-	-	-	-	2
40	银光 Yingguang	2001	-	+	-	-	-	+	-	-	-	-	-	-	2
41	云资粳 41 号 Yunzijing 41	2012	-	-	+	-	-	+	-	-	-	-	-	-	2
42	云粳 2 号 Yunjing 2	-	-	-	+	-	-	+	-	-	-	-	+	-	3
43	云粳 3 号 Yunjing 3	-	-	-	+	-	-	+	-	-	-	-	-	-	2
44	云粳 4 号 Yunjing 4	2001	-	-	+	-	-	+	-	-	-	-	-	-	2
45	云粳 5 号 Yunjing 5	-	-	-	+	-	-	-	-	-	-	-	-	-	1
46	云粳 6 号 Yunjing 6	-	-	+	+	-	-	+	-	-	-	-	-	-	3
47	云粳 7 号 Yunjing 7	-	-	-	+	-	-	+	-	-	-	-	-	-	2
48	云粳 10 号 Yunjing 10	-	-	-	+	-	-	-	-	-	-	-	+	-	2
49	云粳 12 号 Yunjing 12	2005	-	-	+	-	-	+	-	-	-	-	-	-	2
50	云粳 14 号 Yunjing 14	-	-	-	+	-	-	+	-	-	-	-	-	-	2
51	云粳 16 号 Yunjing 16	-	-	-	+	-	-	-	-	-	-	-	+	-	2
52	云粳 17 号 Yunjing 17	-	-	-	+	-	-	+	-	-	-	-	-	-	2
53	云粳 18 号 Yunjing 18	-	-	-	+	-	-	+	-	-	-	-	+	-	3
54	云粳 19 号 Yunjing 19	2010	-	-	+	-	-	+	-	-	-	-	+	-	3
55	云粳 20 号 Yunjing 20	2011	-	-	+	-	-	+	-	-	-	-	-	-	2
56	云粳 21 号 Yunjing 21	-	-	-	+	-	-	+	-	-	-	-	-	-	2
57	云粳 24 号 Yunjing 24	2007	-	-	+	-	-	+	-	-	-	-	-	-	2
58	云粳 25 号 Yunjing 25	2007	-	-	+	-	-	+	-	-	-	-	-	-	2
59	云粳 26 号 Yunjing 26	2010	-	-	+	-	-	+	-	-	-	-	-	-	2
60	云粳 29 号 Yunjing 29	2011	-	-	+	-	-	+	-	-	-	-	-	-	2
61	云粳 30 号 Yunjing 30	2011	-	-	+	-	-	+	-	-	-	-	+	-	3
62	云粳 31 号 Yunjing 31	2011	-	-	+	-	-	+	-	-	-	-	-	-	2
63	云粳 32 号 Yunjing 32	2011	-	-	+	-	-	+	-	-	-	-	-	-	2
64	云粳 35 号 Yunjing 35	2014	+	-	+	-	-	+	-	-	-	-	-	-	3

65	云粳 37 号 Yunjing 37	-	-	-	+	-	-	+	-	-	-	-	-	-	2
66	云粳 38 号 Yunjing 38	2014	-	-	+	-	-	+	-	-	-	-	-	-	2
67	云粳 39 号 Yunjing 39	2014	+	-	+	-	-	+	-	-	-	-	-	-	3
68	云粳 42 号 Yunjing 42	2016	-	+	+	-	-	+	-	-	-	-	-	-	3
69	云粳 43 号 Yunjing 43	2016	-	-	+	-	-	+	-	-	-	-	-	-	2
70	云粳 46 号 Yunjing 46	2018	-	-	+	-	-	+	-	-	-	-	-	-	2
71	云粳 48 号 Yunjing 48	-	-	-	+	-	+	+	-	-	-	-	-	-	3
72	云粳 135 Yunjing 135	-	-	-	+	+	-	+	-	+	-	-	-	-	4
73	云粳 136 Yunjing 136	1983	-	-	-	-	-	+	-	-	-	-	+	-	2
74	云粳优 1 号 Yunjingyou1	2004	-	-	+	-	-	+	-	-	-	-	-	-	2
75	云粳优 5 号 Yunjingyou5	2005	-	-	+	-	-	+	-	-	-	-	-	-	2
76	合系 1 号 Hexi 1	-	-	-	-	-	-	-	-	-	-	-	+	-	1
77	合系 2 号 Hexi 2	1991	-	-	-	-	-	-	-	-	-	-	+	-	1
78	合系 3 号 Hexi 3	-	-	-	+	-	-	+	-	-	-	-	+	-	3
79	合系 4 号 Hexi 4	1990	-	-	+	-	-	+	-	-	-	-	+	-	3
80	合系 5 号 Hexi 5	1990	-	-	-	-	-	-	-	-	-	-	+	-	1
81	合系 6 号 Hexi 6	-	-	-	-	-	-	+	-	-	-	-	+	-	2
82	合系 8 号 Hexi 8	-	-	-	+	-	-	-	-	-	-	-	+	-	2
83	合系 9 号 Hexi 9	-	-	-	-	-	-	-	-	-	-	-	+	-	1
84	合系 10 号 Hexi 10	1990	-	-	+	-	-	+	-	-	-	-	+	-	3
85	合系 12 号 Hexi 12	-	-	-	+	-	-	+	-	-	-	-	+	-	3
86	合系 13 号 Hexi 13	-	-	-	+	-	-	+	-	-	-	-	-	-	2
87	合系 14 号 Hexi 14	-	-	-	-	-	-	-	-	-	-	-	+	-	1
88	合系 15 号 Hexi 15	1993	-	-	+	-	-	-	-	-	-	-	+	-	2
89	合系 16 号 Hexi 16	-	-	-	+	-	-	-	-	-	-	-	-	-	1
90	合系 17 号 Hexi 17	-	-	-	+	-	-	+	-	-	-	-	+	-	3
91	合系 20 号 Hexi 20	-	-	-	+	-	-	+	-	-	-	-	+	-	3
92	合系 22 号 Hexi 22	1991	-	-	-	-	-	+	-	-	-	-	+	-	2
93	合系 23 号 Hexi 23	-	-	-	-	-	-	-	-	-	-	-	+	-	1
94	合系 24 号 Hexi 24	1993	-	-	-	-	-	+	-	-	-	-	+	-	2
95	合系 25 号 Hexi 25	1993	-	-	+	-	-	+	-	-	-	-	-	-	2

96	合系 28 号 Hexi 28	-	-	-	-	-	-	+	-	-	-	-	+	-	2
97	合系 30 号 Hexi 30	1993	-	-	-	-	-	+	-	-	-	-	+	-	2
98	合系 32 号 Hexi 32	-	-	-	-	-	-	+	-	-	-	-	+	-	2
99	合系 34 号 Hexi 34	1997	-	-	+	-	-	+	-	-	-	-	-	-	2
100	合系 35 号 Hexi 35	1997	-	-	-	-	-	+	-	-	-	-	+	-	2
101	合系 38 号 Hexi 38	-	-	-	+	-	-	-	-	-	-	-	+	-	2
102	合系 40 号 Hexi 40	1999	-	-	+	-	-	-	-	-	-	-	+	-	2
103	合系 41 号 Hexi 41	1999	+	-	-	-	-	+	-	-	-	-	+	-	3
104	合系 42 号 Hexi 42	1999	-	-	-	-	-	+	-	-	-	-	-	-	1
105	凤稻 9 号 Fengdao 9	1997	+	-	-	-	-	+	-	-	-	-	+	-	3
106	凤稻 11 号 Fengdao 11	1999	-	+	-	-	-	+	-	-	-	-	+	-	3
107	凤稻 12 号 Fengdao 12	-	-	+	-	-	-	-	-	-	-	-	-	-	1
108	凤稻 14 号 Fengdao 14	2001	-	-	-	-	-	+	-	-	-	-	+	-	2
109	凤稻 15 号 Fengdao 15	2002	-	-	-	-	-	+	-	-	-	-	+	-	2
110	凤稻 16 号 Fengdao 16	2004	+	-	-	-	-	-	-	-	-	-	+	-	2
111	凤稻 17 号 Fengdao 17	2003	+	-	+	-	-	-	-	-	-	-	+	-	3
112	凤稻 18 号 Fengdao 18	2005	-	-	+	-	-	-	-	-	-	-	-	-	1
113	凤稻 19 号 Fengdao 19	2006	-	-	-	-	-	-	-	-	-	-	+	-	1
114	凤稻 20 号 Fengdao 20	2006	-	+	+	-	-	+	-	-	-	-	+	-	4
115	凤稻 21 号 Fengdao 21	2007	+	-	-	-	-	+	-	-	-	-	-	-	2
116	凤稻 23 号 Fengdao 23	2010	-	-	-	-	-	+	-	-	-	-	+	-	2
117	凤稻 29 号 Fengdao 29	2014	-	+	-	-	-	+	-	-	-	-	+	-	3
118	凤稻 30 号 Fengdao 30	2017	+	-	-	-	-	-	-	-	-	-	-	-	1
119	剑粳 3 号 Jianjing 3	2005	-	-	+	-	-	-	-	-	-	-	+	-	2
120	剑粳 6 号 Jianjing 6	2009	-	-	+	-	-	-	-	-	-	-	+	-	2
121	滇榆一号 Dianyu 1	1983	-	-	-	-	-	+	-	-	-	-	-	-	1
122	靖粳 8 号 Jingjing 8	2001	-	-	-	-	-	+	-	-	-	-	+	-	2
123	靖粳 11 号 Jingjing 11	2007	-	-	+	-	-	+	-	-	-	-	-	-	2
124	靖粳 12 号 Jingjing 12	2007	+	-	+	-	-	-	-	-	-	-	-	-	2
125	靖粳 13 号 Jingjing 13	2007	-	-	+	-	-	-	-	-	-	-	-	-	1
126	靖粳 14 号 Jingjing 14	2007	-	-	+	-	-	+	-	-	-	-	+	-	3

127	靖粳 16 号 Jingjing 16	2010	-	-	+	-	-	+	-	-	-	-	-	-	2
128	靖粳 17 号 Jingjing 17	2010	+	-	+	-	-	+	-	-	-	-	+	-	4
129	靖粳 18 号 Jingjing 18	2010	-	-	-	-	-	+	-	-	-	-	+	-	2
130	靖粳 26 号 Jingjing 26	2014	+	-	-	-	-	-	-	-	-	-	-	-	1
131	靖稻 1 号 Jingdao 1	2018	-	-	+	-	-	-	-	-	-	-	+	-	2
132	靖稻 5 号 Jingdao 5	2018	-	-	+	-	-	+	-	-	-	-	-	-	2
133	靖粳优 1 号 Jingjingyou 1	2003	-	-	+	-	-	+	-	-	-	-	+	-	3
134	靖粳优 2 号 Jingjingyou 2	2005	-	-	+	-	-	+	-	-	-	-	+	-	3
135	靖粳优 3 号 Jingjingyou 3	2005	-	-	+	-	-	+	-	-	-	-	-	-	2
136	丽粳 4 号 Lijing 4	-	-	-	-	+	-	+	-	-	-	-	-	-	2
137	丽粳 6 号 Lijing 6	2004	-	-	-	-	-	+	-	-	-	-	-	-	1
138	丽粳 9 号 Lijing 9	2012	-	-	+	-	-	-	-	-	-	-	-	-	1
139	丽粳 10 号 Lijing 10	2009	-	+	+	-	-	-	-	-	-	-	-	-	2
140	丽粳 11 号 Lijing 11	2010	-	-	+	-	-	-	-	-	-	-	-	-	1
141	丽粳 15 号 Lijing 15	2014	-	-	+	-	-	+	-	-	-	-	-	-	2
142	丽粳 18 号 Lijing 18	2018	+	-	-	-	-	+	-	-	-	-	-	-	2
143	丽粳 22 号 Lijing 22	2019	-	-	-	-	-	+	-	-	-	-	+	-	2
144	丽粳 23 号 Lijing 23	2019	-	-	+	-	-	+	-	-	-	-	+	-	3
145	丽粳 314 Lijing 314	2007	-	-	+	-	-	-	-	-	-	-	+	-	2
146	岫粳 12 号 Xiujing 12	2011	+	-	-	-	-	-	-	+	-	-	-	-	2
147	岫粳 18 号 Xiujing 18	2015	-	-	+	-	-	-	-	+	-	-	-	-	2
148	岫粳 22 号 Xiujing 22	2013	-	-	+	+	-	-	-	+	-	-	+	-	4
149	岫粳 26 号 Xiujing 26	2018	-	-	+	-	+	-	-	+	-	-	+	-	4
150	岫粳 28 号 Xiujing 28	2018	-	-	+	-	-	+	-	-	-	-	-	-	2
151	岫粳 29 号 Xiujing 29	2020	-	-	+	-	-	+	-	-	-	-	-	-	2
152	岫 87-15 Xiu 87-15	2003	-	-	-	-	+	+	-	-	-	-	+	-	3
153	岫 191-7 Xiu 191-7	2011	-	-	+	-	+	-	-	-	-	-	+	-	3
154	昌粳 8 号 Changjing 8	2004	-	-	-	-	-	-	-	-	-	-	+	-	1
155	昌粳 9 号 Changjing 9	2007	-	-	-	-	-	+	-	-	-	-	-	-	1
156	隆科 16 号 Longke 16	2015	-	-	+	-	-	+	-	-	-	-	+	-	3
157	玉粳 24 号 Yujing 24	2018	+	-	+	-	-	+	-	-	-	-	-	-	3

158	玉粳 25 号 Yujing 25	2019	-	-	+	+	-	-	-	+	-	-	+	-	4
159	塔粳 3 号 Tajing 3	2014	-	-	+	+	-	-	-	+	-	-	-	-	3
160	粘粳 7 号 Nianjing 7	1993	-	-	-	-	-	-	-	-	-	-	+	-	1
161	锦瑞 4 号 Jinrui 4	2019	-	-	-	+	-	+	-	+	-	-	-	-	3
162	晋宁 78-102 Jinning78-102	-	-	-	-	-	-	+	-	-	-	-	-	-	1
163	西南 175 Xinan 175	-	-	-	-	-	-	-	-	-	-	-	-	-	0

注: +: 检测到靶标基因; -: 未检测到靶标基因

Note: + : The target genes were detected; - : The target genes could not be detected

1.3 12 个抗性基因在云南粳稻品种/系中的构成情况

通过分析 12 个抗性基因的特异性分子标记对 163 份粳稻品种/系的检测结果发现，在云南粳稻育成品种中携带 *Pi54* 基因的频率最高，达 67.48%；*Pib* 和 *Pii* 的分布次之，频率分别为 58.89%和 42.33%；而 *Pita-2*、*Piz-t*、*Pid3*、*Pia* 和 *Pid4* 分布较少，频率分别为 12.26%、9.20%、7.97%、7.97%、2.45%；而 *Pi2*、*Pi9*、*Pi50/Pigm* 以及 *Pi5* 在本研究的粳稻品种/系中均未检测到(图 5)。

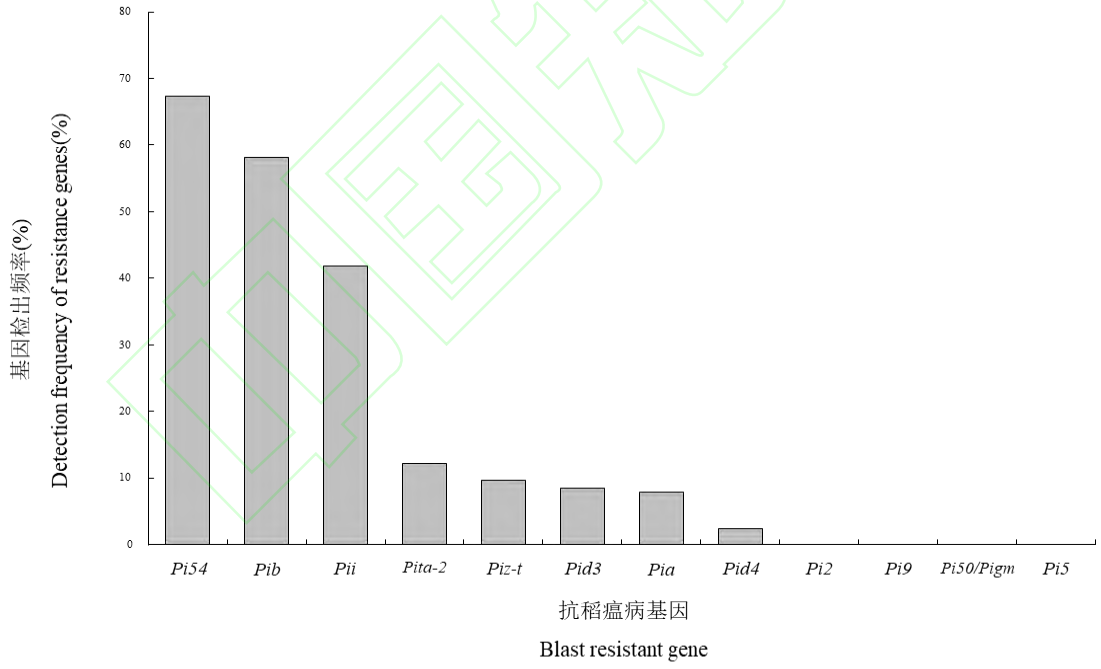


图 5 12 个稻瘟病抗性基因在云南育成粳稻品种中的检出频率

Figure 5 Detection frequency of 12 blast resistance genes in improved *japonica* rice cultivars of Yunan.

1.4 云南省粳稻品种系列携带抗性基因的类型

本研究检测的 163 个粳稻品种/系来自 7 个品种系列，在这 7 个品系中均检测到抗性基因 *Pita2*、*Pib*、*Pi54* 和 *Pii*；均未检测到抗性基因 *Pi2*、*Pi9*、*Pi50/Pigm* 和 *Pi5*；*Pid4* 仅在云粳和岫粳中检测到；*Piz-t* 只在楚粳、云粳和岫粳中检测到；*Pia* 在楚粳、云粳、凤稻和丽粳中检测到；*Pid3* 在合系、凤稻、靖粳中未检

测到。对于 7 个系列品种中存在较为广泛的抗性基因分别是：楚粳为 *Pi54*，岫粳为 *Pib*，云粳、靖粳、丽粳为 *Pi54* 和 *Pib*；合系和凤稻为 *Pii*，且检出频率均在 60%以上。

2 讨论

利用基因特异分子标记或功能标记开展品种的基因构成分析，是明确品种遗传背景最为有效的手段之一(Andersen et al, 2003; Hayashi et al., 2010; Zhai et al., 2011; 王芳权等, 2019; Salgotra and Neal, 2020)。本研究根据已克隆的 *Pi2*、*Pi9*、*Piz-t*、*Pi50/Pigm*、*Pita-2* 和 *Pi5* 等 6 个稻瘟病抗性基因的序列，开发出 6 个抗性基因特异性分子标记，通过品种的检测结果表明这些标记特异性高，扩增效果稳定，可用于稻种抗性基因的检测及分子标记辅助选择育种。在标记开发的基础上，进一步利用 12 个抗性基因的特异分子标记完成云南 20 世纪 80 年代以来育成的 163 个粳稻品种的分析，结果发现 *Pi54*、*Pib* 和 *Pii* 在这些品种中分布最广，持有 *Pita-2*、*Piz-t*、*Pid3*、*Pia* 或 *Pid4* 的品种则相对较少，而 *Pi2*、*Pi9*、*Pi50/Pigm* 和 *Pi5* 基因在这些品种中未检测到。还发现‘楚粳 9 号’、‘楚粳 22 号’和‘西南 175’这 3 个品种不含有本研究中的抗性基因，但前期的研究发现‘楚粳 22 号’在叶、穗瘟的抗性鉴定中均表现抗病(董丽英等, 2018)，表明云南粳稻品种中还含有其它抗性基因，而这些基因是已知或是未知的尚有待于进一步的分析。

宋兆强等(2017)利用 *Pi54* 基因的功能标记对江苏的 60 份资源材料进行基因型鉴定并结合连续两年稻瘟病菌接种鉴定，结果发现 *Pi54* 基因的抗病能力在不断减弱；宛柏杰等(2020)对 *Pi54* 基因的抗性评价研究中发现，含有单个抗性基因 *Pi54* 的水稻品种在江苏已经丧失稻瘟病抗性。有意思的是，前期利用 *Pi54* 基因的特异引物在持有 *Pik-h* 基因的稻瘟病抗性单基因系 KRBLKH-K3、持有 *Pi19* 基因的 IRBL18-A 和持有 *Pik-m* 基因的 IRBLKM-TS 中均鉴定到了 *Pi54* 基因的存在(结果未列出)，而云南粳稻区稻瘟病菌株对持有 *Pi19* 的单基因系 IRBL18-A 的毒性频率达到 90%以上(董丽英等, 2012)，这也反映出无论是 *Pi54* 或是 *Pi19* 均对云南的稻瘟病菌株丧失了抗性，从而表明 *Pi54* 基因在云南已失去利用价值。

早期的研究表明云南粳稻区稻瘟病菌群体对 *Pib* 基因的致病频率比较低(小于 1.8%) (李成云等, 1990)，表明 *Pib* 基因是云南粳稻抗稻瘟病新品种选育的优异基因源，且大量在云南粳稻育种中使用。该基因最早

可追溯到 20 世纪 80 年代育成的品系‘云粳 135’，以其为亲本育成‘合系 4 号’并通过审定后，不同育种单位引进亲本并育成了一系列持有 *Pib* 基因的粳稻品种，而随着持有 *Pib* 基因品种的不断推广，在抗性选择压力下，生产上对 *Pib* 致病的稻瘟病菌增长极快，对 *Pib* 单基因系的毒性频率达到了 67.7%(董丽英等, 2012)；*Pii* 基因在云南粳稻品种中检出率也很高，最早在 1983 年审定的品种‘云粳 136’中检测到，1990 年测定的菌株对其毒性频率为 30%(李成云等, 1990)，到 2012 年已提高至 76.02%(董丽英等, 2012)，表明其基本丧失了抗性；这些结果反映出抗性基因的过度使用造成抗性快速丧失的问题突出。

Pi5 和 *Pii* 基因是位于水稻第 9 染色体上的等位基因(杨勤忠等, 2009; Takagi et al., 2013; Lee et al., 2009)，但 2 个基因的抗谱差异明显，与 *Pii* 相比，*Pi5* 表现出抗谱更广的特点(董丽英等, 2012; 马军韬等, 2017)。本研究通过基因扩增分析发现，Takagi 等(2013)设计特异检测抗性基因 *Pii* 的 CAPS 标记不能将 *Pii* 和 *Pi5* 抗性基因分开，因为 2 个基因能扩增出相同分子量的片段，且持有相同的酶切位点 *Pvu* II。因此本研究根据这 2 个基因的序列差异，在基因内含子区域新开发的 InDel 标记扩增序列中 *Pi5* 基因的序列插入了 4 bp，从而可有效将 *Pii* 和 *Pi5* 基因分开，解决了利用 *Pii* 基因的 CAPS 标记不能区分 2 个基因的问题，此标记可直接用于品种中是否持有靶标基因的检测。

本研究根据基因序列设计的 6 个稻瘟病抗性基因特异性分子标记，可有效检测出品种中是否含有靶标基因，表明这些标记可用于水稻品种中抗性基因的检测分析和分子标记辅助选择抗病育种。利用 12 个稻瘟病抗性基因特异分子标记对云南省近 40 年培育的 163 个粳稻品种/系进行抗性基因分子鉴定，发现云南粳稻品种中有效抗源利用不足，粳稻新品种的选育亟待引入新的抗源，以拓宽云南粳稻新品种的抗性遗传基础。

3 材料与方法

3.1 实验材料

本研究的材料为 1980-2020 年间云南省培育的 163 个常规粳稻品种/系；阳性对照为地谷、谷梅 4 号、特特普和以丽江新团黑谷(LTH)为背景的单基因系 IRBLA-A (*Pia*)、IRBLI-F5 (*Pii*)、IRBLZ5-CA (*Pi2*)、

IRBLZ5-CA® (*Pi2*)、IRBLZT-T (*Piz-t*)、IRBLTA-K1 (*Pita*)、IRBLB-B (*Pib*)、IRBL5-M (*Pi5*)、IRBL9-W (*Pi9*)、IRBLTA2-PI (*Pita-2*)；水稻品种 Arang、Lainokkha、Siboras 和 Terungdaun 用于检测 *Pi50/Pigm* 的同源基因序列。

3.2 引物设计

根据已克隆基因 *Pi2* (基因登录号 DQ352453)、*Pi9* (基因登录号 DQ285630)、*Piz-t* (基因登录号 DQ352040)、*Pi50/Pigm* (基因登录号 KP985761/KU904633)、*Pita-2* (基因登录号 MG385186)和 *Pi5* (基因登录号 EU869185 和 EU869186)的基因序列进行引物设计。对于等位基因 *Pi2*、*Pi9*、*Piz-t* 和 *Pi50*/*Pigm* 的特异引物通过直系同源及旁系同源基因序列比较，寻找基因特异序列位置并进行引物设计；而其中 *Pi50*/*Pigm* 基因的扩增产物，需要进一步进行限制性内切酶 *Ava* II 或 *Sty* I 的酶切，可将其他旁系同源基因扩增片段区分开；*Pita-2* 和 *Pi5* 基因的特异引物则根据抗病与感病品种等位基因间出现插入/缺失(InDel)的两端设计引物进行多态性扩增检测，确定特异引物。

3.3 基因组 DNA 提取

采用 CTAB 法从水稻幼嫩叶片提取基因组 DNA(Warude et al., 2003)，用 1%琼脂糖凝胶电泳检测基因组 DNA 的质量，用 Nanodrop-1000 测定基因组 DNA 的浓度，用 0.1×TE 溶液配制浓度为 20 ng/μL 的 DNA 工作液，并置于-20℃冰箱保存备用。

3.4 PCR 扩增及电泳

本研究所用稻瘟病抗性基因特异性分子标记引物由北京擎科生物科技有限公司昆明分公司合成(表 2)。PCR 反应体系为 20.0 μL，包含 DNA 模板 1.0 μL、10 μmol/L 正反向引物各 1.0 μL、2×Es *Taq* Master Mix(康为世纪) 10.0 μL、ddH_2O 7.0 μL。PCR 反应条件：95℃预变性 3 min；95℃变性 30 s，55℃-63℃退火 30 s (表 2)，72℃延伸 1 min，35 个循环；最终 72℃延伸 5 min。扩增之后，抗性基因 *Pita-2*、*Pi5* 的 PCR 产物采用 8%聚丙烯酰胺凝胶电泳检测；其余 10 个抗性基因的 PCR 产物采用 3%琼脂糖凝胶电泳检测。

表 2 本研究中的引物信息

Table 2 Summary of PCR markers used in this study

目的基因 Target gene	引物名称 Primer name	引物序列 (5'-3') Primer sequence (5'-3')	片段大小 (bp) Fragment size (bp)	限制性内切酶 Restriction enzyme	退火温度 (℃) Annealing temperature	参考文献 Reference
Pib	Pibdom-F Pibdom-R	GAACAATGCCCAAACTTGAGA GGGTCCACATGTCAGTGAGC	365[a]/-[b]		58	Fjellstrom et al., 2004
Pid3	Pid3-dCAPS-1F Pid3-dCAPS-1R	TACTACTCATGGAAGCTAGTTCTC GCAGCACTTCTTGACTACTGTCTGT	178/155	*Xba* Ⅰ	58	Shang et al., 2009
	Pid3-dCAPS-2F Pid3-dCAPS-2R	TACTACTCATGGAAGCTAGTTCTC AGCACTTCTTGACTACTGTCTGCCT	155/178	*Bam*HⅠ	*58*	
Pia	PIA-F PIA-R	GCGACTGACACTTTCAATAGC CGGTAGAGCAATTTAGAAGCAG	189/210		58	Okuyama et al., 2011
Pi54	Pi54-F Pi54-R	CAATCTCCAAAGTTTTCAGG GCTTCAATCACTGCTAGACC	216/359		55	Ramkumar et al., 2011
Pii	Pii-CAPS-F Pii-CAPS-R	TCCAATGCTTCTGAAAGGTAGC TGGAAACATGAACCCATATCCT	113+240/353	*Pvu*Ⅱ	58	Takagi et al., 2013
Pid4	Pid4-GAP-F Pid4-GAP-R	CCGCATCTCCTCCTTCCTCT AGAGATCTCGCTGTTCAGTC	279/352		58	陈智雄, 2018 Chen, 2018
Pita-2	Pita2-InDel-Fw Pita2-InDel-Rv2	TGACCTTCGCAGACCTCATC ATTTCGCAGGCATAAACTCG	117/129		58	本研究 This research
Pi2	Pi2SP1-Fw Pi2SP1-Rv	GCTGCAGGAATCTCAGATGG CAGATTCCAACCTGCACTCG	394/-		63	本研究 This research
Piz-t	PiztSP-Fw PiztSP-Rv	GCTGTGTTATTCTCAGGTATTATT GTAGATCTTCTTCAGGTGCA	150/-		61.4	本研究 This research
Pi9	Pi9SP-Fw Pi9SP-Rv	TCTTCGTTGTAGTAGAAAGTTTGT ATTCCAACCTGCAGCAAGAG	788/-		62	本研究 This research
Pi50/Pigm	Pi50/gmSP-FW	CTGAAGAAGTTCTACTTAC	335/81+254，286+49、108+227、108+178+49	*Ava*Ⅱ, *Sty* Ⅰ	50	本研究 This research
	Pi50/gmSP-Rv	AGCCCAGCCACTTTACTTCC				
Pi5	Pi5-Fw Pi5-Rv	AGAGATAAACCTAATACTTACTT CAATTTTTGTCTATGTAAAGGCA	103/-		55	本研究 This research

注: a: 靶标抗病基因片段长度; b: 感病品种中的片段长度; -: 不能从感病品种中扩增出片段.

Note: a: Expected DNA fragment amplified from cultivars carrying target resistant genes; b: Expected DNA fragment amplified from cultivars without target resistant genes; -: No DNA fragment was amplified from susceptible cultivars.

作者贡献

周伍民是本研究的实验设计者和实验研究的执行人，完成数据分析、论文初稿的写作；刘沛参与数据分析与论文修改；董丽英、刘树芳、张先闻、李迅东和黄立钰参与实验设计和实验结果分析等；杨勤忠是项目的构思者及负责人，指导实验设计、数据分析、论文写作与修改。全体作者都阅读并同意最终的文本。

致谢

本研究由云南省基础研究计划(202001AS070006)、国家自然科学基金(31860524)和云南省科技人才与平台计划(202105AG070001)共同资助。

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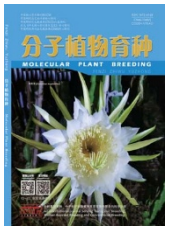
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分子植物育种
Molecular Plant Breeding
ISSN 1672-416X,CN 46-1068/S

《分子植物育种》网络首发论文

题目：蒜芥茄黄萎病抗性相关基因 SsWRKY22 和 SsWRKY33 的克隆与表达分析

作者：尹梦莹，蔚亚楠，龚亚菊，桂敏，鲍锐，黎志彬，吴丽艳，杜光辉

网络首发日期：2021-09-18

引用格式：尹梦莹，蔚亚楠，龚亚菊，桂敏，鲍锐，黎志彬，吴丽艳，杜光辉．蒜芥茄黄萎病抗性相关基因 SsWRKY22 和 SsWRKY33 的克隆与表达分析[J/OL]．分子植物育种．https://kns.cnki.net/kcms/detail/46.1068.s.20210917.1506.012.html



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网络首发时间：2021-09-18 16:34:08
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分子植物育种,
Molecular Plant Breeding,

研究报告

Research Report

蒜芥茄黄萎病抗性相关基因 *SsWRKY22* 和 *SsWRKY33* 的克隆与表达分析

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摘　要　黄萎病是茄子生产中的重要病害之一，严重影响茄子的产量和品质。WRKY 转录因子在植物抗病中扮演着重要的角色，但在茄子黄萎病抗性中的作用不明。本研究从黄萎病高抗材料野生蒜芥茄的转录组数据中，筛选获得 WRKY 家族中的 *SsWRKY22* 和 *SsWRKY33* 基因，利用 PCR 技术克隆获得目的基因，并对其进行生物信息学和表达模式分析。结果表明，*SsWRKY22* 和 *SsWRKY33* 基因的 ORF 序列大小分别为 528 bp 和 1 073 bp，编码 175 个和 357 个氨基酸；蛋白分子量分别为 19.19 kD 和 40.46 kD，均属于亲水性不稳定蛋白，其中，*SsWRKY22* 编码的蛋白属于酸性蛋白，而 *SsWRKY33* 编码的蛋白属于碱性蛋白，亚细胞定位预测两个基因编码的蛋白均定位于细胞核上；此外，SsWRKY22 和 SsWRKY33 分别属于 II 类和 I 类 WRKY 转录因子，具有不同的蛋白结构。系统发育分析结果表明，SsWRKY22 与野生潘那利番茄、番茄和马铃薯中的 WRKY22 亲缘关系较近；而 SsWRKY33 与辣椒中的 WRKY33 亲缘关系最近。利用 qRT-PCR 对 *SsWRKY22* 和 *SsWRKY33* 基因在蒜芥茄不同部位(根，茎，叶)的表达模式进行分析，发现二者的表达具有组织特异性，且在根部的表达量均为最高。在接种黄萎病病菌后，*SsWRKY22* 和 *SsWRKY33* 基因在蒜芥茄根部的表达量显著下调，表明 *SsWRKY22* 和 *SsWRKY33* 可能是蒜芥茄响应黄萎病胁迫的负调控因子。本研究首次克隆了云南野生蒜芥茄的 WRKY 转录因子中的 *SsWRKY22* 和 *SsWRKY33* 基因并解析其表达模式，为后续 *SsWRKY22* 和 *SsWRKY33* 的功能鉴定提供基础。

关键词　蒜芥茄；黄萎病；*SsWRKY22*; *SsWRKY33*；表达模式

基金项目：本研究由国家自然科学基金地区基金项目(31960594)、云南省农业基础研究联合专项面上项目(2018FG001-022)和云南省应用基础研究面上项目(2019FB059)共同资助。

Cloning and Expression Analysis of *Solanum sisymbriifolium* Verticillium Wilt Resistance-Related Genes *SsWRKY22* and *SsWRKY33*

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Abstract Verticillium wilt is a major disease that seriously affects eggplant yield and quality. WRKY transcription factors play a key role in plant resistance, while the knowledge about the resistance to eggplant verticillium wilt are deficient. In this study, two WRKY genes, named *SsWRKY22* and *SsWRKY33*, were screened from the transcriptome database of *Solanum sisymbriifolium* which is highly resistant to verticillium wilt. The target genes were cloned for bioinformatics analysis. Then the expression pattern of two genes were analyzed by qRT-PCR. The results showed that the full length ORF of *SsWRKY22* and *SsWRKY33* are 528 bp and 1 073 bp, encoding 175 and 357 amino acid, respectively. The molecular weights of two proteins are 19.19 kD and 40.46 kD, respectively, which belong to hydrophilic unstable proteins. The SsWRKY22 protein is acidic protein, while the SsWRKY33 protein is basic protein. Subcellular localization predicted that both proteins are located in the nucleus. In addition, SsWRKY22 and SsWRKY33 with different protein structure belong to class Ⅱ and class Ⅰ WRKY transcription factors, respectively. Phylogenetic analysis showed that *SsWRKY22* is closely related to *WRKY22* in *Solanum pennellii*, *S. lycopersicum* and *S. tuberosum*, while the *SsWRKY33* is the most closely related to *WRKY33* in *Capsicum annuum*. The qRT-PCR analysis indicated that the expression levels of *SsWRKY22* and *SsWRKY33* are tissue-specific and the highest in roots. After inoculation by *Verticilliumdahliae*, the expression levels of *SsWRKY22* and *SsWRKY33* were significantly down-regulated in the root, suggesting that both genes may be negative regulatory factors in response to verticillium wilt stress. In this study, WRKY transcription factor genes were firstly cloned and their expression patterns were also analyzed in *S.sisymbriifolium*, which will lay a foundation for the follow-up functional characterization of *SsWRKY22* and *SsWRKY33*.

Key words *Solanum sisymbriifolium*; Verticillium wilt; *SsWRKY22*; *SsWRKY33*; Expression pattern

茄子(*Solanum melongena* L.)是一种经济实惠的大众蔬菜，深受国人喜爱，在中国蔬菜生产中占据着十分重要的地位。随着种植面积的不断扩大，茄子面临的病害问题也愈发严重，由轮枝菌属(*Verticillium*)病原真菌引起的黄萎病(Greensickness)是当前茄子生产中面临的严重威胁之一。培育抗病品种是防治茄子黄萎病的有效办法，挖掘茄子黄萎病抗性基因可以为抗病品种的选育奠定理论基础。

WRKY 家族是植物特有的转录因子家族之一，其功能复杂多样，且调控范围广泛，涵盖植物生长发育和逆境响应等多种生理活动(于永昂等, 2020)。WRKY 转录因子可以通过调节植物防御相关基因的表达水平来影响植物对生物胁迫的抗性(Jiang et al., 2017)。研究发现，*CaWRKY40* 在辣椒青枯病菌抗性应答中起正向调控(Dang et al., 2013)；*NtWRKY40* 过表达使植株对烟草花叶病毒的侵染更加敏感，是抗病负调控因子(刘晶晶等, 2016)；*SpWRKY6* 可以在番茄抗晚疫病菌侵染过程起到正向调节作用(Hong et al., 2018)；茄子 *SmWRKY65* 沉默后植株抗青枯病的能力下降(刘开等, 2021)。与黄萎病抗性相关的 WRKY 转录因子的报道主要集中在棉花上，如 *GbWRKY1* (徐理, 2011)、*GhWRKY22* (雷煜等, 2018)、*GhWRKY40* (张坤, 2015)、*GhWRKY41* (管倩倩, 2019)、*GhWRKY46* (戴鹤, 2015)和 *GhWRKY70* (熊显鹏, 2020)等均与棉花黄萎病抗性相关。而在茄子上，目前仅李笑(2018)克隆获得水茄(*Solanumtorvum*)的 *StWRKY1* 基因，认为其对茄子黄萎病具有抗性；周晓慧等(2016)曾报道过喀西茄(*Solanumaculeatissimum*)中 4 个受黄萎病菌诱导表达的 *WRKY* 基因。虽然，目前茄子中已有黄萎病抗性相关的 WRKY 转录因子的报道，但是与其他植物相比，其黄萎病抗性机理研究较少且并不全面。

野生茄子及其近缘野生种经过长期的自然进化，形成了抗逆、抗虫、抗病等诸多栽培种不具备的优良性状。本实验室前期研究发现云南野生蒜芥茄(*Solanumsisymbriifolium*Lam.)是一个黄萎病高抗材料(吴丽艳等, 2017)。同时，利用转录组测序技术，发现蒜芥茄中多个 WRKY 转录因子在接种黄萎病病菌后发生差异表达(Wu et al., 2019)，推测 WRKY 转录因子在野茄黄萎病抗性中发挥重要作用。本研究筛选了 2 个蒜芥茄接种黄萎病病菌后发生显著差异表达的 WRKY 基因进行克隆、生物信息学分析和表达分析，为深入研究 *SsWRKY22* 和 *SsWRKY33* 基因在蒜芥茄黄萎病抗性中的调控机理提供参考，也为合理利用抗性基因改良茄子种质，实现茄子黄萎病的绿色防控提供理论基础。

1 结果与分析

1.1 *SsWRKY22* 和 *SsWRKY33* 基因的克隆及 ORF 序列分析

经 RT-PCR 扩增，分别获得了 1 237 bp 和 1 169 bp 长度的序列，与预测的 *SsWRKY22* 和 *SsWRKY33* 基因的长度相符(图 1)。DNAMAN 软件比对发现，PCR 产物与转录组测序得到的目的基因的 ORF 序列一致，可用于下一步的研究与分析。ORF finder 序列分析表明，*SsWRKY22* 的完整最大 ORF 序列大小有 528 bp，编码 175 个氨基酸；*SsWRKY33* 的完整最大 ORF 序列大小有 1 073 bp，编码 357 个氨基酸。

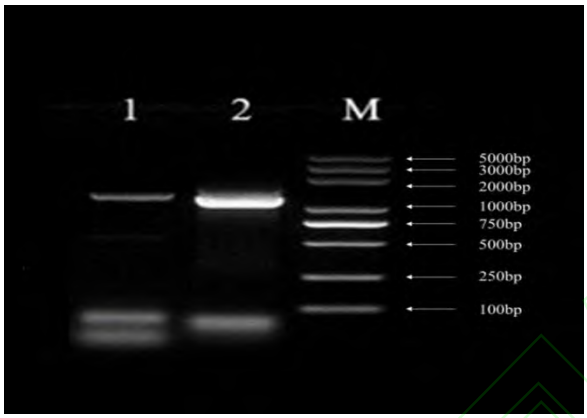


图 1 *SsWRKY22* 和 *SsWRKY33* 电泳检测
注: 1: *SsWRKY22*; 2: *SsWRKY33*; M: DL5000 DNA Marker
Figure 1 The electrophoresis *SsWRKY22* and *SsWRKY33*
Note: 1: *SsWRKY22*; 2: *SsWRKY33*; M: DL5000 DNA Marker

1.2 *SsWRKY22* 和 *SsWRKY33* 的生物信息学分析

Protparam 网站分析结果表明，*SsWRKY22* 编码蛋白的分子式为 $C_{814}H_{1249}N_{237}O_{286}S_8$，理论分子量为 19.19 kD；带正电荷残基总数(Arg+Lys) 19 个，带负电荷残基总数(Asp+Glu) 27 个，理论等电点为 4.89，推测该蛋白为酸性蛋白；不稳定系数(II)为 45.43，大于 40，为不稳定蛋白；该蛋白的亲水性平均值(GRAVY)为-1.033，表明该蛋白亲水性氨基酸的数量高于疏水性氨基酸数量，为亲水性蛋白。*SsWRKY33* 编码蛋白的分子式为 $C_{1708}H_{2668}N_{536}O_{585}S_{12}$，理论分子量为 40.46 kD；带正电荷残基总数(Arg+Lys) 48 个，带负电荷残基总数(Asp+Glu) 44 个，等电点为 8.57；不稳定系数(II)为 58.74；平均亲水性(GRAVY)为-1.311。以上结果表明 *SsWRKY33* 编码的蛋白可能是一个亲水性不稳定碱性蛋白。

应用在线软件 Cell-PLoc 2.0 (http://www.csbio.sjtu.edu.cn/bioinf/Cell-PLoc-2/)对 2 个基因进行亚细胞定位预测，结果表明 *SsWRKY22* 和 *SsWRKY33* 亚细胞定位预测均在细胞核上。

软件 SOPMA 的预测结果显示(图 2)，*SsWRKY22* 编码蛋白的二级结构由 4 种形式组成。其中，α 螺旋占 20.57%，无规则卷曲占 65.14%，延伸链占 8%，β 转角占 6.29%。而在 *SsWRKY33* 编码蛋白的二级结构中，α 螺旋，无规则卷曲，延伸链和 β 转角的占比分别为 9.24%、76.75%、10.92%和 3.08%。SsWRKY22 和 SsWRKY33 中的主要结构均为无规则卷曲。

通过 SWISS-MODEL 网站分别预测 2 个 WRKY 基因编码蛋白的三级结构(图 3)，*SsWRKY22* 和 *SsWRKY33* 编码的蛋白均以 2ayd.1.A 蛋白为模体，相似性分别为 59.65%和 66.67%。

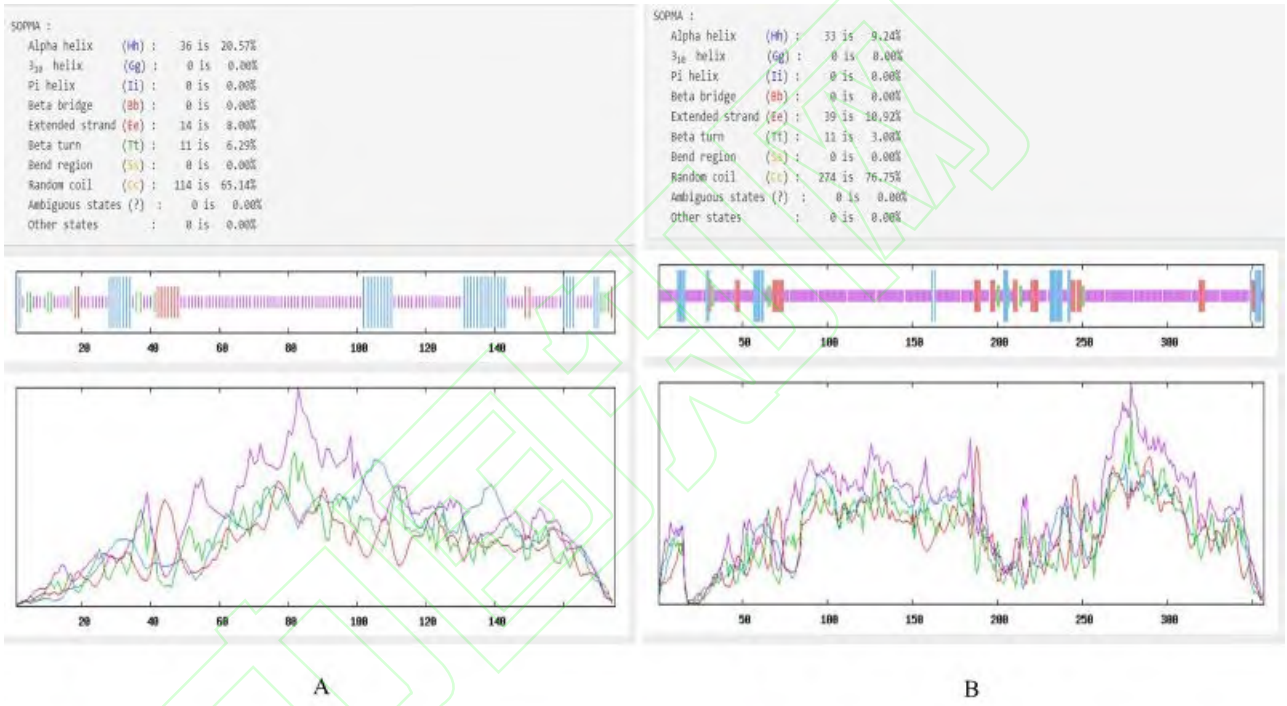


图 2 SsWRKY22 (A)和 SsWRKY33 (B)蛋白的二级结构预测
注：蓝色: α-螺旋；红色：延伸链；绿色: β-转角；紫色：无规则卷曲
Figure 2 Secondary structure prediction of SsWRKY22(A) and SsWRKY33(B) proteins
Note: Blue: α –helix; Red: Extended strand; Green: β-turn; Purple : Random coil

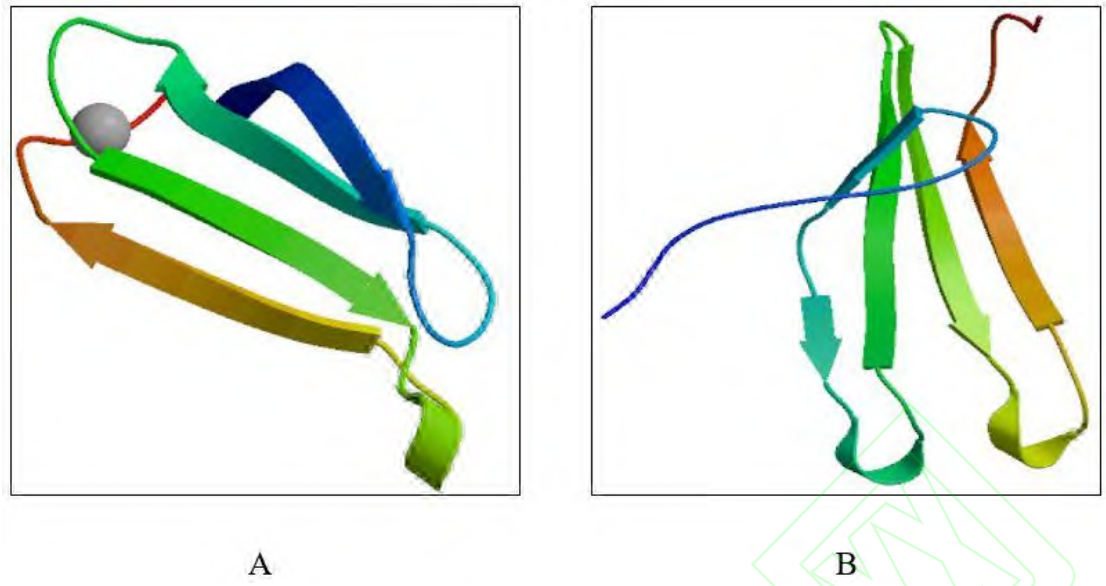


图 3 SsWRKY22 (A)和 SsWRKY33 (B)蛋白的三级结构预测
Figure 3 Prediction of tertiary structures of SsWRKY22 (A) and SsWRKY33 (B) proteins

利用 DNAMAN 分别对蒜芥茄 *SsWRKY22* 和 *SsWRKY33* 以及相似性较高的其他植物的 WRKY 基因编码的蛋白质进行多序列比对分析，发现 SsWRKY22 与野生潘那利番茄等植物的 WRKY 转录因子在编码区前部存在明显的保守结构域，均存在 WRKYGQK 保守基序，还有一个典型的 C_2H_2 型锌指结构($CX_5CX_{23}HX_1H$)，符合Ⅱ类 WRKY 转录因子特征(图 4)。而 SsWRKY33 在编码区的前后两端各存在一个 WRKYGQK 保守基序，且存在典型的 C_2H_2 型锌指结构($CX_{4\text{-}5}CX_{22\text{-}23}HX_1H$)，符合Ⅰ类 WRKY 转录因子特征(图 5)。

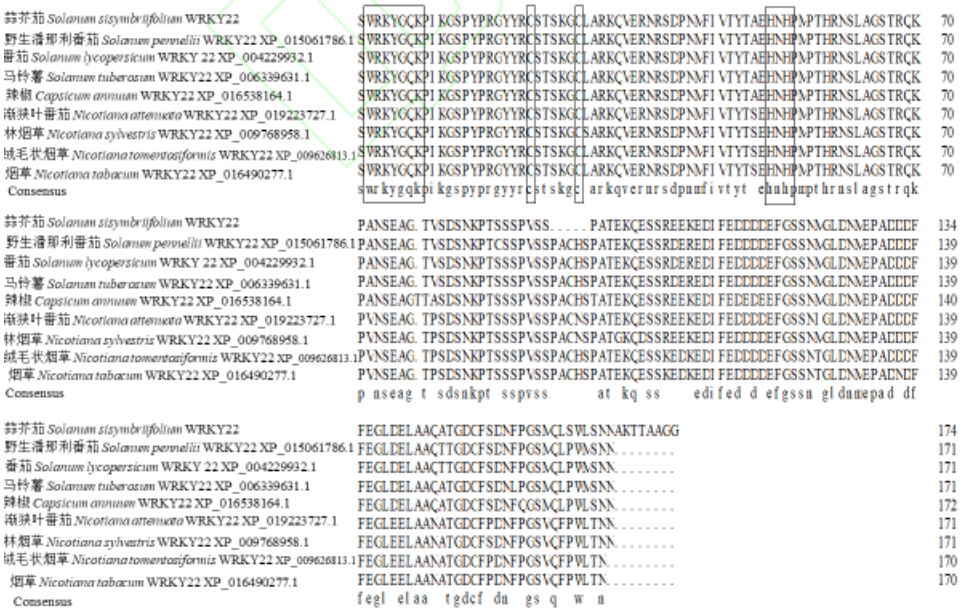


图 4 SsWRKY22 与其他植物的 WRKY22 蛋白质的多序列比对分析
注: 方框: WRKY 保守基序及 C_2H_2 锌指结构

Figure 4 Multiple alignment result of SsWRKY22 and other plant WRKY22 proteins
Note: Box: WRKY conserved sequence and C_2H_2 zinc finger structure

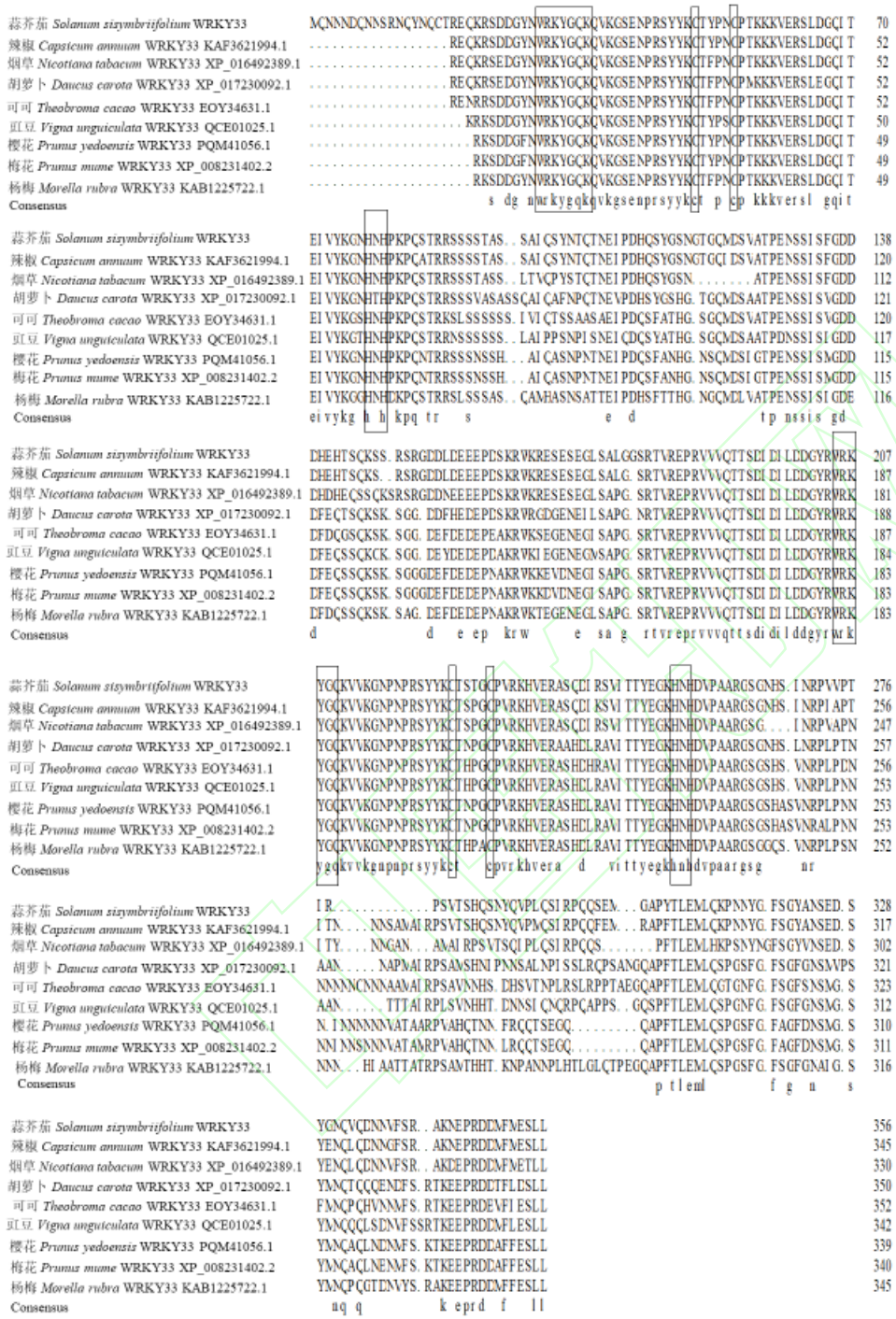


图 5 SsWRKY33 与其他植物的 WRKY33 蛋白质的多序列比对分析
注: 方框: WRKY 保守基序及 C_2H_2 锌指结构
Figure 5 Multiple alignment result of SsWRKY33 and other plant WRKY33 proteins
Note: Box: WRKY conserved sequence and C_2H_2 zinc finger structure

将 *SsWRKY22* 和 *SsWRKY33* 编码的蛋白质序列与 NCBI 在线 BLAST 结果中相似性较高的其他植物的 WRKY 蛋白，利用 MEGA X 软件构建系统进化树(图 6)。进化树分为两大枝，SsWRKY22 和 SsWRKY33

因属于不同的亚组而在不同分支上。*SsWRKY22* 与野生潘那利番茄(*Solanum pennellii*)、番茄(*Solanum lycopersicum*)和马铃薯(*Solanum tuberosum*)中的 *WRKY22* 亲缘关系较近；*SsWRKY33* 与辣椒(*Capsicum annuum*)中的 *WRKY33* 亲缘关系最近。

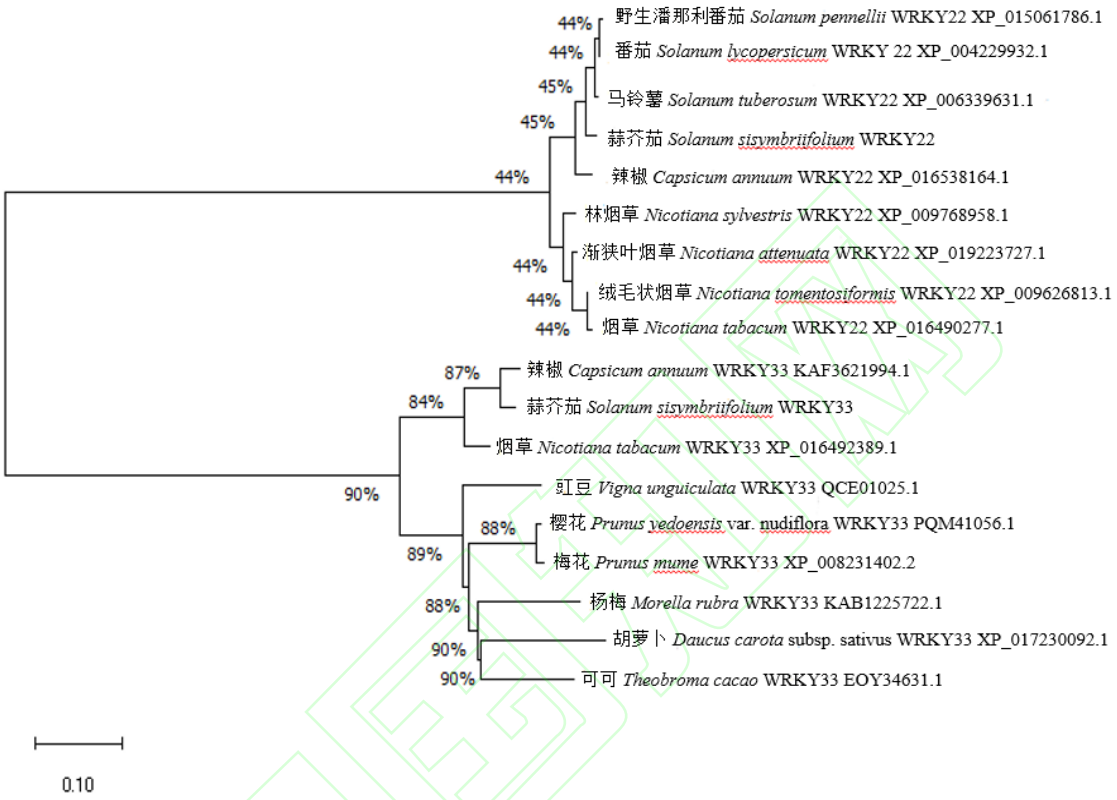


图 6 *SsWRKY22* 和 *SsWRKY33* 与其它物种 WRKY 基因同源序列的系统进化树分析
Figure 6 Phylogenetic tree analysis of homologous sequence of *WRKY* genes in different plant species

1.3 *SsWRKY22* 和 *SsWRKY33* 基因的表达分析

1.3.1 *SsWRKY22* 和 *SsWRKY33* 基因在蒜芥茄不同组织中的表达分析

利用 qRT-PCR 技术分析 *SsWRKY22* 和 *SsWRKY33* 基因在蒜芥茄根、茎、叶不同组织的表达水平。结果显示(图 7)，*SsWRKY22* 和 *SsWRKY33* 在根、茎和叶中都有表达，在根中的表达量最高，具有组织表达差异性。

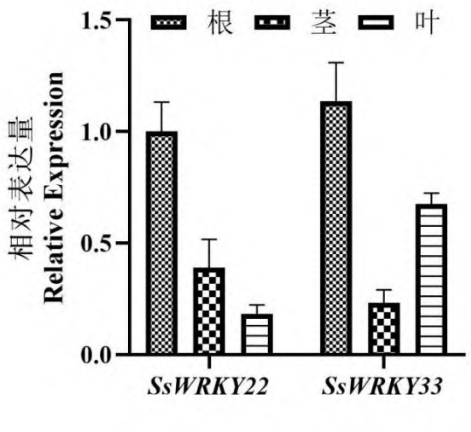


图 7 *SsWRKY22* 和 *SsWRKY33* 在蒜芥茄不同组织中的表达
Figure 7 Expression level of *SsWRKY22* and *SsWRKY33* genes in different tissues of *S.sisymbriifolium*

1.3.2 *SsWRKY22* 和 *SsWRKY33* 基因在蒜芥茄接种病原菌后的表达分析

利用 qRT-PCR 对黄萎病病菌接种 0、24、48 和 72 h 后蒜芥茄根部 *SsWRKY22* 和 *SsWRKY33* 基因的表达水平的变化进行分析。结果表明：黄萎病病菌处理后，*SsWRKY22* 基因(图 8A)的相对表达量在不同时间点均低于对照组，且目的基因的相对表达量显著下调；相较于 0 和 24 h，*SsWRKY33* 基因(图 8B)的相对表达量在接种黄萎病菌 48 h 后迅速减少。

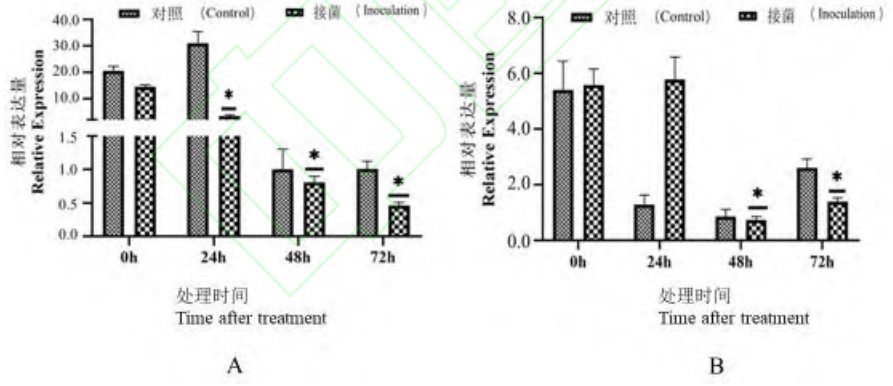


图 8 黄萎病菌接种后 *SsWRKY22* (A)和 *SsWRKY33* (B)的表达模式分析
注: *: 差异显著(P<0.05)
Figure 8 The expression level of *SsWRKY22* (A) and *SsWRKY33* (B) in *S. sisymbriifolium*after inoculation of *V. dahliae*
Note: *: Significant difference (P<0.05)

2 讨论

近年来，国内外学者已经对茄子种质资源中筛选到部分材料开展了黄萎病抗性研究，但主要集中在黄萎病高抗材料托鲁巴姆(*S. torvum* Swartz)上，且多应用于嫁接栽培，有关黄萎病抗性机制的研究甚少。本

研究选择的蒜芥茄同样是茄子黄萎病高抗材料(庄勇和王述彬, 2009; 吴丽艳等, 2017)，但目前其黄萎病抗性机制还不清楚。本研究从蒜芥茄中发掘黄萎病抗性相关基因并进行生物信息学分析和表达分析，不仅能为下一步目的基因的功能鉴定奠定基础，也可以为下一步培育抗病品种，解决茄子黄萎病危害奠定材料基础。

WRKY 转录因子在植物抗黄萎病过程中所发挥的作用一直备受关注，但是茄子中 WRKY 基因和其编码蛋白的功能研究相对较少。本研究根据转录组序列信息设计特异性 PCR 引物，从蒜芥茄中克隆得到 *SsWRKY22* 和 *SsWRKY33* 基因并对其进行生物信息学和表达特征分析。生物信息学分析发现，*SsWRKY22* 编码的蛋白可能是一个亲水性不稳定酸性蛋白。蛋白亚细胞定位预测表明 *SsWRKY22* 编码的蛋白处于细胞核上，这与甜橙 *CsWRKY22* (Long et al., 2021)和辣椒 *CaWRKY22* (Ansar, 2018)亚细胞定位的结果一致。同时，*SsWRKY22* 基因编码的蛋白包含一个保守的 WRKY 结构域，其锌指结构为 C_2H_2 类型，属于第Ⅱ类 WRKY 转录因子。这与已报道的棉花 *GhWRKY22* (雷煜等, 2018)和辣椒 *CaWRKY22*(Ansar, 2018)的分类结果相同。同一类型的 WRKY 转录因子具有相似的逆境诱导表达模式(Dong et al., 2003)，推测 *SsWRKY22* 与 *GhWRKY22* 和 *CaWRKY22* 可能具有相似的生物学功能。同源基因进化分析结果显示，*SsWRKY22* 与野生潘那利番茄、番茄和马铃薯中的 *WRKY22* 亲缘关系较近。本研究中，蒜芥茄 *SsWRKY33* 编码蛋白是一个亲水性不稳定蛋白，且属于第Ⅰ类WRKY转录因子，这与殷丽华等(2020)在小豆中 *VaWRKY33* 的分析结果一致，该特征提示在后续研究其功能时应选择适宜亲水蛋白纯化的条件。同时，*SsWRKY33* 编码蛋白的亚细胞定位预测结果在细胞核上，说明它可能在细胞核中起作用，符合转录因子的特性。同源进化分析表明，*SsWRKY33* 与辣椒中的 *WRKY33* 亲缘关系最近。

WRKY 转录因子家族参与植物抗病、非生物胁迫应答、衰老、种子发育以及其他多种植物发育和激素调控等生理过程(Xu et al., 2020)。目前的研究均表明，WRKY 基因并不是一个组成性表达的基因，而是受各种生物和非生物胁迫因子的诱导表达(Yu et al., 2012; 周汉琛等, 2014; 刘晶晶等, 2016)。其中，*WRKY22* 是植物先天免疫系统 PTI(病原相关分子模式激发的免疫反应)的标志基因，在植物应答生物胁迫中起着关键作用。研究发现，*WRKY22* 可以提高水稻稻瘟病的抗病性(Abbruscato et al., 2012)、棉花黄萎病的抗病性(雷煜等, 2018)、辣椒青枯病的抗病性(Ansar H, 2018)以及提高烟草防御丁香假单胞菌(Ramos et al., 2020)的能力。而在甜橙中，*CsWRKY22* 却可以提高其对溃疡病感病性(Long et al., 2021)。*WRKY33* 基因在棉花黄萎病

(刘建芬, 2019)、大白菜根肿病(韩雪雨等, 2019, 中国园艺学会 2019 年学术年会暨成立 90 周年纪念大会论文摘要集, pp.1)和小豆锈病(殷丽华等, 2020)抗性中发挥正调控的效应。在本研究基因表达分析中发现，与不接种对照相比，接种黄萎病病菌后的蒜芥茄根部的 *SsWRKY22* 和 *SsWRKY33* 基因表达均发生变化，但趋势不同，说明 *SsWRKY22* 和 *SsWRKY33* 基因对黄萎病菌诱导有明显响应，推测蒜芥茄抗黄萎病反应机制中 *SsWRKY22* 和 *SsWRKY33* 基因均有所参与。根部是植株与黄萎病病菌直接接触的部位，受病菌影响最大。蒜芥茄根部的 *SsWRKY22* 和 *SsWRKY33* 基因在接种后均显著下调表达，暗示着这两个基因在蒜芥茄响应黄萎病侵染过程中可能起负调控作用。本研究中 *SsWRKY22* 和 *SsWRKY33* 基因具有组织表达特异性，且表达量最高的部位均为根部，而棉花 *GhWRKY22* 基因在茎部的表达量最高(雷煜等, 2018)，*GhWRKY33* 基因在根部的表达量最高(刘建芬, 2019)，说明不同植物中的 WRKY 基因的组织特异性存在差异。

本研究对蒜芥茄黄萎病抗性相关基因 *SsWRKY22* 和 *SsWRKY33* 的克隆与表达进行了初步分析，但在黄萎病病菌侵染过程中，由 *WRKY22* 和 *WRKY33* 转录因子所介导的蒜芥茄的完整应答过程尚不明确，其上、下游结合因子仍未明确；*WRKY22* 和 *WRKY33* 转录因子在抗黄萎病信号转导途径及其植物激素信号途径中的作用也有待于进一步的研究。

3 材料与方法

3.1 *SsWRKY22* 和 *SsWRKY33* 基因克隆

野生蒜芥茄和黄萎病病原菌大丽轮枝菌菌株(*Verticillium dahlia*Kleb.) QZ-S 均由本实验室提供和保存。采用 OMEGA 公司的 Plant RNA Kit 试剂盒提取蒜芥茄苗期叶片的 RNA，进一步利用 ABM 公司的 5× All-In-One RT MasterMix (with AccuRT Genomic DNA Removal Kit)试剂盒反转录成 cDNA。利用 Primer Premier 5.0 软件设计引物(表 1)，以上述 cDNA 为模板，采用全式金高保真酶(TransTaq DNA Polymerase High Fidelity (HiFi): AP131)进行目的基因 ORF 框的扩增。PCR 产物在 1.0%琼脂糖凝胶电泳进行检测后，将目的基因条带通过全式金胶回收试剂盒(EasyPure Quick Gel Extraction Kit: EG101)进行回收，送昆明擎科生物科技有限公司进行测序；应用 DNAMAN 软件将测序结果与转录组测序结果进行序列比对。

表1 蒜芥茄*SsWRKY22*和*SsWRKY33*基因克隆及表达分析所用引物及其序列
Table 1 Primers used in the gene cloning and expression analysis of *SsWRKY22* and *SsWRKY33* in *S. sisymbriifolium*

基因名称 Gene name	引物名称 Primer name	核苷酸序列(5′-3′) Primer sequence (5′-3′)	用途 Utilization
SsWRKY22	*SsWRKY22-F*	CAAAAACCCATCAAAGGCTCC	基因克隆
	SsWRKY22-R	CTTAAGGTTTGTATGAAGTTTAAAAATACC	Gene cloning
SsWRKY33	*SsWRKY33-F*	TAAAATCAATAGGGACAGCAAGGA	基因克隆

	SsWRKY33-R	CATTTCATAAACTTTGATTCGTGC	Gene cloning
SsWRKY22	*SsWRKY22-F*	CACAACCCAAACARTCTCCTC	表达分析
	SsWRKY22-R	CAACCCTTTGARGTACTRCACC	Expression analysis
SsWRKY33	*SsWRKY33-F*	GATGGAGRAARTATGGWCAAAAAGT	表达分析
	SsWRKY33-R	CTGCWGGAACATCATGGTTGTG	Expression analysis
CYP(内参基因)	*CYP-F*	ATCCTGTCCATGGCTAATGC	表达分析
CYP(reference gene)	*CYP-R*	ATGCCCTCAACAACTTGTCC	Expression analysis

3.2 生物信息分析及系统进化树构建

首先，应用 ORF Finder 程序(http://www.ncbi.nih.gov/gorf/gorf.htmL)进行 *SsWRKY22* 和 *SsWRKY33* 基因开放阅读框架预测；其次，通过 Protparam 网站对 SsWRKY22 和 SsWRKY33 蛋白进行理化性质预测，利用 ProtScale 分析蛋白质的亲疏水性；再次，应用在线软件 Cell-PLoc 2.0 (http://www.csbio.sjtu.edu.cn/bioinf/Cell-PLoc-2/)对目的基因进行亚细胞定位预测；然后，分别运用在线软件 SOPMA 和 SWISS-MODEL 预测目的基因编码蛋白的二级和三级结构；最后，通过 NCBI 的 BLAST 选取与 *SsWRKY22* 和 *SsWRKY33* 编码的蛋白序列同源性较高的 WRKY 蛋白序列，应用 DNAMAN 进行同源序列比对，通过 MEGA X 软件(设置 1 000 次重复，其它均为默认)进行系统发育分析，构建 Neighbor-Joining 系统发育树。

3.3 基因表达分析

用 Primer Premier 5.0 软件设计荧光定量 PCR 引物(表 1)，以 *CYP* 为内参基因，利用实时定量 PCR (qRT-PCR)对蒜芥茄(苗期)不同组织(根，茎，叶)及其接种大丽轮枝菌 0、24、48 和 72 h 后 *SsWRKY2* 和 *SsWRKY33* 基因的表达进行分析，以无菌水处理为对照。接种参照吴丽艳等(2017)的方法。RNA 的提取及反转录按照上述 3.1 中的方法进行。应用北京擎科新业生物技术有限公司生产的 2×TSINGKE® Master qPCR Mix (SYBR Green I)荧光定量 PCR 试剂盒，在 Bio-rad 荧光定量 PCR 仪(CFX Connect, USA)上进行 qRT-PCR 反应，每个样品 3 次技术重复。PCR 反应体系(20 μL)：10 μL 2×TSINGKE® Master qPCR Mix，2 μL 稀释两倍的 cDNA，正、反引物(10 μmol/L)各 0.8 μL，6.4 μL 的 ddH_2O。荧光定量 PCR 扩增条件：95 ℃预变性 1 min，95 ℃变性 10 s，60 ℃退火 10 s，72 ℃延伸 15 s，40 个循环，65 ℃~95 ℃之间检测信号。使用 $2^{-\Delta\Delta CT}$ 方法计算基因的相对表达量，结果均为 3 次重复试验的平均值。采用 Excel 2016 进行数据整理及图表的绘制，使用 SPSS 22.0 软件中的对数据进行显著性分析。

作者贡献

尹梦莹是本研究的实验设计者和实验研究的执行人；蔚亚楠、龚亚菊及桂敏完成数据分析、论文初稿的写作；鲍锐和黎志彬参与实验设计、试验结果分析；吴丽艳和杜光辉是项目的构思者及负责人，指导实验设计、数据分析、论文写作与修改。全体作者都阅读并同意最终的文本

致谢

本研究由国家自然科学基金地区基金项目(31960594)、云南省农业基础研究联合专项面上项目(2018FG001-022)和云南省应用基础研究面上项目(2019FB059)共同资助。

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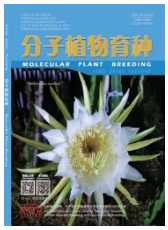
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分子植物育种
Molecular Plant Breeding
ISSN 1672-416X,CN 46-1068/S

《分子植物育种》网络首发论文

题目：铁炮百合‘萨莉’鳞茎各发育时期及不同组织 qPCR 内参基因筛选和验证

作者：张达，李莲莲，吴学尉，王天喜，刘海英，李缘，王丽花

网络首发日期：2021-09-28

引用格式：张达，李莲莲，吴学尉，王天喜，刘海英，李缘，王丽花．铁炮百合‘萨莉’鳞茎各发育时期及不同组织 qPCR 内参基因筛选和验证[J/OL]. 分子植物育种. https://kns.cnki.net/kcms/detail/46.1068.S.20210928.1312.017.html



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分子植物育种，第 XX 卷，第 X 期，第 0000-0000 页
Molecular Plant Breeding, Vol.XX, No.X, 0000-0000

研究报告

Research Report

铁炮百合‘萨莉’鳞茎各发育时期及不同组织 qPCR 内参基因筛选和验证

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摘　要　为筛选铁炮百合杂交系鳞茎不同发育阶段以及相同发育阶段不同组织中稳定表达的内参基因，本研究以铁炮百合观赏品种‘萨莉’为研究对象，采用实时荧光定量 PCR (qPCR)分析百合的 6 个候选的内参基因(*ACT*, *BHLH*, *EF1*, *EIF*, *GAPDH*, *lilyact*)在‘萨莉’鳞片的不同发育时期以及不同组织中的扩增效率，再使用 Normfinder、Bestkeeper、geNorm、比较 ΔCt 法以及 RefFinder 5 种软件或方法对候选内参基因的表达稳定性进行评估。结果表明在所有样品中和在不同发育阶段的鳞茎中都是 *EIF* 和 *BHLH* 综合稳定性最佳，在不同组织中 *GAPDH* 和 *EIF* 的综合稳定性最佳，而在所有样品中及不同组织中，*ACT* 的稳定性最差。本研究所得结果为‘萨莉’鳞茎的不同发育阶段以及不同组织的表达量分析提供了合适的内参基因组合，为其后续基因表达量的研究奠定了基础。

关键词　铁炮百合杂交系；内参基因; qPCR; geNorm；百合

Selection and Validation of Reference Genes for Quantitative RT-PCR Analysis in ‘Sally’

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基金项目：本研究由部省合建项目“低纬高原月季等切花无土栽培技术集成与示范”项目（202102AE090001）和云南省科技厅“珠芽黄魔芋优良品种培育与产业化示范”项目（202004AC10001-A06）共同资助

Abstract In order to select the internal reference genes stably expressed in different developmental stages of longiflorum hybird bulbs and different tissues at the same developmental stage, this study took longiflorum hybird cultivar 'Sally' as the research object, and used Quantitative Real-time PCR (qPCR) to analyze the 6 levels of lily. The amplification efficiency of two candidate internal reference genes (*ACT*, *BHLH*, *EF1*, *EIF*, *GAPDH*, *lilyact)* in different developmental stages of 'Sally' scales and in different tissues, then use Normfinder, Bestkeeper, geNorm, comparative ΔCt method and RefFinder softwares or methods to evaluate the stability of the expression of candidate internal reference genes. The results showed that *EIF* and *BHLH* have the best comprehensive stability in all samples and in bulbs at different developmental stages. And in different tissues,*GAPDH* and *EIF* are most stable. In all samples and different tissues, *ACT* has the worst stability. The results of this study provide a suitable combination of internal reference genes for the analysis of the expression levels of different developmental stages and different tissues of 'Sally' bulbs, which lays the foundation for the subsequent study of gene expression levels.

Keywords Longiflorum hybrids; Reference genes; qPCR; geNorm; *Lilium*

百合(*Lilium* spp.)是百合科(Liliaceae)百合属(*Lilium*)球根花卉植物，是世界四大切花之一(吴学尉等, 2011, 江苏农业科学, 39(3): 197-199; Li et al., 2014)。百合属约有 90 个原生种，主要集中分布在亚洲东部、北美洲、欧洲，同时还有部分分布在寒带地区(Jr and Gianfagna, 2015)。切花用百合种球主要有'东方百合'(Oriental hybrids, O)、'喇叭百合'(Trumpet hybrids, T)、'亚洲百合'(Asiatic hybrids, A)、'铁炮百合'(Longiflorum hybrids, L)和'新铁炮百合'(*L.*×*formolongi* hybrids)5 种类型及以这些类型为基础创制的 OT、LA、OA 和 LO 等杂交品种。这些百合杂交品种大都是原产中国的野生百合杂交而成(吴学尉等, 2007, 北方园艺, (2): 51-52; Wu et al., 2012b)。荧光定量 PCR 可以定量分析 DNA 或 RNA 的拷贝数(陈忠斌和王升启, 2003, 医学研究杂志, 32(6): 51-53)，且由于灵敏度高，准确性好，高通量等特点(Bustin, 2002)而被广泛应用。但是，由于实验中 RNA 模板质量，扩增效率等影响因素的不同，会使得 qPCR 结果与真实表达之间存在误差(Fleige and Pfaffl, 2006)。通常利用内参基因来校正和标准化处理，使得表达量更准确(Zhang et al., 2007)。

ACT、*GAPDH*、*BHLH* 等管家基因尝作为内参基因用于基因水平的校正及均一化(Bustin, 2002)。然而，不同的品种(Qi et al., 2016)、组织(Mallona et al., 2010; Zhang et al., 2013)、发育阶段(Li et al., 2016)以及不同的处理条件(Nicot et al., 2005; Sinha et al., 2015)都会影响这些内参基因的稳定性；因此，在不同的组织，不

同的品种或进行特定的实验处理下测定表达量之前，都应该进行最符合实验条件的内参基因的筛选。在百合中，‘岷江百合’ (Liu et al., 2016)、‘兰州百合’ (Li et al., 2015)、‘野百合’ (Luo et al., 2014)的不同组织，不同胁迫条件下的内参基因已经得到筛选和鉴定。然而，作为切花主用的系列之一，适用于铁炮百合的鳞茎不同发育阶段，不同组织的稳定表达的内参基因组合尚未见报道。

Normfinder (Andersen et al., 2004)、Bestkeeper (Pfaffl et al., 2004)、geNorm (Vandesompele et al., 2002)、比较 ΔCt 法(Silver et al., 2006)被广泛应用于各种植物不同条件下的内参表达稳定性分析(Wan et al., 2010; Xu et al., 2011; Wang et al., 2012; Wu et al., 2012a)。本研究使用 Normfinder、Bestkeeper、geNorm、比较 ΔCt 法以及 RefFinder (http://www.leonxie.com/referencegene.php) 五种软件或方法对 6 个内参基因(*ACT*, *BHLH*, *EF1*, *EIF*, *GAPDH*, *lilyact*)在铁炮百合‘萨莉’鳞茎的不同发育阶段以及不同组织中的表达稳定性进行分析，从中筛选出最适组合。此外，选择淀粉合成限速基因 *AGPase* 的两个亚基基因(*AGPS*, *AGPL1*)做为目的基因计算相对表达量以检测所筛选的内参基因的可靠性。

1 结果与分析

1.1 候选内参基因扩增特异性及扩增效率

本研究的所有内参基因中，*ACT* 略有低峰，且峰值在主峰后，说明有引物二聚体，但总体有 *Ct* 值，故也对其进行稳定性分析。其余引物的溶解曲线均为单峰(图 1)，说明引物特异性较好。有 *Ct* 值的所有引物扩增效率范围 102.63% (*EF1*)~109.09% (*ACT*) (表 1)，拟合回归效果评价指标(R^2)在 0.9850 (*BHLH*)~0.9988 (*EF1*)之间(表 1)。结果符合 qPCR 可信度要求，可以用于进一步的表达分析。

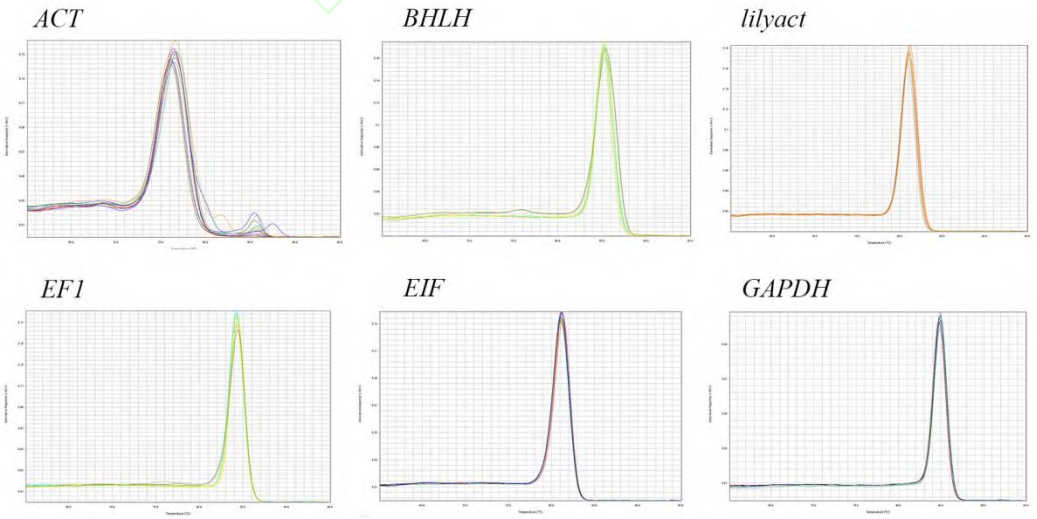


图 1 6 个候选内参的 qPCR 溶解曲线
Figure1 qPCR melting curves of 6 reference genes

表 1 内参基因的扩增效率
Table 1 Amplification efficiencies of reference genes

基因 Genes	斜率 Slope	拟合回归效果评价指标(R^2) Evaluation index of fitting regression effect (R^2)	扩增效率(%) Amplification efficiency (%)
ACT	-3.1217	0.9878	109.09
BHLH	-3.1427	0.9850	108.06
EF1	-3.2605	0.9988	102.63
EIF	-3.1659	0.9933	106.95
GAPDH	-3.2516	0.9977	103.02
lilyact	-3.1720	0.9953	106.66

1.2 候选内参基因的 *Ct* 值

内参基因在不同的发育时期，不同的组织中的表达情况用 *Ct* 值评估，所有内参基因的 *Ct* 值范围为：18.37 (*EIF*)~35.37 (*ACT*)，平均 *Ct* 值的范围为：21.68 (*EIF*)~34.42 (*ACT*) (图 2)。

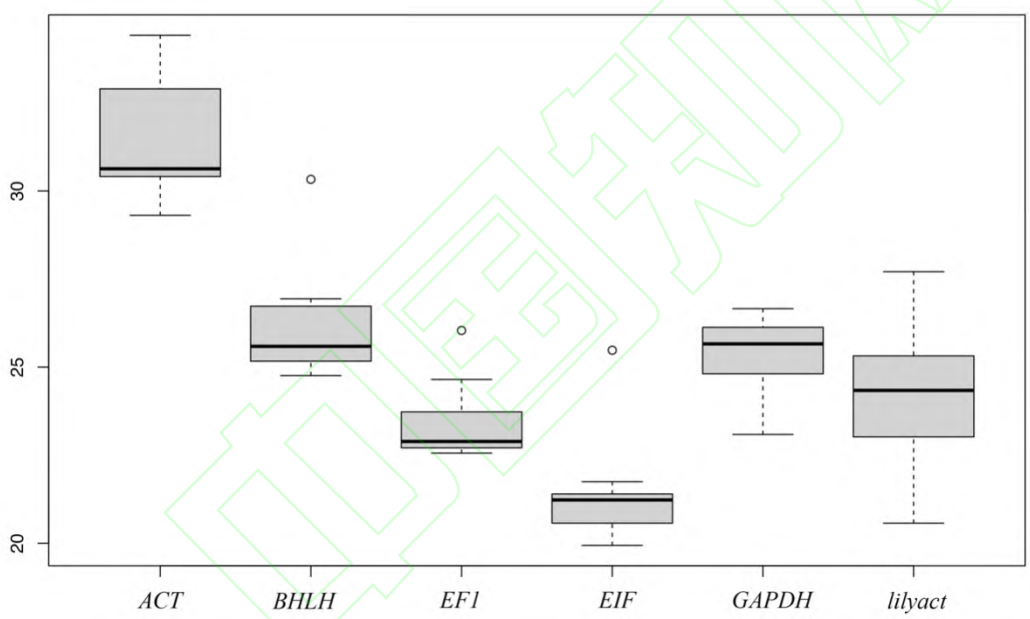


图 2 所有样品的 *Ct* 值分布
注:。: 异常值, 每一个点代表有一个异常值
Figure 2 *Ct* distribution of all samples
Note: 。: An outlier, and each point represents an outlier

1.3 内参稳定性分析

使用上述 5 种方法得到不同发育阶段、不同组织以及总体的内参稳定性分析(表 2; 表 3; 表 4)。发现不同的发育阶段和不同的组织内参基因的稳定性都有所不同。所有样品和不同发育阶段的鳞茎都是 *EIF* 和 *BHLH* 综合稳定性最佳，不同组织间 *GAPDH* 和 *EIF* 的稳定性最佳。

表 2 所有样品内参基因的表达稳定性
Table 2 Expression stability of reference genes in all samples

方法 Methods	稳定性排序 Stability ranking					
Δ CT	*EIF*	*BHLH*	*GAPDH*	*lilyact*	*ACT*	*EF1*
BestKeeper	*EF1*	*GAPDH*	*EIF*	*lilyact*	*BHLH*	*ACT*
Normfinder	*EIF*	*BHLH*	*GAPDH*	*lilyact*	*ACT*	*EF1*
geNorm	*BHLH|EIF*	-	*GAPDH*	*ACT*	*lilyact*	*EF1*
RefFinder	*EIF*	*BHLH*	*GAPDH*	*EF1*	*lilyact*	*ACT*

注: 稳定性排序由左向右稳定性逐渐降低
Note: Stability ranking is stability decreases gradually from left to right

表 3 不同发育阶段内参基因的表达稳定性
Table 3 Expression stability of reference genes in different stages of developmen

方法 Methods	稳定性排序 Stability ranking					
Δ CT	*EIF*	*BHLH*	*GAPDH*	*EF1*	*lilyact*	*ACT*
BestKeeper	*EF1*	*GAPDH*	*EIF*	*BHLH*	*ACT*	*lilyact*
Normfinder	*EIF*	*BHLH*	*GAPDH*	*EF1*	*lilyact*	*ACT*
geNorm	*BHLH|EIF*	-	*GAPDH*	*lilyact*	*EF1*	*ACT*
RefFinder	*EIF*	*BHLH*	*GAPDH*	*EF1*	*lilyact*	*ACT*

注: 稳定性排序由左向右稳定性逐渐降低
Note: Stability ranking is stability decreases gradually from left to right

表 4 不同组织内参基因的表达稳定性
Table 4 Expression stability of reference genes in different tissues

方法 Methods	稳定性排序 Stability ranking					
Δ CT	*GAPDH*	*EIF*	*BHLH*	*lilyact*	*EF1*	*ACT*
BestKeeper	*GAPDH*	*lilyact*	*EF1*	*EIF*	*BHLH*	*ACT*
Normfinder	*GAPDH*	*EIF*	*BHLH*	*lilyact*	*EF1*	*ACT*
geNorm	*BHLH|EIF*	-	*GAPDH*	*lilyact*	*EF1*	*ACT*
RefFinder	*GAPDH*	*EIF*	*BHLH*	*lilyact*	*EF1*	*ACT*

注: 稳定性排序由左向右稳定性逐渐降低
Note: Stability ranking is stability decreases gradually from left to right

1.4 最佳内参基因的稳定性验证

为验证上述结果的可靠性，以所有样品中 *EIF*，*BHLH* 两个最稳定的基因，以及 *ACT* 这一稳定性最差的基因作为内参进行计算，得到的相对表达量(图 3)。从结果可以看出，以 *EIF*、*BHLH* 以及 *EIF*，*BHLH* 同时使用作为内参时的相对表达量，表达模式都几乎没有差异。以 *ACT* 作为内参得到的 *AGPS* 相对表达量与以 *EIF*，*BHLH* 以及 *EIF*，*BHLH* 同时使用作为内参呈现出较大的差异，整体表达模式也不同，前者鳞茎出苗期的表达量只是略高于鳞茎花期及倒苗期的表达量，后者则出苗期的表达量约是倒苗期的 8 倍，花期的 5 倍；得到的 *AGPL1* 不同发育阶段的相对表达量趋势虽然与其他相似，但是相对表达量也与其他内参表现出较大差异。

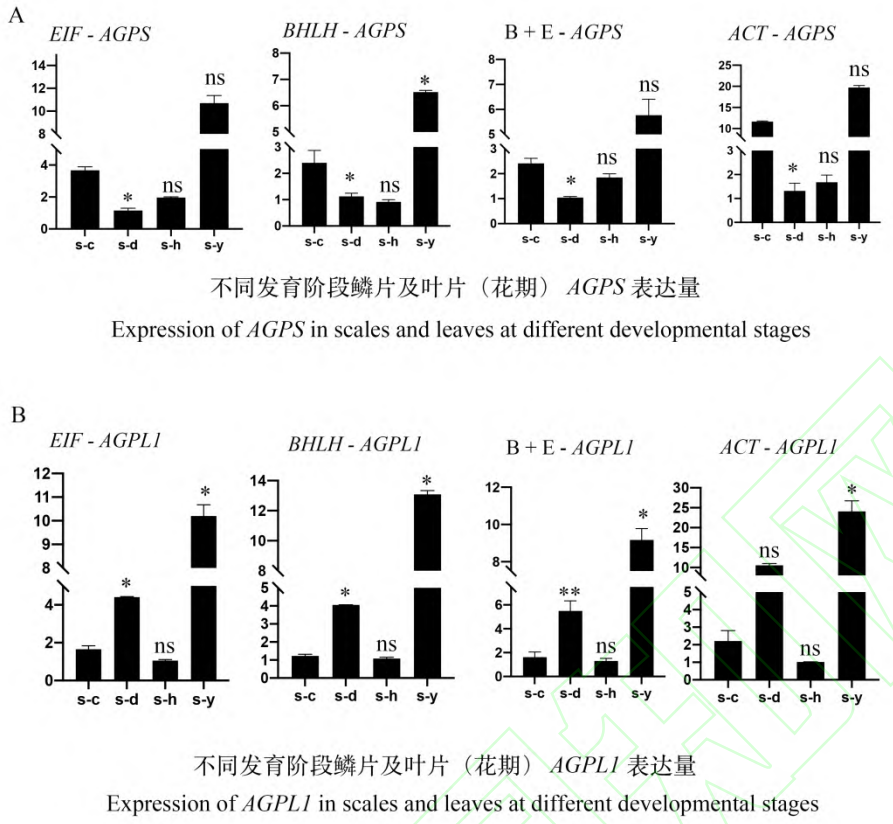


图 3 *AGPS* (A)和 *AGPL1* (B)在不同发育阶段鳞片及花期叶片中的相对表达量
注: s-c, s-d, s-h, s-y 分别代表萨莉的出苗，倒苗，花期，叶片(花期), ns: $P>0.05$, *: $P\leq0.05$, **: $P\leq0.01$
Figure 3 The relative expression of *AGPS* (A) and *AGPL1* (B) in scales and leaves at different developmental stages
Note: s-c, s-d, s-h, s-y represent Sally's emergence, inverted seedling, flowering stage and leaf (flowering stage), ns: $P>0.05$, *: $P\leq0.05$, **: $P\leq0.01$

2 讨论

本研究采用 Normfinder、Bestkeeper、geNorm 等 3 种软件以及比较 ΔCt 法一种方法对候选内参基因的稳定性进行了分析，不同的方法及软件所得到的最佳稳定性内参基因不同。在前人(Tong et al., 2009; Delporte et al., 2015; Mangeot-Peter et al., 2016)的研究中，这种现象也有大量报道，这可能是由于不同软件的统计模型及计算方法的差异导致的。为了进一步对候选内参基因的稳定性进行评估，本研究引用了在线工具 RefFinder 对上述 4 种方法进行综合评估，得到最终的稳定性排序，本研究的内参稳定性排序采取 RefFinder 所确定的稳定性排序。

本研究中所使用的 *ACT* 和 *lilyact* 是不同百合的 *actin* 基因，结果表明 *ACT* 和 *lilyact* 稳定性差异较大，*lilyact* 明显比 *ACT* 更为稳定，且 *Ct* 值明显更小。说明即使是同一个管家基因，在不同的百合中，其序列

和表达也会存在差异，故在没有管家基因序列的情况下，选用亲缘关系越近的种的序列设计引物，结果越准确。

在不同的百合中，内参基因的稳定性差异很大，在兰州百合中(Li et al., 2015)，不同组织中的 *ACT*、*GAPDH* 和 *UBQ* 的组合最为稳定，而 *ACT* 与 *AP4* 及 *ACT* 与 *GAPDH* 的组合则分别适合于不同发育阶段叶片和鳞片，在胁迫处理下的叶片、鳞片和根系中，*FP*、*ACT* 和 *AP4* 的表达最为稳定，其中不同组织中 *GAPDH* 最为稳定这一结果与本研究一致，然而 *ACT* 在本研究中不够稳定，不适合作为内参；在岷江百合中(Liu et al., 2016)，不同发育阶段中的 *EF1*，生物胁迫下的 *SAND*，干旱胁迫下的 *CYP*/*GAPDH*，盐胁迫下的 *TIP41* 最为稳定，这也与本实验的结果相差较大。

本研究为确保所筛选内参稳定性的可靠，进一步使用淀粉合成过程中的关键基因，淀粉合成限速基因 *AGPase* 的两个亚基基因 *AGPS* 及 *AGPL1* 对鳞茎不同发育阶段及不同组织进行了相对表达量计算，结果不仅验证了筛选结果的可靠性，也表明了如果使用不适合的内参基因，甚至会导致最终结果的错误。在进行 qRT-PCR 分析时设计跨内含子的引物可以有效的克服基因组 DNA 残留对结果的影响(Eildermann et al., 2012; Carvalho et al., 2015)。然而，百合属的全基因组测序还未有报道，很难以此方法设计引物。本研究中，为克服基因组 DNA 残留对结果的影响，使用了未反转录的 RNA 作为阴性模板对照(徐雷锋, 2017)，对照的结果溶解曲线不起峰，说明没有残留的基因组 DNA。

综上所述，在不同品种、不同组织的植物材料中最稳定的内参基因是不同的。本研究为铁炮百合鳞茎的不同发育阶段以及不同组织的表达量分析提供了合适的内参基因组合，为其后续基因表达量的研究奠定了基础，提供了科学依据。

3 材料与方法

3.1 样品的采集

本实验所采取的百合鳞片分为 3 个发育阶段，花期即第一朵花开花后的第 2 天进行采集；倒苗期即地上茎完全枯萎后的第 5 天进行采集；出苗即鳞茎出芽后的第 5 天进行采集。本实验所采取的叶片为花期的叶片，采集标准与花期鳞片相同。采样时每个样品采集 3 株百合的组织作为生物学重复材料。

3.2 样品的总 RNA 提取及 cDNA 合成

在液氮冷冻的研钵中，迅速将约 100 mg 鳞片样品(或 200 mg 叶片样品)研磨成粉，收集于离心管中，使用北京擎科生物科技有限公司的多糖多酚植物总 RNA 提取试剂盒(离心柱型, TSP412)进行 RNA 的提取，结果使用微量紫外分光光度计进行浓度的确定含量，使用 1.2%的琼脂糖凝胶电泳检测核酸纯度，提取后的 RNA 置于-80 ℃冰箱保存。取 1 000 ng RNA，使用北京擎科生物科技有限公司的反转录试剂盒(Goldenstar™ RT6 cDNA Synthesis Kit Ver.2)进行基因组 DNA 的去除以及反转录。

3.3 qPCR 内参引物的设计及合成

引物由北京擎科生物科技有限公司进行合成。选取 6 个内参基因序列，使用 Primer Biosoft 的软件 Beacon Designer 8 (http://www.premierbiosoft.com)进行引物设计(表 3)。

表 5 内参基因的引物设计及基因信息
Table 5 Primer design and gene information of reference genes

基因 Genes	引物序列(5’-3’) Primer sequence (5'-3')	NCBI 登录号 NCBI accession number	片段长度(bp) Fragment length (bp)
ACT	F:CAATCTATGAGGGTTATG R:GTAATTCTTCTCA*ACT*GA	KP861871.1	230
BHLH	F:AAGCAAGAGAAGGAGAAC R:G*ACT*GGCATCATCATCTT	KJ543467.1	141
EF1	F:TGAC*ACT*TGCTGATATTG R:TACAGACGGAACAGATTC	KJ543461.1	179
EIF	F:CCGAAGAACAAGGGAAAG R:CCTTCTCATCCTGGTAGT	KP861874.1	262
GAPDH	F:GATGTGTCTGTTGTTGAT R:CCATTCATTGTCATACCA	KP179417.1	240
lilyact	F:GATGCTTATGTTGGTGATGA R:TCGTTGTAGAAGGTGTGAT	JX826390.1	128

3.4 qPCR 反应及体系

使用Takara TB Green® Premix Ex Taq ™ II试剂盒进行实验，反应体系为 20 μL (表 2)，反应条件为预变性 95 ℃，30 s；95 ℃，5 s，60 ℃，34 s，进行 40 个循环。反应在Applied Biosystems® QuantStudio™ 7 Flex 实时荧光定量PCR系统上进行，根据TaKaRa说明书，在 60 ℃收集荧光信号，使用定量PCR仪默认的程序生成溶解曲线。

表 6 qPCR 反应体系
Table 6 qPCR sample volume

试剂 Reagent	使用量(μL) Amount of usage (μL)	终浓度 Final concentration
TB GreenPremix Ex TaqII(Tli RNaseH Plus)(2×)	10	1×
PCR Forward Primer(10 μmol/L)	0.8	0.4 μmol/L
PCR Reverse Primer(10 μmol/L)	0.8	0.4 μmol/L
ROX ReferenceDye II(50×)	0.4	1×
DNA模板 DNA template	2	
ddH_2O	6	

总量 Total	20

3.5 标准曲线绘制及扩增效率

将所有 cDNA 混合作为模板,以每次稀释 2 倍的标准进行 5 次稀释，将原液及稀释的模板进行 qPCR 扩增。再以 *Ct* 值为纵坐标，cDNA 模板浓度的对数值为横坐标，绘制标准曲线，计算扩增效率。

3.6 qPCR 内参的稳定性分析

为表示各个内参基因的表达情况的变化，*Ct* 值使用 R 语言的 boxplot 包以箱线图的形式展现。使用软件 Normfinder、Bestkeeper、geNorm 以及比较 ΔCt 法分析各个发育阶段，不同组织以及总体的内参稳定性分析。此外，使用在线工具 Refinder 对数据对上述四种方式的结果进行综合分析，最终确定内参基因的稳定性次序。

3.7 最佳内参的稳定性验证

选择百合淀粉合成限速基因 *AGPase* 作为目的基因验证内参的稳定性结果是否可靠。选择最稳定的 2 个内参基因以及最不稳定的 1 个内参基因计算鳞茎 3 个不同发育阶段的 *AGPase* 小亚基和大亚基的表达情况。采用 $2^{-\Delta\Delta Ct}$ 法(Livak, and Schmittgen, 2001)计算 *AGPase* 小亚基及大亚基两个基因的相对表达量。

作者贡献

张达是本研究的实验设计者和实验研究的执行人；张达及李莲莲完成数据分析、论文初稿的写作；王天喜及李缘参与实验设计、试验结果分析；吴学尉、王丽花及刘海英是项目的构思者及负责人，指导实验设计、数据分析、论文写作与修改。全体作者都阅读并同意最终的文本。

致谢

本研究由部省合建项目“低纬高原月季等切花无土栽培技术集成与示范”项目(202102AE090001)和云南省科技厅“珠芽黄魔芋优良品种培育与产业化示范”项目(202004AC10001-A06)共同资助。

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文章编号：1005-0906(2021)05-0015-07 DOI: 10.13597/j.cnki.maize.science.20210502

鲜食玉米单倍体诱导率的影响因素研究

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摘 要：选用不同基因类型的鲜食玉米(普甜玉米、超甜玉米、加强甜玉米和糯玉米)共15个材料作为母本,4个不同诱导材料作为父本,分别在景洪冬季和昆明夏季于抽丝后的不同时期进行杂交,统计诱导的单倍体后代,研究不同遗传背景、诱导基因、生态条件以及授粉时期对单倍体诱导率的影响。结果表明,不同的遗传背景、诱导基因、生态条件以及授粉时期对单倍体诱导均有重要影响,昆明夏季进行单倍体诱导的效率显著高于景洪冬季,吐丝早期授粉比晚期授粉的诱导效率要高;不同的母本基因型与不同的诱导系都对单倍体的诱导率产生极显著影响,但同一鲜食玉米类型不同品种的单倍体诱导率表现不同,说明单倍体诱导率的表达不受甜糯基因的影响;父本诱导系的诱导率则表现较为一致,都是诱导系CAU-1和(CAU-1×CAU-5)-B的单倍体诱导率显著高于诱导系(MT1×H780)-B和MT1的诱导率。在进行鲜食玉米单倍体育种时,选用诱导系CAU-1和(CAU-1×CAU-5)-B在昆明夏季于吐丝早期进行诱导,可以大幅提高单倍体的诱导率。

关键词：鲜食玉米;单倍体;诱导率;生态条件;遗传背景;授粉时期
中图分类号：S513.032 **文献标识码**：A

Research on the Influence Factors of the Haploid Inducing Rates in Fresh Maize

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Abstract: In the study, different genotypes of fresh maize (including normal sweet maize, super sweet maize, sugary enhanced maize, and waxy maize), a total of 15 materials as female and four different inducers as male were selected, the crosses were made during different pollination period in Jinghong winter season and Kunming summer season respectively, and the induced haploid offspring were statistical analyzed, finally tried to reveal the influence of different genetic background, different inducer genes, different ecological conditions and different pollination period on haploid inducing rate. The main results were as followed: different genetic background, inducer genes, ecological conditions and pollination periods had important influence on the haploid induction, the haploid inducing efficiency in Kunming summer season was significantly higher than that of in Jinghong winter season, pollination during early stage was significantly higher than that of in late stage, inducers CAU-1 and (CAU-1×CAU-5)-B was significantly higher than that of inducers (MT1×H780)-B and MT1. Therefore, it can be concluded that the haploid inducing rate can be greatly increased by using inducers CAU-1 and (CAU-1×CAU-5)-B, making pollination during early stage in Kunming summer season for fresh maize breeding.

Key words: Fresh maize; Haploid; Inducing rate; Ecological condition; Genetic background; Pollination period

作为现代玉米育种的革命技术,单倍体育种可以快速、高效地固定玉米基因型,只需2~3代即可获得稳定优良的纯合自交系,代替了传统的6~8代连续自交选育,从而大大地提高了玉米育种的效率[1~5]。单倍体育种的操作程序与常规育种基本相同,主要包括诱导、鉴别和加倍,不需要任何特殊设备条件,鉴定方法简单易操作,已成为现代育种技术的重要研究领域[6~10]。单倍体诱导受到被诱导父母

录用日期：2020-11-26
基金项目：云南省应用基础研究计划重点项目(2015FA012)
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致 谢：本研究材料的诱导系1和2由中国农大陈绍江教授提供!

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本材料和诱导环境等方面的影响[11],使得单倍体的诱导率普遍较低,在自然条件下单倍体诱导率只有0.1%~0.2%[12],单倍体诱导率低是目前制约单倍体技术的主要瓶颈。

鲜食玉米是指在乳熟后期至蜡熟初期收获的玉米,根据所携带的基因不同可以分为普甜玉米(*su*$_1$)、超甜玉米(*sh*$_2$/*bt*$_1$)、加强甜玉米(*suse*)和糯玉米(*wx*)等。鲜食玉米蛋白质、氨基酸和维生素等含量高、营养丰富,口味甜、糯、嫩、香,深受消费者喜爱;同时,鲜食玉米还是食品加工业、造纸业、纺织业等工业的重要原材料,具有良好的经济和社会效益[13,14]。我国鲜食玉米种质资源丰富,但对鲜食玉米的研究起步较晚,且主要以常规育种手段为主,导致鲜食玉米的育种效率较低[15];再加上鲜食玉米的遗传背景不同于普通玉米,目前有关鲜食玉米的单倍体育种研究开展得较少。本研究选用不同基因类型的鲜食玉米作为母本,和不同的诱导材料在不同生态气候条件和不同授粉时期进行杂交诱导,研究不同遗传背景、诱导基因、生态气候条件以及授粉时期对鲜食玉米单倍体诱导率的影响,分析鲜食玉米单倍体诱导率的影响因素,为将单倍体技术应用于鲜食玉米自交系选育、提高鲜食玉米育种效率、加快鲜食玉米种质创新提供理论指导。

1 材料与方法

1.1 试验材料

表1 鲜食玉米单倍体诱导研究的母本材料

Table 1 Maternal materials for haploid inducing of fresh maize

序号 No.	类型 Type	品种名称 Variety name
1	普 甜	泰国晶晶乐
2		花超甜糯(金银粟)
3		超甜脆黄玉米
4	超 甜	双色先蜜
5		美珍204
6		华珍
7		绿色超人
8	加强甜	珠贝粒
9		甜单10号
10		中甜2号
11	糯玉米	燕禾金2000
12		安徽白糯
13	普通玉米	会单四号
14		裕强玉1号
15		云瑞8号

根据不同鲜食玉米基因类型,本研究分别选择普甜、超甜、加强甜和糯玉米共4种类型12个不同鲜食玉米品种做杂交诱导的母本材料,同时设置3个普通玉米杂交种作为对照;根据前期鉴定结果选择农艺性状优良、标记明显和诱导率高的诱导系及其杂交后代共计4个做杂交诱导的父本材料(表1、表2)。

表2 鲜食玉米单倍体诱导研究的诱导材料

Table 2 Inducer materials for haploid inducing of fresh maize

诱导系 Inducer	系 谱 Pedigree
1	CAU-1
2	(CAU-1×CAU-5)-B
3	(MT1×H780)-B
4	MT1

1.2 试验方法

上述材料分别于2017年11月种植于景洪市嘎洒镇和2018年5月种植于昆明市安宁县街,每个材料种植60株,试验期间田间管理同一般大田玉米。开花前所有单株套袋并挂牌记载抽丝日期,根据抽丝日期于抽丝后2~10 d分别取4个诱导系花粉授粉并记载授粉日期和对应的诱导系,不同母本材料的不同抽丝期与不同诱导系各杂交2~3穗;分单穗收获上述杂交果穗,根据子粒标记(胚乳有色、胚无色)进行单倍体子粒的鉴定,按照不同母本材料、不同抽丝期、不同诱导系进行统计。对根据子粒标记鉴定出的单倍体每个母本分别选20粒进行育苗移栽,根据植株颜色、形态长势、花粉育性等确定真实单倍体植株;再根据单倍体子粒总数和杂交子粒总数,确定不同鲜食玉米、不同诱导材料和不同生态条件以及不同授粉时期获得的单倍体比例。

1.3 数据处理与分析

数据用Excel进行整理后利用SPSS 25.0和DPS 14.5软件进行方差分析和多重比较。

2 结果与分析

2.1 不同生态条件对单倍体诱导率的影响

15个母本材料与4个诱导系杂交得到的共60个组合的单倍体诱导率,分别按景洪冬季和昆明夏季2种生态条件进行统计(表3),方差分析结果表明(表4),60个组合之间的诱导率达到极显著差异,2种生态条件间的诱导率达到显著差异。60个组合的诱导率在0.56%~21.03%,其中组合母本13×

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表3　60个组合在两种生态条件下的单倍体诱导率

Table 3　The haploid induction rate of the 60 crosses in two ecological locations

组 合 Cross	母本材料 Maternal material	诱导系 Inducer	诱导率(%) Induction		组 合 Cross	母本材料 Maternal material	诱导系 Inducer	诱导率(%) Induction	
			景洪冬季 Jinghong winter	昆明夏季 Kunming summer				景洪冬季 Jinghong winter	昆明夏季 Kunming summer
1	1	1	1.23	3.00	31	8	3	6.14	3.71
2	1	2	1.30	1.47	32	8	4	5.11	5.30
3	1	3	0.63	0.48	33	9	1	8.67	7.92
4	1	4	2.04	0.92	34	9	2	9.53	9.53
5	2	1	13.34	10.63	35	9	3	4.12	7.56
6	2	2	16.24	10.16	36	9	4	4.01	6.69
7	2	3	17.99	15.08	37	10	1	8.47	14.72
8	2	4	14.43	6.66	38	10	2	13.02	14.19
9	3	1	8.67	5.37	39	10	3	2.28	7.51
10	3	2	11.19	8.96	40	10	4	7.14	7.14
11	3	3	3.65	3.65	41	11	1	8.50	12.36
12	3	4	3.86	3.51	42	11	2	7.21	6.80
13	4	1	8.67	10.64	43	11	3	3.84	4.39
14	4	2	9.98	9.59	44	11	4	3.14	4.17
15	4	3	12.94	14.08	45	12	1	0.00	1.89
16	4	4	9.85	6.54	46	12	2	0.00	1.89
17	5	1	8.67	6.45	47	12	3	0.73	2.15
18	5	2	4.81	4.23	48	12	4	0.00	1.62
19	5	3	3.88	11.10	49	13	1	13.71	28.35
20	5	4	7.84	4.03	50	13	2	9.40	19.17
21	6	1	12.17	13.50	51	13	3	8.31	6.20
22	6	2	17.54	15.33	52	13	4	4.40	3.87
23	6	3	9.82	6.39	53	14	1	11.52	14.16
24	6	4	4.75	5.12	54	14	2	9.05	19.71
25	7	1	7.21	10.43	55	14	3	2.53	2.53
26	7	2	6.25	12.50	56	14	4	3.21	2.70
27	7	3	2.50	5.42	57	15	1	11.70	17.13
28	7	4	1.56	7.76	58	15	2	7.48	17.60
29	8	1	7.50	7.50	59	15	3	4.74	4.74
30	8	2	8.21	8.21	60	15	4	2.59	4.88

表4　60个组合在两种生态条件下单倍体诱导率的方差分析表

Table 4　ANOVA of the haploid inducing rate for the 60 crosses in two ecological conditions

变异来源 Variation source	平方和 SS	自由度 df	均 方 MS	*F*值 *F* value
组　合	0.2548	59	0.0043	5.369**
生态条件	0.0041	1	0.0041	5.08*
误　差	0.0475	59	0.0008	
总变异	0.3063	119		

注:*表示0.05水平下差异显著,**表示0.01水平下差异显著。下表同。

Note: * means significant difference at 5%, ** means significant difference at 1%. The same as below.

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诱导系1、母本2×诱导系3等17个组合的诱导率较高，都在10%以上；组合母本1×诱导系4、母本12×诱导系3等7个组合的诱导率较低，平均在2%以下。昆明夏季的诱导率平均为8.15%，显著高于景洪冬季的诱导率。不同的母本与诱导系杂交的组合之间以及不同生态条件间的诱导率存在着显著至极显著的差异，有必要进一步分析不同母本基因型与不同诱导系在不同生态条件下对单倍体诱导率的影响。

2.2 不同母本基因型与不同诱导系对单倍体诱导率的影响

2.2.1 景洪冬季的单倍体诱导率

将15个母本材料与4个诱导系在景洪冬季杂交得到的单倍体分别按母本和诱导系进行统计，经方差分析结果表明(表5)，15个母本材料之间以及4个诱导系之间的单倍体诱导率均达到极显著差异。15个母本材料的平均单倍体诱导率在0.18%～15.50%，其中单倍体诱导率最高的是母本2，其次是母本6；母本1和12的单倍体诱导率最低，分别为1.30%和0.18%。在景洪冬季的生态条件下，虽然不同母本材料对单倍体诱导率有极显著影响，但除了加强甜玉米和普通玉米不同品种之间的单倍体诱导率表现较为一致之外，其余类型鲜食玉米的不同品种之间的诱导率差异较大。诱导系1和2的单倍体诱导率较高，都在8.00%之上；诱导系3和4的单倍体诱导率较低，都在6.00%以下。在景洪冬季的生态条件下，用诱导系1和2做父本的单倍体诱导率极显著高于用诱导系3和4做父本的单倍体诱导率。

表5 15个母本材料与4个诱导系在景洪冬季的单倍体诱导率的方差分析表

Table 5 ANOVA of the haploid inducing rate of the 15 maternal materials and 4 inducers in Jinghong winter season

变异来源 Variation source	平方和 SS	自由度 df	均 方 df	*F*值 *F* value
母本材料	0.077 2	14	0.005 5	8.694**
诱导系	0.018 1	3	0.006 0	9.508**
误 差	0.026 7	42	0.000 6	
总变异	0.122 0	59		

2.2.2 昆明夏季的单倍体诱导率

将15个母本材料与4个诱导系在昆明夏季杂交得到的单倍体分别按母本和诱导系进行统计，经方差分析结果表明(表6)，15个母本材料之间以及4个诱导系之间的单倍体诱导率均达到极显著差异。15个母本材料的平均单倍体诱导率在1.47%～14.40%，其中母本13的单倍体诱导率最高，单倍体诱导率最低的是母本12和母本1。在昆明夏季的生态条件下，除了普甜玉米3个品种之间的单倍体诱导率差异显著之外，其余类型鲜食玉米和普通玉米不同品种之间的单倍体诱导率表现较为一致。诱导系1和2的单倍体诱导率较高，都在10.00%以上；诱导系3和4的诱导率较低，分别为6.33%和4.73%。在昆明夏季的生态条件下，用诱导系1和2做父本的单倍体诱导率极显著高于用诱导系3和4做父本的单倍体诱导率。

表6 15个母本材料与4个诱导系在昆明夏季的单倍体诱导率的方差分析表

Table 6 ANOVA of the haploid inducing rate of the 15 maternal materials and 4 inducers in Kunming summer season

变异来源 Variation source	平方和 SS	自由度 df	均 方 MS	*F*值 *F* value
母本材料	0.069 1	14	0.004 9	3.054**
诱导系	0.043 3	3	0.014 4	8.947**
误 差	0.067 8	42	0.001 6	
总变异	0.180 2	59		

从对景洪冬季与昆明夏季的方差分析来看，不同的母本基因型与不同的诱导系都对单倍体的诱导率产生极显著影响。但由于生态条件的影响，同一母本材料的诱导率在不同的生态条件下的诱导率不同，进一步分析可以发现，种质基础偏热带的材料在景洪冬季表现较好，种质基础偏温带的材料在昆明夏季表现较好。虽然同一诱导系的诱导率在景洪冬季和昆明夏季之间不同，但都是昆明的诱导率高于

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景洪的诱导率,而且都是诱导系1和2的诱导率极显著高于诱导率3和4的诱导率,因此不论在景洪冬季还是昆明夏季,选用诱导系1和2可获得较高的单倍体诱导率。

2.3　不同授粉期对单倍体诱导率的影响

2.3.1　景洪冬季的单倍体诱导率

将15个母本材料与4个诱导系在景洪冬季杂交得到的单倍体按母本和授粉时期进行统计(表7),经方差分析结果表明(表8),不同的母本材料之间与不同的授粉时期之间的单倍体诱导率均达到了极显著差异。在3个授粉时期中,抽丝后2~4 d授粉的单倍体诱导率最高,平均诱导率为8.25%;其次为抽丝后5~7 d授粉的,平均诱导率为7.14%,但两者之间没有显著差异;抽丝后8~10 d授粉的诱导率最低,为5.12%,与以上2个时期授粉达显著差异。

表7　15个母本材料的3个授粉时期在景洪冬季的单倍体诱导率

Table 7　The haploid induction rate of 15 maternal materials and 3 pollination periods in Jinghong winter　　%

母本材料 Maternal material	诱导率 Induction		
	抽丝后2~4 d 2 to 4 days after silk	抽丝后5~7 d 5 to 7 days after silk	抽丝后8~10 d 8 to 10 days after silk
1	2.04	0.93	1.49
2	17.02	18.97	7.17
3	9.49	5.14	5.38
4	10.00	12.09	10.69
5	6.10	5.55	5.55
6	9.16	10.89	5.38
7	4.29	2.22	2.37
8	8.23	4.56	5.38
9	8.40	4.25	5.38
10	11.67	9.76	3.99
11	5.62	6.74	5.24
12	0.00	0.00	1.22
13	14.22	8.97	7.60
14	9.10	9.15	6.89
15	8.36	7.95	3.10

表8　15个母本材料与3个授粉时期在景洪冬季的单倍体诱导率的方差分析表

Table 8　ANOVA of the haploid inducing rate of 15 maternal materials and 3 pollination periods in Jinghong winter season

变异来源 Variation source	平方和 SS	自由度 df	均 方 MS	*F*值 *F* value
母本材料	0.054 5	14	0.003 9	8.145**
授粉时期	0.007 5	2	0.003 8	7.875**
误　差	0.013 4	28	0.000 5	
总变异	0.075 5	44		

2.3.2　昆明夏季的单倍体诱导率

将15个母本材料与4个诱导系在昆明夏季杂交得到的单倍体按母本和授粉时期进行统计(表9),经方差分析结果表明(表10),不同的母本材料之间与不同的授粉时期之间的单倍体诱导率均达到了极显著差异。在3个授粉时期中,抽丝后5~7 d授粉的单倍体诱导率最高,平均诱导率为8.24%;其次为抽丝后2~4 d授粉的,平均诱导率为8.18%,但两者之间没有显著差异;抽丝后8~10 d的诱导率最低,为5.05%,与以上2个时期授粉达显著差异。

无论是景洪冬季还是昆明夏季,不同的授粉时期间的单倍体诱导率均达到了极显著差异;3个授粉时期中,抽丝后2~4 d与抽丝后5~7 d授粉的单倍体诱导率较高,显著高于抽丝后8~10 d授粉的单倍体诱导率,因此早期授粉比晚期授粉的单倍体诱导率要高。

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表9 15个母本材料的3个授粉时期在昆明夏季的单倍体诱导率

Table 9 The haploid induction rate of 15 maternal materials and 3 pollination periods in Kunming summer %

母本材料 Maternal material	诱导率 Induction		
	抽丝后2~4 d 2 to 4 days after silk	抽丝后5~7 d 5 to 7 days after silk	抽丝后8~10 d 8 to 10 days after silk
1	1.03	0.78	3.00
2	14.57	12.71	7.84
3	4.53	5.56	2.58
4	12.91	14.25	5.18
5	6.42	11.86	6.26
6	7.96	11.57	2.38
7	10.40	8.73	5.05
8	4.31	4.24	5.91
9	7.95	6.01	5.69
10	15.10	7.68	9.83
11	7.17	7.14	5.05
12	1.53	1.99	1.68
13	7.47	12.85	6.18
14	10.04	9.27	5.05
15	11.25	8.93	4.10

表10 15个母本材料与3个授粉时期在昆明夏季的单倍体诱导率的方差分析表

Table 10 ANOVA of the haploid inducing rate of 15 maternal materials and 3 pollination periods in Kunming summer season

变异来源 Variation source	平方和 SS	自由度 df	均 方 MS	*F*值 *F* value
母本材料	0.038 8	14	0.002 8	4.809**
授粉时期	0.01	2	0.005	8.641**
误差	0.016 1	28	0.000 6	
总变异	0.064 9	44		

3 结论与讨论

3.1 不同生态条件的单倍体诱导率

根据Rober等的报道,不同的环境条件下的单倍体诱导率之间存在很大差异。Eder和Chalyk[16]的研究结果与Rober不同,并没发现环境会对诱导率产生影响。Prigge等[17]的研究结果与Rober的基本一致,不同环境下的单倍体诱导率不同。黎亮等在长期的试验结果中,证实了在海南冬季进行单倍体诱导的效果要比在北京夏季进行的效果要好。慈佳宾等[18]分别在长春、榆树和白城3种不同生态环境下研究玉米的单倍体诱导率,得出不同生态条件对玉米单倍体的诱导率影响显著。

本研究通过分析发现,昆明夏季的单倍体诱导率显著高于景洪冬季,说明不同的生态条件会对单倍体的诱导率产生影响。分析其原因可能与当地的气候条件和研究材料有关,景洪属于热带湿润季风气候,冬季气温较高、日照时间短、基本无雨;昆明属于亚热带高原季风气候,夏季气候温和、日照时间长、雨水较多、温差较大。本研究材料以温带种质为主,对昆明的气候条件表现更为适应。因此,在进行玉米单倍体育种时,需考虑到生态条件对诱导率和研究材料的影响,选择适宜的生态环境可以提高单倍体的育种效率。

3.2 不同母本基因型与不同诱导系的单倍体诱导率

张如养等[19]利用6个玉米单倍体诱导系对6个玉米自交系通过杂交进行单倍体的诱导,结果表明,不同诱导系对同一自交系进行杂交与不同自交系由相同诱导系进行杂交,单倍体诱导率存在显著差异。秦燕等[20]在糯玉米单倍体诱导研究中得出,单倍体诱导率受母本基础材料和父本诱导系影响均较

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大。本研究通过分析也得出,不同父母本的杂交组合诱导率差别很大,说明单倍体诱导率因父母本的遗传背景不同而存在较大差异。不同母本基因型间单倍体的诱导率差异显著,这是由于母本基因型所产生的雌配子中有害基因的概率不同所造成的,农艺性状改良的材料作母本比未经改良材料产生单倍体的频率要高[21]。

虽然母本不同的遗传背景对单倍体诱导率有极显著影响,但本研究中同一鲜食玉米基因类型的不同品种的单倍体诱导率却表现不同,与普通玉米相比也并未因携带特定甜糯基因而有不同的表现,说明单倍体诱导率的表达不受甜糯基因的影响,由此推断甜糯基因与单倍体诱导率之间并无相关关系。与母本遗传背景对单倍体诱导率的影响相比,父本不同的诱导基因对单倍体诱导率的影响可能更大也更稳定。本研究中的4个诱导材料在不同的生态条件下表现较为一致,无论在景洪冬季还是昆明夏季,诱导系1和2的单倍体诱导率都显著高于诱导系3和4的诱导率。单倍体诱导性状是一个由2个主效QTL加8个微效QTL控制的并受环境影响的复杂性状,诱导系本身聚合的QTL位点越多,诱导能力就越高[22,23]。因此,选择诱导能力高、遗传标记明显、散粉性好、抗病性强等综合性状优良的父本诱导系,更有可能获得高频率的单倍体后代。

3.3　不同授粉时期的单倍体诱导率

授粉时期对单倍体诱导率的影响,不同的研究者得到的结论不同。有的研究结果表明,延迟授粉可以提高玉米单倍体的诱导率[24]。有研究结果表明,授粉时期越长、单倍体诱导率越低[25~26]。本研究发现,不同授粉时期的单倍体诱导率达到了显著的差异,早期授粉的单倍体诱导率要比晚期授粉的高。造成以上研究结果差异的原因,可能与研究地点的气候条件以及采用的研究材料不同有关。

高效地进行单倍体诱导对玉米单倍体育种至关重要。本研究得出,单倍体诱导率受到不同生态条件、不同母本基因型和不同诱导系以及不同授粉时期等因素的影响,在进行鲜食玉米单倍体育种过程中,选择适合的生态条件、农艺性状优良的母本材料和高频诱导系以及最佳授粉时间,可以大幅提高鲜食玉米单倍体育种的效率。

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云南大学学报(自然科学版), 2022, 44(3):627~635 DOI: 10.7540/j.ynu.20210137
Journal of Yunnan University: Natural Sciences Edition

咖啡园土壤硝化作用研究

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摘要: 为探究云南咖啡园土壤硝化作用及其主要影响因素, 采集云南省保山市隆阳区潞江坝和德宏州芒市 2 个主产区 5 个咖啡园土壤进行培养试验, 并分析土壤性质和土壤氨氧化微生物特征对硝化作用的影响. 结果表明, 供试土壤培养期间 (0~7 d) 的净硝化速率为 1.83 ~ 7.42 mg·kg^{-1}·d^{-1}, 土壤 pH 是影响土壤净硝化速率的重要因子, 两者呈极显著正相关关系 (p<0.01). 培养结束后, 净硝化速率高的土样, pH 值显著下降, 表明硝化作用会加剧土壤酸化. 在供试土壤中, 氨氧化古菌 (ammonia-oxidizing archaea, AOA) 数量高于氨氧化细菌 (ammonia-oxidizing bacteria, AOB), 氨氧化古菌 Group I.1 a-associated (*Candidatus Nitrosotalea*) 相对丰度在所有土壤硝化细菌中最高, 其相对丰度与净硝化速率没有直接关系, 但与有效磷和速效钾含量呈极显著正相关 (p<0.01). 高 pH 值土壤净硝化速率较高, 由此引起的 pH 值下降风险较大, 应通过改善土壤缓冲性, 达到构建氮素养分库和减少酸化的目的.

关键词: 咖啡园土壤; 净硝化速率; 氨氧化古菌; 氨氧化细菌
中图分类号: S154.1 **文献标志码:** A **文章编号:** 0258−7971(2022)03−0627−09

硝化作用是土壤氮素转化的关键过程, 决定着植物可利用氮素形态的数量和相对比例. 就农业生产和环境保护领域而言, 硝化过程产生的 NO_3^-极易淋失并造成水体污染; NO_3^-是反硝化作用的底物, 土壤中 NO_3^-的增加会加速土壤氮素的气体损失和温室气体 N_2O 的排放, 氮素流失也导致氮肥的使用量增加, 生产成本上升; 硝化过程 H^+的释放和累积可引起土壤酸化, 继而导致土壤退化并使作物减产[1]. 因此, 了解硝化过程及影响硝化作用的主导因素, 对生态系统健康和农业生产力提高都有重要意义.

酸性土壤的硝化活性一般较弱[2-3], 但长期施用氮肥和有机肥会明显提高酸性土壤硝化活性[4-5], 导致酸化加剧[6-7]. 因此, 深入研究酸性土壤硝化作用, 是退化土壤修复和土壤质量提升的重要基础. 咖啡是云南重要的经济作物, 种植面积和产量占全国的 98% 以上[8], 是云南省热区边疆农民的主要收入来源之一, 对云南省的经济社会发展和边疆乡村振兴具有重要影响. 有研究表明, 云南咖啡主产区超过 50% 的土壤 pH 值已低于适宜范围 5.5 ~ 6.5[9], 土壤酸化趋势明显. 云南咖啡产区气温高, 雨量大, 高温多雨会加快土壤氮素转化, 硝化作用强烈[10-12], 由此带来的氮素损失和土壤酸化风险较高. 因此以咖啡园土壤为典型材料开展硝化作用研究, 通过测定净硝化速率和氨氧化微生物, 阐明云南热区咖啡园土壤硝化作用特征及其关键影响因子, 能为该区域防治土壤酸化提供科学依据, 预期成果还可以为其它类型土壤酸化研究提供参考.

1 材料与方法

1.1 土壤采集区概况 云南热区总面积 81 110 km^2 左右, 占云南省土地总面积的 21%, ≥10 ℃ 年积温 6 000 ℃ 以上, 年平均气温≥17.5 ℃, 年降雨量 600 ~ 2 700 mm, 5—10 月为雨季, 降雨量占全年的 86%, 年日可照时数 1 750 ~ 2 250 h. 土壤类型主要是砖红壤、赤红壤、燥红壤和褐红壤, pH=4.1 ~ 6.5.

云南咖啡产区主要分布在怒江、澜沧江、红河、金沙江等流域海拔 500 ~ 1 800 m 之间的热区, 南部

收稿日期: 2021-04-02; 接受日期: 2021-10-29; 网络出版日期: 2021-11-13
基金项目: 国家自然科学基金(41661071); 国家重点研发计划(2018YFD0201108, 2020YFD1001202).
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与越南、老挝、缅甸接壤,北部抵达金沙江流域. 土壤采集区域设置在保山市潞江坝和德宏州芒市. 保山市潞江坝是南亚热带干热河谷气候,德宏州芒市属于南亚热带季风湿热气候.

1.2 土壤样品采集 2017 年咖啡采摘结束后,选择种植年限 5 ~ 20 a 咖啡园土壤,按 S 形取样法取 10 个点合成 1 个混合样,每块取样地重复采集 3 次,采集深度 0 ~ 20 cm 耕层. 采集的土样存于冰盒中带回实验室,存于 4 ℃ 冷库中备用. 供试土壤基本情况见表 1.

表 1 供试土壤基本信息和理化性质

Tab. 1 Basic information and physicochemical properties of the soil samples

编号	种植年限/a	采样地点	w(有机碳)/(g·kg^{-1})	w(全氮)/(g·kg^{-1})	w(有效磷)/(mg·kg^{-1})	w(速效钾)/(mg·kg^{-1})	pH
A	5	潞江坝(25°2′49″N,98°50′5″E)	13.7±0.70a	0.45±0.07a	123±3.76b	365±0.30b	5.04±0.01d
B	8	潞江坝(25°1′56″N,98°50′26″E)	17.2±1.21b	0.63±0.09ab	213±9.23c	374±20.2b	4.72±0.02b
C	5	芒市(24°32′49″N,98°27′5″E)	30.8±1.36d	1.10±0.09c	91.0±6.69a	159±1.00a	4.44±0.01a
D	6	芒市(24°21′46″N,98°27′5″E)	27.5±0.36b	0.80±0.02b	104±6.28ab	513±28.0d	4.67±0.03b
E	20	芒市(24°21′55″N,98°25′27″E)	19.5±0.06b	0.60±0.02ab	85.3±7.28a	162±3.86a	4.86±0.07c

不同采样点同一列不同字母表示处理间差异达到显著水平(P<0.05)

1.3 土壤硝化作用培养实验 将保存于 4 ℃ 冷库中备用的新鲜土样取出,称取 40 g 置于 250 mL 三角瓶中,调节土壤含水量至 45% 最大持水量,用透气保水膜封口,在 30 ℃ 黑暗条件下预培养 1 周以激活微生物,每个处理重复 3 次. 预培养结束后的土样中加入硫酸铵,铵态氮 (NH^+−N) 添加量为 20 mg·kg^{-1},同时把土壤含水量调至 60% 最大持水量,于 30 ℃ 黑暗条件下继续培养,在第 0、1、2、4、7 d 测定土样中的 NH_4^+−N、硝态氮 (NO_3^-−N) 质量比,其中 0 d 为加入硫酸铵 2 h 后采样,净硝化速率计算见文献 [13].

1.4 土壤性质和 DNA 分析 全氮:凯氏定氮法;有机碳:低温外热重铬酸钾−容量法测定有机质,参考张维理等[14] 计算方法将有机质换算成有机碳;速效钾:乙酸铵提取−火焰光度计法;有效磷:碳酸氢钠浸提−钼蓝比色法;土壤 pH:液土质量比为 5∶1,pH 仪 (inolabpH7300) 测定. 土壤 NH_4^+−N:按液土质量比 5∶1 加入 100 mL 浓度为 2 mol·L^{-1} KCl 浸提,靛酚蓝比色法测定;土壤 NO_3^-−N:紫外分光光度法测定[15];土壤最大持水量:把过 20 目筛(孔径 0.85 mm)的风干土样 20 g 置于用棉球塞住的漏斗中,加去离子水浸泡 2 h,加盖,除去棉塞,让水分自由下渗,放置过夜后,测定土壤质量含水量作为土壤最大持水量.

土壤 DNA 提取及 PCR 扩增及测序:土壤基因组 DNA 提取试剂盒 (天根生化科技有限公司,北京) 提取样本的微生物基因组 DNA. 根据测序的土壤细菌 16S rRNA 基因 V4 区选择特异引物 515F 和 806R 进行 PCR 扩增[16]. PCR 反应体系如下 (50 μL):2×Phusion Master Mix 25 μL, 515F Primer (10 μmol/L) 2.5 μL, 806R Primer (10 μmol/L) 2.5 μL, 1 ng/μL gDNA 10 μL (5-10 ng) , H_2O 10 μL. PCR 反应程序为:98 ℃ 预变性 30 s;98 ℃ 10 s;50 ℃ 30 s;72 ℃ 30 s,循环 30 次;72 ℃ 延伸 5 min. 利用 2% 的琼脂糖胶电泳和 GeneJET 胶回收试剂盒 (Thermo Scientific, USA) 对 PCR 产物进行纯化回收. 随后,PCR 产物送至北京诺禾致源科技股份有限公司进行文库构建和高通量测序(基于 IonS5TMXL 测序平台).

1.5 土壤数据分析 IonS5^{TM}XL 测序数据经过 Cutadapt (v1.9.1) 软件过滤和按 barcode 拆分样本后,利用 Uparse 软件 (v7.0.1001) 进行 OTUs (Operational Taxonomic Units) 聚类和物种分类分析[17]. 根据 OTUs 聚类结果,利用 Silva 数据库[17],对每个 OTU 的代表序列进行物种注释. 同时,对 OTUs 进行基于物种的丰度分布情况分析,根据所有样品在属水平上的物种注释及丰度信息,筛选氨氧化微生物 (AOA 和 AOB) 和亚硝酸盐氧化细菌 (NOB),从物种层面进行 5 个样品聚类,绘制成热图. 热图对应的值为每一行物种相对丰度经过标准化处理后得到的 Z 值,即一个样品在某个分类上的 Z 值为样品在该

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分类上的相对丰度和所有样品在该分类的平均相对丰度的差除以所有样品在该分类上的标准差所得到的值.

利用 SPSS 25.0 进行数据分析, 多重比较采用 Duncan 检验, 相关分析采用 Pearson 相关系数表示, 多元线性回归分析采用后退法, 通径系数即多元线性回归中的标准回归系数.

2　结果与分析

2.1　土壤硝化特征　添加 NH_4^+源后, 除土样 B 外, 其余土样的 NH_4^+–N 质量比在培养期间随时间增加下降, 特别在培养 2 d 后, 土样 A、D 和 E 的 NH_4^+–N 质量比下降较为迅速, 土样 C 则下降幅度较小, 可能是硝化作用受到了抑制. 培养期间, 土样 B 的 NH_4^+–N 质量比增加, 可能与矿化作用较强有关(图 1(a)), 其原因有待进一步研究. 培养初始, 土样 C 的 NO_3^-–N 质量比最高, 且 NO_3^-–N 质量比在培养过程中小幅增加, 其余土样在培养 2 d 内 NO_3^-–N 质量比增幅较快, 培养 2 ~ 4 d 时, NO_3^-–N 质量比增加缓慢, 土样 D 还出现下降趋势, 可能发生了微生物固定. 培养 4 d 后, 除土样 C 外, 其余土样的 NO_3^-–N 质量比又开始增加, 其中, 土样 E 增幅最大 (图 1(b)).

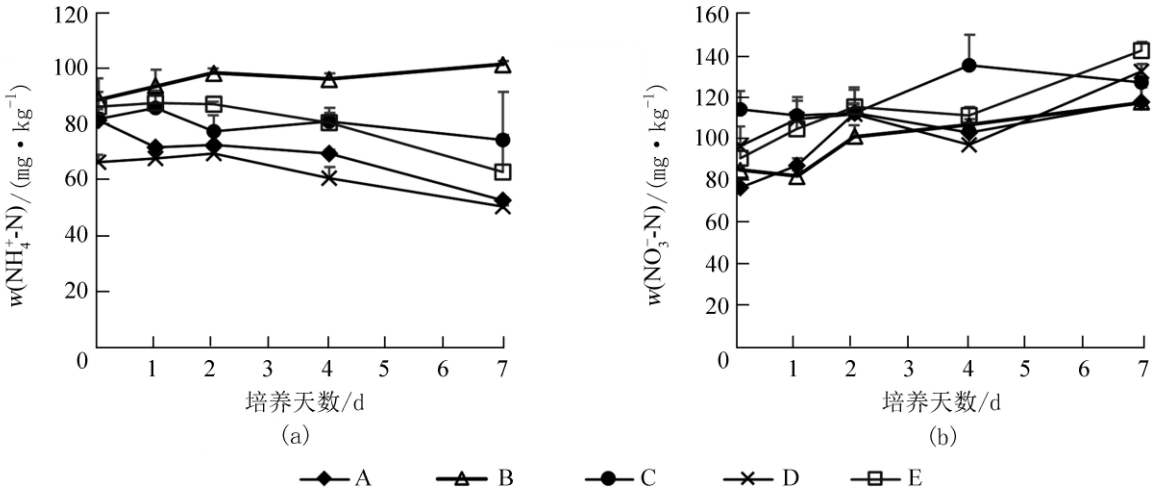


图 1　培养期间 NH_4^+–N (a) 和 NO_3^-–N (b) 质量比动态变化

Fig. 1　Dynamics of soil NH_4^+–N (a) and NO_3^-–N (b) content in the incubation experiment

5 个土样 0 ~ 7 d 净硝化速率如图 2 所示, 5 个土样的净硝化速率大小顺序为 E (7.42 $mg·kg^{-1}·d^{-1}$) > A (5.91 $mg·kg^{-1}·d^{-1}$) >D (5.11 $mg·kg^{-1}·d^{-1}$) >B (4.69 $mg·kg^{-1}·d^{-1}$) >C (1.83 $mg·kg^{-1}·d^{-1}$). 结合表 1 土壤性质, 土样 C 的 pH 值最低, 为 4.44, 说明低 pH 值能抑制土壤硝化作用.

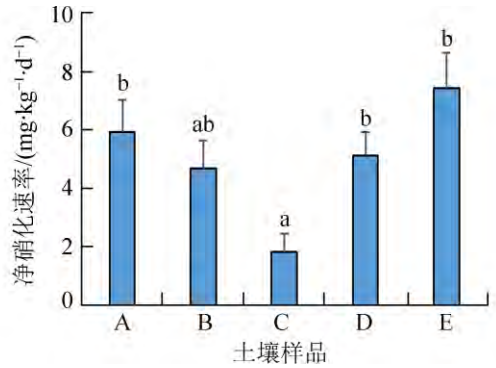


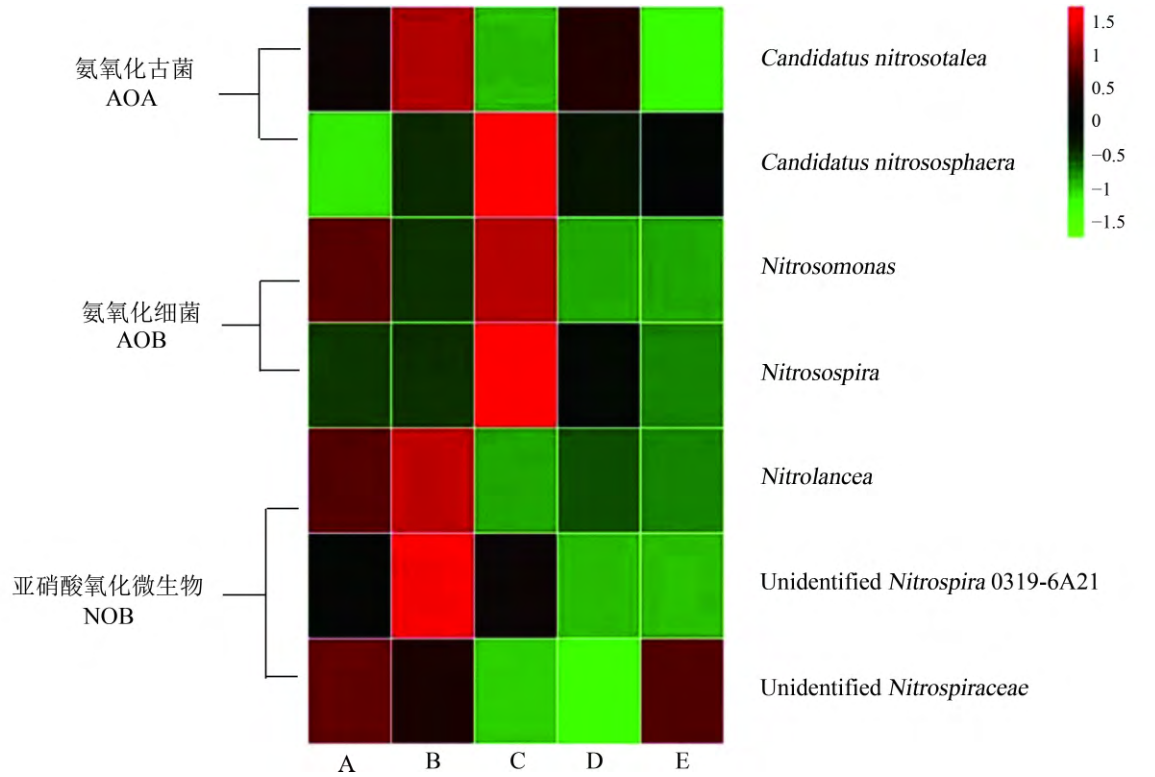
图 2　各供试土样 0～7 d 的净硝化速率

Fig. 2　The net N nitrification rates of the experimented soils from 0 to 7 day

2.2　氨氧化微生物和硝化细菌的相对丰度　利用 5 个咖啡种植地土样的微生物样品 16S rDNA 扩增子测序数据[18], 根据物种注释结果筛选硝化作用相关微生物, 结果发现 5 个咖啡种植地土样是主要由氨氧化古菌(Ammonia-oxidizing archaea, AOA)、氨氧化细菌(Ammonia-oxidizing bacteria, AOB)和亚硝化氧化细菌(Nitrite oxidizing bacteria, NOB)的 7 个属进行硝化作用(图 3). 其中, 氨氧化古菌(AOA)可能是云南热区咖啡种植地土壤中起主要氨氧化作用的微生物[18].

硝化作用相关微生物的相对丰度在不同产区和种植年份均存在明显差异(图 3). 其中, *Candidatus Nitrosotalea* 相对丰度在样点 B 最高, 样点 C 和 E 最低, 样点 A 和 D 居中 (图 4). 从土壤性质来看(表 1), 氨氧化古菌相对丰度与有效磷含量变化一

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横向为样品名，纵向为微生物注释信息，热图对应的值为每一行微生物相对丰度经标准化处理后的 Z 值（由绿到红，相对丰度逐渐升高）

图 3 云南热区咖啡种植地土壤中氨氧化古菌和硝化细菌的相对丰度热图

Fig. 3 Heatmap of relative abundance of ammonia-oxidizing archaea and nitrifying bacteria in coffee-growing regions of Tropical Yunnan

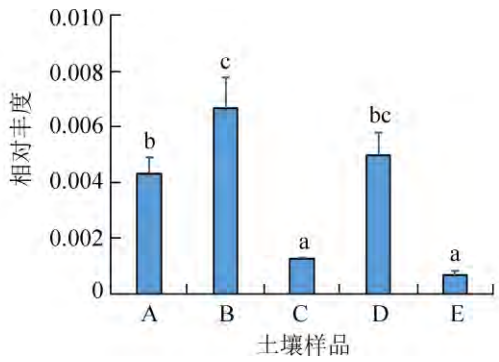


图 4 供试土壤氨氧化古菌 *Candidatus nitrosotalea* 相对丰度

Fig. 4 Relative abundance of *Candidatus nitrosotalea* (group I.1a-associated ammonia-oxidizing archaea) in soil samples

致，并且，有效磷和速效钾含量高的样点 A、B 和 D，其氨氧化古菌相对丰度高于有效磷和速效钾含量的低的样点 C 和 E.

2.3 净硝化速率与土壤性质的关系 从表 2 可知，净硝化速率与土壤 pH 值呈极显著正相关关系（$p<0.01$），说明低 pH 值能抑制硝化作用. 有机碳和全氮与净硝化速率分别呈极显著（$p<0.01$）和显著负相关关系（$p<0.05$），并都与土壤 pH 值呈极显著负相关（$p<0.01$），可能与低 pH 值条件下微生物活性受到抑制，导致碳氮转化速率下降有关. 氨氧化古菌相对丰度与有效磷和速效钾均呈极显著正相关关系（$p<0.01$），因为磷、钾能改善微生物生存的营养环境，最终，氨氧化古菌丰度得以增加. 有机碳与全氮呈极显著正相关（$p<0.01$），有机氮一般占全氮的 98% 以上，土壤氮的含量与供应取决于有机质的积累和分解.

将净硝化速率与土壤性质作逐步回归分析，得到 $y = -0.004\ x_1 + 6.225\ x_2 - 26.180$ ($r^2 = 0.776$)，x_1 为速效钾，x_2 为 pH 值，通径系数为 x_2 (0.891) > x_1 (0.359)，对净硝化速率影响最大的土壤因子是土壤 pH 值，两者呈正相关关系.

2.4 土壤酸度变化 供试土样在硝化培养期间的 pH 值变化见表 3. 培养 1 d 时，一部分略微升高 (土样 A、B 和 C)，另一部分土样 pH 值保持不变 (土样 D 和 E). 培养 2 d 时，土样 A、B 和 C 的 pH 值下降，土样 D 和 E 则小幅升高或基本不变. 培养 4 d 时，除土样 C 的 pH 值增加外，其余土样的 pH 值在

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表 2　净硝化速率与土壤性质的相关性

Tab. 2　Correlation between net nitrification rate and physicochemical properties with soil samples

项目	净硝化速率	*Candidatus Nitrosotalea* 相对丰度	*w*(有机碳)	*w*(全氮)	*w*(有效磷)	*w*(速效钾)	pH
净硝化速率	1	−0.17	−0.67**	−0.64*	−0.21	−0.15	0.81**
*Candidatus Nitrosotalea*相对丰度		1	−0.30	−0.35	0.73**	0.81**	0.20
w(有机碳)			1	0.83**	−0.46	−0.13	−0.85**
w(全氮)				1	−0.31	−0.28	−0.88**
w(有效磷)					1	0.37	0.10
w(速效钾)						1	0.23
pH							1

*表示相关达到显著水平(P<0.05), **表示相关达到极显著水平(P<0.01).

下降. 至培养 7 d 时, 全部土样的 pH 值都有所降低. 整个培养期间内, 5 个土样 pH 值均下降, 幅度分别为 0.20、0.22、0.05、0.04 和 0.14. 结合图 2 来看, 除土样 D 外, 有较高净硝化速率的土样 A (5.91 mg·kg^{-1}·d^{-1})、B (4.69 mg·kg^{-1}·d^{-1}) 和 E (7.42 mg·kg^{-1}·d^{-1}), 其 ΔpH 分别为 0.20、0.22 和 0.14, 显著高于净硝化速率低的土样 C (1.83 mg·kg^{-1}·d^{-1}) 的 0.05. 土样 D 的净硝化速率为 5.11 mg·kg^{-1}·d^{-1}, 但 ΔpH 仅为 0.04, 说明其对酸的缓冲能力较强. 结合表 1, 土样 D 速效钾含量极高, K^+是土壤盐基离子组分之一, 可能其较高的缓冲性能缓解了土壤酸化, 具体原因有待进一步研究.

表 3　供试土壤培养期间 pH 值变化特征

Tab. 3　Dynamics of pH values of the soil samples during the incubation experiment

编号	土壤pH					ΔpH[a]
	0 d	1 d	2 d	4 d	7 d	
A	5.24±0.05	5.45±0.03	5.36±0.03	5.32±0.02	5.04±0.01	0.20±0.05b[b]
B	4.95±0.04	4.99±0.02	4.92±0.03	4.93±0.02	4.73±0.03	0.22±0.05b
C	4.69±0.04	4.77±0.02	4.66±0.04	4.75±0.01	4.64±0.01	0.05±0.04a
D	4.51±0.02	4.50±0.06	4.56±0.03	4.53±0.01	4.47±0.01	0.04±0.02a
E	4.60±0.01	4.62±0.02	4.61±0.02	4.59±0.01	4.47±0.01	0.14±0.01ab

[a]: ΔpH表示第7天土壤pH与第0天土壤pH之差; 同一列不同字母表示处理间差异达到显著水平(P<0.05).

3　讨论

土壤 pH 值是影响硝化作用的重要因子. 一般认为低 pH 值能抑制土壤硝化作用[19-20], 但 pH> 4.5 的酸性土壤硝化速率明显增强[21]. 本试验条件下, 初始 pH 值在 4.67 ~ 5.04 的土样(土样 A、B、D 和 E), 净硝化速率显著高于初始 pH 值 4.44 的土样(土样 C), 通径分析表明土壤 pH 是影响净硝化速率最主要的因素, 与前人研究结果一致[13, 22]. 酸性土壤硝化活性提高的主要原因是长期施用氮肥和有机肥, 由于 NH_3 或 NH_4^+硝化成 NO_3^-会在其第一个反应步骤, 即氨氧化过程中产生 2 个 H^+, 硝化作用增强, 加速了土壤酸化[5-6, 23]. 本试验条件下, 硝化培养结束时, 净硝化速率高的土样 pH 值下降 0.14 ~ 0.22, 显著高于净硝化速率低的土样的 0.05, 与前人的报道的 pH=4.34 ~ 5.14 的土壤其酸化速率与硝化速率呈显著线性关系一致[24-25].

盐基离子淋溶是土壤酸化的重要原因[26], 盐基离子的组成和数量是 pH 缓冲容量增加的主要机制[27-28], 如果盐基离子与致酸离子(交换性 H^+、

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Al^{3+})发生交换反应,潜性酸含量可能下降,利于减缓酸化. 本试验条件下,净硝化速率较高的土样 D,硝化培养结束时,pH 仅下降 0.04,无酸化现象,推测与其速效钾含量较高有关. 研究表明,土壤 pH 值与交换性盐基含量呈显著正相关关系[29-30],土壤交换性酸的减少与交换性盐基的释放呈线性关系[31-32],由于缺乏更详细的结果,需进一步加强盐基离子与土壤酸化方面的研究.

氨氧化过程主要由氨氧化细菌(AOB)和氨氧化古菌(AOA)驱动. 酸性红壤中 AOA 比 AOB 与硝化作用的关系更为密切[33-34],并且有了 AOA 是酸性土壤活性氨氧化微生物的直接证据[35]. 本试验条件下,在属水平上的细菌群落的物种丰度聚类分析表明,相对丰度排名前 35 的属,仅发现 AOA Group I.1 a-associated(*Candidatus Nitrosotalea*),AOB 的排名在 80 位以后,说明低 pH 土壤中 AOA 数量占主导优势. 尽管本试验条件下 AOA 相对丰度与净硝化速率没有关系,但给出了中等酸度土壤(pH=4.44 ~ 5.04)AOA 数量高于 AOB 的证据.

4 结论

土壤 pH 是影响咖啡园酸性土壤硝化作用的重要因子,低 pH 值能抑制土壤硝化作用,土壤 pH 值与净硝化速率呈正相关关系,净硝化速率高的土壤酸化明显. 咖啡园酸性土壤 AOA 相对丰度高于 AOB. 在净硝化速率高的土壤中存在酸化不明显现象,通过调控盐基离子增加土壤对酸的缓冲能力的作用机制和实践需要进一步研究.

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Research on nitrification of coffee planting field soils

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Abstract: Soil nitrification was studied with the coffee planting soils from Mangshi and Lujiangba, major producing area of Yunnan Province. To study nitrification and influencing factors, 5 coffee planting soil samples were collected. An incubation experiment was conducted to analyze the effects of soil properties and characteristics of ammonia oxidation microbial on nitrification. The result showed that the net N nitrification rates were 1.83−7.42 $mg \cdot kg^{-1} \cdot d^{-1}$ in a 0−7 days incubation experiment. The soil pH value was important factor for the net nitrification rates, which were positively correlated with pH ($p<0.01$). Higher net N nitrification rates reduced soil pH values and were responsible for the soil acidification in experimented acid soils. In the test soil, numbers were noticeably higher for ammonia-oxidizing archaea than for ammonia-oxidizing bacteria, suggesting that the relative abundance of *Candidatus nitrosotalea* (group I.1a-associated ammonia-oxidizing archaea) was the highest in all soil ammonia oxidizing microorganisms and nitrite oxidizing bacteria. There was no significant correction between relative abundance of *Candidatus nitrosotalea* and net N nitrification rates, while net nitrification rates were positively with the olsen-P ($p<0.01$) and available-K ($p<0.01$). As result of strong nitrification with high pH value, ammonium nitrogen is nitrified into nitrate nitrogen, which is readily accumulated and/or soil acidification. It is, therefore, essential to regulate soil buffer capacity to a proper level so as to achieve the target of constructing a nitrogen nutrient pool and reducing.

Key words: coffee planting soil; net nitrification rate; ammonia-oxidizing archaea; ammonia-oxidizing bacteria

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中国农业科技导报，2021，23(10)：15-24
Journal of Agricultural Science and Technology

玉米叶夹角的遗传与分子调控研究进展

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摘　要：培育高产品种是玉米育种的主要目标之一，而叶夹角是株型选择进而提高产量的重要指标，对叶夹角的形成及相关基因的克隆和分子机理解析是开展耐密高产玉米品种选育的基础。从叶夹角对玉米生长发育的影响，叶夹角的形态建成、遗传基础、分子调控机制及 QTL 定位和基因克隆等方面归纳了国内外对玉米叶夹角的遗传与分子调控研究进展，总结现有研究存在的问题，初步提出未来玉米叶夹角的主要研究方向，为利用分子标记辅助选择培育耐密型品种，提高玉米产量潜力提供信息参考。

关键词：玉米；叶夹角；形态建成；调控机制；QTL 定位

doi：10.13304/j.nykjdb.2021.0121

中图分类号：S513　　**文献标识码**：A　　**文章编号**：1008-0864(2021)10-0015-10

Advances in Genetic Researches and Molecular Regulation Mechanism of Leaf Angle in Maize

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Abstract: The major goal of maize breeding is to develop high yielding hybrids for various agro-ecological and socio-economic conditions. The leaf angle plays an important role in maize plant architecture. Understanding the formation of leaf angle, gene cloning and molecular mechanism controlling leaf angles are essential for the development of maize hybrids with high-yielding and tolerance to high density. This article summarized the progress on morphological formation, genetic basis, regulatory mechanism, and QTL mapping and gene cloning of the leaf angle in maize. The unexplained genetic and biochemical bases were clarified and some suggestions for the investigation in genetics and molecular regulation mechanism of leaf angle of maize in the future were provided, which were useful for the development of hybrids with tolerance to high density via marker-assisted selection to improve the yield potential in maize breeding program.

Key words: *Zea mays* L.; leaf angle; morphology formation of plant type; regulatory mechanism; QTL mapping

玉米(*Zea mays* L.)是世界上广泛种植的作物，也是我国种植面积较大的粮食作物。高产是我国玉米育种的首要目标，提高单产是增加作物总产量的主要方式，加大种植密度是提高单产的重要方法，而选育株型紧凑的高产杂交种进行合理密植是提高玉米产量最经济有效的措施之一。Mock 和 Pearce[1]最早提出玉米理想株型及具体指标，随后理想株型育种成为玉米育种的重要研究方向，先后育成了一系列株型紧凑、耐密性强的玉米品种，使种植密度逐渐提高，单产水平不断提升。美国玉米主产区的种植密度在 20 世纪 30 年代仅为 30 000 株·hm^{-2}，而目前已提升到90 000 株·hm^{-2}以上，耐密品种的选育和推广是美国玉米产量稳居世界前列的重要原因。随着耐密品种(先锋公司 P1197YHR)的选育以及保护性耕作等栽培技术的应用，玉米创下了 41 340 kg·hm^{-2}的高产记录(美国国家玉米种植协会，National Corn Growers Association，NCGA)。我国掖单 2 号、掖单

收稿日期：2021-02-02；**接受日期**：2021-04-11

基金项目：国家自然科学基金项目(31661143004，U1602261 和 31961143014)；国家重点研发计划项目(2016YFD0101803)；云南大学研究生科研创新基金项目(201915)。

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13号、DH3719、郑单958等耐密品种的成功选育为玉米高产育种提供了新途径，显著推动了我国现代玉米育种进步[3-4]。最近，Tian等[2]从玉米野生祖先种大刍草中克隆了控制玉米紧凑株型、密植增产的关键基因，建立了玉米紧凑株型的分子调控网络，揭示了玉米密植株型的分子调控机制。

耐密优良品种的选育是解决我国耕地面积减少与玉米供需矛盾的有效途径。叶夹角作为耐密品种的重要外在形态指标，直接决定了玉米在高密度种植条件下对避荫综合症的抵抗能力，对于改善群体冠层结构，增强光能的利用率，减小避荫综合症的诱发因素，促进光合产物在源、流、库之间的高效合理运转，增加光合产物的积累和提高产量具有重要的作用。本文从叶夹角对玉米株型、光合作用、种植密度与产量的影响，以及叶夹角的形态建成、遗传、分子调控机制、QTL定位与基因克隆研究等方面综述了叶夹角形成的分子机理和遗传研究进展，分析了当前研究存在的问题，并提出了未来玉米叶夹角研究的主要方向，为耐密理想株型分子标记辅助选择提供理论依据，在玉米高密度育种及株型育种中也具有重要的实际应用价值。

1 叶夹角对玉米生长发育的影响

1.1 叶夹角对玉米株型的影响

叶夹角即叶片与主茎之间的夹角，是决定玉米株型的主要因素[5]。根据叶夹角的差异，玉米株型分为平展型、紧凑型和中间型。平展型玉米植株高大、叶片宽大，穗位以上叶片与主茎之间的夹角大于45°，叶片平伸、顶尖下垂，单株生产潜力高，不耐密植；紧凑型玉米植株稍小、叶片窄小，穗位以上叶片与主茎之间的夹角小于30°，叶片直立向上，单株生产潜力低、耐密植，群体生产潜力高；中间型玉米介于紧凑型和平展型之间，穗位以上叶片与主茎之间的夹角在30°~45°之间。其中紧凑型玉米植株叶片直立向上，冠层截光能力低，群体透光率高，有利于中下部叶片获得更多光照，使叶片高效地利用光能，进而提高植株净光合速率和群体生产力，增加种植密度。最早培育出的耐密玉米杂交种先锋3306，其显著的特点是冠层叶片直立、与地面间的夹角逐渐增大，因此能够有效截获光合辐射[6-7]，进一步模拟试验和田间试验均表明，冠层叶片直立的杂交种都具有显著的产量优势，说明合理的叶片结构分布对冠层形态有重要决定作用。在高密度种植条件下，紧凑植株具有最大的光截获叶面积指数，能够有效地促进籽粒灌浆中的氮素积累，因此产量显著高于平展型植株。由于较小的叶夹角有利于降低冠层截光率，提高群体光能利用率和群体种植密度，从而提高产量，同时为了适应高密度的种植，叶夹角也变得更加直立[2]。当群体冠层随种植密度增加变得拥挤时，直立的叶子可以通过减少遮荫和保持光截获效率以最大限度地提高光合作用，实现高密度种植条件下的高产目标，因此较小的叶夹角是玉米育种最为重要的靶标。

1.2 叶夹角对玉米光合作用的影响

叶长、叶宽、叶夹角和叶片形态是决定玉米株型紧凑程度的主要性状，直接影响玉米群体冠层截光能力和群体光能利用率[6,8]。当叶面积指数最高，叶夹角接近90°时，光合作用最强，光能利用率最高。由于高产品种的株高较低、叶夹角较小、穗上叶较少，叶向值、叶面积指数和单位面积内种植群体数量较大，因此，在高密度种植条件下，中下层叶片也能获得光照，增加产量。黄收兵等[9]提出，株型紧凑、穗下叶层间距小、穗位低、叶夹角小等性状为理想株型，其具有冠层叶片排列紧密、透光率和光能利用率高等优势，因此有助于增加种植密度，提高产量。上述研究结果一致表明，减小叶夹角是构建理想株型、提高产量的重要途径之一。

1.3 叶夹角对玉米种植密度与产量的影响

叶夹角对玉米种植密度有重要决定作用。Pendleton等[7]比较C103×Hy组合近等基因系的产量差异，结果表明，携带*liguleless2*(*lg2*)基因的近等基因系叶片直立，具有较好的冠层结构以及较高的光能利用率，产量比其他近等基因系高41.2%，此外将平展型品种先锋3306改良为叶片直立的紧凑型品种，其光截获效率和产量均显著增加。随后Lambert和Johnson[6]用B14×h43和Oh43×R177的RILs比较普通重组系(叶夹角35°)、*liguleless 1*(*lg1*)基因型和*lg2*基因型玉米的产量，结果表明，在75 000和90 000株·hm^{-2}的高密度种植下，*lg2*基因型的产量显著高于普通重

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组系,说明叶夹角与玉米种植密度及产量密切相关。*lg1* 基因型叶片直立,为高密度种植的理想株型,而 *lg2* 叶夹角较 *lg1* 大,但其光截获效率高,因此也具有较高的产量。李登海等[10]将平展型玉米叶片改造成紧凑型,产量增加 21%,而将紧凑型品种改造成平展型,产量减少 20%,并且紧凑型品种 DH3719 于 2005 年以 105 000 株•hm^{-2} 的种植密度创下了 21 042 kg•hm^{-2}的夏玉米高产纪录,较掖单 13 号 1989 年创造的 16 444 kg•hm^{-2} 的世界夏玉米纪录超出4 584 kg•hm^{-2},将单产纪录提高了 27.96%。近几十年玉米株型育种取得显著成效,与 20 世纪中早期育成品种相比,现代玉米杂交种叶片直立且叶面积较大,光截获效率增加了 14%,增产 20%左右。此外,Ku 等[8]提出,叶面积指数大于 3,上部叶片直立为玉米最佳株型组合类型。随着具有直立叶的自交系 B73 在杂交种选育中的广泛利用,玉米杂交种的叶夹角逐年下降,特别是 34N44、P1197YHR 等叶夹角小、耐密品种的成功选育及推广应用为玉米增产做出了重要贡献,说明减小叶夹角或降低叶向值是通过株型改良提高玉米产量的重要途径。

2　叶夹角的形成机制

2.1　叶夹角形态建成

叶片发育起始于茎顶端分生组织(stem apical meristem,SAM)周边的初始细胞,包括叶原基的形成和极性轴向的建立。叶原基由顶端分生组织中心“干细胞”在生长素诱导下形成成簇分布的外缘叶原初生细胞,然后在遗传背景和环境的互作效应下,叶原基建立基-顶轴(叶的基部-顶端)、腹-背轴(茎的近轴面-远轴面)、中-边轴(叶的主脉-边缘)3 个极性轴向,该过程为叶片发育和形态建成的关键时期,其中叶夹角主要由叶片与叶鞘之间的叶环所调控,叶环包括叶舌与叶耳,其形成发生在基-顶轴的极性建立过程中[11]。叶夹角与叶耳及中脉的机械支撑强度有关[12],因此,解析叶环发育基因的调控机制有助于从分子水平阐明叶夹角形成的遗传基础。

2.2　叶夹角遗传研究

叶夹角的广义和狭义遗传力均较高,并且以加性效应为主,同时受非加性效应的影响。蔡一林等[13]认为,叶夹角的遗传符合加性遗传模型,因此以加性效应为主,也有研究显示,叶夹角由多基因或多位点控制,并且受非加性基因的影响[5]。Chen 等[14]采用 Griffing Ⅱ双列杂交进行研究,发现叶夹角的遗传符合加性-显性-上位性模型。也有研究表明,叶夹角主要由加性效应和母本效应所决定。赖仲铭等[15]发现,在叶夹角遗传效应中,一般配合力作用较大,而特殊配合力的影响较小,说明玉米叶夹角的杂种优势不突出。综上所述,叶夹角的遗传效应以加性效应为主,但不同材料间由于遗传背景的影响,可能存在显性、上位性及母本效应,因此可通过遗传育种,选择叶夹角较小的植株,通过株型改良提高玉米产量。

3　叶夹角形成的分子调控机制

3.1　叶夹角形态发育的调控机制

近年来,国内外对玉米叶片形态发育相关基因的调控进行了较多研究。*KNOX* 基因家族 *rs1* 位于顶端分生组织处,维持细胞分化,其中 *Knotted-1*(*Kn1*)基因是首个从植物中发现的 *KNOX1* 亚基因家族,其与 *Knox7* 同属于 KNOX 家族,在顶端分生组织特异性表达,调控细胞分化向膨大生长的转变,形成特定的叶向,此外在基-顶轴的极性形成过程中,*Kn1* 通常与 *lg3*、*lg4*、*Knox8* 相互作用进而调控叶片发育[16]。研究表明,KNOX 还能促进细胞分裂素(CTK)合成基因上调表达,抑制 GA3 和 GA20 氧化酶合成,可以促进 SAM 细胞分裂形成叶原基,并且增加 GA 的含量有利于解除 DELLA 对叶片发育的抑制作用[16]。*lg3* 和 *lg4* 基因半显性突变主要在叶鞘中表达,但是 *lg3* 隐性突变对叶片发育的影响较小,可能原因是 *lg3* 与 *KNOX* 基因家族存在一定的功能冗余[17]。此外,TCP 家族的 *Wab1* 基因的显性突变会造成异位表达,导致叶舌缺失,进而影响基-顶轴极性的建立[18]。

lg1、*lg2* 和 *lgn* 对叶舌及叶耳的发育具有调控作用。Johnston 等[19]利用 RNA-seq 技术对突变体 *lg1* 和野生型的第六和第七叶原基、上部叶片及下部叶鞘进行比较,共检测到 96 个差异表达基因,其中 34 个差异表达基因在野生型中上调表达,而在 *lg1* 突变体中下调表达。进一步研究发现,在叶夹角的形态建成中,*lg1* 基因首先激活

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PIN1a 在叶环区的表达,调控生长素和叶片-鞘边界基因如 *CUC2* 和 *lg3* 之间相互作用,阻碍边界基因的细胞表达进而形成叶舌。在边界分化过程中,*lg3* 与 *BEL14* 或 *BEL12* 对叶舌细胞分裂有互作效应,而 PIN1a 蛋白介导的生长素促进了边界细胞的分裂形成叶舌。最近,Tian 等[2] 克隆了两个控制玉米紧凑株型、密植增产的关键基因 *UPA1*(*upright plant architecture1*) 和 *UPA2*(*upright plant architecture2*),其中 *UPA2* 的功能变异是由于 2 bp 核苷酸插入/缺失,该插入/缺失为顺式调控元件的突变进而调控下游 9.5 kb 的 B3 转录因子 *ZmRAVL1* 的表达,UPA1 主要调控 BRs 合成途径中 *brd1* 基因的表达。进一步功能分析发现,大刍草 *UPA2* 基因与玉米叶片发育基因 *DRL1* 的结合能力更强,而 *DRL1* 可与玉米无叶舌基因 *lg1* 互作进而抑制 *lg1* 对 *ZmRAVL1* 的激活,引起下游 *brd1* 基因的表达下调,从而降低叶环处内源 BRs 表达水平,影响叶耳细胞的增殖,最终形成较小的叶夹角。

3.2 叶夹角形成的激素调节机制

叶夹角的形态建成与植物激素特别是油菜素内酯(brassinosteroids, BRs)、生长素(auxin, IAA) 和赤霉素(gibberellin, GA) 密切相关。在玉米、水稻以及高粱中,国内外鉴定出大量的 BRs 合成、信号传递相关基因,其中参与 BRs 信号传导的基因突变体大多表现为叶夹角较小。Makarevitch 等[20] 研究油菜素内酯 C-6 氧化酶对玉米生长发育的影响发现,细胞色素 P450 家族 *Brd1* 基因的无义突变会影响 BRs 的合成,引起植株矮化、叶片呈波浪状扭曲、叶舌变小、叶耳增大,并且叶耳与叶片的边界消失,进而影响叶夹角的大小。Best 等[21] 鉴定出 BRs 合成途径的另一个基因 *na2*,该基因突变同样影响 BRs 的合成,导致株高矮化、叶片形态化及叶夹角变大。此外,玉米 2 号染色体上 *qLA2* 的候选基因 *ZmILI1*,通过与下游基因 *lg1* 的启动子结合后激活 *lg1* 的表达,从而调控叶夹角。此外 *ZmILI1* 和 *CYP90D1* 形成负调控,维持玉米中 BRs 的平衡[22]。通过 RNAi 干扰 BRs 受体的同源基因表达,转基因植株更绿、株型更紧凑且植株形态扭曲,叶舌与叶耳均变小。上述研究表明,BRs 对叶夹角的形成具有重要调控作用。

IAA 也参与调控玉米叶夹角的形成和发育,其作用机制较复杂。Moon 等[23] 通过生长素定向运输蛋白的荧光成像分析,发现生长素在叶片-叶鞘边界的叶舌形成部位累积,说明生长素与叶舌的形成有关。此外叶夹角基因 *LAZY1* 通过调节生长素的极性运输,进而影响细胞发育使叶夹角发生改变[24-25]。Fellner 等[26] 比较现代玉米品种 3394 与早期育成品种 307 对生长素的响应情况发现,现代品种 3394 的叶夹角更小,其对生长素敏感性减弱,因此品种的适应性性更强,主要原因是 3394 的叶夹角较小,生长素结合蛋白基因 *ABP4* 的表达量随之下降,因此对生长素的敏感性减弱。

赤霉素对叶夹角的形态建成有一定影响。Kong 等[12] 对自交系 B73 和 986 进行比较,从叶夹角较小的 986 中鉴定出 7 个与细胞分裂素(CK) 降解相关基因、19 个乙烯(ETH) 和 23 个 GA 信号传导相关基因,这些基因调控细胞分裂并促进细胞的伸长,进而影响叶夹角的形成。Best 等[21] 研究表明,GA 突变体、BRs 突变体以及 GA-BR 双突变体与普通植株间株高、叶夹角、分蘖以及育性均存在显著差异,特别是两种激素的双突变体株型与育性均有较大差异,说明 GA 和 BRs 基因间的互作可能对叶夹角的形成有重要调控作用。

4 叶夹角的 QTL 定位与基因克隆研究

4.1 叶夹角的 QTL 定位研究

连锁分析和关联分析是研究农作物复杂数量性状的主要方法,国内外对玉米叶夹角相关 QTL 进行了大量的研究(表 1)。Mickelson 等[27] 最早对叶夹角进行研究,用 B73 和 Mo17 构建重组自交系群体(RILs),采用复合区间作图法在两个试点共检测到 9 个叶夹角 QTL,其中位于第 1 和 7 号染色体上的主效 QTL 分别解释表型变异的 20.6%和 27.7%。Guo 等[3] 用 10 个不同自交系作为供体亲本,以农系 531 为受体亲本构建染色体片段导入系,鉴定出 4 个叶夹角发育相关 QTL,其中位于第 2、9 及 10 号染色体上 QTL 分别解释了 12.7%、14.1%和 26.5%的表型变异。Tian 等[5] 利用 5 000 份 RILs 组成的巢式关联作图(nested association mapping,NAM) 群体,在 9 个环境下进行表型鉴定,通过玉米全基因组关联分析,从 160

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万个遗传位点鉴定叶舌基因的变异类型及叶夹角 QTL/基因,共检测到 30 个叶夹角 QTL,解释总遗传变异的 74.8%。Li 等[49] 利用 GBS(genotyping by-sequencing) 简化测序技术分析 3 个 RILs 群体的基因型,在 6 个环境下对 3 个株型叶夹角相关性状进行分析,共检测到 17 个叶夹角 QTL 位点,

表 1　玉米叶夹角 QTL 定位汇总

Table 1　QTL summary of leaf angle in maize

组合名称 Cross	群体类型 Type of population	群体大小 Population size	QTLs 数目 No. of QTLs	贡献率 Phenotypic variation/%	参考文献 Reference
B73×莫 17 B73×Mo17	S_5 RILs	180	10	31.2~39.2	[27]
H21×莫 17 和自 330×K36 H21×Mo17/Zi330×K36	$F_{2:3}$ RILs	120/114	9	8.4~16.8	[28]
掖 478×丹 340 Ye 478×Dan 340	$F_{2:3}$ RILs	397	6	2.9~11.2	[1]
豫 82×沈 137 Yu 82×Shen 137	$F_{2:3}$ RILs	299	3	7.3~20.4	[8]
B73 和莫 17 等 B73/Mo17 etc.	NAM	~5 000	30	2.9~19.6	[5]
豫 82×豫 87-1 Yu 82×Yu 87-1	$F_{2:3}$ RILs	256	5	7.3~18.4	[29]
B73×莫 17 B73×Mo17	RILs	93	3	10.4~16.4	[30]
郑 58、87-1、PH6WC 和自 330 Z58/87-1//PH6WC/Zi 330	CP	228	13	5.1~20.0	[31]
B73 等 B73 etc.	RILs/DH	320	12	—	[32]
D132×豫 82 D132×Yu 82	BC_5F_{1-2}, BC_3F_3, BC_4F_{2-3}	10 628	1	57.6	[24]
CY5×YL106	$F_{2:3}$ RILs	144	11	6.2~31.9	[14]
D276、D72、A188 和交 51 D276, D72, A188, Jiao 51	F_1	277	14	2.3~7.8	[33]
R08×掖 478 R08×Ye 478	$F_{2:3}$ RILs	266	14	1.3~13.0	[34]
获白×黄早四、旅 28×黄早四等 HUOBAI×HZSHZS, YU28×HZS, etc.	F_7 RILs	183/172/183	17	2.0~11.4	[35]
许 178×K12 Xu 178×K12	F_7 RILs	150	34	5.0~22.8	[36]
豫 82、豫 87-1、综 3 等 Yu 82, Yu 87-1, Zong 3, etc.	RILs	215/223/ 208/212	21	5.0~25.6	[37]
B73×By804 等 B73×By804, etc.	ROAM	~1 880	149	4.3~23.8	[38]
郑 58×HD568 Zheng 58×HD568	F_{10}RILs	220	17	3.0~16.5	[39]
B73×BY804	RILs	196	24	—	[40]
廊黄×TS141、昌 7-2×TS141 Langhuang×TS141, Chang 7-2×TS141	$F_2/F_{2:3}$	202/218	33	3.6~20.6	[41]
B73×川农 1212 B73×SICAU1212	RILs	199	56	0.4~20.1	[42]
莫 17×X26-4 Mo 17×X26-4	BC_2F_5	191	2	9.2~10.9	[43]
B73×PHW30、莫 17×PHW30 B73×PHW30, Mo 17×PHW30	$F_2/F_{2:3}$	125/125	12	7.5~17.2	[44]
郑 683-1×ZPH1388 或 ZPH5 或东 237/或昌 7-2 Zheng 683-1×ZPH1388 or ZPH5 or Dong 237/Chang 7-2	BC_3F_5	53/53/48/61	14	6.5~28.6	[45]
B73×郑 58 B73×Zheng 58	$F_{3:4}$ RILs	165	8	4.3~14.2	[46]
P014×E1312	RILs	179	9	3.2~22.8	[47]
廊黄×TS141、昌 7-2×TS141 Langhuang×TS141, Chang 7-2×TS141	$F_2/F_{2:3}$	202/218	27	4.3~21.4	[48]

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能够解释表型变异的2.0%~11.4%。李威亚[43]以玉米大刍草渗入系BC_2F_5群体为研究材料，在第2染色体定位到两个叶夹角QTL(*qLA2-1*和*qLA2-2*)，分别解释表型变异的9.2%和10.9%，进一步研究发现，这两个叶夹角QTL来自大刍草。郭书磊等[50]利用Meta-QTL统计方法对不同实验、不同群体的株型相关QTL进行整合，在已报道的592个QTL中，鉴定出17个一致性叶夹角QTL，显著缩小了QTL置信区间，提高了QTL的定位精度。Dzievit等[44]通过单个群体或跨群体的联合连锁分析，检测到495个株型相关QTL，能解释表型变异的0.4%~85.1%。进一步通过Meta-QTL分析了325个非重叠QTL，找到58个真实性叶夹角QTL，分布在玉米的10条染色体上，其中叶夹角相关的热点区域主要分布在1、2、3、7和10染色体上。

上述研究表明，玉米RILs群体、NAM群体、大刍草群体及SSR标记、SNP标记、GBS技术等均广泛用于叶夹角的QTL定位研究。国内外学者对现有QTL进行整合分析，明确了研究目标的重点区域与选择范围，相关结果为叶夹角基因的挖掘和克隆提供了重要信息。然而不同研究选用的材料不同，定位到的QTL存在显著差异，说明叶夹角QTL受遗传背景的影响较大，未来仍需利用不同材料定位新的叶夹角QTL。此外，现有叶夹角QTL定位的研究材料均为温带玉米种质，温带材料间遗传差异较小，其所构建的群体分离模糊，所报道的玉米叶夹角QTL效应普遍较小，不够稳定。此外，遗传群体与育种群体的不一致更加限制了遗传分析结果的育种价值。

4.2 玉米叶夹角相关基因的克隆

目前已克隆的玉米叶夹角相关基因有12个(表2)。Schneeberger等[51]最早利用转座子诱变法克隆了KNOX家族基因*rsl*，该基因编码RS1蛋白，与KN1结构域高度同源，*rsl*基因在顶端分生组织中周期性表达，并且主要在顶端分生组织叶片形成的叶环区以及花序和花分生组织形成的区域表达，时空表达分析显示*rsl*基因影响基部-末端轴向的形成过程。与野生型相比，*rsl*基因在突变体植株的叶原基中异位表达，影响叶舌和叶鞘的形成进而决定叶夹角的大小。随后，用相同的转座因子标签法克隆了*lg1*、*lg2*、*lg3*和*lgn*基因。Ku等[54]用豫82×沈137的$F_{2:3}$家系，图位克隆了叶夹角基因*ZmTAC1*，其位于2号染色体*qLA2*区域，与水稻*OsTAC1*基因高度同源，编码263个氨基酸组成的功能未知蛋白。RT-PCR分析发现，*ZmTAC1*在叶鞘中的表达量最高，叶片和茎尖中较低。进一步研究发现，在紧凑型自交系豫82中，5'-UTR端序列为“CTCC”，在平展型沈137中，5'-UTR端为“CCCC”，该序列差异引起*ZmTAC1*基因在不同品种之间的不同表达，从而影响叶夹角的大小。此后，用相同的方法图位克隆了*ZmCLA4*、*nana plant2*、*ZmILI1*、*UPA2/UPA1*、*ZmIBH1-1*等叶夹角基因。

玉米叶夹角基因的克隆为解析玉米叶夹角的遗传基础提供了新思路和新方法，最近基于CRISPR/Cas9系统，利用RNA介导的基因组定向编辑技术，对玉米自交系的*lg1*基因进行编辑并成功筛选到突变体[56]，为利用*lg1*基因改变叶夹角的大小开辟了新途径。但叶夹角性状由多基因调控，调控方式比较复杂，单基因在表型控制方面贡献效率较低，目前成功用于玉米新品种选育的叶夹角基因仅见于*lg1*和*lg2*。因此，在玉米叶夹角的发育调控研究中，亟待开展关键基因之间的网络调控研究，为玉米株型分子育种提供理论依据与技术支撑。

5 展望

我国玉米消费量的增长不可逆转，提高单产的压力越来越大，充分发掘玉米遗传资源的潜在优势，在育种技术升级的基础上，培育高产高效的玉米新品种，提高单位面积的产量是保障我国粮食安全的战略措施。近年来，株型育种已成为玉米遗传育种研究的重点领域，玉米叶夹角对高密度种植及产量具有决定作用，因此对调控叶夹角发育的机理及遗传研究已成为我国玉米研究的热点。

国内外关于玉米株型形态及分子调控机理的大量研究认为，植株中层的叶片大而宽，能截获更多的光能，光合作用强，叶绿素含量比上层和下层的含量高，尤其是在灌浆期，对玉米籽粒的形成过程影响较大。然而中部叶片能否得到充足的光照条件，取决于上层叶的叶夹角大小，随着太阳一天中的运动变化，如果穗上叶紧凑，有利于延长上层

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表 2　已克隆的玉米叶夹角相关基因

Table 2　Cloned gene related to leaf angle

基因 Gene	染色体 Chr.	表达部位 Expression tissue	功能注释 Annotation	克隆方法 Cloning method	参考文献 Reference
rs1	Chr. 7	SAM	编码含有同源异型盒结构域的转录调控因子 Encode a transcription regulator with homeobox domain	突变克隆 Mutation cloning	[51]
lg1	Chr. 2	叶舌区 Ligule	编码 SPL 蛋白 Encode SQUAMOSA promoter-binding protein-like	突变克隆 Mutation cloning	[52]
lg2	Chr. 3	叶原基 Leaf primordia	编码碱性亮氨酸拉链蛋白,与叶原基-叶环的形成有关 Encode a basic leucine zipper protein which regulates the formation of leaf primordium and ring	突变克隆 Mutation cloning	[53]
lg3	Chr. 3	叶鞘与叶片连接部位 Junction of the leaf blade and the sheath	KNOX 转录因子 KNOX transcription factor	突变克隆 Mutation cloning	[17]
lg n	Chr. 9	叶枕叶舌 Pulvinus and ligule	Serine/threonine 激酶,调控叶耳叶舌的发育 Serine / threonine kinase regulate the development of auricle and ligule	突变克隆 Mutation cloning	[23]
ZmTAC1	Chr. 2	叶鞘/叶片/茎尖 Leaf sheath, blade, and stem	编码一个 263 个氨基酸的未知功能蛋白质 Encode an unknown function protein comprised with 277 amino acids	QTL-克隆 QTL cloning	[54]
ZmCLA4	Chr. 4	SAM	水稻 OsLAZY1 的同源序列 Homologous sequence of OsLAZY1 of *Oryza sativa* L.	QTL-克隆 QTL cloning	[24]
nana plant2	Chr. 5	未知 Unknown	拟南芥 DWF1 的同源序列 Homologous sequence of DWF1 of *Arabidopsis thaliana*	QTL-克隆 QTL cloning	[21]
ZmILI1	Chr. 2	未知 Unknown	编码 HLH-DNA 结合域超家族蛋白 Encode helix-loop-helix (HLH) DNA-binding domain superfamily proteins	QTL-克隆 QTL cloning	[22]
UPA2/UPA1	Chr. 2/Chr. 1	叶片 Leaf	调控转录因子 *ZmRAVL1* 的表达/参与 BRs 合成 Regulate the expression of transcription factor *ZmRAVL1* and affect brassinosteroids (BRs) synthesis	QTL-克隆 QTL cloning	[2]
ZmIBH1-1	Chr. 2	叶原基 Leaf primordia	aHLH 转录因子 aHLH transcription factor	QTL-克隆 QTL cloning	[55]

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叶的受光时长,提高光合速率,并且减少了对中层及下层叶片的遮挡,有效促进了中层叶片叶绿素的积累,进而提高了玉米产量。因此,玉米叶夹角的形态建成及调控对玉米冠层结构及光合效率有重要决定作用,成为国内外研究的重点领域。目前已解克隆了一系列叶夹角调控基因,如 *lg1* 和 *lg2* 基因已成功应用于育种实践。然而由于玉米光合指标多为次级性状,与产量性状的相关性较低且重复性较差,加之玉米植株高大,增加了对玉米生理性状准确鉴定的难度,因此叶夹角形成的生理调控有待进一步研究。叶夹角的大小主要与叶片中脉的机械支持强度有关,挖掘影响叶脉强度的相关基因及解析其遗传机制,为改良叶夹角进一步提供有用信息。然而现有研究主要集中于叶原基、叶舌和叶耳的发育及油菜素内酯的代谢等,对叶脉强度及紧凑型与平展型植株间的生理性状差异比较等研究相对较少,亟待进一步加强。

目前国内外对玉米叶夹角的遗传规律及 QTL 定位方面开展了较多研究并取得显著进展,但仍存在以下问题:①不同研究选用的材料不同,定位到的 QTL 存在显著差异,说明叶夹角 QTL 受遗传背景的影响较大,未来仍需利用不同材料定位新的叶夹角 QTL;②现有叶夹角的 QTL 定位研究材料均为温带玉米种质,温带材料间遗传差异较小,所报道的玉米叶夹角 QTL 效应普遍较小,在今后的遗传研究中,迫切需要从遗传变异丰富的热带玉米中挖掘玉米叶夹角 QTL,拓宽玉米种质基础;③由于叶夹角 QTL 定位的亲本群体无法覆盖全部玉米种质,早期分子标记多为 SSR 标记,密度较低,检测到叶夹角相关 QTL 有限,并且定位到的大多 QTL 置信区间遗传距离较大,进一步精细定位后未能鉴定出目标基因并开发功能标记,因此难以应用到分子标记辅助选择等育种实践。

高通量、高密度分子标记检测技术的进步,以及生物信息学和统计学的发展,为深入研究玉米叶夹角的遗传机理展现了广阔的前景。未来玉米叶夹角的遗传研究有待进一步加强:①目前已完成全基因组测序的玉米种质包括 73、Mo17、W22、SK、PH207、*mexicana*、HZS[25],建议今后结合现有的 QTL 定位结果,利用生物信息学比较叶夹角 QTL 热点区域的序列差异,挖掘叶夹角相关候选基因并进行功能验证;②在已有 QTL 初定位结果的基础上,精细定位叶夹角相关候选基因,并解析其功能;③根据已克隆的叶夹角相关基因的序列,开发功能标记,结合分子标记的前景和背景选择,进行育种应用;④根据水稻、高粱及二穗短柄草中控制叶夹角发育关键基因的序列,在玉米 B73、Mo17 和 SK 的基因组中寻找相同物理位置或功能相似的基因,利用 CRISPR/Cas9 技术进行基因编辑,筛选突变体,挖掘叶夹角发育关键基因。在上述研究的基础上还应深入研究基因间的互作,解析其信号转导通路,阐明叶夹角形成的分子机理,对玉米株型育种及高密度栽培等均具有重要的实际应用价值。

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福建农业学报 2021, 36（11）: 1288−1294 http://www.fjnyxb.cn
Fujian Journal of Agricultural Sciences doi: 10.19303/j.issn.1008-0384.2021.11.005

吕凤仙，李崇娟，杨鼎，等. 菜薹和芥蓝种间杂交胚挽救技术研究［J］. 福建农业学报，2021，36（11）：1288−1294.
LYU F X, LI C J, YANG D, et al. Embryo Rescue of Interspecific *Brassica campestris* x *B. albograbra* Hybrids［J］. *Fujian Journal of Agricultural Sciences*，2021，36（11）：1288−1294.

菜薹和芥蓝种间杂交胚挽救技术研究

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摘　要：【目的】 获得适合菜薹和芥蓝种间杂种的胚挽救实验方案。**【方法】** 以菜薹（菜心）作为母本，芥蓝作为父本，进行种间杂交，对种间杂种进行人工胚挽救培养，设置 8 种不同成分的胚挽救培养基，6 个不同取材时间，4 种增殖培养基，9 种不同成分生根培养基。**【结果】** ERM_4（MS+6-BA 0.5 mg·L^{-1}+NAA 0.5 mg·L^{-1}+KT 1.0 mg·L^{-1}+AC 1.0 g·L^{-1}）出胚率达 26.32%，为最佳胚挽救培养基；授粉后第 12 天取材，出胚率达 26.32%，为最适宜取材时间；DM_1（MS+6-BA 0.9 mg·L^{-1}+NAA 0.1 mg·L^{-1}）培养基增殖系数最大，为 5.37，为最佳增殖培养基；$RM_{4\text{-}2}$（1/2MS+IBA0.5 mg·L^{-1}）培养基生根率为 37.21%，平均生根时间为 8.33 d，生根最快，为最佳生根培养基。**【结论】** 菜薹与芥蓝种间杂交，于授粉后 12 d 取材，胚珠接种在 ERM_4 培养基上，出胚后接种在 DM_1 培养基上增殖，在 $RM_{4\text{-}2}$ 培养基上生根，能够显著提高种间杂种的获得率。

关键词： 菜薹；芥蓝；种间杂交；胚挽救；增殖；不定根诱导

中图分类号： S 634　　**文献标志码：** A　　**文章编号：** 1008-0384（2021）11−1288−07

Embryo Rescue of Interspecific *Brassica campestris* x *B. albograbra* Hybrids

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Abstract: 【Objective】 Methods suitable for embryo rescue in the interspecific hybridization between *Brassica campestris* and *B. albograbra* were investigated.【Method】 The interspecific crossing was carried out using *B. campestris* L. as female parent and *B. albograbra* L. H. Bailey as male parent. An embryo rescue culture for the hybridization was conducted with 8 different media at 6 sampling times followed subsequently by 4 proliferation media and 9 rooting media.【Result】 The embryo genesis rate at 26.32% of ERM_4 medium consisting of MS, 0.5mg 6-BA·L^{-1}, 0.5mg NAA·L^{-1}, 1.0mg KT·L^{-1}, and 1.0g AC·L^{-1} was the highest among all. The samples collected 12d after pollination had the best rate of 26.32%, DM_1 (i.e., MS+ 0.9mg 6-BA·L^{-1}+0.1mg NAA·L^{-1}) the greatest proliferation coefficient of 5.37, and $RM_{4\text{-}2}$ (i.e., 1/2MS+ 0.5mg IBA·L^{-1}) the top rate of 37.21% and average 8.33d on rooting.【Conclusion】 The optimum conditions for the embryo rescue in the interspecific hybridization between *B. campestris* L. and *B. albograbra* L. H. Bailey included sampling at 12d after pollination and following by culture on ERM_4 medium for embryo induction, on DM_1 for ovule proliferation, and on $RM_{4\text{-}2}$ for seedling rooting.

Key words: *Brassica campestris* L.; *Brassica albograbra* L. H. Bailey; interspecies cross; embryo rescue; proliferation; adventitious root induction

收稿日期： 2021−05−31 初稿；2021−10−14 修改稿

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基金项目： 云南省“万人计划”产业领军人才项目（YNWR-CYJS-2018-040）；国家大宗蔬菜产业技术体系昆明综合试验站项目（CARS-23-G37）；云南省科技计划重大科技专项（2019ZG001）

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0　引言

【研究意义】菜薹（俗称菜心，*Brassica campestris* L., AA, 2n=20），和芥蓝（*Brassica albograbra* L. H. Bailey, CC, 2n=18）均属十字花科芸薹属中不同种的重要薹用蔬菜。菜薹是由易抽薹白菜类蔬菜材料，经过长期选育而来的[1]。芥蓝在我国的栽培历史悠久，但是遗传育种的研究较为薄弱[2]，生产上推广应用的品种普遍存在产量低、品质差、病虫害严重等问题[3]。由于二者种质资源相对较少，遗传背景狭窄，单靠种内杂交，很难在现有基础上取得突破性进展。通过种间杂交，可以创制新品种，获得有用的育种材料。但二者存在杂交不亲和，不能得到后代，需采用人工胚挽救等方法才能获得菜薹和芥蓝种间杂交的后代。【前人研究进展】通过远缘杂交，可以创造新的杂种优势和新物种、新材料，但远缘杂交过程中存在诸多生殖障碍。邱德勃等[4]在芸薹属和萝卜属植物的远缘杂交中发现，胚乳在发育过程中解体，导致远缘杂种胚的败育，是受精后不亲和以及无法形成成熟种子的主要原因，Chrungu[5]研究发现，幼胚的离体培养是克服不亲和性的有效途径。梁红[6]对菜薹与甘蓝正反交，通过杂种胚离体培养，获得杂交优势显著的种间杂种。满红[7]以二倍体菜薹、四倍体菜薹、二倍体芥蓝、四倍体芥蓝为材料，经人工授粉杂交，通过子房培养和胚珠培养，创建了菜薹和芥蓝的种间异源四倍体和异源三倍体种质。乔海云[8]以菜薹和芥蓝为试验材料，运用蕾期授粉结合胚挽救技术获得菜薹与芥菜的种间杂种，为芸薹种和甘蓝种种质创新和遗传研究奠定基础。魏晓云等[9]采用二倍体菜薹和二倍体芥蓝杂交，筛选出亲和性较高的材料。【本研究切入点】前人的研究中虽已经获得菜薹和芥蓝的种间杂种，但对菜薹和芥蓝种间杂种胚挽救过程中，培养基成分、取材时间等细节进行筛选的研究有待深入进行。本试验通过对菜薹和芥蓝种间杂种胚挽救各个过程进行详细研究，以提高种间杂种的获得率。【拟解决的关键问题】通过设置不同的取材时间，不同生长调节剂配比，不同培养基成分，利用出胚率、增殖系数、生根率、生根时间等指标对各设定进行评估，筛选出最适合菜薹×芥蓝种间杂种胚挽救的实验方案，为菜薹和芥蓝育种种质的创制奠定基础。

1　材料与方法

1.1　试验材料

供试材料菜薹 CX20000 和芥蓝 JL20000 均由云南省农业科学院园艺所十字花科课题组提供。

1.2　试验方法

1.2.1　种间杂交　试验材料于 2020 年 6 月 1 日播种，7 月 8 日定植于云南省农业科学院十字花科课题实验基地塑料大棚内，常规栽培管理，菜薹于种间杂交。于杂交前 2 d 摘除父母本花序上已开放花朵，并套袋隔离，套袋父母本开花当天，对菜薹套袋分枝上未开放且未露柱头的花蕾进行剥蕾去雄，授以芥蓝套袋分枝上当日开放的花粉，配制 CX20000×JL20000 杂交组合，授粉后套袋隔离，严防父本以外的花粉污染。

1.2.2　不同培养方式　人工去雄授粉后，于适宜时间，取部分杂交后胚珠进行胚挽救培养，留取一部分在植株上继续生长，进行常规培养，比较两种方式的出胚率。

1.2.3　不同胚挽救培养基　大田授粉后，于授粉后第 12 天，选取有明显膨大胚珠的子房，用自来水冲洗 2～3 遍，于超净工作台内用 75% 酒精浸泡 50 s，将酒精倒出后用 10% 次氯酸钠浸泡 15 min，接着用无菌水冲洗 3 次，最后用无菌水浸泡 3 min。用解剖刀沿子房腹缝线和背缝线处轻轻切开，用镊子挑取饱满且呈绿色的胚珠，分别接种到 ERM_1、ERM_2、ERM_3、ERM_4、ERM_5、ERM_6、ERM_7、ERM_8 培养基上（表 1），每种培养基均设置 3 次重复，每次重复接种 25～35 个胚珠，接种后胚珠在温度（25±2）℃、光照 12 $h \cdot d^{-1}$、光照强度 1 500 lx 的条件下培养[8]。接种 1 周后进行观察，30 d 后统计出胚率，筛选适合的胚挽救培养基。

1.2.4　不同取材时间　大田授粉后，分别于授粉后 10、11、12、13、14、15 d，选取有明显膨大胚珠的子房，消毒、灭菌后，挑取饱满且呈绿色的胚珠，接种到 ERM_4 培养基上，每个取材时间设置 3 次重复，每次重复接种 30～130 个胚珠，接种后胚珠在温度（25±2）℃、光照 12 $h \cdot d^{-1}$、光照强度 1 500 lx 的条件下培养 1 周后进行观察，30 d 后统计出胚率，观察不同取材时间出胚率，筛选最适宜的取材时间。

1.2.5　不同增殖培养基　成活并成功分化的胚珠分别转移至 DM_1、DM_2、DM_3、DM_4 培养基上进行增殖分化（表 2），每种培养基设置 3 次重复，每次重复接种 25-27 个不定芽，观察增殖情况，并统计各培养基的增殖系数，筛选适宜的增殖培养基。

增殖系数=出芽数/原有芽数

1.2.6　不同生根培养基　胚珠在增殖培养基上分化至具有完整植株形态后，转接至 1/2MS 培养基上继续培养，长至 3 对真叶后，转移至生长素种类和质

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表 1 菜薹×芥蓝种间杂种胚挽救培养基成分

Table 1 Components in embryo rescue medium for interspecific *B. campestris* × *B. albograbra* hybrids

编号 No.	基本培养基 Minimal medium	6-BA质量浓度 6-BA mass concentration/（mg·L^{-1}）	NAA质量浓度 NAA mass concentration/（mg·L^{-1}）	KT质量浓度 KT mass concentration/（mg·L^{-1}）	AC含量 AC content/（g·L^{-1}）
ERM_1	MS	0.00	0.00	0.00	1.00
ERM_2	MS	0.30	0.00	0.00	1.00
ERM_3	MS	0.50	0.00	0.00	1.00
ERM_4	MS	0.50	0.50	1.00	1.00
ERM_5	MS	1.50	0.50	1.00	1.00
ERM_6	MS	3.00	0.50	1.00	1.00
ERM_7	MS	4.50	0.50	1.00	1.00
ERM_8	MS	5.00	0.50	1.00	1.00

表 2 菜薹×芥蓝种间杂种增殖培养基成分

Table 2 Components in propagation medium for interspecific *B. campestris* × *B. albograbra* hybrids

编号 No.	基本培养基 Minimal medium	6-BA质量浓度 6-BA mass concentration/（mg·L^{-1}）	NAA质量浓度 NAA mass concentration/（mg·L^{-1}）
DM_1	MS	0.90	0.10
DM_2	1/2MS	0.90	0.10
DM_3	MS	0.50	0.01
DM_4	1/2MS	0.50	0.01

量浓度不同的 RM 培养基（表 3），每种培养基设置 3 次重复，每次重复接种 8～16 株幼苗，观察统计各培养基的生根情况。根长适宜的幼苗定植于含育苗基质的苗钵后，在温度 20～25 ℃，湿度 80% 条件下进行炼苗，待幼苗生长情况稳定后，移栽至红土∶过筛腐殖土=1∶1 比例混合土内，常规肥水管理。定植后的杂种植株，统计其成活率。结合生根情况与幼苗成活率，筛选最适宜的生根培养基。

1.3 种间杂种形态学鉴定

成活后的杂种植株，在正当繁茂时观察其叶形、叶色、叶表面特征、花器官的颜色大小等方性状，并分别与菜薹和芥蓝两亲本进行比较，鉴定杂种的真实性。

1.4 数据分析

利用 SPSS23.0 软件对试验数据进行差异性分析。

2 结果与分析

2.1 胚挽救和常规培养的比较

菜薹×芥蓝种间杂交后，在母本植株上自然生长进行常规培养，获得种子数为 0，而胚挽救培养成功获得了种间杂种植株，证明菜薹×芥蓝种间杂交后代不结籽，需通过人工胚挽救培养，才能成功获得杂种。

表 3 菜薹×芥蓝种间杂种生根培养基成分

Table 3 Components in rooting medium for interspecific *B. campestris* × *B. albograbra* hybrids

编号 No.	基本培养基 Minimal medium	NAA质量浓度 NAA mass concentration/（mg·L^{-1}）	IBA质量浓度 IBA mass concentration/（mg·L^{-1}）	IAA质量浓度 IAA mass concentration/（mg·L^{-1}）
RM_{1-1}	MS	0.20	0.00	0.00
RM_{1-2}		0.50	0.00	0.00
RM_{2-1}		0.00	0.20	0.00
RM_{2-2}		0.00	0.50	0.00
RM_{3-1}		0.00	0.00	0.20
RM_{3-2}		0.00	0.00	0.50
RM_{4-1}	1/2MS	0.20	0.00	0.00
RM_{4-2}		0.00	0.50	0.00
RM_{4-3}		0.00	0.00	0.50

2.2 不同培养基成分对出胚的影响

不同成分培养基出胚率如表 4 所示，共接种杂种胚珠 730 粒，出胚总数 75 粒，其中未添加生长调节剂的 ERM_1 培养基上，未见胚珠萌发；ERM_2 和 ERM_3 胚珠萌发率较低，分别为 1.33% 和 2.56%，且差异不显著；ERM_4 培养基上出胚率显著高于其他培养基，为 26.32%；ERM_5、ERM_6、ERM_7、ERM_8 培养基随着添加的 6-BA 浓度逐渐增大，出胚率从 18.18% 逐渐降低至 7.77%；因此 ERM_4（MS+6-BA 0.5 mg·L^{-1}+ NAA 0.5 mg·L^{-1}+KT 1.0 mg·L^{-1}+AC 1.0 g·L^{-1}）出胚率最高，为最佳胚挽救培养基。萌发后的杂种胚均生长良好，接种 20 d 后出胚情况、40 d 后成苗情况、成活胚珠增殖情况均如图 1 所示。

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表 4　菜薹×芥蓝杂种胚珠在不同培养基上出胚率

Table 4　Embryogenesis rate of *B. campestris* × *B. albograbra* hybrid ovules on different media

编号 No.	胚珠培养数 Number of ovules	成胚数 Number of embryogenesis	出胚率 Embryogenesis rate/%
ERM_1	105	0	0.00±0.00 f
ERM_2	75	1	1.33±0.02 ef
ERM_3	78	2	2.56±1.25 e
ERM_4	95	25	26.32±0.78 a
ERM_5	88	16	18.18±0.93 b
ERM_6	85	13	15.29±1.00 c
ERM_7	101	10	9.90±0.89 d
ERM_8	103	8	7.77±0.90 d

注：不同小写字母表示差异达0.05显著水平。下同。

Note: Different lowercase letters indicate significant difference at $P<0.05$. Same for the following.



A　　B　　C

注：A，胚珠萌发；B，胚挽救幼苗；C，成活胚增殖分化

Note: A: Ovule germination; B: embryo rescued seedling; C: proliferation and differentiation of survival embryos.

图 1　菜薹×芥蓝种间杂种胚挽救情况

Fig. 1　Embryo rescue of interspecific *B. campestris* × *B. albograbra* hybrids

2.3　不同取材时间对出胚的影响

如表 5 所示，不同取材时间的胚珠，在 ERM_4 培养基上出胚率均存在差异，授粉后第 10 天取材，由于胚珠过小，依赖母体而离体培养很难成活，胚珠萌发率只有 4.44%；授粉后第 12 天取材出胚率最高，为 26.32%，且与其他取材时间相比差异显著；授粉 12 天以后取材，由于杂种胚逐渐败育，出胚率逐渐减小，第 15 天仅有 3.33%。因此授粉后第 12 天为菜薹×芥蓝杂种胚最适宜的取材时间。

表 5　菜薹×芥蓝不同取材时间杂种胚珠出胚率

Table 5　Embryogenesis rate of *B. campestris* × *B. albograbra* hybrid ovules with differed sampling time

授粉后天数 Days after pollination/d	胚珠培养数 Number of ovules	出胚数 Number of embryogenesis	出胚率 Embryogenesis rate/%
10	90	4	4.44±1.93 b
11	88	9	10.23±1.97 b
12	95	25	26.32±2.77 a
13	382	40	10.47±0.92 b
14	150	10	6.67±3.34 b
15	60	2	3.33±2.89 c

2.4　不同增殖培养基的增殖效果

从表 6 可见，不同培养基增殖效果不同，增殖系数越大，增殖分化效果越好。DM_1 增殖系数为 5.37，显著高于其他培养基；DM_2 和 DM_3 增殖系数无显著差异，分别为 4.02、4.15；DM4 的增殖系数显著小于其他培养基，为 2.90。因此 DM_1（MS+ 6-BA 0.9 $mg·L^{-1}$+ NAA 0.1 $mg·L^{-1}$）为菜薹×芥蓝种间杂种的最佳增殖培养基。

表 6　菜薹×芥蓝种间杂种成活胚珠在不同培养基上增殖分化情况

Table 6　Proliferation and differentiation of viable ovules in *B. campestris* × *B. albograbra* hybrids on different media

编号 No.	原有芽数 Number of original bud	出芽数 Number of budding	增殖系数 Multiplication coefficient
DM_1	79	424	5.37±0.05 a
DM_2	76	306	4.02±0.38 b
DM_3	80	332	4.15±0.25 b
DM_4	78	226	2.90±0.15 c

2.5　不同生根培养基对生根效果的影响

从表 7 可知，以不同基本培养基，添加不同种类和不同浓度生长素，生根效果均存在差异。生根率方面，最高的为 RM_{4-1} 和 RM_{1-1}，分别为 44.44% 和 40.74%，二者相比无显著差异，但显著高于其他培养基；其次是 RM_{4-2}，为 37.21%，与 RM_{1-1} 差异不显著。生根时间方面，RM_{4-2} 平均生根时间为 8.33 d，生根最快，且与其他培养基平均生根时间差异显著。在不同培养基上成功生根后的幼苗，经炼苗、移栽后，均未出现死苗现象，幼苗移栽成活率为 100%。

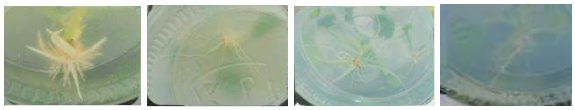
从图 2 可知，添加不同种类生长素，诱导的根形态也存在差异，添加 NAA 培养基，幼苗生长的根，表现为短且粗壮、无须根，添加 IBA 和 IAA 诱导的根，表现为细长。以 MS 培养基为基本培养基时，各培养基均出现部分苗产生盐害的现象（图 3），盐害苗叶片部分黄化且逐渐脱落，将 MS 培养基更换为 1/2MS 培养基时，这一现象得到缓解（图 4）。综上所述，幼苗移栽成活率未受不同生根培养基影响，RM_{4-1}、RM_{1-1} 和 RM_{4-2} 的生根率较高，RM_{4-1} 和 RM_{4-2} 培养基上生长的幼苗质量较高，RM_{4-2} 生根时间最短，生根最快，适用于组培苗的快速繁殖，因此 RM_{4-2}（1/2MS+IBA 0.5 $mg·L^{-1}$）为菜薹×芥蓝种间杂种胚挽救幼苗的最佳生根培养基。

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表 7 菜薹×芥蓝种间杂种不同培养基生根情况

Table 7 Rooting of interspecific *B. campestris* × *B. albograbra* hybrids on different media

编号 No.	培养幼苗数 Number of cultured seedlings	生根幼苗数 Number of rooting seedlings	生根率 Rate of rooting/%	平均生根时间 Mean rooting time/d	移栽后成活幼苗 Number of survived seedlings after transplanting	移栽成活率 Rate of transplanting survival/%
RM_{1-1}	27	11	40.74±2.20 ab	11.00±1.00 b	11	100
RM_{1-2}	28	4	14.29±3.07 e	11.33±0.58 b	4	100
RM_{2-1}	24	6	25.00±3.13 cd	8.67±0.58 b	6	100
RM_{2-2}	25	9	36.00±2.57 bc	8.67±0.58 b	9	100
RM_{3-1}	27	5	18.52±3.03 d	14.33±1.53 a	5	100
RM_{3-2}	27	7	25.93±2.75 c	15.00±1.00 a	7	100
RM_{4-1}	45	20	44.44±1.93 a	11.33±0.58 b	20	100
RM_{4-2}	43	16	37.21±1.47 cd	8.33±0.58 c	16	100
RM_{4-3}	47	13	27.66±1.54 c	14.67±1.53 a	13	100



注：A：NAA 生根效果；B：IBA 生根效果；C：IAA 生根效果；D：未加激素 MS 培养基生根效果

Note: A: Rooting affected by NAA; B: rooting affected by IBA; C: rooting affected by IAA; D: seedling rooting on MS without hormone addition.

图 2 菜薹×芥蓝种间杂种生根过程不同生长素生长果

Fig. 2 Effects of auxins on growth during rooting of interspecific *B. campestris* × *B. albograbra* hybrids



图 3 菜薹×芥蓝种间杂种生根过程盐害现象

Fig. 3 Damage induced by salt at rooting stage of interspecific *B. campestris* × *B. albograbra* hybrids



图 4 菜薹×芥蓝种间杂种生根过程

Fig. 4 Rooting of interspecific *B. campestris* × *B. albograbra* hybrids

2.6 种间杂种形态学表现

对菜薹×芥蓝种间杂种进行形态学观察并与菜薹和芥蓝各性状进行比较。以菜薹作为母本的种间杂种植株，植株形态如图 5 所示，主薹表面有棱，叶片形状为长卵圆，叶片颜色为深绿，与母本菜薹更为接近，主薹的颜色为浅绿，且有少量蜡粉，与父本芥蓝更为接近。杂种植株花器官形态如图 6 所示，花朵颜色与父本相同，为白色，花朵大小介于



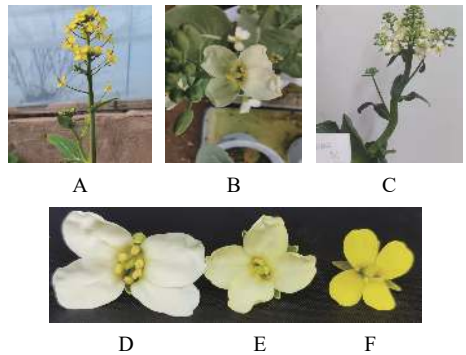
A B C D

注：A，菜薹；B、C，种间杂种；D，芥蓝

Note：A: *B. campestris*; B and C: interspecific hybrid; D: *B. albograbra*.

图 5 菜薹×芥蓝种间杂种植株形态

Fig. 5 Morphology of interspecific *B. campestris* × *B. albograbra* hybrid plant



A B C

D E F

注：A：F 菜薹；B：E 种间杂种；C：D 芥蓝

Note: A and F: *B. campestris*; B and E: interspecific hybrid; C and D: *B. albograbra*.

图 6 菜薹×芥蓝种间杂种花器官形态

Fig. 6 Morphology of floral organ of interspecific *B. campestris* × *B. albograbra* hybrid

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父母本之间。综合上述形态学表现，初步判断所得植株为菜薹和芥蓝种间杂交所得真杂种。

3　讨论与结论

影响杂交亲和性的因素主要包括杂交组合方式和亲本的基因型[10]，而亲缘关系越近的两种植物杂交，越容易获得成功[11]。刘爱华[12]、栗茂腾[13]、李宗芸[14]、宋运淳[15]等学者的研究结果都表明芸薹属 A 和 C 基因组较 A 和 B 以及 B 和 C 之间亲缘关系较近，本试验进行远缘杂交的两亲本作物菜薹和芥蓝，分别为芸薹属 A 组和 C 组染色体中的重要薹用蔬菜，成功获得了菜薹×芥蓝的种间杂种。李明山[16]、张国庆[17]等学者的研究结果表明，胚挽救技术能够克服杂交的不亲和性，本试验中进行的种间杂交常规培养无法获得种子，采用胚挽救技术获得了杂交种，证明胚挽救技术能克服杂交不亲和性的困难。

受父母本基因型的影响，授粉后的不同时间胚珠发育程度不同，谭亚飞[18]利用白菜型油菜黄籽沙逊和芥蓝进行种间杂交，胚挽救实验结果表明授粉后第 11 天为最佳取材时间，出胚率最高。而本试验中，受母本菜薹影响，最佳取材时间为授粉后第 12 天。Harberd[19]在甘蓝×白菜的杂交研究中发现，取材时间和培养基种类都会影响胚挽救技术的结果。满红[7]对四倍体菜薹和四倍体芥蓝的种间杂种进行子房培养，最佳的取材时间为授粉后 7 d，得胚率为 1.53 个胚珠/子房。本试验中授粉后 10~15 d 取材，进行胚珠培养，每个取材时间的出胚率各不相同，且授粉后第 12 天出胚率最高，为 26.32%，而菜薹×芥蓝的胚珠，在 5 种不同的培养基上萌发情况也不同，以 ERM_4 培养基（MS+6-BA 0.5 mg·L^{-1}+NAA 0.5 mg·L^{-1}+KT 1.0 mg·L^{-1}+AC 1.0 g·L^{-1}）萌发率最好，出胚率为 26.32%，显著高于其他培养基。

植物根系的形成与诸多因素有关，植物生长调节剂起着关键作用[20]，当生长调节剂浓度高于细胞分裂素时，有利于不定根的诱导。本试验中在 1/2MS 和 MS 中分别添加不同浓度的 NAA、IBA 和 IAA，试验数据表明，以 $RM_{4\text{-}1}$（1/2MS+NAA 0.2 mg·L^{-1}）生根率最高，高于添加 IBA 和 IAA 的培养基。这一结果与张以忠[21]的 IAA 与 IBA 诱导荞麦生根的效应好于 NAA 有差异，但与王健省[22]的 NAA对双瓣茉莉水培扦插生根的效果明显优于 IAA 一致，据吴大忠[23]的研究结果表明，不同的无性系生根所需的生长调节剂种类和浓度存在较大的差异，可能是不同植物诱导生根适宜的生长调节剂种类不同。另外，本试验通过添加不同浓度生长调节剂诱导生根发现，$RM_{4\text{-}2}$（1/2MS+IBA 0.5 mg·L^{-1}）诱导生根最快，适宜进行快繁，实际应用中应根据具体需求，选择适宜的生根培养基成分。

本研究筛选出了适宜菜薹和芥蓝种间杂种的胚挽救实验方案，为种间杂种的选育奠定了基础。但通过此试验获得的杂交种为异源二倍体，减数分裂时染色体无法正常联会配对，从而无法形成配子，不具有育性，要得到能正常繁殖的杂交种，还需进行人工加倍等试验，克服种间杂种的不育性，选育出具有杂种优势的种间杂种。

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引用格式：胡庆毅，陈本佳，杨立凡，等 . 植物激素调控顶端分生组织发育的研究进展 [J]. 湖南农业科学，2022（1）：105-110.
DOI:10.16498/j.cnki.hnnykx.2022.001.027

植物激素调控顶端分生组织发育的研究进展

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摘　要：植物分生组织的发育主要依赖于植物体内多功能干细胞群体，并受多种激素的共同调控，其中生长素和细胞分裂素调控通路及其相互作用是调控分生组织形成和分化的主要因素。了解植物分生组织的形态建成及信号途径相关分子机制，将加深人们对植物生长发育调控的认识，为植物资源进一步开发和利用提供理论基础。为此，综述了植物顶端分生组织的结构特征及核心调控基因，对植物激素（生长素、细胞分裂素、赤霉素、脱落酸、独脚金内酯、茉莉酸）协同调控植物分生组织发育的分子机制进行了深入阐述，并展望了该领域今后的主要研究方向。

关键词：植物激素；分生组织；调控途径；干细胞；综述
中图分类号：Q942.5　　**文献标识码**：A　　**文章编号**：1006-060X（2022）01-0105-06

Progress in Phytohormones Regulating Apical Meristem Development

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Abstract：The development of plant meristems mainly relies on multifunctional stem-cell populations in plants and is controlled by a variety of hormones. Furthermore, the regulation pathways and interactions of auxin and cytokinin are the main regulatory factors to the formation and differentiation of meristems. Investigating the morphogenesis of plant meristems and related molecular mechanisms of signal pathways will deepen our understanding of the regulation of plant growth and development, and provide a theoretical basis for the further development and utilization of plant resources. Thus, the structure character and core regulatory pathways of plant apical meristem were reviewed, and the molecular regulatory mechanisms of phytohormones (auxin, cytokinin, gibberellins, abscisic acid, strigolactone, jasmonic acid) synergistically regulating the development of plant meristem were further elaborated. The research directions in the future were discussed in this paper.

Key words：plant hormones; meristem; regulation approach; stem cells; review

高等植物的器官形成离不开分生组织（meristem）。分生组织是在植物体的一定部位，具有持续或周期性分裂能力的细胞群，又被称为植物干细胞。由其分裂产生的细胞排列紧密，无细胞间隙；其中一小部分仍保持高度分裂的能力，大部分则陆续长大并分化为具有一定形态特征和生理功能的细胞，构成植物体的其他各种组织，使器官得以生长或新生 [1]。分生组织在植物体内根据分布位置主要分为顶端分生组织和侧生分生组织。其中，顶端分生组织发育为根、茎，使植株得以伸长生长，侧生分生组织则形成腋芽（axillary bud meristem，AM）和侧根（lateral root，LR），进而产生新的分枝并重新建立顶端分生组织和侧生分生组织。在高等植物的发育中，胚胎从纵轴两端到胚胎两端分别形成特殊区域，即茎端分生组织（stem apical meristem，SAM）和根端分生组织（root apical meristem，RAM）[1]。分生组织的存在保证了植物整个生长发育过程的可塑性，通过干细胞的分裂和分化产生新器官所需要的子细胞 [2]。

收稿日期：2021-10-10
基金项目：云南省基础研究项目（202001BB050050）；云南省教育厅科学研究基金研究生项目（2021Y046）
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1　植物顶端分生组织的结构特征及核心调控基因

分生组织的发育主要依赖于植物干细胞的分化，干细胞分化后的一部分子细胞经分裂分化形成新器官，一部分始终保持自身未分化状态，并在分生组织中心区域内维持干细胞的自我更新 [3-4]。

SAM 形成于胚胎发育的早期阶段，结构上分为中央分生组织区（central zone，CZ）、肋状分生组织区（rib zone，RZ）和外缘分生组织区（peripheral zone，PZ）。CZ 区由全能干细胞组成，处于未分化状态，细胞分裂周期长，分裂频率低，由外向内分为 L_1、L_2、L_3 这 3 个不同的胚性细胞层，分别形成表皮、皮下组织以及芽的相关内部器官；RZ 区为维管分生组织提供细胞支持；PZ 区是进一步细胞分裂、分化发育成为侧生器官的核心区域 [5]。CZ 区需要提供干细胞补充到 PZ 区和 RZ 区，CZ 区和 RZ 区之间还存在一个组织中心（Organization center，OC）区域，它介导不同区域间信息的传递和干细胞的维持（图 1A）。

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SAM 中央区的组织中心细胞和其上方的干细胞构成的干细胞微环境是维持分生组织的结构基础。*wuschel*（*WUS*）基因受生长素的诱导，是体细胞胚胎发生过程中干细胞更新的关键，并维持未分化干细胞自我更新的平衡 [6]。WUS 蛋白作为建立 SAM 的一个重要调控因子，在 WUS 表达区域内维持 SAM 组织中心干细胞的数量，同时和 *clavata3*（*CLV3*）形成负反馈调节机制以维持 SAM 的大小 [7]；*STM*（*SHOOT MERISTEMLESS*）在维持茎端分生组织中干细胞的产生也是必需的，它在整个茎端分生组织中的表达阻止了干细胞向器官特异性细胞的转化 [8-9]。

RAM 邻近根冠，由根端静止中心（quiescent center，QC）与根端干细胞区域组成。QC 位于 RAM 的根端并在该区域衍生出根端干细胞，根端干细胞产生子细胞，子细胞随后分化成为过渡区细胞的成员（图 1B）。因此，干细胞分裂和分化速度之间的平衡对维持根端分生组织至关重要。在静止中心的形成和根部干细胞的建立过程中，主要受依赖生长素信号的 *plethora*（*PLT*）途径和不依赖生长素信号的 *short-root*（*SHR*）/*scarecrow*（*SCR*）途径的调控 [10-12]。其中 *PLT* 基因编码了含有 *AP2*（*apetala2*）结构域的转录因子，可以促进细胞分裂；*SHR* 激活 *SCR* 的转录，*SCR* 通过细胞自主性机制维持 QC 的稳定 [13]。QC 作为根发育中心的同时也是 RAM 确立和维持的必要条件，在胚根或侧根形成的早期原基细胞中开始分裂，最终形成根分生组织的早期结构。侧根分生组织是从根顶端分生组织外更远的内皮层开始的，在分生组织的 OC 内干细胞分裂分化形成侧根原基（lateral root primordia，LRP），继而发育成为 LR。研究发现 *wuschel homeobox5*（*WOX5*，在 QC 细胞中表达）和 *PLTs*（在 QC 周围的干细胞中表达）是干细胞形成和维持的主要调节因子，在生长素信号下游起作用，以

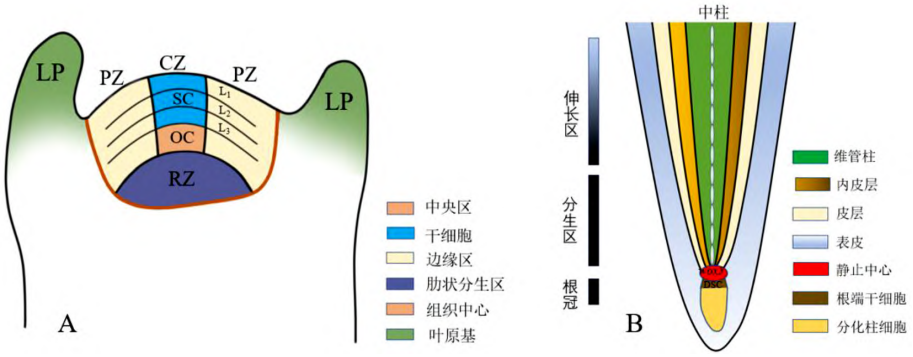


图 1 植物茎端（A）和根端（B）分生组织

维持根端干细胞的活性 [14]。

2 生长素、细胞分裂素调控分生组织的作用及相关信号途径

生长素（auxin）和细胞分裂素（cytokinin，CTK）是植物体内 2 类重要有机化合物，调节着细胞分化、生长方向、发育过程以及植物生命活动等过程。植物形态结构的形成是植物适应环境并长期进化的结果，植物在生长发育过程中，感受外界环境信号，传递并影响生长素和细胞分裂素等激素的合成、运输、信号传递和代谢，进而影响植物分生组织的维持和分化。生长素在 SAM 中抑制细胞分裂而促进细胞分化，而在 RAM 中，生长素引导分生组织细胞分裂；细胞分裂素在 SAM 和 RAM 中均可以通过抑制生长素响应信号阻碍细胞分裂，并促进细胞从分裂状态向分化状态转变 [15-16]。

2.1 生长素对植物分生组织的调控

2.1.1 生长素的合成及运输 生长素（auxin）在植物体内主要以吲哚 -3- 乙酸（indole-3-acetic acid，IAA）形式存在 [17]。IAA 的生物合成主要有色氨酸依赖型和非色氨酸依赖型 2 种途径。其中，色氨酸依赖型途径是植物体内 IAA 的主要合成途径，TAA1（tryptophan aminotransferase of arabidopsis 1，TAA 家族）催化色氨酸生成吲哚丙酮酸（indole-3-pyruvate，IPA），进而由 YUCCA（YUC 家族）催化 IPA 生成 IAA，并参与器官的形成 [18]。生长素除了在植物体内原位合成发挥作用外，还能运输到其他部位，使植物组织器官的生长素浓度产生差异，进而发挥作用 [19]。研究表明，生长素运输包括长距离运输和短距离运输，主要有吲哚 -3- 乙酰胺（IAM）途径、吲哚 -3- 乙醛肟（IAQx）途径、色胺（TAM）途径和吲哚 -3- 丙酮酸（IPA）途径 4 种运输途径。长距离运输是指生长素从合成部位（如茎端分生组织）运输到其他组织的运输，例如顶端优势的作用机制：生长素在茎顶端分生组织合成，向下运输，进而抑制侧生分生组织的生长发育；短距离运输为细胞之间的单向极性运输，主要通过生长素转运蛋白 pinformeds（PINs）进行生长素细胞间运输，形成生长素的梯度分布从而调控分生组织发育 [20-21]。

2.1.2 生长素信号调控途径 生长素合成并运输至分生组织区域达到最适水平，通过 Aux/IAA-ARF 信号

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途径激活下游生长素响应基因，从而调节分生组织的发育[22]。在分生组织中，生长素可以通过抑制分生组织细胞分化基因 *shoot meristimless*（*STM*）的表达，进而抑制拟南芥 A 型调节因子 *ARR7*/*ARR15* 和叶极性基因 *PHAN* 的同源基因 *AS1*、*AS2*（*asymmetric leaves1*、*asymmetric leaves2*）在分生组织 CZ 区和 PZ 区的表达，最终抑制器官原基的产生（图 2）。

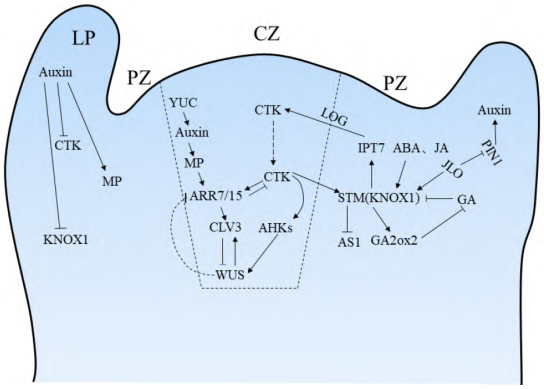


图 2　植物激素和相关基因的调控关系

高浓度的生长素会抑制植株侧生分生组织的发育，而适当的生长素浓度则可促进侧生分生组织的发育，并通过向外运输 PINs 加快侧原基的形成。生长素还可以调控根的形成、增殖、伸长和分化，通过 *PLT*、*ARF*、*SCF*、*LBD* 等基因来维持 RAM 的稳定性，并调控侧根分生组织的发生。*LBD* 家族转录因子 *JLO*（jagged lateral organs）一方面可以抑制 *PIN1* 的表达使生长素在内皮层积累，促进中柱鞘细胞的增生使其发育成侧根原基[16,23]，另一方面可以通过调控生长素受体 *TIR1*、*AFB1* 和同源异型盒基因 *KNOX1* 的表达，控制茎端分生组织的生长发育[23]。生长素响应因子 *ARF10*/*ARF16* 负调控 *wuschel-related homeobox5*（*WOX5*），并将其表达区域限制在 QC 区域，从而抑制 *PLT* 基因的表达以影响干细胞的分化，进而影响根端分生组织的形成及发育。生长素还可通过负调控 SCF^{TIR1} 复合物的形成抑制 *ARF7*/*ARF19* 的表达，进而促进细胞在根端分生组织的不对称分裂以形成侧根原基[16,24]。

2.2　细胞分裂素对植物分生组织的调控

2.2.1　细胞分裂素的合成及运输　细胞分裂素（cytokinin，CTK）在种子萌发、细胞分化和器官发育等植物发育和生理过程中都发挥了重要作用[25]。细胞分裂素关键合成酶主要有异戊烯基转移酶 IPT、细胞色素单氧酶 P450（CYP735A1、CYP735A2）和细胞分裂素激活酶 LOG[26]。在分生组织区域，细胞分裂素的生物合成还受 *STM* 的影响，*STM* 通过诱导 *IPT7* 的表达来促进细胞分裂素的生物合成[27]。在 SAM 中细胞分裂素通过 KNOX1（knotted1-like homeobox1）蛋白介导运输，并且负调控生长素的转运从而影响叶原基的形成，同时还通过 KNOX1 维持着分生组织的活性，调控植物器官的形态建成[27]。

2.2.2　细胞分裂素信号调控途径　细胞分裂素主要通过依赖 CLV3 和依赖 AHKs 的信号调控途径，进而影响 *STM*、*IPT7* 的表达量，实现对植物分生组织的调控。细胞分裂素以 AHKs 介导的途径诱导 *WUS* 和 *CLV3*，进而促进 SAM 的形成[28]。同时，细胞分裂素激活酶 LOG 可将细胞分裂素前体的一种非活性反式玉米素 - 糖苷转化为活性细胞分裂素，在 STM 启动子的控制下，促进细胞分裂素合成关键基因 *IPT7* 的表达，从而促使侧芽的形成（图 2）。

拟南芥中有 14 种细胞分裂素信号响应蛋白，其中有 8 种 A 型调节蛋白 ARR 是细胞分裂素转导的负调节因子，有 6 种 B 型调节蛋白 ARR（ARR1/2/10/12/14/18）是细胞分裂素响应的正向调节因子[29]。细胞分裂素 B 型应答因子可直接激活茎尖干细胞，并稳定 *WUS* 基因的表达水平，从而维持干细胞的活性[30]。细胞分裂素通过 AHK2/AHK4 依赖途径和 CLV 依赖途径来诱导 *WUS* 基因的表达，从而实现对茎端分生组织的调控；而 *WUS* 基因在异位表达时却能够激活 *CLV3* 的表达，导致茎分生组织增大；说明二者之间通过负反馈调节机制共同调控分生组织的发生[31]。WUS 蛋白从组织中心 OC 区域转移到 CZ 区，在 CZ 区中它直接与 *CLV3* 启动子结合影响 *CLV3* 的表达[32]。

3　生长素和细胞分裂素互作调控分生组织的发生与发育

生长素和细胞分裂素间的拮抗作用是顶端分生组织或侧生分生组织器官发育起始的关键调控因素[24]。在植物分生组织的发生过程中，生长素、细胞分裂素的相互作用主要是通过 *WUS-CLV3* 的反馈调节机制、不同比例的 CTK/IAA 水平、B-ARR 和 A-ARR 介导的激素响应及运输等一系列途径来调控分生组织和相关器官的形态建成（图 2）。

在植物 SAM 中，生长素通过影响 *STM* 的表达来上调细胞分裂素合成关键基因 *IPT7* 的表达，促进细胞分裂素的合成，并激活 AHKs 途径和细胞分裂素信号应答基因 *ARR7*/*ARR15* 以促进 *WUS-CLV3* 通路间接调控干细胞的增殖分化（图 2）；WUS 可以直接抑制 *ARR7*/*ARR15* 的表达，是细胞分裂素信号的有效调节因子，还可直接调控细胞分裂素和生长素相关基因，控制生长素和细胞分裂素之间的平衡[33-34]。在植物 RAM 中，MP 是激活 QC 特异性 *WOX5* 基因和生长素响应基因 *PLT* 的调节因子，调节生长素信号介导分生组织模式的建立。另外，生长素可以通过单肽转录因子（*monopteros*，*MP*）介导的途径来抑制细胞分裂素

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的生物合成，从而触发器官原基的启动；而 *MP*/*ARF5* 作为生长素信号的整合因子，使生长素信号和细胞分裂素信号在调控网络中有机结合以参与 SAM 的形成和维持 [8]。

SAM 的形成与生长素的梯度和 *WUS* 表达水平密切相关，细胞分裂素的局部合成是细胞分裂素区域分布和 *WUS* 表达所必需的，是体细胞胚胎发生过程中 SAM 形成的必要条件。细胞分裂素可以调控生长素的合成、运输并在侧原基起始时期扰乱生长素输出载体 PIN 的表达模式，从而间接调控生长素的合成。细胞分裂素 B 类调节因子 B-ARR 则通过抑制 *YUCCA* 基因的表达来降低生长素的积累；另外，B-ARR 还可以结合到生长素合成基因 *TAA1* 启动子上，激活 *TAA1* 的表达从而调控生长素的生物合成 [35]。

侧生分生组织发育也受生长素和细胞分裂素共同作用调控。拟南芥叶腋处 *ATH1* 基因通过诱导分生组织 *STM* 的表达，维持了叶腋分生细胞的特征；另外，ATH1 蛋白能够与 STM 蛋白发生互作，形成一个 *STM* 基因的自激活回路 [36]。ARR7/ARR15 在生长素的介导下负调控细胞分裂素的产生，PIN 蛋白介导的生长素运输也会抑制细胞分裂素的产生，从而影响侧生器官的形成。例如：*TB1* 基因在水稻、玉米中对侧枝的发育起负调控作用，主要通过调控生长素的运输来调节生长素的水平，并参与腋分生组织的调节过程 [37]；在拟南芥叶腋处，随着生长素和细胞分裂素水平的提高，*SPS*（*supershoot*）和 *BUS*（*bushy*）基因的表达水平提高，并通过反馈调节相关激素水平来调控植株腋分生组织的发育 [38-39]。

在 RAM 中由 AHK3/ARR1 介导的细胞分裂素响应信号途径通过生长素抑制因子 short hypocotyl 2（SHY2）负调控 IPTs 的表达，从而影响细胞分裂素的合成，来促进根的伸长 [40]。在侧根分生组织发生过程中，维管柱内中柱鞘对生长素的运输和信号感知的差异将引发不对称的细胞分裂，从而形成侧根原基，继而形成侧根。在这一过程中细胞分裂素通过抑制生长素转运蛋白 PINs 的表达以及生长素的梯度分布来负调控 LRP 的发生 [41]。CTK 通过依赖细胞分裂素受体 AHKs 途径介导细胞分裂素响应信号，控制分生组织的大小，并负调控 *PIN* 基因，从而促进侧根形成 [8]。拟南芥细胞分裂素 B 型调节因子 ARR1 介导 RAM 调控以及侧生分生组织的形成，ARR1 在中柱鞘激活 *SHY2* 的表达，以抑制生长素转运蛋白 PINs 的运输，从而减缓侧根原基的形成，并抑制侧根分生组织的发生 [42]。

4 其他植物激素对分生组织发生的影响

脱落酸（abscisic acid，ABA）除了在植物抗逆境和胁迫耐受性中发挥重要作用外，还可以通过脱落酸转运蛋白 NRT1、DTX50、ABCG25 等将 ABA 运输至植物生长发育的各个部位，从而实现对植物生长的调控 [43]。在植物正常生长时，顶端形成的生长素促进相关诱导基因的表达和蛋白的抑制，而在顶端优势去除之后，顶端不能合成足够的 IAA，细胞分裂素合成基因 *IPT* 开始表达并大量合成细胞分裂素，降低 ABA 水平，从而解除腋芽的休眠，促进芽的生长。

茉莉酸（jasmonate acid，JA）通过茉莉素转运蛋白 JAT1/ABCG16 的运输使其在核内外形成浓度差，从而抑制主根的伸长 [25]。茉莉素通过激活生长素生物合成基因 *anthranilate synthase a1*（*ASA1*）的表达来调控生长素的主动运输，从而控制侧根的形成。而茉莉素响应因子 MYC2 抑制生长素响应基因 *PLT1/2* 的表达，并调控 IAA-PLT1/2 介导的 RAM 的形成。另外，茉莉素可以诱导受伤部位快速积累生长素，从而改变损伤处的植物干细胞功能，并形成侧原基 [44]，具体表现为 MYC2 结合在 *PLT1/2* 的启动子上抑制其表达，从而影响主根的伸长，促进侧根的发育。同时茉莉酸调控转录因子 *ERF109* 可以促进生长素合成基因 *YUUC2* 的表达，从而促进生长素的生物合成以促进侧原基的形成 [45]。

赤霉素（gibberellin，GA）在分生组织发育中也发挥了重要作用。STM 可以激活茎端分生组织和侧生分生组织边界的 *GA2ox2*（*GA20-oxidases2*）的表达，导致赤霉素的活性丧失，从而影响侧生器官原基的形成 [46]。在 SAM 中 KNOX1 抑制赤霉素的合成而促进细胞分裂素的合成以维持分生组织的大小，是植物分生组织发育的关键调控因子 [47]；相反的是，细胞分裂素通过刺激 *GA2ox2* 的表达并负调节 *KNOX1*，而促进侧生分生组织的发育 [48]。

独脚金内酯（strigolactones，SLs）作为一类由类胡萝卜素衍生而来的新型植物激素，在植物根部产生，并沿茎干向上运输，从而抑制植物的分枝发育。水稻 *D3*/*14*/*53*（*dwarf3*/*14*/*53*）作为独角金内酯的受体基因和 F-box 家族基因的 *MAX*（*more axillary growth*）一起参与 SL 的生物合成和信号转导。*D3*/*314*/*53*、*MAX1*/*2*/*4* 则通过调控独角金内酯的合成和转导来抑制分枝的发育，*D10* 编码的类胡萝卜素裂解双加氧酶 8（*OsCCD8*）受生长素的诱导，从而控制水稻侧芽向外伸长，而 *OsMAX1* 则通过编码 *OsCCD7* 来抑制水稻腋芽分生组织的生长 [49]。

5 植物激素调控分生组织的展望

分生组织的维持和发育是器官形态建成的基础，同时也是挽救植物机械损伤或自然损伤，以保证植物正常生长和作物产量的一种植物生存机制。在植物

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分生组织的发育过程中，生长素和细胞分裂素通过相互协同或者拮抗作用来共同调控分生组织的维持与发育，并且通过调控生长素 / 细胞分裂素的不同水平来实现对 SAM、RAM 的促进或者抑制。在整个分生组织的发育过程中 ABA、GA、JA、SLs 等通过协同或拮抗生长素和细胞分裂素的合成、运输，响应信号途径，从而实现对分生组织发育的调控。目前众多研究已经揭示了植物激素在细胞增殖和新器官产生中的重要作用，初步明确了维持干细胞和植物器官发育的平衡机制，并且对于生长素和细胞分裂素的合成、运输、信号转导途径及其调控顶端分生组织发育的机制已经比较清晰。

在植物分生组织发育的调控途径中除了文中所述几种激素的调控途径外，还存在其他的信号调控途径的协同作用。在植物的整个发育进程中，所有器官的产生都来自于分生组织内细胞的分裂、分化活动，并贯穿于植物生长发育的整个生长周期。因此，进一步明确顶端分生组织的调控方式对植物生长发育意义重大。植物体内生长素的梯度分布影响了侧根、侧芽的起始以及侧根原基的发育，但生长素梯度的差异是如何转化为不同的信号输出并影响侧生器官的形成还有待进一步研究；脱落酸是否受生长素表达水平的限制或诱导降解为非活性物质从而影响植物分生组织的形成，这一机制还不清楚；独角金内酯除了调控植物侧枝的形成外，还通过抑制植物下胚轴的生长来限制茎的伸长，它通过抑制生长素的合成来影响茎的负向地性的作用机制目前正在解析中。另外，植物激素调控分生组织形态建成的途径仅在拟南芥等模式植物中研究得较为清楚，但对复杂植物组织分化现象的解释还不够，而更加深入研究植物激素调控分生组织发生及发育的分子机制，并将之扩展至各作物中去，将为进一步提升农作物产量和品质提供遗传和理论支撑。

目前，利用野生近缘种培育多年生作物是解决农业生产和生态环境安全的重要手段，具有广阔的应用前景。一年生作物（栽培种）收获后，腋芽再生率或成活率低，表现为随作物驯化过程慢慢被弱化，腋芽再生相关机制及野生种向栽培种的驯化机制还缺乏相关研究。分生组织的形成在作物驯化过程中受到人工选择的影响，从而衍生了许多对分生组织调控途径的研究，期待解析更多关键调控途径来进一步提高生产效率和作物产量。因此，综述植物激素对顶端分生组织发生和发育的调控，对未来研究植物顶端优势的去除、植物腋芽的再生以及越冬休眠机制具有指导性的意义。

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南方农业学报 *Journal of Southern Agriculture* 2022,53(1):47-56
ISSN 2095-1191; CODEN NNXAAB
http://www.nfnyxb.com
DOI:10.3969/j.issn.2095-1191.2022.01.006

云南粳型地方稻资源抗稻瘟病鉴定评价

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摘要：【目的】鉴定和筛选获得云南粳型地方稻抗稻瘟病种质资源，明确其稻瘟病抗性及抗病基因分布，为合理利用水稻抗稻瘟病种质资源及其布局提供参考。【方法】采用病圃自然鉴定与人工接种相结合的方法，对150份云南粳型地方稻资源的稻瘟病抗性进行鉴定，并利用9个已克隆基因（*Pid4*、*Pi25*、*Pi35*、*Pi36*、*Pikm*、*Pigm*、*Pi37*、*Pi56*、*Pi33*）和2个已定位基因（*Pi27*、*Pi23*）对供试稻种资源进行基因型检测，明确抗病基因在抗稻瘟病材料中的分布。【结果】从150份水稻材料中共鉴定出抗稻瘟病（叶瘟或穗颈瘟）材料45份，45份材料中携带*Pi25*、*Pi23*、*Pikm*、*Pi35*、*Pi36*、*Pi33*、*Pi27*、*Pi37*、*Pid4*、*Pigm*抗性基因的材料分别有34份（75.6%）、25（55.6%）、25份（55.6%）、22份（48.9%）、14份（31.1%）、14份（31.1%）、12份（26.7%）、10份（22.2%）、6份（13.3%）和5份（11.1%），未检测到含有*Pi56*的材料。45份抗性材料含有的抗性基因数量介于1~7，大部分含有3~5个，其中携带3个抗性基因材料13份占28.9%，携带4个抗性基因材料8份占17.8%，携带5个抗性基因材料11份占24.4%。抗病能力较强的材料黑壳谷携带6个抗病基因（*Pi25*、*Pi35*、*Pi36*、*Pikm*、*Pi37*和*Pi33*），光壳花糯携带5个抗病基因（*Pi35*、*Pi36*、*Pikm*、*Pi37*和*Pi33*）。【结论】研究获得45份云南粳型抗稻瘟病地方稻材料，鉴定获得2份（黑壳谷和光壳花糯）全生育期抗叶瘟和穗颈瘟材料，这2份抗性材料均携带*Pi35-Pi36-Pikm-Pi37-Pi33*基因组合，可作为抗稻瘟病分子育种的优异抗源材料。

关键词：水稻；稻瘟病；表型鉴定；基因分析；云南省

中图分类号：S435.111.41 **文献标志码：**A **文章编号：**2095-1191(2022)01-0047-10

Evaluation of resistance to rice blast of japonica rice landrace resources in Yunnan

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Abstract:【Objective】Yunnan japonica rice blast resistant germplasm resources were obtained through identification and screening. Furthermore, the distribution of rice blast resistance genes in Yunnan japonica local rice resources were studied, which provided reference for rational utilization and distribution of rice blast resistance germplasm resources. 【Method】The blast resistance of Yunnan japonica local rice resources was identified by natural identification in nursery and artificial inoculation. Rice blast resistance of 150 Yunnan japonica local rice resources was identified. Nine cloned genes (*Pid4*, *Pi25*, *Pi35*, *Pi36*, *Pikm*, *Pigm*, *Pi37*, *Pi56*, *Pi33*) and two localized genes (*Pi27*, *Pi23*) were used to detect the genotypes of the tested rice germplasms to determine the distribution of resistance genes in rice blast resistance materials. 【Result】A total of 45 rice materials resistant to rice blast (leaf blast or panicle neck blast) were identified from 150 rice materials. The numbers of resistant materials carrying resistance genes *Pi25*, *Pi23*, *Pikm*, *Pi35*, *Pi36*, *Pi33*, *Pi27*, *Pi37*, *Pid4* and *Pigm* were 34 (75.6%), 25 (55.6%), 25 (55.6%), 22 (48.9%), 14 (31.1%), 14 (31.1%), 12 (26.7%), 10 (22.2%), 6 (13.3%) and 5 (11.1%). No material containing *Pi56* was detected. The number of resistance genes in 45 resistant materials ranged from 1 to 7, and most of them contained 3 to 5 resistance genes, including 13 materials carr-

收稿日期：2022-01-10
基金项目：云南省青年拔尖人才专项（YNWR-QNBJ-2018-284）；云南省颜龙安院士专家工作站项目（202005AF150032）；云南大学研究生科研创新基金项目（2020325）
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ying three resistance genes(28.9%), 8 materials carrying 4 resistance genes(17.8%) and 11 materials carrying 5 resistance genes(24.4%). Heikegu with strong disease resistance carried 6 resistance genes (*Pi25*, *Pi35*, *Pi36*, *Pikm*, *Pi37* and *Pi33*), and Guangkehuanuo carried 5 resistance genes (*Pi35*, *Pi36*, *Pikm*, *Pi37* and *Pi33*).【Conclusion】In this study, 45 japonica rice blast resistant materials in Yunnan were obtained. And 2 materials with resistance to leaf blast and neck blast were identified as Heikegu and Guangkehuanuo during the whole growth period they the gene combination of *Pi35-Pi36-Pikm-Pi37-Pi33*. This study can provide resistance source materials for molecular breeding and gene mapping of resistance to rice blast.

Key words: rice; rice blast; phenotypic identification; gene analysis; Yunnan

Foundation items: Yunnan Talents Special Project(YNWR-QNBJ-2018-284); Project of Yan Long'an Academician Workstation of Yunnan(202005AF150032); Graduate Research Innovation Project of Yunnan University(2020325)

0 引言

【研究意义】水稻(*Oryza sativa* L.)是全球约1/2人口的主粮。由子囊真菌(*Magna-porthe oryzae*)引起的稻瘟病被称为"水稻癌症",其严重影响水稻产量和稻米品质,对世界粮食安全构成严重威胁。在25~28 ℃、空气湿度达96%的高温高湿环境下最易发生稻瘟病(张小花等,2021)。稻瘟菌具有遗传复杂性、致病多样性和变异高度性的特点,水稻品种经过长时间种植易丧失抗性而成为感病品种(Wilson and Talbot,2009;徐志健等,2020)。云南是水稻的起源和演化中心之一,具有丰富且遗传多样性水平高的稻种资源(陈越等,2019),由于特殊的地理隔离环境,这些资源外观表型差异大,长期的稻种隔离进化或演化,在多种多样病原菌的胁迫下,极易演化出多种多样的抗稻瘟病基因,是拓宽现代栽培稻抗稻瘟病遗传基础的重要资源。在云南省水稻品种审定中稻瘟病实行一票否决制。基于绿色发展理念,优化栽培管理措施、栽种抗稻瘟病品种是防治稻瘟病的有效手段(温小红等,2013),而开展水稻种质资源的抗病性评价,综合选育出适合当地栽培的高产优质抗病新品系(新品种),是水稻抗性资源利用的基础工作。抗病基因被挖掘、定位和克隆为水稻抗稻瘟病育种提供了丰富的抗源(殷得所等,2011)。利用基因序列信息,发展特异性功能标记,可快速检测品种的抗稻瘟病基因型(何重等,2014),对全面了解我国水稻抗稻瘟病的基因型及其合理布局与轮换具有重要意义。【前人研究进展】国内外对稻瘟病抗性基因已有较系统深入的研究,随着抗瘟基因定位、克隆及分子标记的发展,分子标记辅助育种的效率得以大幅度提高(王生轩等,2017)。近年来已从水稻种质资源中筛选和鉴定出一批优良的抗稻瘟病资源,并利用分子标记技术鉴定和定位出约100多个稻瘟病抗性基因(徐小金,2016),鉴定的抗稻瘟病QTLs有500多个(Li et al.,2019),其中已克隆37个NBS-LRR基因*Pit*、*Pi37*、*Pish*、*Pi35*、*Pi64*、*Pib*、*Pi63*、*Pi2*、*Pi9*、*Piz-t*、*Pigm*、*Pid3*、*Pid3 -A4*、*Pi25*、*Pi50*、*Pi36*、*Pi33*、*Pi5*、*Pii*、*Pi56*、*Pia*、*Pi-CO3911*、*Pi54*、*Pi54rh*、*Pi54of*、*Pik*、*Pi-1*、*Pikh*、*Pikm*、*Pikp*、*Pike*、*Piks*、*Pb1*、*Pi-ta*、*Pid4*、*rbr2*)、*Pi48*(杨红等,2008;盛浩闻,2014;陈智雄,2018;Chen et al.,2018;高清等,2021)及6个其他结构类型基因*bsr-d1*(MYB转录因子)、*pi21*(富含脯氨酸蛋白)、*Pid2*(RLK)、*bsr-k1*(富含TPRs蛋白)、*Ptr*(富含ARM蛋白)、*Pi65*(LRR-RLK)(高清等,2021;Wang et al.,2021)。利用抗性基因的功能标记或紧密连锁分子标记,可准确地开展水稻种质资源抗稻瘟病基因型鉴定工作,已相继从云南地方稻资源中鉴定出一批含有抗稻瘟病基因的种质,如董丽英等(2012)利用由24个稻瘟病抗性单基因系组成的鉴别材料对云南稻区的稻瘟病菌群体致病性进行分析,结果发现*Piz-t*、*Pi9*和*Pi5*等基因是改良品种抗病性的重要基因源;在云南籼稻区对稻瘟病菌抗性频率高于80%的基因主要有*Pi9*、*Pi5*、*Pik-h*、*Pita-2*、*Piz-5*、*Pita*和*Piz*,在粳稻区对稻瘟病菌抗性频率高于80%的基因主要有*Pi9*、*Pi5*、*Piz-t*和*Pi20*;李进斌等(2012b)对云南省176份地方水稻品种进行*Pita*和*Pib*基因型检测,发现携带*Pita*或*Pib*基因的品种对稻瘟病的抗性较好;孙一丁等(2018)研究5个抗稻瘟病主效基因在云南省抗稻瘟病育种中的利用状况,用分子标记对云南省推广品种和优异地方稻种进行了分子检测,发现*Pik-h*、*Pi5*和*Pita*在地方品种中出现频率较高;马继琼等(2021)研究结果表明,*Pib*、*Pikh*、*Piz*、*Pi9*和*Pi5*等5个基因在云南籼粳稻交错区—温暖粳稻亚区具有广泛的利用价值。【本研究切入点】前人虽然已有对云南地方稻资源进行抗稻瘟病鉴定的报道,但往往仅限于单一的叶瘟或穗颈瘟鉴定;对材料既进行实验室和田间自然鉴定、又进行苗期和孕穗期接菌鉴定的较少见,且云南地方稻材料中是否携带9个已克隆基因*Pid4*、*Pi25*、

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Pi35、*Pi36*、*Pikm*、*Pigm*、*Pi37*、*Pi56*、*Pi33*和2个已定位基因*Pi27*、*Pi23*的研究尚未可知。【拟解决的关键问题】采用多种表型鉴定与基因型鉴定相结合的方法，对150份云南粳型地方稻抗稻瘟病及其所携带9个已克隆基因（*Pid4*、*Pi25*、*Pi35*、*Pi36*、*Pikm*、*Pigm*、*Pi37*、*Pi56*、*Pi33*）和2个已定位基因（*Pi27*、*Pi23*）情况进行鉴定，明确供试云南粳型地方稻资源的稻瘟病抗性及本研究所检测基因的分布，以鉴定和筛选获得云南粳型地方稻抗稻瘟病种质资源，为合理利用水稻抗稻瘟病种质资源及其布局提供参考。

1 材料与方法

1.1 试验材料

供试水稻材料为云南省农业科学院生物技术与种质资源研究所提供的150份来源于云南16个州（市）的粳型地方稻资源，以蒙古稻为诱发材料，以感病品种丽江新团黑谷为阴性对照。供试稻瘟病菌菌株：供试菌株8个，为云南强致病稻瘟病菌株491、66b、540、129、179、463、YN355和226，由云南省农业科学院环境资源研究所杨勤忠老师和云南农业大学植物保护学院李成云老师提供。

1.2 试验方法

1.2.1 *稻瘟病菌培养和孢子悬浮液制备* 分别采用PDA培养基（Liu and Stützel，2004）（去皮马铃薯200 g、葡萄糖10 g、琼脂15 g、蒸馏水1000 mL）和番茄燕麦产孢培养基（张国珍等，2002）（燕麦片40 g、番茄汁150 mL、琼脂20 g、蒸馏水1000 mL）进行稻瘟病菌菌丝培养及孢子培养。制备稻瘟病菌孢子悬浮液时，先在产孢的培养皿中加入5 mL灭菌水，用棉签或载玻片刮洗，洗下培养基表面的分生孢子，过滤收集孢子悬浮液，菌液用纱布（或专用滤布）过滤，滤液在100倍显微镜下观察有20~50个孢子时可供苗期喷雾接菌和孕穗期注射接菌使用（李进斌等，2012a）。

1.2.2 *稻瘟病菌的苗期接种鉴定* 将供试水稻材料浸种至露白，分批播于50孔加深育苗盘（54.0 cm×28.0 cm×9.5 cm）中，以丽江新团黑谷为感病对照，每个材料播种16株，2.5叶期每盘施0.5 g尿素（李进斌等，2012a），1 d后追施第2次，施肥7 d后用加入0.2% Tween-20的混合菌悬浮液（66b、129、491、179、463、226、540和YN355）进行喷雾接种（夏小东等，2011）。接种后的稻苗置于26~28 ℃、湿度95%的人工气候箱中暗处理24 h，随后在12 h光照：12 h黑暗、温湿度保持不变的环境下培养，7 d后进行调查。

1.2.3 *稻瘟病菌的自然诱发鉴定和接种鉴定* 在云南省玉溪市易门县实验基地田间自然诱发鉴定圃对参试材料进行苗期和孕穗期自然诱发鉴定及孕穗期人工注射接种鉴定。

人工接种鉴定时考虑到8个菌工作量较大，因此只用3个致病菌株491、66b和540在参试材料孕穗期时对破肚前3~4 d的穗包进行人工注射接菌法鉴定穗颈瘟。每个菌株分别接种10株，每穗接种5~10 mL菌液，接种20 d或成熟后进行调查。

1.2.4 *发病调查标准* 参照国际水稻研究所叶瘟抗性评价分级标准对发病情况进行调查，按照病情指数=∑（各级病株数×该病级值）/（调查总株数×最高级值）×100进行病情指数计算。病情指数为0表示高抗（HR），0~5.0%为抗（R），5.1%~15.0%为中抗（MR），15.1%~25.0%为中感（MS），25.1%~50.0%为感（S），≥50.1%为高感（HS）（何奕霏等，2021）。人工注射接种鉴定穗颈瘟参考GB/T 15790—2009《稻瘟病测报调查规范》制定的稻瘟病9级（以穗为单位）标准进行调查（董丽英等，2018），计算出穗平均损失率后对应9级发病等级。注射接种鉴定抗1~3个接种稻瘟病菌株记为抗，感3个菌株时记为感。

1.2.5 *抗性基因的分子标记检测* 在水稻分蘖期或拔节期，取新鲜水稻叶片用试剂盒的方法提取DNA。抗性基因分子标记序列见表1，PCR检测方法参照表1的相关文献。以丽江新团黑谷为阴性对照，引物均由北京擎科新业生物技术有限公司合成。

1.3 统计分析

采用Excel 2016对数据进行整理和作图。采用SPSS 23.0对不同水稻品种含有的抗瘟基因数与抗性频率间的相关性进行分析。

2 结果与分析

2.1 稻瘟病表型鉴定结果

自然诱发和人工接种鉴定结果显示蒙古稻和丽江新团黑谷发病均达9级，表明试验结果数据可靠。叶瘟发病情况如图1~图3，以自然诱发和喷雾接菌均中抗及以上的材料记为抗叶瘟，反之记为感叶瘟。自然诱发鉴定出抗叶瘟材料5份、中抗材料56份、中感材料53份、感病材料32份、高感材料4份；室内人工接种鉴定共鉴定出抗叶瘟材料2份、中抗材料22份、中感材料63份、感病材料63份。综合自然诱发和人工喷雾2种方法接种鉴定叶瘟，共鉴定出叶瘟发病等级在0~4级、病情指数在15%以下的抗叶瘟材料8份。

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表 1　PCR引物信息
Table 1　PCR primers information

目的基因 Target gene	引物名称 Primer name	引物序列 Primer sequence	片段大小(bp) Product size	退火温度(℃) Annealing temperature	分子标记类型 Molecular marker type	参考文献 Reference
Pid4	*Pid4*-GAPF	5'-CCGCATCTCCTCCTTCCTCT-3'	279 bp(抗)/352 bp(感)	58	Indel功能标记	Chen et al., 2018
	Pid4-GAPR	5'-AGAGATCTCGCTGTTCAGTC-3'				
Pi25	Si13070C-F	5'-ATAACGTGTGTCCATAACAGT-3'	149 bp(抗)/110 bp(感)	55	功能标记	朱玉君等,2010
	Si13070C-R	5'-TTAATGACTCGTGTATAGAGC-3'				
Pi35	*Pi35*-PF	5'-GCGTACAGCGTTCGCTCTATA-3'	–	55	基因内特异SNP共显性	徐华山等,2016
	Pi35-PR	5'-CTTCAAAAGCTCACAACCA-3'	317 bp PF-PR(T型)			
	Pi35-NF	5'-CCGTCCTCCCTCCAGCATATATGTA TAAGG GATGCAGGATAAAGAATGAA-3'	535 bp PF-NR(共有带)			
	Pi35-NR	5'-CTGCAAAACAAAGGAAACGTG-3'	266 bp NF-NR(G型)			
Pi36	*Pi36*F	5'-CAATGTGTGACTTGTGCGGACT-3'	1036 bp(抗)	68	特异引物	靳春鹏等,2011
	*Pi36*R	5'-TCTTCCATCTCGGATTTCGTGT-3'				
Pikm	Dkm1-F	5'-CTGGAGAGCTTCCGTGTCGAC-3'	223 bp(抗)	62	功能标记	Costanzo and Jia,2010
	Dkm1-R	5'-TCTTCACGACGTCAATGGTGGC-3'				
Pigm	*Pigm*F	5'-CCTTGTTCCTCCTGCTATCG-3'	约220 bp(抗),谷梅4号为阳性对照	56	共分离标记	–
	*Pigm*R	5'-TCTCGCTGTTCAGTCTTGGA-3'				
Pigm	M143104F	5'-CCTTGTTCCTCCTGCTATC-3'	215 bp(抗)/288 bp(感)	55	功能标记	李友发等,2021
	M143104R	5'-ATCTCGCTGTTCAGTCTTG-3'				
Pi37	*Pi37*-1F	5'-AATGAACTGAAGACCATGCTGGA-3'	600 bp(抗)	53	特异引物	王丽丽等, 2017
	Pi37-1R	5'-TGGAAGTACATCTCGTCTAGAAG-3'				
Pi37	*Pi37*F	5'-TCTTGAGGGTCCCAGTGTAC-3'	1149 bp(抗)	68	特异引物	谷思涵等,2020
	*Pi37*R	5'-CGAACAGTGGCTGGTATCTC-3'				
Pi56	RM24022F	5'-TCCTCTCAACCATGTACGGAATAGC-3'	298 bp(抗)	58	连锁标记	王晓玲等,2021
	RM24022R	5'-CGATGTGTTTATGTCCGCAATCC-3'				
Pi56	*Pi56*F	5'-ATGGAAACTTGGCTTGCTG-3'	1156 bp(抗)	62	全长引物	本研究
	*Pi56*R	5'-CTATGAGTTCACTATGTGGAGGCT-3'				
Pi33	RM3507F	5'-ACCCCTATCGATCAACCCTC-3'	123 bp(抗)	58	连锁标记	Berruyer et al., 2003
	RM3507R	5'-TTCGTTTGGTGTTAGGGGC-3'				
Pi27	RM151-F	5'-GGCTGCTCATCAGCTGCATGCG-3'	197 bp(抗)	55	连锁标记	Zhu et al.,2004
	RM151-R	5'-TCGGCAGTGGTAGAGTTTGATCTGC-3'				
Pi27	RM259-F	5'-TGGAGTTTGAGAGGAGGG-3'	162 bp(抗)	62	连锁标记	Zhu et al.,2004
	RM259-R	5'-CTTGTTGCATGGTGCCATGT-3'				
Pi23	RM164F	5'-TCTTGCCCGTCACTGCAGATATCC-3'	246 bp(抗)	60	连锁标记	Ahn et al.,1996
	RM164R	5'-GCAGCCCTAATGCTACAATTCTTC-3'				

穗颈瘟鉴定结果(图4),自然诱发鉴定发现高抗材料6份,抗病材料28份,中抗材料46份,中感材料29份,感病材料20份,高感材料21份。注射接种鉴定发现150份供试材料中同时抗491、66b和540菌株的材料7份;抗2个菌株的材料18份,其中抗491和540菌株的材料8份,抗66b和540菌株的材料5份,抗491和66b菌株的材料5份;只抗1个菌株的材料43份,其中只抗491菌株的材料13份,只抗66b菌株的材料12份,只抗540菌株的材料18份;不抗3个菌株的材料82份。综合自然诱发鉴定和人工注射接种鉴定结果,自然诱发鉴定在0~3级且至少抗1个接种稻瘟病菌株的材料统计为抗穗颈瘟,共鉴定出抗穗颈瘟材料39份。

综合叶瘟和穗颈瘟鉴定结果,共筛选出表型抗稻瘟病(叶瘟或穗颈瘟)材料45份,感稻瘟病材料105份。45份抗稻瘟病材料中抗穗颈瘟、感叶瘟材料37份,占供试材料的24.7%,抗叶瘟、感穗颈瘟材料6份,占供试材料的4.0%,既抗叶瘟又抗穗颈瘟材料2份(黑壳谷和光壳花糯),占供试材料的1.3%,该2份材料可作为抗稻瘟病育种优异抗性亲本(表2)。

2.2　抗性基因在参试水稻材料中的分布

对从云南粳型地方稻材料中筛选出的45份表型抗叶瘟或穗颈瘟材料进行分子标记检测,根据11个抗稻瘟病基因的特异性功能标记或紧密连锁分子标记进行PCR扩增,以丽江新团黑谷为阴性对照,以谷梅4号为*Pigm*基因的阳性对照(Deng et al.,2006),分析11个基因及其同源基因在45份材料中的分布情况。结果表明,携带*Pi25*、*Pi23*、*Pikm*、*Pi35*、*Pi36*、*Pi33*、*Pi27*、*Pi37*、*Pid4*和*Pigm*抗性基因的抗性供试材料分别有34份(75.6%)、25份(55.6%)、25份(55.6%)、22份(48.9%)、14份(31.1%)、14份(31.1%)、12份(26.7%)、10份(22.2%)、6份(13.3%)和5份(11.1%)。在45份供试抗性材料中,11个抗性基因的分布以*Pi25*、*Pi23*和*Pikm*最多,*Pid4*和*Pigm*较少,未检测到含*Pi56*的材料。没有材料同时含有11个检测抗性基

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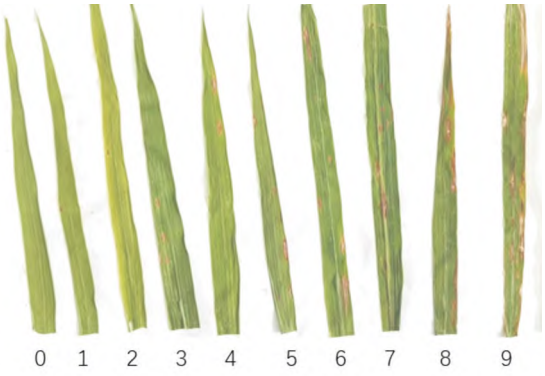


图 1　田间叶瘟发病情况调查(0~9级)
Fig.1　Investigation on the incidence of leaf blast in the field (grades 0-9)



图 2　自然诱发叶瘟蒙古稻发病情况
Fig.2　Incidence of naturally induced leaf blast in Menggudao Rice



图 3　自然诱发叶瘟供试材料感病病斑
Fig.3　Typical disease spots of naturally induced leaf blast

因,也没有材料不含任何抗性基因。

2.3　携带抗性基因数量与水稻的抗病性

45份抗性材料含有的抗性基因数量介于1~7个,大部分含有3~5个,其中携带1个抗性基因的材料2份占4.4%,携带2个抗性基因的材料7份占15.6%,携带3个抗性基因的材料13份占28.9%,携带4个抗性基因的材料8份占17.8%,携带5个抗性基因的材料11份占24.4%,携带6个抗性基因的材料3份占6.7%,携带7个抗性基因的材料1份占2.2%。抗病能力较强的材料黑壳谷携带6个抗病基因(*Pi25*、*Pi35*、*Pi36*、*Pikm*、*Pi37*和*Pi33*),光壳花糯携带5个抗病基因(*Pi35*、*Pi36*、*Pikm*、*Pi37*和*Pi33*)(表3)。

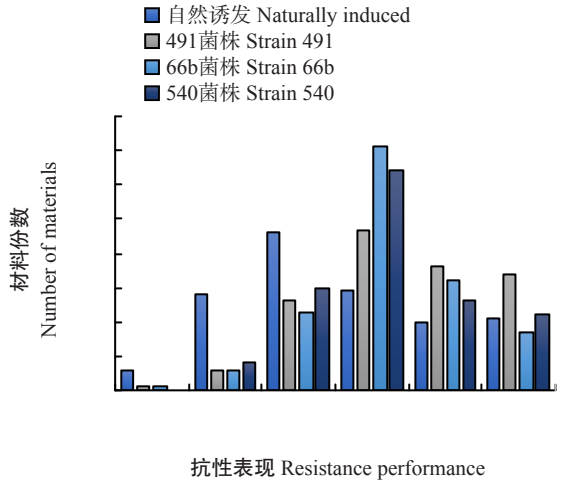


图 4　穗颈瘟鉴定结果
Fig.4　Identification of neck blast

参试材料的抗病性大致随着携带抗性基因数量的增多呈上升趋势。利用SPSS 23.0对45份材料携带抗性基因数量与叶瘟、穗颈瘟抗性水平(R记为1,S记为0)进行Pearson相关性分析,结果显示抗性基因数量与叶瘟和穗颈瘟抗性水平的相关系数分别为0.133和0.019,表明携带的抗性基因数量与叶瘟和穗颈瘟抗性水平均无显著相关(P>0.05)。

3　讨论

研究表明,培育广谱持久抗稻瘟病品种和抗病品种合理布局是绿色防控稻瘟病最经济、有效的措施,而开展稻瘟病抗性品种鉴定是筛选优良抗病品种和抗瘟基因的重要基础。本研究从150份云南粳型地方稻材料中综合鉴定获得45份抗性种质资源,其中,抗穗颈瘟材料39份,抗叶瘟材料8份,既抗叶瘟又抗穗颈瘟材料2份,为黑壳谷和光壳花糯。本研究鉴定的抗稻瘟病资源对云南现有稻瘟病菌具有显著抗性,可作为稻瘟病育种和抗病基因研究的优异抗源材料。因水稻品种抗病性差异、稻瘟病生理小种侵染能力强弱、抗病鉴定生态区不同和气温年际间差异等,在稻种资源叶瘟抗性与穗颈瘟抗性关系的研究结果中有一定差异,叶瘟抗性变化较穗颈瘟大(陈福如等,2006)。本研究发现水稻的叶瘟抗性与穗颈瘟抗性并非一一对应,感叶瘟的材料到后期不一定就感穗颈瘟,与殷春渊等(2017)的研究结论一

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表 2　表型鉴定中筛选出的45份抗稻瘟病材料

Table 2　45 blast resistant materials selected from phenotypic identification

编号 Serial number	材料 Material	叶瘟抗性 Leaf blast resistance			穗颈瘟抗性 Neck blast resistance					
		自然诱发叶瘟 Naturally induced leaf blast	喷雾接菌叶瘟 Bacterial Leaf blast by spray	叶瘟评价 Evaluation of leaf blast	自然诱发穗颈瘟 Naturally induced panicle neck blast	接种491菌株 Inoculated strain 491	接种66b菌株 Inoculated strain 66b	接种540菌株 Inoculated strain 540	抗已知菌株 Resistant strain	穗颈瘟评价 Evaluation of neck blast
G246	光壳花糯	MR	R	R	R	R	R	R	491、66b、540	R
G234	黑壳谷	MR	MR	R	MR	S	S	R	540	R
G62	高脚糯	MS	S	S	R	R	S	S	491	R
G68	饵块谷(一)	MS	S	S	MR	S	S	R	540	R
G70	小红脚	MR	S	S	R	S	R	R	66b、540	R
G72	大白糯(1)	S	S	S	R	S	R	R	66b、540	R
G127	毫卡	MR	MS	S	MR	R	S	R	491、540	R
G128	毫咙早	MR	MS	S	R	S	S	R	540	R
G137	昆明红	MS	MS	S	MR	R	R	S	491、66b	R
G182	检棵红	MS	MS	S	MR	R	R	R	491、66b、540	R
G238	金包银	HS	MR	S	MR	R	S	R	491、540	R
G262	毫啊(老鸦谷)	MR	MS	S	MR	S	S	R	540	R
G280	乌九糯	MR	S	S	MR	R	S	S	491	R
G281	香谷(秆黄壳)	MR	S	S	MR	S	S	R	540	R
G294	黄金谷	MR	S	S	MR	R	S	S	491	R
G296	糯白谷	MS	S	S	MR	S	S	R	540	R
G480	本地红谷	R	MS	S	MR	S	S	R	540	R
G481	失名	MR	S	S	HR	S	R	S	66b	R
G110	大白谷	MR	S	S	MR	S	S	R	540	R
G239	长毛红糯	S	MR	S	MR	R	R	S	491、66b	R
G31	大红糯	MR	MS	S	R	S	S	R	540	R
G55	糯谷	S	MS	S	MR	S	S	R	540	R
G82	白山谷	MR	MS	S	R	S	R	S	66b	R
G85	小花糯	MR	S	S	MR	S	R	R	66b、540	R
G106	毫甘哟	MR	S	S	MR	R	S	R	491、540	R
G163	白长毛谷	MR	MS	S	MR	R	S	S	491	R
G165	鸡叫糯	MR	MS	S	MR	S	R	S	66b	R
G237	红壳谷(秆黄壳)	MS	MR	S	MR	R	S	R	491、540	R
G247	黄绿糯	MR	S	S	MR	R	S	R	491	R
G248	冷浸谷(二)	S	S	S	HR	S	R	S	66b	R
G269	金包银	MR	S	S	MR	S	R	R	66b、540	R
G320	白果糯	S	MS	S	MR	R	S	R	491、540	R
G354	老来红糯谷	MS	S	S	MR	R	S	S	491	R
G391	细烂地谷	S	MS	S	R	R	R	S	491、66b	R
G392	小黑咀	MS	MS	S	R	R	R	R	491、66b、540	R
G402	香糯	HS	MR	S	R	S	R	S	66b	R
G404	水杂谷	MR	S	S	R	R	R	S	491、66b	R
G406	黄壳糯	MS	MS	S	R	R	S	R	491、540	R
G472	选6号	MR	MS	S	R	R	S	S	491	R
G65	老来红(一)	MR	MR	R	MS	S	S	S		S
G188	毫糯浪	MR	MR	R	S	S	S	R	540	S
G254	黑地糯	MR	MR	R	S	R	S	R	491、540	S
G142	香糯(二)	MR	MR	R	MS	S	S	S		S
G263	矮棵红谷(完)	MR	R	R	MR	S	S	S		S
G399	香谷	MR	MR	R	MS	S	S	S		S

抗叶瘟：该材料自然诱发和混合接种8个强致病菌株的菌悬液处理鉴定结果均为中抗及以上；抗穗颈瘟：该材料在5年自然诱发穗颈瘟为中抗及以上且分别注射接种491、66b和540菌株后抗1~3个已知菌株

Resistance to leaf blast showed that the results of bacterial suspension treatment of 8 strongly pathogenic strains naturally induced and mixed with this material were moderately resistant and above, while resistance to panicle neck blast for 5 years was moderately resistant to neck blast and resistant to 1-3 known strains after inoculation with strains 491, 66b and 540, respectively

致。从整个表型鉴定结果来看，筛选出的抗叶瘟材料少于抗穗颈瘟材料，可能是因为叶瘟与穗颈瘟鉴定试验的严格程度、接种环境和接种菌株数量的不同而导致。与穗颈瘟田间鉴定相比，叶瘟鉴定是在严格控温控湿的人工气候室内喷雾接种8个菌株的混合菌悬液，不但人为控制了发病条件，而且喷雾接种的菌株数量比注射接种的菌株数量多。

一定程度上，聚合多个抗性基因有利于拓宽品

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表 3　45份表型抗叶瘟或穗颈瘟材料基因型检测结果

Table 3　Genotypic detection of 45 phenotypic materials resistant to leaf blast or neck blast

编号 Serial number	材料 Material	携带基因数 Number of gene	*Pid4*	*Pi25*	*Pi35*	*Pi36*	*Pikm*	*Pigm*	*Pi27*	*Pi37*	*Pi56*	*Pi23*	*Pi33*
G234	黑壳谷	6	−	+	+	+	+	−	−	+	−	−	+
G246	光壳花糯	5	−	−	+	+	+	−	−	+	−	−	+
G62	高脚糯	2	−	+	−	−	−	−	−	−	−	+	−
G68	饵块谷(一)	3	−	−	+	−	+	−	−	−	−	+	−
G70	小红脚	2	−	−	−	−	+	−	−	−	−	+	−
G72	大白糯(1)	4	−	+	+	+	−	−	−	−	−	−	+
G127	毫卡	3	−	+	−	−	+	−	+	−	−	−	−
G128	毫咙早	5	+	+	−	−	+	−	+	−	−	+	−
G137	昆明红	4	+	−	−	−	+	+	−	−	−	+	−
G182	检棵红	3	−	+	−	−	−	−	+	−	−	+	−
G238	金包银	5	−	+	+	+	+	−	−	+	−	−	−
G262	毫啊(老鸦谷)	5	−	+	+	+	+	−	−	−	−	−	+
G280	乌九糯	1	−	−	−	−	−	−	−	−	−	+	−
G281	香谷(秆黄壳)	4	−	+	−	−	+	−	+	−	−	+	−
G294	黄金谷	4	+	+	−	−	−	+	−	−	−	+	−
G296	糯白谷	3	−	+	−	−	+	−	−	−	−	+	−
G480	本地红谷	6	+	+	+	−	+	+	−	+	−	−	−
G481	失名	3	−	+	−	−	+	−	−	−	−	+	−
G110	大白谷	3	−	−	+	−	+	−	−	−	−	−	+
G239	长毛红糯	3	−	−	−	+	−	−	−	+	−	−	+
G31	大红糯	3	−	+	+	−	−	−	−	−	−	−	+
G55	糯谷	3	−	+	+	−	−	−	−	+	−	−	−
G82	白山谷	3	−	+	−	−	+	−	−	−	−	+	−
G85	小花糯	6	+	+	+	−	+	+	−	−	−	+	−
G106	毫甘哟	4	−	+	−	−	+	−	+	−	−	+	−
G163	白长毛谷	5	−	+	+	+	+	−	−	+	−	−	−
G165	鸡叫糯	2	−	+	−	−	+	−	−	−	−	−	−
G237	红壳谷(秆黄壳)	5	−	+	+	+	−	−	−	+	−	−	+
G247	黄绿糯	1	−	−	−	−	−	−	−	−	−	+	−
G248	冷浸谷(二)	3	−	+	+	−	−	−	−	−	−	−	+
G269	金包银	3	−	+	−	−	−	−	+	−	−	+	−
G320	白果糯	5	−	+	+	+	−	−	−	+	−	−	+
G354	老来红糯谷	5	−	+	−	+	+	−	+	−	−	+	−
G391	细烂地谷	4	−	+	+	−	+	−	−	−	−	+	−
G392	小黑咀	2	−	+	+	−	−	−	−	−	−	−	−
G402	香糯	5	−	+	+	+	−	−	−	+	−	−	+
G404	水杂谷	3	−	+	−	−	−	−	+	−	−	+	−
G406	黄壳糯	5	−	+	+	+	−	−	−	−	−	+	+
G472	选6号	4	−	+	−	+	+	−	−	−	−	+	−
G65	老来红(一)	7	+	+	+	−	+	+	+	−	−	+	−
G188	毫糯浪	4	−	+	−	−	+	−	+	−	−	+	−
G254	黑地糯	5	−	+	+	+	+	−	−	−	−	−	+
G142	香糯(二)	2	−	−	+	−	−	−	−	−	−	−	+
G263	矮棵红谷(完)	2	−	−	−	−	−	−	+	−	−	+	−
G399	香谷	2	−	−	−	−	−	−	+	−	−	+	−

+：表示携带抗病等位片段；−：表示携带感病等位片段

+: Indicated carrying disease resistant allelic fragment; −: Indicated carrying disease susceptible allelic fragment

种的抗谱，提高材料的抗病性，可作为培育稻瘟病持久抗性的有效方法（张银霞等，2016）。但携带基因数量不是影响抗性的唯一因素，供试材料来源、遗传背景、基因组合类型和聚合方式以及基因之间的互作均会影响稻瘟病抗性（邓钊等，2022）。王丽丽等（2017）研究认为抗瘟基因聚合与抗病性并非完全正相关，本研究对不同抗稻瘟病基因的抗病效应进行相关性分析，发现抗性基因数量与材料的稻瘟病抗性无显著相关，即表型抗感与携带抗性基因个数并非一一对应关系。其中，从2份抗叶瘟和穗颈瘟材料（黑壳谷和光壳花糯）发现*Pi35-Pi36-Pikm-Pi37-Pi33*基因组合在本研究中表现抗病能力最强，黑壳谷比光壳花糯多携带1个抗瘟基因*Pi25*，但表型抗叶瘟和穗颈瘟能力弱于光壳花糯，说明抗稻瘟病基因间的互作关系十分复杂，抗稻瘟病基因的聚合在一定程度上能提高水稻抗性，但水稻抗瘟能力的提高并不是简单的抗瘟基因叠加的结果。因此，对抗稻瘟病水稻亲本的选择，应从田间筛选出抗病性较强的亲

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本作为抗稻瘟病育种的候选亲本。

4　结论

通过表型鉴定与基因分析相结合的方法从150份来自云南多地的粳型地方稻资源进行抗稻瘟病分析，鉴定出表型抗稻瘟病（叶瘟或穗颈瘟）材料45份，其中黑壳谷和光壳花糯2份材料在表型鉴定中既抗叶瘟又抗穗颈瘟，携带*Pi35-Pi36-Pikm-Pi37-Pi33*基因组合的抗病能力最强，可作为抗稻瘟病分子育种的优异抗源材料。

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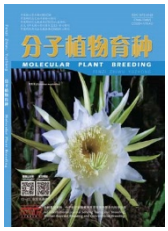
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分子植物育种
Molecular Plant Breeding
ISSN 1672-416X,CN 46-1068/S

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题目：马铃薯晚疫病抗性基因及其病原菌无毒基因研究进展
作者：尹润金，霍超，曹继芬，隋启君，黄斌全，赵志坚
网络首发日期：2022-03-29
引用格式：尹润金，霍超，曹继芬，隋启君，黄斌全，赵志坚．马铃薯晚疫病抗性基因及其病原菌无毒基因研究进展[J/OL]．分子植物育种．
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网络首发时间：2022-03-29 10:23:21
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评述与展望

Review and Progress

马铃薯晚疫病抗性基因及其病原菌无毒基因研究进展

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摘　要　晚疫病是全球马铃薯生产最重要的卵菌病害，可引起大幅减产并造成严重的经济损失。抗病育种是防控晚疫病最经济有效的措施，近年来马铃薯晚疫病抗性基因、晚疫病菌无毒基因的研究取得了显著进展，为马铃薯抗晚疫病分子标记辅助育种、基因工程育种及马铃薯晚疫病精准防控提供了广阔的前景和新的视野。本研究从马铃薯晚疫病主效抗性基因定位与克隆、病原菌无毒基因、抗病基因与无毒基因的互作等方面进行综述，并据此提出了进一步开展的研究建议，这可为马铃薯抗病育种和晚疫病防控提供科学参考。

关键词　马铃薯；晚疫病菌；抗性基因；无毒基因；抗病育种

Recent Advances in Resistance Genes of Potato Late Blight and Avirulence Genes of *Phytophthora infestans*

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Abstract　Late blight is the most important oomycete disease on potato production in worldwide, which can cause serious yield and economic losses. Resistant potato breeding is the most economical and effective strategy for late blight management. In recent years, remarkable progress has been made in research on potato resistance genes and cognate avirulence genes of *Phytophthora infestans*, which provides broad prospects and new insights for molecular marker-assisted breeding, gene engineering breeding and precision control of late blight. Here, we reviewed the location and cloning of potato major resistance genes against late blight, cognate avirulence genes of *Phytophthora infestans*, the interaction between resistance genes and avirulence genes, respectively. We also

基金项目：本研究由国家重点研发计划项目（2021YFE0109600）、云南省农业基础研究联合专项重点基金(2018FG001-001)、云南省重点研发计划项目(202102AE090019)和云南省种子种业联合实验室项目共同资助

proposed further research suggestions based on these advances. It could provide important scientific reference for potato resistance breeding and late blight management.

Keyword Potato; *Phytophthora infestans*; Resistance gene; Avirulence genes; Resistance breeding

马铃薯(*Solanum tubersum* L.)起源于南美洲，是世界上继水稻、小麦、玉米之后的第四大粮食作物，同时也是粮、菜、饲兼用型农作物。中国是世界第一大马铃薯生产国，马铃薯不仅在保证国家粮食安全方面有重要作用，在脱贫攻坚方面也扮演重要角色。由晚疫病菌(*Phytophthora infestans*)引起的晚疫病是全球马铃薯生产的毁灭性病害。现代植物病理学诞生于对马铃薯晚疫病病因的研究。时至今日，晚疫病菌造成的马铃薯晚疫病仍然是最严重的作物病害之一，晚疫病依旧在全球各地的马铃薯产区发生和流行，引起巨大的经济损失(Fry et al., 2015)。中国马铃薯年均种植面积约 5.40×10^6 hm^2，但平均单产仅为 1.54×10^4 kg/hm^2，低于世界平均水平，晚疫病的危害是中国平均亩产偏低的主要限制因素。该病在各生态种植区的常年发生面积为 2.05×10^6 hm^2，偏重或大流行年份可占播种面积的 46%以上，中国每年因晚疫病减产10%~30%，严重流行时可达 50%以上或至绝产(黄冲和刘万才, 2016)。

选育抗病品种是防控植物病害最经济有效的措施。病原菌与寄主协同进化，相互对抗，晚疫病菌基因组较大，在选择压力下，变异进化快，容易快速克服品种的抗性，从而导致抗性丧失，防治比较困难。目前已经鉴定的马铃薯主效抗性基因多达 70 多个，但绝大多数抗性基因已经失效。幸运的是，近年来随着基因组学和抗性基因富集测序 Renseq (Resistance gene enrichment sequencing)等新技术的发展，一些具有广谱抗性的基因从马铃薯野生种中被鉴定和克隆，并通过多基因聚合的方式转移到栽培品种中，在晚疫病抗病育种中发挥着重要作用。

本研究简要对马铃薯晚疫病抗性基因定位与克隆、病原菌无毒基因鉴定、抗病基因与无毒基因的识别机制等方面进行了综述，对研究马铃薯晚疫病抗性基因及病原菌无毒基因具有重要借鉴，并据此提出了进一步开展抗病育种的研究建议，以期为现阶段马铃薯抗晚疫病育种和晚疫病精准防控提供科学参考。

1 马铃薯晚疫病抗性基因定位与克隆

1.1 马铃薯晚疫病抗性基因定位

由于马铃薯晚疫病的流行性和毁灭性，许多植物病理学家和育种学家通过各种方法鉴定相关的抗性基因。经过多年的搜集和鉴定不同的抗源，已经发现并鉴定了大量的马铃薯主效抗性基因(Rodewald and

Trognitz, 2013)。采用基因工程育种技术，可以将抗性基因导入马铃薯中，从而快速培育抗性品种，因此，发现新的晚疫病广谱抗性基因是当前国内外研究的一个重要热点。早在 20 世纪初的马铃薯晚疫病抗病育种过程中，最先受到育种者关注的是携带晚疫病抗性基因的墨西哥野生种 *Solanum demissum*(2n=6x=72)，并从该野生种中鉴定出 11 个主效抗病基因(*R1-R11*)成功转育到栽培种中，这些基因大部分被定位到染色体上，其中，*R1* 和 *R2* 分别定位在V和IV号染色体上(Li et al., 1998; Ballvora et al., 2002)。*R3*、*R5*、*R6*、*R7*、*R9*、*R10* 和 *R11* 被定位在XI号染色体上(El-Kharbotly et al., 1996; Huang et al., 2004, 2005)，*R8* 定位于IX染色体(Jiang et al., 2018)。由于 *R1-R11* 是小种专化抗性基因，目前生产上使用的马铃薯品种主要依赖于这些基因的抗病，随着使用年限的增加，很容易随着晚疫病菌生理小种的快速变异而丧失抗性。

目前定位的抗性基因大部分集中在第 V、VI、VIII、IX、XI 这五条染色体上，从具有田间广谱抗性的墨西哥野生种 *S. bulbocastanum* 鉴定出的 *Rpi-blb1/RB* 定位于VIII号染色体上(Van der Vossen et al., 2003)。*Rpi-blb2* 定位于VI号染色体上与番茄的 *Mi-1* 基因位点相同的区域(Van der Vossen et al., 2005)。*Rpi-blb3* 和 *Rpi-abpt* 定位于IV号染色体上，来源于野生种 *S. berthaultii* 的 *Rpi-ber1* 和 *Rpi-ber2* 被定位在X号染色体上，来源于 *S. edinense* 的 *Rpi-edn1.1* 和 *Rpi-edn1.2*，以及来源于 *S. hjertingii* 的 *Rpi-hjt1.1*，*Rpi-hjt1.2* 和 *Rpi-hjt1.3* 都被定位在IV号染色体上(Rauscher et al., 2006; Lokossou et al., 2009)，来源于 *S. venturii* 的 *Rpi-vnt1.1* 和 *Rpi-vnt1.3* 被定位在IX号染色体上(Foster et al., 2009)，来源于 *S. ruiz-ceballosii* 的 *Rpi-rzc1* 定位在第X号染色体上(Śliwka et al., 2012)。此外，研究者从 *S. pinnatisectum*、*S. microdontum*、*S. phureja* 及 *S.okadae* 等其它野生种中定位了更多的晚疫病抗性基因，如 *Rpi1*、*Rpi-mcd1*、*Rpi-phu1* 及 *Rpi-oka1* 等(Kuhl et al., 2001; Śliwka et al., 2010)。Verzaux 等(2011)在野生种 *S. avilesii* 中定位了位于 11 号染色体的晚疫病抗性基因 *Rpi-avl1*，并筛选到共分离的分子标记用于辅助育种，促进了马铃薯抗病育种选择的速度和效率。

1.2马铃薯晚疫病抗性基因克隆

随着基因组学和现代分子生物学技术的快速发展，利用抗病基因图位克隆结合比较基因组学、抗性基因富集测序 Renseq 以及病原菌效应子组学等方法，从多个马铃薯野生种质资源中鉴定和克隆了多个新的抗病基因(表 1)。如 *R1*、*R3a*、*R3b* 是通过图位法被克隆出的晚疫病抗性基因(Ballvora et al., 2002)，*RB/Rpi-blb1* 是通过图位法从 *S. bulbocastanum* 中克隆出来的第一个广谱抗性基因(Song et al, 2003; Van der Vossen et al., 2003)，而 *Rpi-blb2* 是利用比较基因组学从 *S. bulbocastanum* 中被克隆出来的广谱抗病性~~的~~基因。这些已克

隆的马铃薯晚疫病抗性基因，均属于胞内免疫受体，为一类包含核苷酸结合结构域和亮氨酸富集重复区(NB-LR)的受体类蛋白(Nucleotide-binding domain and Leucine-rich repeat Receptors, NLRs)，这些抗性蛋白通过识别进入到植物细胞内的病原菌效应蛋白，触发寄主植物的抗病性。

根据目前对中国马铃薯晚疫病菌群体生理小种的监测，除 *R8* 外，*R1-R11* 几乎失去了马铃薯抗病育种中的利用价值(曹继芬等, 2017, 中国作物学会马铃薯专业委员会会议论文集, pp.461-466)。虽然单个小种专化抗性基因容易被晚疫病菌克服，但不同的广谱抗性基因聚合会赋予植株持久抗性。Zhu 等(2012)将 3 个克隆自不同野生种的 R 基因 *Rpi-sto1* (*S. stoloniferum*)，*Rpi-vnt1.1* (*S. venturii*) 和 *Rpi-blb3* (*S. bulbocastanum*) 聚合到感病马铃薯品种中，发现三重 *Rpi* 基因转化感病品种的抗性谱是来自三个单独 *Rpi* 基因抗谱的总和，可以显著提高马铃薯晚疫病的抗性，结果表明植物中广谱 *Rpi* 基因的叠加有助于马铃薯在田间的抗性持久性。Jones 等(2014)利用从野生种中克隆的晚疫病抗性基因 *Rpi-mcq1* 和 *Rpi-vnt1.1* 转化到马铃薯感病品种 Désirée 中，发现在田间能表现出持久的晚疫病抗性。Jo 等(2014)将 *Rpi-vnt1.1* 和 *Rpi-sto1* 顺式连接，通过无选择标记转入 3 个不同的马铃薯品种中获得了广谱晚疫病抗性。Ghislain 等(2018)将 3 个广谱抗性基因 *RB* (*Rpi-blb1*)、*Rpi-blb2* 和 *Rpi- vnt1.1* 聚合到非洲的两个马铃薯感病品种中，当所有 3 个 *Rpi* 基因叠加时，极端抗性的频率从单个 *Rpi* 基因的 3% 到 9% 上升到 75%。聚合 *Rpi* 基因的品种不用施用任何杀菌剂而提供了完全的晚疫病抗性，产量比以前提高了 3~4 倍。

近年来，科学家们探索了与马铃薯相关的多种野生茄科植物抗性基因的多样性，研究发现，广泛种植的野生龙葵的祖先少花龙葵(*Solanum americanum*)是晚疫病新抗性基因的极好来源。*Rpi-amr3* 就是首个从少花龙葵中克隆到的晚疫病抗性基因，将其转到栽培马铃薯中表现出高水平的晚疫病抗性(Witek et al., 2016)。最近 Witek 等(2021)又通过传统图位克隆法以及最新的基于三代测序的抗病基因富集测序、混池抗病基因富集测序(BSA-RenSeq)等多种技术从二倍体少花龙葵中克隆了晚疫病抗性基因 *Rpi-amr1*，该基因编码具有四个外显子的带有 NB-CC (coiled-coil) 结构域的抗性蛋白。对 19 种来自不同国家的晚疫病菌小种均有抗性，是目前唯一已知的没有被任何小种克服的晚疫病最强抗性基因。因此，原始栽培种和野生种中广谱抗性新基因的不断挖掘和克隆为马铃薯晚疫病抗病新品种的选育及抗源的持久利用奠定了重要基础。

2 马铃薯晚疫病菌已克隆的无毒基因

晚疫病原菌分泌质外体效应蛋白(Apoplastic effector)和细胞质效应蛋白(Cytoplasmic effector)去干扰、操纵寄主免疫反应，从而促进病原菌侵染。目前晚疫病菌已鉴定的所有无毒基因均为编码蛋白中具有RXLR(R: 精氨酸; X: 任意氨基酸; L: 亮氨酸)基序的小分子细胞质效应蛋白，一般由N端信号肽、RXLR motif序列和C端功能区三部分组成。N端信号肽作用是促使效应蛋白分泌到疫霉菌胞外，成熟效应蛋白的信号肽在分泌前被切除。保守的RXLR基序在介导卵菌效应蛋白转运到寄主细胞内有关键作用，是必需的保守基序 (Whisson et al., 2007)，C端是功能区，是干扰、操控寄主细胞免疫的区域，或是被寄主植物抗病蛋白~~质~~识别的区域。在寄主植物有抗病蛋白存在的情况下，效应蛋白C端功能区能够被抗病蛋白识别从而激活植物的免疫反应，植物表现为抗病 (Dou and Zhou, 2012)。

晚疫病菌效应分子RXLR模体的发现，大大加速了新无毒基因的鉴定。基于图位克隆和效应分子组学的方法，先后报道的晚疫病菌无毒基因已达20余个，能被对应的植物免疫受体NLR(抗性基因)识别，激发寄主抗病。已鉴定的无毒基因包括：*Avr3a* (Armstrong et al., 2005)、*Avrblb1/Avrsto1* (Vleeshouwers et al., 2008)、*Avr4*(Van Poppel et al., 2008)、*Avrblb2*(Oh et al., 2009)、*Avrvnt1*(Pel, 2010)、*Avr3b*(Li et al., 2011)、*Avr10a*、*AvrCap1.1*、*AvrCap1.2* (Rietman, 2011)、*AvrSmira1/ Avr9a*、*AvrSmira2*(Rietman et al., 2012)、*Avr2*(Saunders et al., 2012)、*Avr8*(Jo,2013)、*Avr1*(Du et al.,2015b)、*Avredn2*(Chatziavgerinos, 2015)、*Avrchc1*、*Avrchc2*(Haverkort et al., 2016)、*Avramr1*(Lin et al., 2020)、*Avramr3*(Lin et al., 2021)。

*Avr3a*是第一个从马铃薯晚疫病菌中克隆到的RXLR效应分子，编码147个氨基酸的小分子分泌蛋白，能够被抗性基因*R3a*识别引起寄主植物过敏性坏死(HR)反应。AVR3a可以抑制激发子INF1等触发的细胞坏死(ICD)，是晚疫病菌的核心毒性因子，在功能上是非冗余的，靶向和稳定植物U-box E3连接酶CMPG1，进而操纵植物正常泛素蛋白酶体降解系统，在晚疫病菌侵染的活体营养阶段阻止植物细胞死亡。但AVR3a $^{KI/Y147del}$突变体不能抑制 ICD 或与 CMPG1 相互作用或稳定 CMPG1 (Armstrong et al., 2005)。

*Avrblb2*编码100个氨基酸，主要定位于植物细胞膜并在晚疫病菌侵入形成的吸器周围积累，通过靶向寄主植物木瓜蛋白酶样半胱氨酸蛋白酶C14并特异性阻止其分泌到质外体中。AVRblb2的定位对其毒性功能至关重要，其与C14结合并改变其亚细胞分布，显著增强植物对晚疫病菌的感病性，显示了一种独特的反防御策略(Bozkurt et al., 2011)。AVRblb2与抗性蛋白Rpi-blb2的互作识别依赖

SGT1，不需要水杨酸、茉莉酸及乙烯介导的信号传导途径(Oh et al., 2014)。最近Naveed等(2019)发现AVRblb2通过与植物细胞质膜上的钙调蛋白互作干扰植物防御相关的Ca^{2+}信号传导。

*Avr1*编码208个氨基酸，在蛋白的C末端含有W和Y功能域，可以抑制激发子CRN2触发的细胞坏死，而且其对CRN2诱导的细胞死亡抑制取决于T区。研究发现抗性蛋白R1定位于寄主细胞膜和细胞核，R1对晚疫病菌抗性的激活需要AVR1和R1共同定位于细胞核才能触发HR(Du et al., 2015a)。AVR1在植物体内与胞外分泌复合体(Exocyst)亚基SEC5互作，AVR1蛋白的T区对于与SEC5的互作至关重要，二者互作后抑制了SEC5的表达，干扰了囊泡运输，从而导致病程相关蛋白PR-1分泌和胼胝质沉积减少，促使植物免疫能力下降(Du et al.,2015b)。

*Avr2*编码114个氨基酸，在晚疫病菌侵染过程中优先积累于吸器周围。AVR2与寄主植物磷酸BSU-LIKE PROTEIN1 (BSL1)结合后与抗性蛋白R2形成三元复合物，从而触发寄主抗性，BSL是R2识别AVR2所必须的，而能克服R2抗性的晚疫病菌毒性菌株中则表达了一种无法识别的AVR2-like (A2L)形式。A2L可以与BSL1结合，但不会促进BSL1与R2的关联 (Saunders et al., 2012)。AVR2可通过上调转录因子bHLH来激活植物油菜素内酯激素信号传导，间接抑制激发子INF1介导的植物先天免疫反应，从而促使植物感病(Turnbull et al., 2017)。

*Avrblb1*编码152个氨基酸，C端含有一个W-motif，此区域是抗性蛋白Rpi-blb1(RB)识别AVRblb1并引起过敏性坏死(HR)反应的关键区域(Champouret et al., 2009)。AVRblb1在晚疫病菌群体中存在大量的突变体，大多数的IPI-O突变体能被Rpi-blb1识别引发抗性，但是IPI-O4能够逃避Rpi-blb1的侦测。研究还发现IPI-O4阻断IPI-O1的识别，导致RB (Rpi-blb1)介导的抗性失活(Chen et al., 2012)。最近报道晚疫病菌AVRblb1的突变体 IPI-O4 通过影响抗性蛋白Rpi-blb1和IPI-O1的蛋白互作状态抑制免疫反应(Zhao and Song, 2021)。

目前多数无毒基因与其抗性基因的识别模式、在植物体内互作的寄主靶标蛋白以及如何操纵寄主免疫的分子机理尚不清楚，还需要进一步开展深入研究。

3晚疫病抗性基因与病原菌无毒基因的互作

3.1基因对基因假说

马铃薯－晚疫病菌的互作符合“基因对基因”假说(Flor, 1971)，即植物的抗病基因(*R, NLR*)与病原菌的无

毒基因(*Avr*)存在着一一对应的关系，其核心内容指抗病反应是寄主的抗病蛋白识别病原菌效应蛋白(无毒蛋白)激发寄主植物程序性细胞死亡的结果，否则病原菌致病，寄主植物感病。因此抗病基因能否持久有效在很大程度上取决于病原群体的进化潜力。如果病原菌持有的效应分子(无毒基因)发生变异，逃避了寄主植物对应抗性基因的侦测和识别，则表明抗性基因被病原菌克服，寄主抗性“丧失”，变得感病(Thrall et al., 2016)。根据“基因对基因”假说，通过NLR-AVR互作产生过敏性细胞坏死反应(hypersensitive Response, HR)可确定马铃薯和病原菌中是否含有对应的抗性基因和无毒基因。因此，利用晚疫病菌核心RXLR效应子组可以从抗晚疫病的茄科野生种中快速高效鉴定和克隆新的抗病基因，为抗病育种工作提供新的思路。同时，通过对病原菌群体中无毒基因变异进化的监测，可准确地判断对应抗性基因是否失效和能否持久利用，为马铃薯抗性基因在新品种选育中的合理利用提供理论依据。

3.2晚疫病菌无毒基因逃避抗性基因识别的方式

抗性蛋白(NLRs)与无毒蛋白(AVRs)通过直接或间接方式识别，激活引起寄主植物强烈的免疫反应被称作效应蛋白触发的免疫(effector-triggered immunity, ETI)。一旦病原菌无毒蛋白发生变异逃避抗性蛋白的侦测，则不能激活ETI，导致抗性基因失效。当前已报道的卵菌RXLR效应分子可通过点突变、移码突变、缺失、转录沉默等方式进化，进而逃避抗性蛋白的识别(Anderson et al., 2015; Dong and Ma, 2021)。研究发现，马铃薯晚疫病菌以各种方式适应与其匹配的效应分子来克服抗性基因介导的抗性。已知的晚疫病菌无毒基因也通过点突变、移码突变、缺失、转录沉默等进化机制逃避对应抗性基因的侦测。因此对病原菌群体中无毒基因灾变的风险监测是晚疫病抗性基因持久利用和抗病育种的重要内容。

3.2.1点突变

晚疫病菌中无毒基因*Avr2*、*Avr3a*、*Avr3b*、*Avr4*、*Avrblbl*、*Avrblb2*、*AvrSmira1*主要以点突变的方式进化，*Avr3a*是第一个从晚疫病菌中克隆到的RXLR类无毒基因。它以AVR3a^{KI}和AVR3a^{EM}两种形式存在，两者之间由于19、80和103位氨基酸的差异，导致AVR3a^{EM}逃避了R3a抗性蛋白的识别(Armstrong et al., 2005)。*Avrblb2*包含24种突变体，集中在C端氨基酸发生突变，69位氨基酸是Rpi-blb2识别的关键位点，当该位点为撷氨酸(V)、丙氨酸(A)或异亮氨酸(I)情况下，能够被相应的抗性基因*Rpi-blb2*识别，当为苯丙氨酸(F)情况时，则逃避了抗性基因的识别(Oh et al., 2009)。*Avrblbl* 的突变体IPI-O1发生点突变，129位的亮氨酸突变为脯氨酸，则逃避了抗性基因*RB*(*Rpi-blb1*)的识别，不能诱导HR，而其他单氨基酸突变体仍然能够诱导 HR

(Chen et al., 2012)。

3.2.2移码突变

在克服抗性基因*R4*的马铃薯晚疫病菌中，由于*Avr4*的毒性等位基因在ΔT^{12} 和ΔT^{196}两个单碱基对缺失，导致移码突变和提前引入中止密码子而提前翻译结束，使自身丧失功能从而逃避抗性基因*R4*的识别(Van Poppel et al., 2008)。研究发现，*Avr4*在其编码蛋白C端结构域中含有三个W基序和一个Y基序。如果将*Avr4*突变为含有W2和W1或者W2和W3 motif 的结构时，携带*R4*抗性基因的马铃薯植株产生坏死反应(Van Poppel et al., 2009)。

3.2.3缺失

在晚疫病菌中，*Avr1*、*Avr2*、*Avr3b* 和 *Avrblb1* 存在基因缺失的情况。无毒晚疫病菌中研究发现，能够克服马铃薯抗性蛋白 R1 的一个马铃薯晚疫病菌毒性菌株中缺失 *Avr1* 基因。但在另一个基因座上发现了与 *Avr1* 高度同源的 *Avr1-like* 基因，AVR1-like 与 AVR1 同源性为 82%，AVR1-like 的 *C* 端缺失 38 个氨基酸，从而导致不能被 R1 识别(Du et al., 2015)。对 *Avr2* 研究发现，所有无毒菌株都含有 *Avr2*，而在所有检测的毒性菌株中缺失 *Avr2*，从毒性菌株中发现了与 *Avr2* 相差 13 个氨基酸的 *Avr2-like* 基因，且其中 8 个位于 C 端的 *Avr2-like* 不能被 R2 识别引起过敏性反应，AVR2 和 AVR2-like 的差异解释了晚疫病菌逃避 R2 识别的变异机制(Gilroy et al., 2011)。在晚疫病菌群体中鉴定了 *Avrblb1* 的 16 个突变体，类型 I 和 II 虽然高度多样化，但是均可被抗性基因 *Rpi-blb1* 识别激发细胞死亡。而且还发现来源于晚疫病菌起源中心墨西哥的两个菌株缺失了类型 I，从而克服了 *Rpi-blb1* 的抗性(Champouret et al., 2009)。

表 1 目前已鉴定和克隆的晚疫病抗性基因及其对应的无毒基因
Table 1 Resistance genes against late blight and their cognate avirulence genes of *Phytophthora infestans*

抗病基因 R gene	来源种 Species	染色体 Chromo-some	类型 Class	抗性谱 Resistance spectrum	已克隆的无毒基因 Cloned avirulence genes	参考文献 References
Ph-5-1	醋栗番茄 *Solanum pimpinellifolium*	I				Merk et al., 2012
Ph-4	多毛番茄 *Solanum habrochaites*	II				Nowicki et al., 2012
Rpi-amr3	少花龙葵 *Solanum americanum*	IV	CC-NB-LRR	广谱抗性 Broad-spectrum resistance	*Avramr3*	Witek et al., 2016
Rpi-blb3	球栗薯 *Solanum bulbocastanum*	IV	LZ-NB-LRR	小种专化抗性 Race-specific resistance	*Avr2*	Park et al., 2005a; Lokossou et al., 2009
Rpi-abpt	球栗薯 *Solanum bulbocastanum*	IV	LZ-NB-LRR	小种专化抗性 Race-specific resistance	*Avr2*	Park et al., 2005b; Lokossou et al., 2009
Rpi-bst1	*Solanum brachistotricum*	IV				Hein et al., 2009
RD	落果薯 *Solanum demissum*	IV				Zhang et al., 2014b
*Rpi-chc*l	*Solanum chacoense*	IV	CC-NB-LRR	广谱抗性 Broad-spectrum resistance	*Avrchc1*	Vossen et al., 2017
Rpi-demf1	落果薯 *Solanum demissum*	IV				Danan et al., 2011
Rpi-edn1	*Solanum edinense*	IV	CC-NB-LRR	小种专化抗性 Race-specific resistance	*Avr2*	Lokossou et al., 2009
Rpi-hjt1.1	*Solanum hjertingii*	IV	CC-NB-LRR	小种专化抗性 Race-specific resistance	*Avr2*	Lokossou et al., 2009
Rpi-hjt1.2	*Solanum hjertingii*	IV	CC-NB-LRR	小种专化抗性 Race-specific resistance	*Avr2*	Lokossou et al., 2009
Rpi-hjt1.3	*Solanum hjertingii*	IV	CC-NB-LRR	小种专化抗性 Race-specific resistance	*Avr2*	Lokossou et al., 2009
R2	落果薯 *Solanum demissum*	XI	CC-NB-LRR	小种专化抗性 race-specific resistance	*Avr2*	Lokossou et al., 2009
R2-like	*Solanum edinense*	IV	LZ-NB-LRR	小种专化抗性 Race-specific resistance	*Avr2*	Lokossou et al., 2009
Rpi-mcd		IV/X				Sandbrink et al., 2000
Rpi-mcd1	*Solanum microdontum*	IV	CC-NB-LRR	小种专化抗性 Race-specific resistance	*Avr2*	Tan et al.,2008; Lokossou et al., 2009
Rpi-oka2	*Solanum okade*	IV				Huang, 2005
Rpi-snk1.1	*Solanum schenckii*	IV	CC-NB-LRR	小种专化抗性 Race-specific resistance	*Avr2*	Lokossou et al., 2009
Rpi-snk1.2	*Solanum schenckii*	IV	CC-NB-LRR	小种专化抗性 Race-specific resistance	*Avr2*	Lokossou et al., 2009
Rpi-neo1	*Solanum neorossii*	IV/VII				Huang, 2005
R1	落果薯 *Solanum demissum*	V	CC-NB-LRR	小种专化抗性 race-specific	*Avr1*	Ballvora et al., 2002

				resistance		
Rpi-blb2	球栗薯 *Solanum bulbocastanum*	VI	CC-NB-LRR	广谱抗性 Broad-spectrum resistance	*Avrblb2*	Van der Vossen et al.,2005
Rpi-ver1	*Solanum verrucosum*	VI				Jacobs et al., 2010
Ph-1	*Solanum pimpinellifolium*	VII				Foolad et al., 2014
Rpi1(Rpi-pnt1)	*Solanum pinnatisectum*	VII				Kuhl et al., 2001
Rpi2	*Solanum pinnatisectum*	VII				Yang et al.,2017
Rpi-mch1	*Solanum microdontum*	VII				Śliwka et al., 2012
Rpi-blb1, RB,	球栗薯 *Solanum bulbocastanum*	VIII	CC-NB-LRR	广谱抗性 broad-spectrum resistance	*Avrblb1*	Van der Vossen et al., 2003
Rpi-pta1	*Solanum papita*	VIII	CC-NB-LRR	广谱抗性 broad-spectrum resistance	*Avrsto1*	Vleeshouwers et al., 2008; Wang et al., 2008
Rpi-plt1	匍枝薯 *Solanum stoloniferum*	VIII				Wang et al.,2008
Rpi-sto1	*Solanum stoloniferum*	VIII	CC-NB-LRR	广谱抗性 broad-spectrum resistance	*Avrsto1*	Vleeshouwers et al., 2008; Wang et al., 2008
Rpi-bt1	球栗薯 *Solanum bulbocastanum*	VIII				Oosumi et al., 2009
R8	落果薯 *Solanum demissum*	IX	CC-NB-LRR	小种专化抗性 race-specific resistance	*Avr8*	Vossen et al., 2016
R9	落果薯 *Solanum demissum*	IX	NB-LRR			Huang, 2005
R9a	落果薯 *Solanum demissum*	IX	CC-NB-LRR	广谱抗性 broad-spectrum resistance	*Avr9a/ Avrblb2*	Jo et al., 2015
Rpi-crp1	*Solanum caripense*	IX				Trognitz and Trognitz, 2005
Rpi-edn2	*Solanum edinense*	IX	CC-NB-LRR	广谱抗性 broad-spectrum resistance	*Avredn2*	Verzaux, 2010; Haverkor et al., 2016
Rpi-hcb1.1	*Solanum huancabambense*	IX		小种专化抗性 race-specific resistance	*Avr2*	Aguilera-Galvez et al., 2020
Rpi-hcb1.2	*Solanum huancabambense*	IX		小种专化抗性 race-specific resistance	*Avr2*	Aguilera-Galvez et al., 2020
Rpi-mcq1, Rpi-moc1	*Solanum mochiquense*	IX	CC-NB-LRR	小种专化抗性 race-specific resistance	*Avr2*	Jones et al., 2014
Rpi-oka1	*Solanum okade*	IX				Nowicki et al., 2012
Rpi-vnt1.1, Rpi-phu1	*Solanum venturii*	IX	CC-NB-LRR	广谱抗性 broad-spectrum resistance	*Avrvnt1*	Foster et al., 2009
Rpi-vnt1.2	*Solanum venturii*	IX	CC-NB-LRR	广谱抗性 broad-spectrum resistance	*Avrvnt1*	Foster et al., 2009
Rpi-vnt1.3	*Solanum venturi*	IX	CC-NB-LRR	广谱抗性 broad-spectrum resistance	*Avrvnt1*	Foster et al., 2009
Rpi-Smira2	*Solanum tuberosumcv.Sarpo Mira*	IX		广谱抗性 broad-spectrum resistance	*AvrSmira2/Avr8*	Rietman et al., 2012
Rpi-dlc1	*Solanum dulcamara*	IX		小种专化抗性 race-specific resistance		Golas et al., 2010

Ph-3	*Solanum pimpinellifolium*	IX	CC-NBS-LRR	广谱抗性 broad-spectrum resistance		Zhang et al., 2014a
Rpi-ber	*Solanum berthaultii*	X		广谱抗性 broad-spectrum resistance	*Avrchc1*	Rauscher et al., 2006
Rpi-ber1	*Solanum berthaultii*	X				Rauscher et al., 2006
Rpi-ber2	*Solanum berthaultii*	X				Rauscher et al., 2006
Rpi-dlc2	*Solanum dulcamara*	X				Golas et al., 2013
Rpi-rzc1	*Solanum ruiz-ceballosii*	X				Śliwka et al., 2012
Rpi-chc1	*Solanum chacoense*	X	CC-NB-LRR	广谱抗性 broad-spectrum resistance	*Avrchc1*	Vossen et al., 2017
Rpi-chc2	*Solanum chacoense*	X	CC-NB-LRR	广谱抗性 broad-spectrum resistance	*Avrchc2*	Haverkor et al., 2016
Rpi-tar1	*Solanum tarijense*	X		广谱抗性 broad-spectrum resistance	*Avrchc1*	Van der Vossen et al., 2003; Haverkor et al., 2016
Ph-2	*Solanum pimpinellifolium*	X				Moreau et al., 1998
Ph-5-2	*Solanum pimpinellifolium*	X				Merk et al., 2012
R3a	落果薯 *Solanum demissum*	XI	CC-NB-LRR	小种专化抗性 race-specific resistance	*Avr3a*	Huang et al., 2004
R3b	落果薯 *Solanum demissum*	XI	CC-NB-LRR	小种专化抗性 race-specific resistance	*Avr3b*	Li et al., 2011
R4	落果薯 *Solanum demissum*	XI		小种专化抗性 race-specific resistance	*Avr4*	Van Poppel et al., 2009
R5	落果薯 *Solanum demissum*	XI		小种专化抗性 race-specific resistance		Huang, 2005
R6	落果薯 *Solanum demissum*	XI		小种专化抗性 race-specific resistance		Huang, 2005
R7	落果薯 *Solanum demissum*	XI		小种专化抗性 race-specific resistance		Huang, 2005
R10	落果薯 *Solanum demissum*	XI		小种专化抗性 race-specific resistance	*Avr10*	Huang, 2005
R11	*落果薯* *Solanum demissum*	XI		小种专化抗性 race-specific resistance		Huang, 2005
Rpi-amr1	少花龙葵 *Solanum americanum*	XI	CC-NB-LRR	广谱抗性 broad-spectrum resistance	*Avramr1*	Witek et al., 2021
Rpi-cap1	*Solanum capsicibaccatum*	XI				Jacobs et al., 2010
Rpi-edn3	*Solanum edinense*					Verzaux, 2010
Rpi-pcs	*Solanum paucissectum*	XI				Villamon et al., 2005
Rpi-pta2	*Solanum papita*	XI				Vleeshouwers et al., 2008; Wang et al., 2008
Rpi-pur1	*Solanum piurae*	XI				Rietman, 2011
Rpi-qum1	*Solanum circaeifolium*	XI				Verzaux, 2010
Rpi-sto2	*Solanum stoloniferum*	XI	CC-NB-LRR		*Avr3a*	Vleeshouwers et al., 2008; Wang et al., 2008
Rpi-Smira1	*Solanum tuberosumcv.Sarpo Mira*	XI		小种专化抗性 race-specific resistance	*AvrSmira1/Avr9a*	Rietman et al., 2012

Rpi-vnt2	*Solanum venturii*	XI	Rietman, 2011
Rpi-avl1	*Solanum avilesii*	XI	Verzaux et al.,2011

注:灰色标注为已克隆的抗性基因, *Rpi:* 晚疫病菌抗性基因
Note: Cloned resistance genes were marked in gray, *Rpi: R*esistance gene to *Phytophthora infestans*

3.2.4转录沉默

通过基因沉默方式而逃避抗性基因识别的有*Avrvnt1*和*Avr3b*。研究发现南美洲菌株P13626编码完整的*Avrvnt1*，但接种P13626能侵染含有抗性基因*Rpi-vnt1.1*的马铃薯。研究者认为菌株P13626中*Avrvnt1*未转录表达从而逃避了寄主*Rpi-vnt1.1*的识别，即无毒基因*Avrvnt1*的沉默使含有*Rpi-vnt1.1*的马铃薯抗性品种变得感病(Pais et al., 2018)。通过分析来自中国北方菌株HB150和中国南方菌株HN1602感染期间的全基因组变异和基因表达水平，发现*Avr3b*(PITG_18215) 在HN1602中缺失，但在HB1501中存在，通过RT-PCR检测的结果证明了*Avr3b*基因沉默是一种识别逃避机制，这在*Avr3b*中前所未有。且两个菌株都能侵染含有*R3a*和*R3b*的马铃薯植株，研究者推测*Avr3b*沉默可能是由于HB1501菌株的表观遗传变异所致，基因沉默是晚疫病菌用来破坏寄主抗性的一种灵活策略(Zhang et al., 2021)。

4展望

晚疫病菌属于进化潜力最高、风险最大、最易克服单个抗病基因或少数几个抗病基因的累加系的病原之一。来源于*S. demissum*中的小种专化抗性*R1~R11*基因渗入到栽培种中选育的抗病品种经过多年的推广和种植，除*R8*外，绝大多数的抗病基因已经被晚疫病菌高频率克服，使得抗源匮乏、抗病育种的难度增大，马铃薯安全生产受到威胁。幸运的是，随着基因组学、效应子组学以及高通量测序、Ren-seq等先进技术的快速发展，马铃薯晚疫病广谱抗性新基因鉴定和克隆、马铃薯—晚疫病菌互作机制的研究取得了显著进展。截止目前，国内外已鉴定和克隆了大量的抗性基因和无毒基因，并对二者间的识别和免疫机制开展了解析，这些研究进展为马铃薯晚疫病抗病育种和病害防控提供了新思路和新材料。很多研究者把已知抗性基因运用到抗晚疫病育种实践中，获得了一批具有广谱抗性又高产的马铃薯新材料，并已在农业生产中被广泛利用(周倩等, 2016)。聚合多个晚疫病抗性基因并在时空上合理地部署可以将杀菌剂的使用量减少 80% 以上，甚至不喷施任何杀菌剂也能使马铃薯产量提高3~4倍(Haverkort et al., 2016; Ghislain et al., 2018)。

当前马铃薯抗性基因与晚疫病菌无毒基因之间精细互作、调控寄主免疫机制的报道仍旧较少。为了科学鉴选和利用持久抗病的理想靶标，还需要从以下几方面开展研究：(1)加强马铃薯抗性基因与无毒基因的相互作用研究，深入解析马铃薯广谱抗性的分子机理，为抗性基因的合理利用提供理论依据。(2)继续挖掘

和克隆马铃薯野生种和茄属近缘种中潜在的晚疫病广谱新抗源，通过基于CRISPR-Cas系统的基因编辑技术创新可杂交利用的高抗亲本材料，加速抗病育种进程。(3)通过基因工程途径进行多基因聚合，合理组合已克隆的广谱抗性基因，培育马铃薯高抗新品种或创造新种质。(4)对晚疫病菌重要无毒基因灾变风险进行长期监测，研判对应抗性基因抗性失效的风险，精准布局抗性基因，通过抗性管理确保最大限度地延长抗性的寿命。(5)深入研究晚疫病菌操纵马铃薯免疫的分子机理，除NLR基因外，不断发掘新的寄主/非寄主免疫受体和靶标，通过基因编辑技术精准改良马铃薯抗性，实现马铃薯产业的绿色可持续发展。

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尹润金是综述的主要撰写人，完成论文资料收集和分析，以及初稿写作；霍超和曹继芬参与资料的收集和讨论；隋启君和黄斌全指导论文写作，赵志坚是综述的构思者及负责人，指导论文的写作与修改。全体作者都阅读并同意最终的文本。

致谢

本研究由国家重点研发计划项目(2021YFE0109600)、云南省农业基础研究联合专项重点基金(2018FG001-001)、云南省重点研发计划项目(202102AE090019)和云南省种子种业联合实验室项目共同资助。

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食用菌学报© 2022.29(2):39～47

DOI:10.16488/j.cnki.1005－9873.2022.02.005

羊肚菌产量与种袋营养变化关系

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摘　要：设置不同培养料配方(麦粒含量分别为0、25%、50%、75%和100%，其余为木屑，命名为M0、M1、M2、M3和M4)和不同种袋放置数量(8、12、14和16)处理组合20个，栽培3个六妹羊肚菌(*Morchella sextelata*)菌株YAASJNLM1-31、YAASJNLM6-6和YAASJNLM6-20，测定产量；测定菌株YAASJNLM1-31接种前和采收后种袋基质的总干重、有机碳和总氮含量，探索种袋栽培条件下种袋基质消耗、有机碳消耗和总氮消耗与羊肚菌产量之间的关系。结果表明：对于菌株YAASJNLM1-31，种袋配方为M1时，种袋放置数量为16时的产量较高；种袋配方为M4时，种袋放置数量为12、14和16时的产量较高；种袋放置数量为14和16时，配方M1～M4的产量较高。对于菌株YAASJNLM6-6，种袋放置数量为8时，配方M2～M4的产量较高；种袋放置数量为12时，配方M1～M4的产量较高；种袋放置数量为14时，配方M2、M3的产量较高。对于菌株YAASJNLM6-20，种袋放置数量为8时，配方M1～M4产量较高。对于菌株YAASJNLM1-31，不同处理种袋基质消耗比例(40.81%～64.75%)差别较大，纯木屑配方、纯麦粒配方种袋基质消耗比例分别为40.81%～41.73%、49.65%～50.44%；种袋基质有机碳消耗和总氮消耗均与产量显著正相关。研究结果为优化羊肚菌种袋栽培方法提供参考。

关键词：六妹羊肚菌；种袋；配方；产量；碳氮比

Relationship Between Morel Yield and Nutrient Consumption in Spawn Bottles

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Abstract: A novel morel cultivation mode using spawn bottles was developed. In the new mode, mycelial blocks were inoculated into transparent glass jars (560 mL each) filled with cultivation substrate (spawn bottle), cultivated at 18 ℃ in darkness for 3 d to allow mycelial growth, and then the spawn bottles were placed on planting land with the mouth down on the ground and cultivated until harvest. Different cultivation substrates in combination with different spawn bottle numbers per cultivation plot of 1.5 m×1.5 m were studied on three strains of *Morchella sextelata*, YAASJNLM1-31, YAASJNLM6-6 and YAASJNLM6-20, respectively. Different cultivation substrates comprised different proportions of wheat grain and sawdust, including 100% sawdust (M0), 25% wheat grain＋75% sawdust (M1), 50% wheat grain＋50% sawdust (M2), 75% wheat grain＋75% sawdust (M3) and 100% wheat grain (M4), respectively. Different spawn bottle placements were 8, 12, 14 and 16 bottles per cultivation plot of 1.5 m×1.5 m, respectively. Each strain was cultivated in all 20 combinations of the above conditions, and then measured for yield and nutrient

收稿日期：2021-12-21 **原稿**；2022-01-13 **修改稿**

基金项目：国家现代农业产业技术体系建设专项(CARS-20)和国家自然科学基金(31960621)

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consumption in spawn bottles. The results showed that the yield of YAASJNLM1-31 was high using M1 and 16 bottles per cultivation plot. When cultivated on M4, the yield of YAASJNLM1-31 was high under 12, 14 and 16 bottles per cultivation plot. When 14 or 16 bottles were placed on a cultivation plot, the yield of YAASJNLM1-31 was high on M1-M4. For YAASJNLM6-6, high yields were obtained under the following combinations: M2×8 bottles per cultivation plot, M3×8 bottles per cultivation plot, M4×8 bottles per cultivation plot, M1×12 bottles per cultivation plot, M2×12 bottles per cultivation plot, M3×12 bottles per cultivation plot, M4×12 bottles per cultivation plot, M2×14 bottles per cultivation plot, and M3×14 bottles per cultivation plot. For YAASJNLM6-20, high yields were obtained under M1-M4 with 8 bottles per cultivation plot. For YAASJNLM1-31, the substrate consumption ratio of each treatment group varied greatly (40.81%－64.75%). The consumption ratio of YAASJNLM1-31 on M0 was 40.81%－41.73%, and the consumption ratio of YAASJNLM1-31 on M4 was 49.65%－50.44%. Both organic carbon consumption and total nitrogen consumption were positively correlated with yield. The above results provided a reference to improve morel cultivation mode.

Key words: *Morchella sextelata*; spawn bottle; formula; yield; C:N ratio

羊肚菌(*Morchella* spp.)隶属于子囊菌门(Ascomycota)、盘菌纲(pezizomycetes)、盘菌目(Pezizales)、羊肚菌科(Morchellaceae)、羊肚菌属(*Morchella*),又名羊肚菜、羊肚蘑等,因其菌盖呈羊肚状而得名[1]。羊肚菌味道鲜美,营养丰富,是著名的食用菌之一,因深受国内外消费者喜爱,所以市场需求日益增加和野生产量逐年减少的矛盾越来越突出,人工栽培羊肚菌是解决这一矛盾的唯一手段,相关研究工作从未间断。1982 年,OWER[2]在羊肚菌营养生长阶段供给养分促其形成菌核,并以菌核接种培养料,通过浸水诱发菌核萌发菌丝,形成原基,进而通过控制环境条件促使原基分化,形成子实体,首次实现羊肚菌室内人工栽培。外源营养袋栽培解决了羊肚菌人工栽培条件下不能重复出菇的技术难题[3],使我国羊肚菌人工栽培商业化发生质的飞跃,使羊肚菌菌种筛选和栽培技术革新研究变得可行,羊肚菌栽培规模也得以迅速扩大。围绕提高羊肚菌产量和降低生产成本,对营养袋的配方、用量、使用时间和方式、作用机制等已开展一些研究[4-10]。目前,制备营养袋对麦粒的需求量越来越大,一方面不符合我国食用菌产业发展"不争粮"的初衷,另一方面土壤中残留大量麦粒易导致污染和连作障碍。羊肚菌产量不稳定、生产成本较高、营养袋污染率较高、栽培连作障碍等问题仍制约羊肚菌产业健康以及可持续发展。

赵永昌等[11-12]研发的种袋栽培方法利用活力旺盛的羊肚菌菌种封住种袋口,避免放置种袋后土壤中的杂菌污染种袋基质,污染率较低;同时可实现羊肚菌菌丝在土壤和种袋基质中同时生长,种袋中营养成分向土壤中生长的菌丝输送,达到营养高效利用并提高产量目的,种袋栽培方法有利于进一步开展羊肚菌栽培过程中对营养利用研究。虽然羊肚菌有可能在营养袋上形成原基[13],但不能在营养基质上形成子实体仍是未解之谜,这也是羊肚菌高产稳产的制约因素之一。笔者采用不同配比麦粒与木屑制备种袋,结合不同种袋放置数量,栽培 3 个六妹羊肚菌(*M. sextelata*)菌株,研究种袋配方和放置数量对产量的影响;测定菌株 YAASJNLM1-31 接种前和采收后种袋基质的总干重、有机碳和总氮含量,探索种袋中主要营养成分在羊肚菌栽培过程中的作用和变化,为优化种袋栽培方法提供参考。

1 材料与方法

1.1 供试菌株

六妹羊肚菌(*M. sextelata*)菌株 YAASJNLM1-31、YAASJNLM6-6 和 YAASJNLM6-20 为具有 *MAT1-1* 和 *MAT1-2* 交配型的异核体单孢分离菌株,保存于云南省农业科学院大型真菌菌种库。

1.2 培养基

YPD 培养基:20 g 葡萄糖、2 g 酵母浸粉、2 g 蛋白胨、12.5 g 琼脂粉、1 L 蒸馏水,pH 自然。

原种培养料:70%麦粒、30%细木屑(直径≤0.2 cm),含水量 55%～60%,pH 自然。

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1.3 栽培方法

1.3.1 场地

实验在云南省农业科学院晋宁科研基地开展,该地地势平坦,沙壤土有机质含量高,适宜羊肚菌生长。实验大棚为平棚,棚高 1.8 m,长度和宽度依据地形而定,每隔 2 m 用竹杆支撑,采用 6 针加密黑色遮阳网覆盖。土壤翻耕前清除杂草,667 m^2 撒生石灰 150 kg,用旋耕机翻耕土壤,开沟做墒,宽1.5 m,长度依据地形而定,沟宽 0.5 m,沟深 0.2 m。大棚内设置 180 个实验区,每个实验区栽培面积为 1.5 m×1.5 m,小区间距为 0.5 m,各实验区的地膜独立覆盖。

1.3.2 原种制备

分别称量制备原种的麦粒和木屑,麦粒放入 40 ℃水中浸泡,水量以高出麦粒 10 cm 为宜,浸泡期间检查麦粒是否透心,浸泡时间与环境温度相关,一般为 22～24 h,麦粒透心时控水滤干;木屑为渥堆 5 个月的阔叶树木屑,渥堆发酵的木屑水溶物含量有所增加,为防止木屑中水溶物损失,木屑采用二次给水方式加湿,边搅拌边用雨洒式龙头给水,以地面不见流水为准,搅拌均匀后盖薄膜保湿,12 h 翻堆一次,翻堆过程中仍用雨洒式龙头适当给水,预湿时间为 24 h,两次给水量为木屑的 50%左右($W:W$);将控水滤干的麦粒与预湿的木屑混匀,装聚丙烯袋(14 cm×28 cm),每袋装量 250～300 g(湿重),121 ℃灭菌 2 h,冷却至室温,每袋接种 1 个 2 cm×2 cm 菌丝块(供试菌株接种直径为 9 cm YPD 培养基平板,20 ℃避光培养 6 d),20 ℃避光培养 8 d。

1.3.3 种袋制备

制备种袋的 5 种不同配方(命名为 M0、M1、M2、M3 和 M4)麦粒含量分别为 0、25%、50%、75%和 100%,其余为木屑(表 1),为方便观察,不同培养料(湿重 250～400 g)分别混匀后装在 560 mL 广口玻璃瓶中,121 ℃灭菌 2 h,冷却至室温后在超净工作台上接种 5～7 g 原种,18 ℃避光培养 3 d,此时菌丝萌发并向种袋基质中生长,种袋口向下放置时菌种和培养料不易分离。每个处理 3 个重复。

表 1　实验设置

Table 1　Experimental design

配方 Formula	种袋放置数量 Number of spawn bottles per 1.5 m×1.5 m cultivation area			
	8	12	14	16
M0	M0B08	M0B12	M0B14	M0B16
M1	M1B08	M1B12	M1B14	M1B16
M2	M2B08	M2B12	M2B14	M2B16
M3	M3B08	M3B12	M3B14	M3B16
M4	M4B08	M4B12	M4B14	M4B16

M0、M1、M2、M3 和 M4 麦粒含量分别为 0、25%、50%、75%和 100%,其余为木屑

M0, M1, M2, M3 and M4 contained 0, 25%, 50%, 75% and 100% wheat grain, respectively, and the rest in M0-M3 was sawdust

1.3.4 种袋放置方法

地表下 10 cm 处温度低于 20 ℃时,每个厢面排 5 条滴灌水带,将厢面均匀分为 4 列,土壤含水量低于 18%时,栽培前 2 d 对土壤进行滴灌预湿,预湿后土壤含水量不超过 20%。将培养好的种袋口向下放在厢面两条滴灌水带中间,每种配方种袋的放置数量分别为 8、12、14、16,每个处理在 3 个实验区实施;每个实验区放 4 列种袋,种袋放置数量为 8 时,每列放 2 个;种袋放置数量为 12 时,每列放 3 个;种袋放置数量为 14 时,第 1 列和第 3 列放 4 个,第 2 列和第 4 列放 3 个;种袋放置数量为 16 时,每列放 4 个。种袋放置时用力压以直立不倒为宜,之后用白色地膜覆盖厢面。

1.3.5 出菇管理与采收

控制土壤含水量 15%～25%,60 d 左右菌霜消失,滴灌补水刺激出菇,约 10 d 后原基出现,控制土壤含水量 20%～30%、湿度 75%～85%、气温 20 ℃以下、地温 13～17 ℃,10～14 d 后原基分化成

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幼菇(高 2 cm 左右),约 10 d 后子实体成熟,即子实体由深灰色变为黑褐色,菌盖脊与凹坑棱廓分明,开始采收,采收 3 次,为期 1 个月,每个处理采收 3 次鲜菇的质量为产量,单位为 $g \cdot m^{-2}$,每个处理 3 次重复。

1.4 种袋基质消耗比例测定

将未接种菌袋的基质取出烘干至恒重,计算接种前种袋基质总干重;羊肚菌采收后,将栽培菌株 YAASJNLM1-31 的种袋中基质分别回收称重(采收后种袋基质总湿重),每个处理取 500 g 基质烘干至恒重(取样干重),每个处理 3 次重复,计算采收后种袋基质总干重和含水量,然后计算基质消耗比例,计算公式如下:

$$\text{接种前种袋基质总干重/g} = \text{接种前一个种袋基质干重} \times \text{种袋放置数量}$$

$$\text{采收后种袋基质总干重/g} = \text{取样干重}/500 \times \text{采收后种袋基质总湿重}$$

$$\text{采收后种袋基质含水量} = \frac{(500 - \text{取样干重})}{500} \times 100\%$$

$$\text{基质消耗比例} = \frac{(\text{接种前种袋基质总干重} - \text{采收后种袋基质总干重})}{\text{接种前种袋基质总干重}} \times 100\%$$

1.5 有机碳和总氮消耗测定

基质烘干至恒重,分别采用重铬酸钾容量法和凯氏定氮法测定有机碳和总氮含量[14],以肥料的质量分数表示;根据接种前和采收后种袋基质有机碳和总氮质量,分别计算不同处理种袋基质有机碳和总氮消耗,计算公式如下:

$$\text{有机碳含量} = \frac{C \times (V - V_0) \times 0.003 \times 1.5 \times D}{m \times (1 - X_0)} \times 100\%$$

公式中 C 表示硫酸亚铁标准溶液的摩尔浓度,单位为 $mol \cdot L^{-1}$;V、V_0 分别表示样品测定、空白实验时消耗硫酸亚铁标准溶液的体积,单位为 mL;0.003 为四分之一碳原子的摩尔质量,单位为 $g \cdot mol^{-1}$;1.5为氧化校正系数;D 表示分取倍数,定容体积/分取体积=250/50=5;m 表示风干样品质量,单位为 g;X_0 表示风干样品含水量,单位为%

$$\text{总氮含量} = \frac{C \times (V - V_0) \times 0.014 \times D}{m \times (1 - X_0)} \times 100\%$$

公式中 C 表示酸标准溶液的摩尔浓度,单位为 $mol \cdot L^{-1}$;V、V_0 分别表示样品测定、空白实验时消耗标准酸溶液的体积,单位为 mL;0.014 为氮的摩尔质量,单位为 $g \cdot mol^{-1}$;D 表示分取倍数,定容体积/分取体积=50/5=5;m 表示风干样质量,单位为 g;X_0 表示风干样含水量,单位为%

$$\text{接种前种袋基质有机碳(总氮)质量/g} = \text{接种前种袋基质总干重} \times \text{接种前种袋基质有机碳(总氮)含量}$$

$$\text{采收后种袋基质有机碳(总氮)质量/g} = \text{采收后种袋基质总干重} \times \text{采收后种袋基质有机碳(总氮)含量}$$

$$\text{有机碳消耗/g} = \text{接种前种袋基质有机碳质量} - \text{采收后种袋基质有机碳质量}$$

$$\text{总氮消耗/g} = \text{接种前种袋基质总氮质量} - \text{采收后种袋基质总氮质量}$$

1.6 数据分析

利用 SPSS 26.0 软件的 ANOVA 和 Duncan 对不同处理的产量进行多重比较分析,对有机碳、总氮消耗与产量进行 Pearson 相关性分析[15]。

2 结果与分析

2.1 种袋配方和放置数量对羊肚菌产量的影响

种袋放置 27 d 后开始出现分生孢子,麦粒添加量为 0 的配方 M0 原基出现时间较晚,第 100 天才开始出现原基;其他配方第 83 天开始出现原基。如表 2 所示,对于菌株 YAASJNLM1-31,种袋配方为 M1 时,种袋放置数量为 8 时产量较低,种袋放置数量为 16 时产量较高;种袋配方为 M4 时,种袋放置数量为 8 时产量较低,其他种袋放置数量的产量较高;种袋放置数量为 14 和 16 时,M0 配方的产量较低,其他配方产量较高。对于菌株 YAASJNLM6-6,种袋放置数量为 8 时,M0 配方的产量较低,M2~

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M4 配方的产量较高;种袋放置数量为 12 时,M0 配方的产量较低,M1～M4 配方的产量较高;种袋放置数量为 14 时,M0 配方的产量较低,M2、M3 配方的产量较高。对于菌株 YAASJNLM6-20,种袋放置数量为 8 时,M0 产量较低,其他配方产量较高。

表 2　种袋配方和放置数量对羊肚菌产量的影响

Table 2　Effect of cultivation substrate formula and number of spawn bottles on morel yield

菌株 Strain	配方 Formula	种袋数量 Number of spawn bottles per 1.5 m×1.5 m cultivation plot			
		8	12	14	16
YAASJNLM1-31	M0	2.0±2.0* A	2.0±2.0 * A	5.7±4.7 bA	6.7±6.5 bA
	M1	74.7±11.4* B	171.0±88.6 * AB	197.0±110.2 aAB	250.0±91.1 aA
	M2	98.7±67.0 *✦	147.7±20.0 *✦	257.0±73.7 a✦	312.3±148.4 a✦
	M3	155.0±32.7* A	235.0±71.6 * A	297.0±203.2 aA	359.3±117.1 aA
	M4	116.7±30.0 * B	182.3±26.1 * A	217.3±19.0 aA	283.7±87.4 aA
YAASJNLM6-6	M0	2.0±0.0 bA	8.0±5.6 bA	11.3±7.5 * A	13.0±11.0 bA
	M1	67.3±47.4 abA	103.0±24.6 aA	126.7±14.0 * A	109.7±79.1 abA
	M2	126.3±65.5 aA	183.7±54.0 aA	187.3±35.1 * A	206.0±129.3 aA
	M3	126.0±71.5 aA	215.0±126.1 aA	228.0±108.3 * A	191.3±48.8 aA
	M4	108.0±20.9 aA	146.0±70.2 aA	112.7±34.5 * A	97.0±34.8 abA
YAASJNLM6-20	M0	0 b✦	1.3±2.3*✦	2.7±1.2*✦	14.0±19.0 a✦
	M1	50.3±30.1 aA	84.3±72.6 * A	100.7±74.0 * A	145.0±119.2 aA
	M2	97.7±27.6 a✦	122.3±111.8*✦	154.3±41.0 *✦	160.0±137.2 a✦
	M3	106.0±57.0 aA	155.7±29.7 * A	145.0±34.2 * A	180.7±106.3 aA
	M4	91.0±54.8 aA	82.0±19.2 * A	98.3±82.5 * A	87.3±74.4 aA

产量为平均值±标准差(n=3),单位为 $g \cdot m^{-2}$;同一菌株同列不同小写字母表示差异显著 $P<0.05$;同一菌株同行不同大写字母表示差异显著 $P<0.05$;*:同一菌株同列数据比较方差不齐;✦:同一菌株同行数据比较方差不齐

Values are means±SD (n=3) and the unit is $g \cdot m^{-2}$; different lowercase letters in the same column indicate a significant difference between different formulas for the same strain at $P<0.05$; different uppercase letters in the same row indicate a significant difference between different numbers of spawn bottles for the same strain at $P<0.05$; *: uneven variance between different formulas in the same column for the same strain; ✦: uneven variance between different numbers of spawn bottles in the same row

2.2 种袋基质营养消耗情况

如表 3 所示,接种前不同配方每个种袋干物质质量为 132.6～141.0 g,有机碳含量为 60.0%～65.3%,总氮含量为 0.51%～1.69%;纯木屑配方的有机碳含量高于其他配方,而总氮含量又低于其他配方。菌株 YAASJNLM1-31 不同处理基质消耗比例(40.81%～64.75%)差别较大,纯木屑配方、纯麦粒配方基质消耗比例分别为 40.81%～41.73%、49.65%～50.44%(表 4)。纯木屑配方 M0 也有基质消耗,说明六妹羊肚菌可以利用木屑,基质消耗比例较低的原因可能是纯木屑总氮含量较低,一定程度影响菌丝生长和营养输送。纯麦粒配方 M4 的菌丝较稀疏、分支多、颜色发黄,营养利用效率低可能与纯麦粒营养单一以及通气性差不利于羊肚菌菌丝生长有关。

2.3 种袋基质有机碳和总氮消耗与产量的关系

接种前和采收后种袋基质的有机碳和总氮含量不同,采收后种袋基质的有机碳和总氮含量分别为 46.9%～66.4%和 0.85%～3.22%(表 5);接种前和采收后种袋基质的组成也不同,采收后种袋基质含有大量羊肚菌菌丝体,菌丝体的碳氮比明显低于基质,这可能是种袋基质中总氮含量升高的原因。所有处理有机碳消耗均为正值;纯木屑配方 M0 部分处理的总氮消耗为负值。双变量 Pearson 检验结果显示,种袋基质有机碳消耗与产量呈显著正相关($r=0.810$, $P<0.01$),总氮消耗与产量也呈显著正相关($r=0.652$, $P<0.01$)(表 6)。

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表 3 接种前种袋基质干重、有机碳和总氮含量

Table 3 Dry weight, organic carbon content and total nitrogen content of cultivation substrate in a spawn bottle before inoculation

配方 Formula	干重 Dry weight/g	有机碳含量 Organic carbon content/%	总氮含量 Total nitrogen content/%
M0	132.6	65.3	0.51
M1	134.6	61.7	1.02
M2	135.0	61.7	1.36
M3	138.5	60.0	1.44
M4	141.0	61.3	1.69

表 4 菌株 YAASJNLM1-31 采收后种袋基质消耗情况

Table 4 Consumption of substrate in a spawn bottle by YAASJNLM1-31 at harvest

处理 Treatment	采收后 At harvest				接种前总干重 Total dry weight before inoculation/g	基质消耗比例 Substrate consumption ratio/%
	总湿重 Total fresh weight/g	取样干重 Sampled dry weight/g	含水量 Water content/%	总干重 Total dry weight/g		
M0B08	1437±60	219±2	56.20	628±21	1061	40.81
M0B12	2160±70	215±3	57.00	927±17	1591	41.73
M0B14	2563±110	213±8	57.40	1091±7	1856	41.22
M0B16	2903±100	215±5	57.00	1246±28	2122	41.28
M1B08	1173±21	222±2	55.60	520±8	1077	51.72
M1B12	1707±35	219±6	56.20	746±7	1615	53.81
M1B14	2060±56	226±2	54.80	930±19	1884	50.64
M1B16	2370±20	224±1	55.20	1060±9	2154	50.79
M2B08	883±42	231±25	53.80	408±57	1080	62.22
M2B12	1240±118	232±36	53.60	571±52	1620	64.75
M2B14	1573±15	228±27	54.40	717±88	1890	62.06
M2B16	1777±40	222±25	55.60	790±89	2160	63.43
M3B08	890±75	258±3	48.40	460±44	1108	58.48
M3B12	1353±74	257±10	48.60	695±29	1662	58.18
M3B14	1580±165	261±2	47.80	825±82	1939	57.45
M3B16	1717±176	252±7	49.60	864±64	2216	61.01
M4B08	1040±44	269±10	46.20	559±6	1128	50.44
M4B12	1550±56	272±14	45.60	843±58	1692	50.18
M4B14	1847±81	266±3	46.80	981±34	1974	50.30
M4B16	2077±115	274±5	45.20	1136±42	2256	49.65

采收后种袋基质总湿重、取样干重和总干重为平均值±标准差(n=3)

Values for total fresh weight, sampled dry weight and total dry weight are means±SD (n=3)

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表 5　菌株 YAASJNLM1-31 种袋基质有机碳、总氮消耗和产量

Table 5　Organic carbon consumption, total nitrogen consumption and yield of YAASJNLM1-31

处理 Treatment	接种前 Before inoculation				采收后 At harvest						有机碳消耗 Organic carbon consumption/g	总氮消耗 Total nitrogen consumption/g	A/B	产量 Yield /(g·m^{-2})
	总干重 Total dry weight/g	有机碳质量 Organic carbon/g	总氮质量 Total nitrogen/g	C:N (A)	总干重 Total dry weight/g	有机碳 Organic carbon 含量 Content/%	有机碳 Organic carbon 质量 Weight/g	总氮 Total nitrogen 含量 Content/%	总氮 Total nitrogen 质量 Weight/g	C:N (B)				
M0B08	1060.8	692.6	5.4	128	628.0±20.5	64.4±2.7	404.0±29.7	0.97±0.19	6.1±1.3	66	287.9±29.7	−0.7±1.3	1.9	2.0±2.0
M0B12	1591.2	1038.9	8.2	120	927.0±17.3	66.4±4.2	616.4±50.2	0.88±0.10	8.2±1.1	75	422.4±41.0	−0.1±1.1	1.6	2.0±2.0
M0B14	1856.4	1212.0	9.5	128	1090.7±7.1	64.2±1.9	700.1±22.3	0.89±0.26	9.7±2.9	72	512.0±22.3	−0.2±2.9	1.8	5.7±4.7
M0B16	2121.6	1385.2	10.9	127	1246.0±27.8	64.8±2.4	807.7±42.2	0.85±0.12	10.6±1.6	76	577.4±42.2	0.2±1.5	1.7	6.7±6.5
M1B08	1076.8	664.3	11.0	60	520.3±8.1	59.4±0.8	309.2±5.3	1.53±0.30	8.0±1.7	39	355.0±5.4	3.0±1.7	1.6	74.7±11.4
M1B12	1615.2	996.4	16.5	60	746.3±6.7	60.9±1.9	454.3±10.5	1.44±0.33	10.7±2.4	42	542.1±10.5	5.8±2.4	1.4	171.0±88.6
M1B14	1884.4	1162.5	19.2	61	929.7±19.3	61.4±1.4	570.7±22.6	1.30±0.09	12.1±1.1	47	591.8±22.6	7.1±1.1	1.3	197.0±110.2
M1B16	2153.6	1328.5	22.0	60	1060.3±9.5	61.1±0.9	648.3±8.6	1.35±0.15	14.3±1.5	45	680.2±8.6	7.7±1.5	1.3	250.0±91.1
M2B08	1080.0	666.2	14.7	45	408.0±56.8	49.5±2.9	202.7±37.3	2.02±0.27	8.3±2.1	24	463.5±37.3	6.4±2.1	1.9	98.7±67.0
M2B12	1620.0	999.3	22.0	45	571.3±51.8	52.4±5.9	301.5±59.8	2.15±0.28	12.4±2.7	24	697.8±59.7	9.6±2.6	1.9	147.7±20.0
M2B14	1890.0	1165.9	25.7	45	716.7±88.2	46.9±1.5	335.8±33.6	2.15±0.28	15.5±3.7	22	830.1±33.6	10.2±3.7	2.1	257.0±73.7
M2B16	2160.0	1332.4	29.4	45	790.0±89.2	55.0±4.5	433.3±48.5	2.27±0.04	17.9±2.0	24	899.1±48.5	11.4±2.0	1.9	312.3±148.4
M3B08	1108.0	665.0	15.9	42	460.0±44.2	50.3±4.1	230.9±25.0	2.89±0.22	13.4±2.3	17	434.1±25.0	2.5±2.3	2.4	155.0±32.7
M3B12	1662.0	997.5	23.9	42	695.0±29.2	52.3±2.9	362.9±9.0	2.88±0.10	20.0±1.3	18	634.6±9.0	3.9±1.4	2.3	235.0±71.6
M3B14	1939.0	1163.7	27.8	42	825.3±81.5	52.5±5.4	430.3±18.5	2.85±0.25	23.5±2.3	18	733.4±18.5	4.4±2.3	2.3	297.0±203.2
M3B16	2216.0	1330.0	31.8	42	863.7±63.8	53.0±2.5	456.7±14.1	2.73±0.16	23.6±2.8	19	873.3±14.1	8.2±2.8	2.2	359.3±117.1
M4B08	1128.0	691.6	19.1	36	559.0±5.6	58.8±1.4	328.4±4.2	3.15±0.24	17.6±1.6	19	363.2±4.2	1.4±1.6	1.9	116.7±30.0
M4B12	1692.0	1037.4	28.6	36	842.7±58.4	62.9±1.0	529.5±31.5	3.13±0.39	26.4±3.7	20	507.9±31.5	2.2±3.7	1.8	182.3±26.1
M4B14	1974.0	1210.3	33.4	36	981.0±34.4	62.0±0.7	608.3±27.7	3.22±0.39	31.7±4.8	19	601.9±27.7	1.7±4.8	1.9	217.3±19.0
M4B16	2256.0	1383.2	38.1	36	1136.3±42.2	60.6±2.6	688.9±43.5	3.21±0.25	36.5±3.6	19	694.3±43.5	1.6±3.6	1.9	283.7±87.4

表 6　菌株 YAASJNLM1-31 有机碳和总氮消耗与产量的相关性

Table 6　Correlation between YAASJNLM1-31 yield and consumption of organic carbon and total nitrogen

类别 Category	皮尔逊相关性 Pearson's correlation
有机碳消耗与产量 Organic carbon consumption and yield	0.810 **
总氮消耗与产量 Total nitrogen consumption and yield	0.652 **

**:显著相关($P<0.01$)

**: extremely significant at $P<0.01$

3　讨论

羊肚菌采收结束后,5 种配方的基质消耗为 40.81%～65.75%,这些基质消耗一部分通过菌丝生长产生气体释放到环境中;一部分以菌丝体及其代谢产物的形式进入土壤,种袋栽培技术可有效避免种袋基质污染[12],因为采用种袋栽培时羊肚菌菌丝体能够较好地双向生长,在种袋基质中生长的同时利用种袋营养向土壤中生长,有利于营养的高效利用,有机碳和总氮消耗与产量正相关。笔者积极探索种袋中麦粒与木屑不同配比及种袋放置数量对羊肚菌产量的影响,但种袋配方是否适宜梯棱羊肚菌

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(*M. importuna*)生长还有待探究。另外,木屑的成本较高,且过度砍伐林木会对环境造成破坏,利用棉籽壳、秸秆、玉米芯、谷壳等农业废弃物制备种袋,既能减少羊肚菌栽培的原料成本,又能缓解农业废弃物带来的环境污染问题,在种袋营养利用和营养成分消耗研究基础上,有必要对羊肚菌栽培的种袋配方进行研究。对于羊肚菌菌株 YAASJNLM1-31,配方 M0 产量最低,随种袋中麦粒添加量的增加,产量有增加趋势;纯麦粒种袋的产量并不是最高,可能和种袋的通透性有关;产量随种袋放置数量的增多有增加趋势,可能是向土壤提供的养分增多有利于羊肚菌生长。种袋基质有机碳、总氮消耗和产量的关系表明,配方 M3 产量高且接种前和采收后种袋基质碳氮比变化较大,推测接种前种袋基质的碳氮比为 40∶1左右较适宜,有利于基质利用和提高产量;种袋麦粒用量对生产成本的影响较大,综合考虑产量与成本,适宜的麦粒用量为 50%~75%。

本研究结果表明,羊肚菌生长发育过程中种袋内碳源和氮源的消耗比例不同,对于氮源含量较低的纯木屑配方,还出现总氮反向输送的问题,即种袋向土壤输送有机碳营养,供菌丝生长,而氮营养存在双向输送且部分处理种袋的总氮消耗为负值。TAN 等[16]认为羊肚菌在营养袋栽培过程中菌丝体与其他微生物一起从土壤中获得氮,这些氮被用来合成参与底物分解的酶。本研究结果表明,种袋基质中氮源不足、不利于羊肚菌生长发育时,会发生种袋基质总氮质量净增加,即进入种袋的总氮质量比输出的总氮质量多。与营养袋栽培法比较,种袋栽培法中种袋基质中生长的羊肚菌菌丝来自菌种而不是土壤,外源微生物(包括细菌和真菌)进入种袋基质的机会较小,两种栽培方法中氮源利用和输送的机制有待于进一步研究。笔者调查发现羊肚菌栽培不出菇、低产和绝产的田地除连作地外,多数为前茬种植豆类作物或叶菜类蔬菜的田地,这些田地土壤的总氮含量比周边土壤高 50%~300%,这些田地影响羊肚菌出菇的原因尚不清楚。羊肚菌生长发育过程中氮的输送机制值得深入探讨,营养基质中氮源种类和土壤中氮含量是否影响羊肚菌生长发育有待进一步研究。

本研究结果表明,羊肚菌栽培时种袋基质的适宜 C∶N 约为40∶1,目前羊肚菌种袋或营养袋基质中麦粒占比差别较大,根据适宜的 C∶N 调整基质配方,减少麦粒用量,有利于羊肚菌产业的健康发展,同时也可以减少基质对环境的影响。种袋采用纯木屑的 M0 配方(C∶N 为120∶1)时,虽然能出菇,也有40%左右营养消耗,但羊肚菌生育期长、产量低,说明羊肚菌生长速率以及对木屑的利用效率均较低,不宜采用纯木屑制备种袋栽培羊肚菌,同样木质素、纤维素含量高的棉籽壳、谷壳也不太适宜制备种袋,可采用速效碳源含量较高的玉米芯、食用菌菌渣、发酵的农作物秸秆等制备种袋,本研究结果提示研发新型种袋或营养袋基质有助于降低羊肚菌生产成本。

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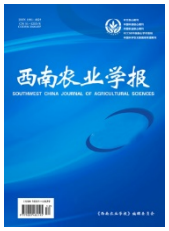
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西南农业学报
Southwest China Journal of Agricultural Sciences
ISSN 1001-4829,CN 51-1213/S

《西南农业学报》网络首发论文

题目：干旱胁迫对甘蔗光合生理特性的影响
作者：刘硕，樊仙，全怡吉，杨绍林，李如丹，邓军，张跃彬
收稿日期：2021-06-18
网络首发日期：2022-07-21
引用格式：刘硕，樊仙，全怡吉，杨绍林，李如丹，邓军，张跃彬．干旱胁迫对甘蔗光合生理特性的影响[J/OL]．西南农业学报.
https://kns.cnki.net/kcms/detail/51.1213.S.20220721.0831.008.html



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网络首发时间：2022-07-21 15:13:34
网络首发地址：https://kns.cnki.net/kcms/detail/51.1213.S.20220721.0831.008.html

西南农业学报 2022, 35（8）
收稿日期：2021 – 06 – 18
基金项目：国家重点研发计划资助（2020YFD1000600）；国家现代农业产业技术体系资助（CARS-170205）
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干旱胁迫对甘蔗光合生理特性的影响

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摘　要：【目的】研究干旱胁迫下甘蔗的光合及生理作用对提高甘蔗品质与产量具有重要理论意义。【方法】以第十四轮全国区试品种中 3 个品种云蔗 11-1074、云瑞 12-263、粤甘 52 号和新台糖 22 号（ROC22）为供试材料，研究干旱胁迫及复水条件下甘蔗形态特征、光合作用以及生理反应的变化规律。采用盆栽试验，通过人工称重控制土壤含水量，在甘蔗伸长期设定干旱 3d、干旱 6d、干旱 10d 和复水 10d4 个处理，测定甘蔗光合指标和生理指标，并通过隶属函数法对 4 个品种的抗旱性进行评价。【结果】经干旱 10d 后，各参试品种绿叶率、叶片含水率显著降低。光合指标中净光合速率、气孔导度、蒸腾速率下降，叶绿素荧光参数 F_v/F_m 和 F_v/F_o 降低，叶绿素含量显著降低，胞间 CO_2 浓度上升。4 个品种中粤甘 52 号光合指标受影响最大。干旱胁迫 10 d 时 4 个甘蔗品种的抗氧化酶活性整体增加，CAT 和 GR 活性变化更加明显，可溶性糖含量有所提升，可溶性蛋白含量先增加后减少，MDA 含量逐渐升高。云瑞 12-263 的 SOD 和 CAT 活性较高；ROC22 有着较高的 GR 活性；云蔗 11-1074 的 MDA 含量显著高于其它 3 个品种，云瑞 12-263 含量最低。复水后，4 个品种受干旱胁迫的影响得到一定缓解，粤甘 52 号光合能力恢复性较强但其它方面则未有好的表现。在生长能力方面，ROC22 和云瑞 12-263 受干旱胁迫影响小，云蔗 11-1074 和粤甘 52 号受影响较大。结合平均隶属函数值对甘蔗抗旱性进行排序为：云瑞 12-263＞ROC22＞云蔗 11-1074＞粤甘 52 号。【结论】干旱胁迫条件对 4 个甘蔗品种的形态变化、光合作用、抗氧化酶活性均产生影响，参试品种中云瑞 12-263 的各项光合指标均高于其它 3 个品种，可以对此品种进行进一步研究挖掘抗旱基因用于育种。

关键词：甘蔗；干旱胁迫；光合作用；抗氧化酶活性；渗透调节

中图分类号：S566　　**文献标识码：A**

Effects of drought stress on photosynthetic and physiological characteristics of sugarcane

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Abstract:【Objective】Study of the effects of drought stress on photosynthetic and physiological activities of sugarcane is important for improving the quality and yield of sugarcane.【Method】In this study, three varieties Yunzhe 11-1074, Yunrui 12-263, Yuegan 52 from the fourteenth round of national regional trials and a control variety Xintaitang 22 (ROC22) were used as test materials. The effects of drought stress and rewatering on its morphological characteristics, photosynthesis and physiological responses were studied. Pot experiment was established. Soil moisture content was evaluated by manual weighing. Four treatments including underdrought for 3d, drought for 6d, drought for 10dand rewatering for 10dduring the elongation period were set.The photosynthetic and physiological indexes of sugarcane were measured, and the drought resistance of the four varieties was evaluated by membership function method.【Result】After 10 d of drought, the green leaf ratio and leaf moisture content of the tested varieties decreased significantly. Photosynthetic indicators P_n, G_s, and T_r decreased, chlorophyll fluorescence parameters F_v/F_m and F_v/F_o decreased, chlorophyll content decreased significantly, but the C_i increased. Among the four varieties, the photosynthesis of Yuegan 52 was the most affected. Under drought stress for 10 days, the activities of antioxidant enzymes of the four sugarcane varieties increased overall. The changes of catalase(CAT) and glutathione reductase(GR) activities were more detectable, that the content of soluble sugar increased, the content of soluble protein increased first and then decreased, and the content of malondialdehyde(MDA) increased gradually. The superoxide dismutase(SOD) and CAT activities of Yunrui 12-263 were higher; ROC22 had higher GR activity; the MDA content of Yunzhe 11-1074 was significantly higher than that of the other three varieties, and the content of Yunrui 12-263 was the lowest. After rewatering, the effects of drought stress on the four varieties were relieved to a certain extent, and the photosynthetic capacity of Yuegan 52 was relatively recoverable, but other parameters did not recover. In terms of growth ability, ROC22 and Yunrui 12-263 were less affected by drought stress, while Yunzhe 11-1074 and Yuegan 52 were more affected. Combined with the average membership function value, the drought resistance of sugarcane was ranked as follows: Yunrui 12-263>ROC22> Yunzhe 11-1074>Yuegan 52.【Conclusion】Drought stress affected the morphological changes, photosynthesis and antioxidant enzyme activities of the four sugarcane varieties. The photosynthetic indexes of Yunrui 12-263 in the tested varieties were higher than those of the other three varieties. So, this variety can be further studied to broaden drought-resistant genes for breeding.

Key words: Sugarcane; Drought stress; Photosynthesis; Antioxidant enzyme activity; Osmotic regulation

【研究意义】甘蔗是中国种植面积最大的糖料作物，以甘蔗为原料的糖产量占我国食糖供应总量 85%以上[1]。随着全球气候的变化，干旱成为影响甘蔗生长最严重的非生物胁迫之一。在中国，甘蔗主要种植在旱坡地，蔗区旱地面积达到 70%以上[2]。夏秋季是甘蔗生长的关键时期—伸长期，随着秋旱发生的日益频发，甘蔗产量和糖分受到严重影响。因此，研究甘蔗伸长期的抗旱特性，了解土壤水分胁迫对甘蔗光合及生理生化指标的影响，对抗旱甘蔗品种选择、提高产量具有指导意义。干旱胁迫下，由于植物体内水分状态发生改变，导致植物生理脱水，造成植物呼吸作用紊乱、膜伤害、代谢失调等一系列损害，最终使得

植物生长抑制、萎蔫、局部坏死或全株死亡[3]。【前人研究进展】大量对甘蔗的研究表明，干旱胁迫导致甘蔗株高变矮、单茎重、叶片含水量下降[4－6]，叶片枯萎程度加重，光合作用面积减小并减弱光合作用，使甘蔗株高生长变慢，产量降低[7]；甘蔗脯氨酸、丙二醛、可溶性糖含量上升[8－9]；可溶性蛋白在干旱初期含量上升，后期随着干旱增强降低[10]；超氧化物歧化酶、过氧化氢酶、过氧化物酶、谷胱甘肽还原酶等酶活性升高[11－13]；干旱胁迫引起甘蔗光合速率、蒸腾速率、气孔导度和叶绿素含量降低，胞间二氧化碳浓度上升[2, 14]；光系统 II（PSII）活性 F_v/F_o、光系统 II 光化学最大效率 F_v/F_m 随水分胁迫强度增强而下降[15－16]。在甘蔗抗旱性鉴定的指标选取方面，有研究认为叶绿素值 SPAD[17]、叶绿素荧光参数[18－19]、渗透物质[20]、抗氧化酶活性[21]等可以作为评价甘蔗抗旱性的主要指标。【本研究切入点】粤甘 52 号、云蔗 11-1074、云瑞 12-263 是中国第十四轮区试的新品种，ROC22 是中国主栽品种，通过对新品种和老品种之间的比较，探究品种间对干旱胁迫的表现。【拟解决的关键问题】本研究对 4 个不同基因型甘蔗在人工气候温室内采用干旱胁迫－复水法，通过探究甘蔗在伸长期不同水分胁迫下相关生理生化指标及光合作用的变化趋势和规律，并对试验品种进行抗旱性评价，对甘蔗抗旱品种的鉴定、选育提供理论参考。

1 材料与方法

1.1 试验材料

所选品种为粤甘 52 号、云蔗 11-1074、云瑞 12-263 和 ROC22，由云南省农业科学院甘蔗研究所提供。

1.2 试验设计

试验地位于云南省开远市云南省农业科学院甘蔗研究所第一科研基地。将粤甘 52 号、云蔗 11-1074、云瑞 12-263 和对照 ROC22 号用桶进行栽植(桶规格为直径 40 cm×高度 35 cm），每桶分别种植 4 个单芽，出苗后选取健康蔗苗定苗 3 株进行常规管理。于 2020 年 9 月甘蔗伸长期时对长势一致的甘蔗开始进行干旱胁迫，设置自然干旱 3、6、10 d 及复水 10 d 4 个处理。每个处理 15 株，均设置正常浇水作为对照。

1.3 试验方法

分别在各处理日上午 9:00-11:00 测定植株的光合参数并取样。甘蔗叶片的光合速率（P_n）、蒸腾速率（T_r）、气孔导度(G_s)和胞间二氧化碳浓度（C_i）由 LI-6400 便携式光合仪测定。叶绿素含量采用手持 SPAD-502 测定+1 叶 SPAD 值。使用 Handy Pea 进行叶绿素荧光参数的测定。叶片含水率用称重法测定，超氧化物歧化酶（SOD）活性用 WAT-8 法测定[22]，过氧化物酶（POD）活性用微量法测定[23]，过氧化氢酶（CAT）用钼酸铵比色法测定[24]，丙二醛（MDA）用硫代巴比妥酸法测定[25]，脯氨酸（Pro）用磺基水杨酸法测定[26]，可溶性糖（SS）用蒽酮比色法测定[27]，可溶性蛋白（SP)用 BCA 法测定[28]，谷胱甘肽还原酶（GR）活性采用试剂盒进行测定。在各处理取样前测量株高并记录绿叶数和总叶数，计算绿叶率。

1.4 数据统计分析

根据以下公式计算各品种所有指标的隶属函数值，以各品种平均隶属函数值作为抗旱性评价的依据[29]。

$$\mu(X_i)=\frac{X_i-X_{i\min}}{X_{i\max}-X_{i\min}} \quad (1)$$

$$或\ \ \mu(X_i)=1-\frac{X_i-X_{i\min}}{X_{i\max}-X_{i\min}} \quad (2)$$

其中，X_i 是某指标测定值；$X_{i\min}$ 和 $X_{i\max}$ 分别为四个甘蔗品种中某一指标内的最小值和最大值。当所测指标与抗旱性为正相关时，用（1）式计算，反之使用（2）式计算。平均隶属函数值越高的品种抗旱性越强，反之抗旱性越弱。

数据采用软件 SPSS 25.0 进行统计和分析，Origin2019 进行作图。

2 结果与分析

2.1 干旱胁迫对甘蔗形态特征的影响

从表 1 可以看出 4 个甘蔗品种绿叶率的变化，正常供水下绿叶率均达到 70%以上。干旱胁迫下 4 个品种的绿叶率均随着干旱时间的增长显著降低，在复水后显著提升。云瑞 12-263 在正常供水时绿叶率为 4 个品种中最低，为 74.49%，但在干旱胁迫 10 d 时绿叶率为 45.18%，显著高于其它 3 个品种。粤甘 52 号在干旱 3 d 时绿叶率显著高于其它品种，在干旱 10 d 时绿叶率为 4 个品种中最低，仅为 13.39%。复水后，ROC22 和云瑞 12-263 的绿叶率恢复，与干旱 6 d 时无显著差异，说明复水情况较好，云蔗 11-1074 与粤甘 52 号的绿叶率虽然显著高于干旱 10 d，但低于干旱 6 d，恢复能力较弱。

表 1 干旱胁迫对甘蔗绿叶率的影响

Table1 Effect of drought stress on green leaf ratioof sugarcane

胁迫时期 Stress stage	干旱胁迫绿叶率(%)Green leaf ratioof drought stress				对照绿叶率(%)Green leaf ratioof CK			
	新台糖 22 号 ROC22	云蔗 11-1074 Yunzhe 11-1074	云瑞 12-263 Yunrui 12-263	粤甘 52 号 Yuegan 52	新台糖 22 号 ROC22	云蔗 11-1074 Yunzhe 11-1074	云瑞 12-263 Yunrui 12-263	粤甘 52 号 Yuegan 52
干旱 3d Drought for 3 d	84.37±4.41Ab	76.08±1.82Ab	65.13±4.02Ac	96.18±5.25Aa	96.00±0.72Aa	83.98±2.56Ab	76.33±3.63Ac	98.33±1.92Aa
干旱 6d Drought for 6 d,	55.81±2.37Ba	59.81±4.52Ba	51.45±6.02Ba	56.60±5.00Ba	94.24±1.49Aa	85.70±1.31Ab	78.50±2.94Ac	94.07±2.26Ba
干旱 10d Drought for 10 d	35.34±2.16Cc	41.42±1.70Db	45.18±1.97Ca	13.39±2.15Dd	85.38±5.86Ba	83.56±4.24Aa	74.49±3.24Ab	88.25±1.40Ca

复水 10d Rewatering for 10 d	50.72±6.36Ba	53.42±1.86Ca	52.47±2.11Ba	23.59±1.87Cb	92.75±5.01Aa	82.97±1.80Ab	74.56±6.03Ac	88.69±1.43Cab

注：大写字母表示同一品种内不同处理的植株差异(p<0.01)，小写字母表示品种之间不同处理的植株差异(p<0.05)。下同。

Note: Capital letters indicate plant differences in different treatments within the same variety (p<0.01).Lowercase letters indicate plant differences in different treatments between varieties (p<0.05). The same as below.

甘蔗是一种高光效 C4 植物，本研究中，正常供水下叶片含水率达 80%以上。从表 2 可以看出，干旱胁迫后期会对甘蔗叶片含水率影响显著。干旱 3 d 时 4 个品种叶片含水率无显著差异，在干旱 10 d 时叶片含水率均显著低于其它处理，其中粤甘 52 号的叶片含水率显著低于其它 3 个品种。复水后，4 个品种的叶片含水率均显著提升。

表 2 干旱胁迫对甘蔗叶片含水率的影响

Table 2 Effect of drought stress onrelative moisture content of leaves

胁迫时期 Stress stage	干旱胁迫叶片含水率（%）Leaf moisture content of drought stress				对照叶片含水率（%）Leaf moisture content of CK			
	新台糖 22 号 ROC22	云蔗 11-1074 Yunzhe 11-1074	云瑞 12-263 Yunrui 12-263	粤甘 52 号 Yuegan 52	新台糖 22 号 ROC22	云蔗 11-1074 Yunzhe 11-1074	云瑞 12-263 Yunrui 12-263	粤甘 52 号 Yuegan 52
干旱 3 d Drought for 3 d	77.92±4.75Aa	78.77±0.71Aa	77.04±3.71Aa	80.73±3.60Aa	80.55±0.66Ab	86.12±3.23Ba	85.73±1.66Ba	88.33±0.31Ba
干旱 6 d Drought for 6 d,	69.42±1.71Bc	72.41±1.07Bb	72.72±0.67Ab	77.49±0.76Aa	84.33±2.59Ab	94.09±0.78Aa	85.90±0.40Bb	91.46±1.21ABa
干旱 10 d Drought for 10 d	63.10±1.39Ca	65.54±0.83Ca	67.90±1.65Ba	30.10±4.25Cb	82.77±1.25Ab	93.11±0.85Aa	90.85±0.59Aa	91.37±3.97ABa
复水 10 d Rewatering for 10 d	69.63±1.50Ba	71.78±4.78Ba	75.13±1.74Aa	40.13±2.25Bb	84.16±2.40Ab	91.29±2.88Aa	90.16±0.77Aa	94.43±1.14Aa

从表 3 可以看出，受干旱胁迫影响 4 个品种的株高生长速率均低于对照。干旱胁迫下 ROC22 和云瑞 12-263 的株高生长速率高于云蔗 11-1074 和粤甘 52 号。干旱 10d 时，粤甘 52 号生长速率最低，仅为 0.13，ROC22 最高，为 0.71。复水后，云瑞 12-263 株高生长速率最高为 0.93，云蔗 11-1074 最低为 0.38。

表 3 干旱胁迫对株高生长速率的影响

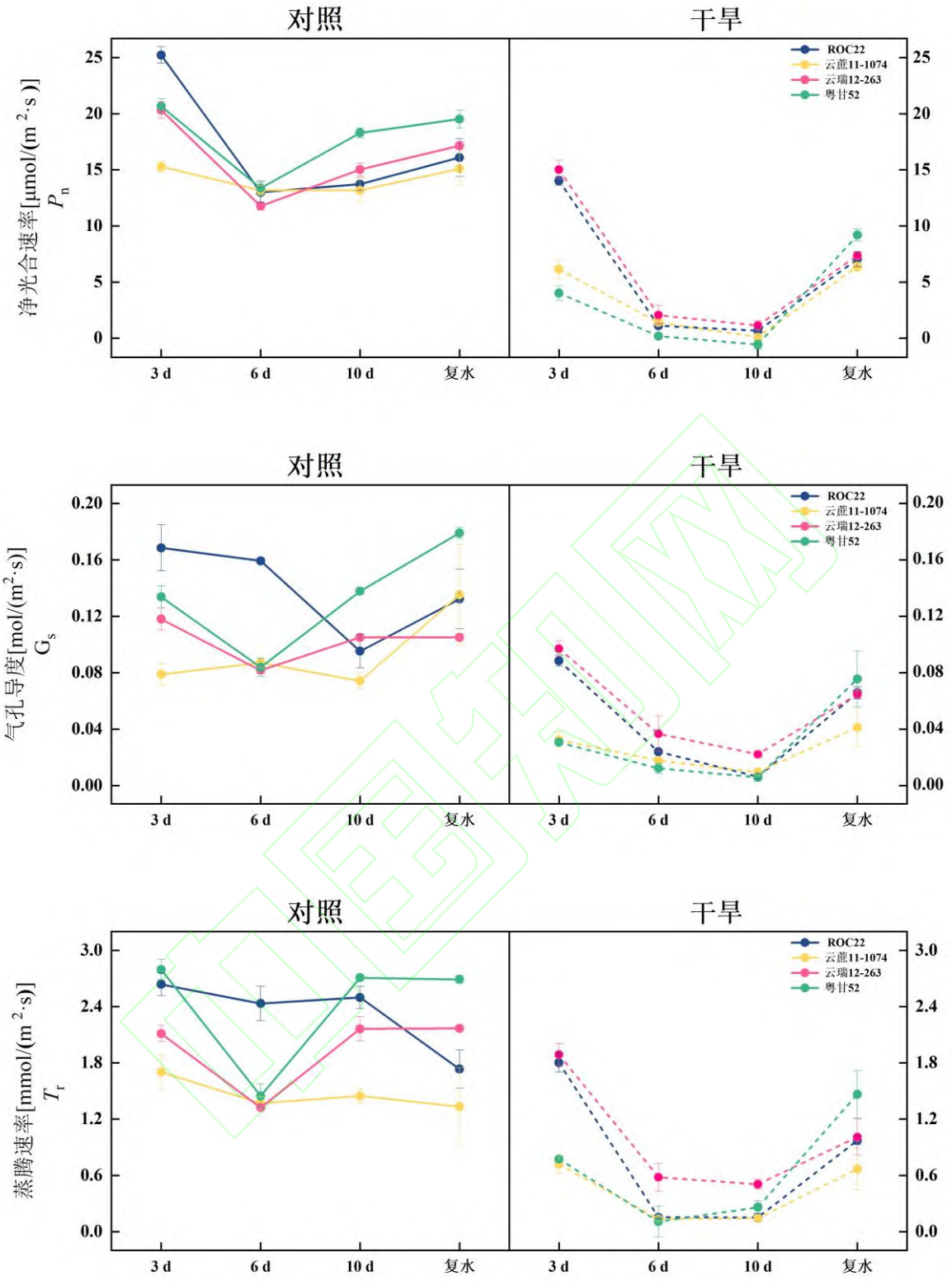
Table 3 The effect of drought stress on the growth rate of plant height

胁迫时期	干旱胁迫株高生长速率 Growth rate of plant height of	对照株高生长速率 Growth rate of plant height of CK

Stress stage	drought stress							
	新台糖 22 号 ROC22	云蔗 11-1074 Yunzhe 11-1074	云瑞 12-263 Yunrui 12-263	粤甘 52 号 Yuegan 52	新台糖 22 号 ROC22	云蔗 11-1074 Yunzhe 11-1074	云瑞 12-263 Yunrui 12-263	粤甘 52 号 Yuegan 52
干旱 3~6d Drought for 3~6 d	1.22	0.33	0.50	0.33	2.78	1.44	1.78	2.67
干旱 6~10d Drought for 6~10 d	0.71	0.19	0.44	0.13	0.92	1.17	0.92	1.08
复水 10d Rewatering for 10 d	0.78	0.38	0.93	0.43	0.73	0.83	1.27	0.97

2.2 干旱胁迫对甘蔗光合作用的影响

由图 1 可以看出，在干旱胁迫下 4 个甘蔗品种的净光合速率、气孔导度和蒸腾速率均低于正常浇水对照。干旱胁迫下，4 个品种的净光合速、气孔导度和蒸腾速率率均随着干旱加剧后复水而呈现出先降低后增加的趋势。其中粤甘 52 号的净光合速率和气孔导度在干旱 3、6、10 d 3 个处理中是 4 个品种中最低的，复水后其净光合速率和气孔导度恢复至 4 个品种中的最高值，分别达到了 9.18 μmol/(m^2·s)和 0.076 mol/(m^2·s)。云瑞 12-263 的净光合速率和气孔导度在干旱 3、6、10 d 3 个处理中均为最高。云蔗 11-1074 在正常和干旱期间的净光合速率、气孔导度和蒸腾速率均处于较低水平。ROC22 作为对照品种，在干旱 10d 时净光合速率、气孔导度、蒸腾速率和胞间 CO_2 浓度分别为 0.66 μmol/(m^2·s)、0.006 mol/(m^2·s)、 0.151 mmol/(m^2·s)和 292.75 μmol/mol。随着干旱胁迫气孔关闭，4 个品种的胞间 CO_2 浓度随着干旱加剧而不断上升，其中粤甘 52 号上升最高在干旱 10d 时为 459.00 μmol/mol，云蔗 11-1074 最低为 290.75 μmol/mol。复水后，4 个品种的胞间 CO_2 浓度均有所降低。


对照
干旱
ROC22
云蔗11-1074
云瑞12-263
粤甘52
净光合速率[μmol/(m²·s)]
P_n
气孔导度[mol/(m²·s)]
G_s
蒸腾速率[mmol/(m²·s)]
T_r
3 d
6 d
10 d
复水

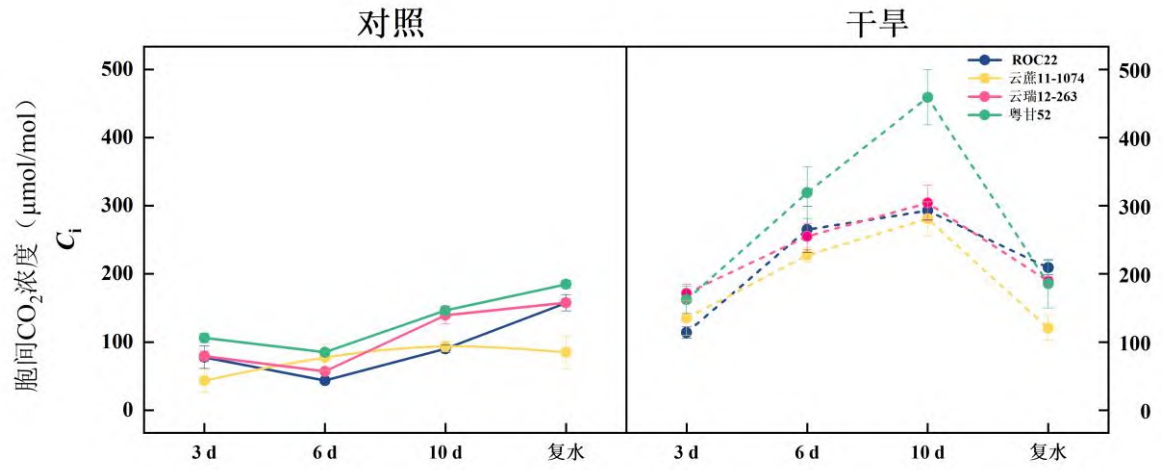
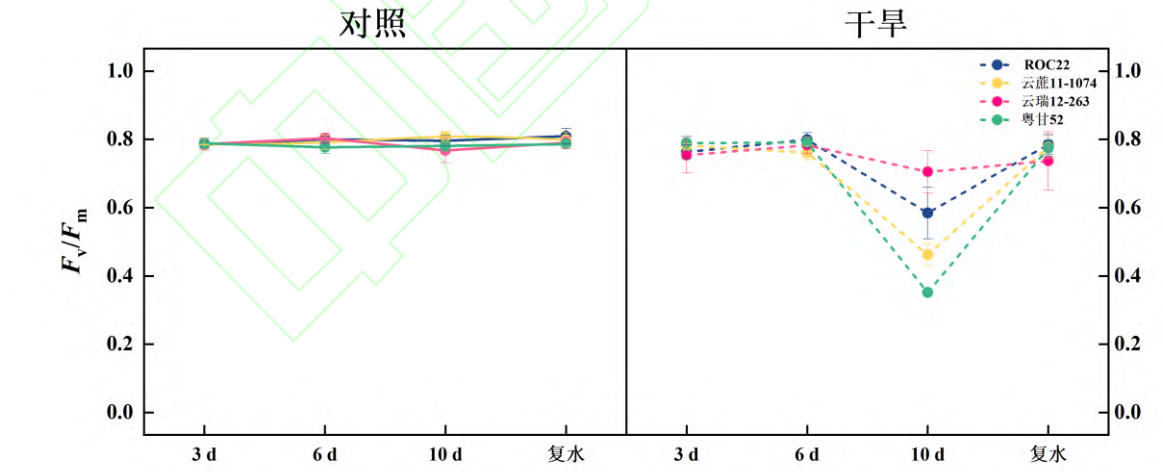


图 1 干旱胁迫对甘蔗光合作用的影响

Fig.1 Effect of drought stress on photosynthesis of sugarcane

由图 2 可以看出，各参试品种在不同干旱胁迫时期下的叶绿素荧光 F_v/F_m 和 F_v/F_o 的变化情况。正常浇水时，4 个品种的 F_v/F_m 和 F_v/F_o 无较大变化。在干旱 6 d 至干旱 10 d 时 4 个品种 F_v/F_m 降低，ROC22、云蔗 11-1074、云瑞 12-263 和粤甘 52 号的降幅分别为 26.84%、39.31%、9.96%、55.62%，其中粤甘 52 号降幅最大，云瑞 12-263 降幅最小。F_v/F_o 在干旱胁迫下也受到影响，在干旱 6 d 至干旱 10 d 时 ROC22、云蔗 11-1074、云瑞 12-263 和粤甘 52 号的降幅分别为 58.10%、71.77%、25.20%、75.06%，其中云瑞 12-263 降幅最小。复水后，4 个品种 F_v/F_m 和 F_v/F_o 均有所回升。



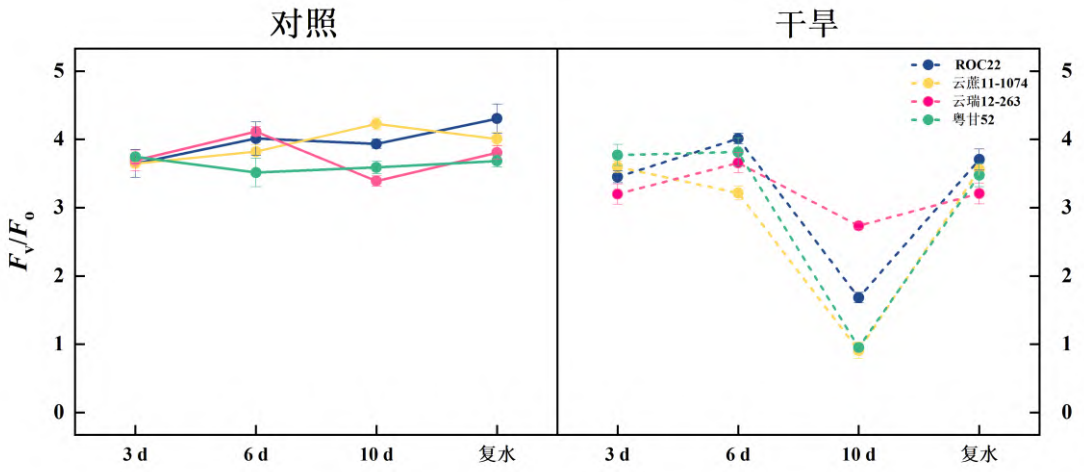


图 2 干旱胁迫对甘蔗叶绿素荧光的影响

Fig.2 Effect of drought stress on chlorophyll fluorescence of sugarcane

水分胁迫下叶绿体膨胀，排列紊乱，基质片层模糊，基粒间连接松弛，类囊体层肿胀或解体，叶绿素含量下降[30]。由图 3 可以看出，正常浇水情况下，4 个时期内各品种的 SPAD 均无显著差异。干旱胁迫时，除云瑞 12-263 在 4 个时期 SPAD 无显著差异外，其它 3 个品种在干旱 10 d 时均显著低于干旱 3 d 和 6 d。在干旱 10 d 时，4 个品种的叶绿素均达到最低值，分别为 28.89、27.20、32.87 和 17.44。SPAD 排序为云瑞 12-263＞ROC22＞云蔗 11-1074＞粤甘 52 号。

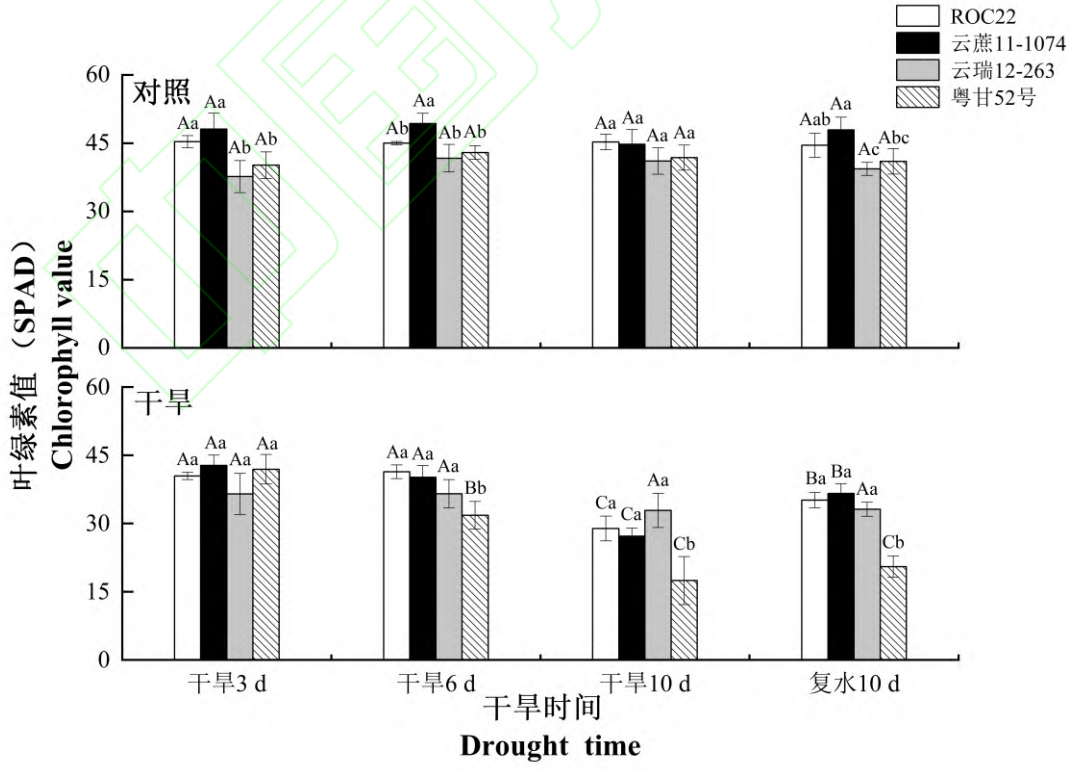


图 3 干旱胁迫对甘蔗叶绿素含量的影响

Fig.3 Effect of drought stress on Chlorophyll content of sugarcane

2.3 干旱胁迫对甘蔗抗氧化酶活性的影响

由图 4 可以看出，干旱胁迫下甘蔗抗氧化酶活性的变化规律。干旱胁迫导致 4 个品种的 SOD 活性不断提高，并在干旱 10 d 时达到最高值，显著高于干旱 3 d 和 6 d，复水后显著降低。云瑞 12-263 的 SOD 活性在干旱胁迫 3、6、10 d 和复水 10 d 4 个处理中均显著高于 ROC22、云蔗 11-1074 和粤甘 52 号。ROC22 和云瑞 12-263 在干旱 6、10 d 时 POD 活性均没有显著差异，粤甘 52 号在干旱 6、10 d 和复水 10 d 的 POD 活性分别为 814.25、984.47、650.02 U/g，均显著高于其它 3 个品种，在干旱胁迫 6、10 d 时 3 个品种并无显著差异。干旱胁迫下 4 个品种的 CAT 活性在干旱 10d 时显著高于其它处理。云瑞 12-263 在 4 个处理的 CAT 活性为 77.64、79.03、81.76 和 80.88 U/g，均显著高于其它 3 个品种，云蔗 11-1074 在干旱 3、6 d 时 CAT 活性最低，到干旱 10 d 时增幅达到 204.73%。GR 活性在干旱 10 d 时显著高于其它处理。ROC22 在干旱 10 d 时 GR 活性显著高于其它品种，为 522.75 nmol/min/g。

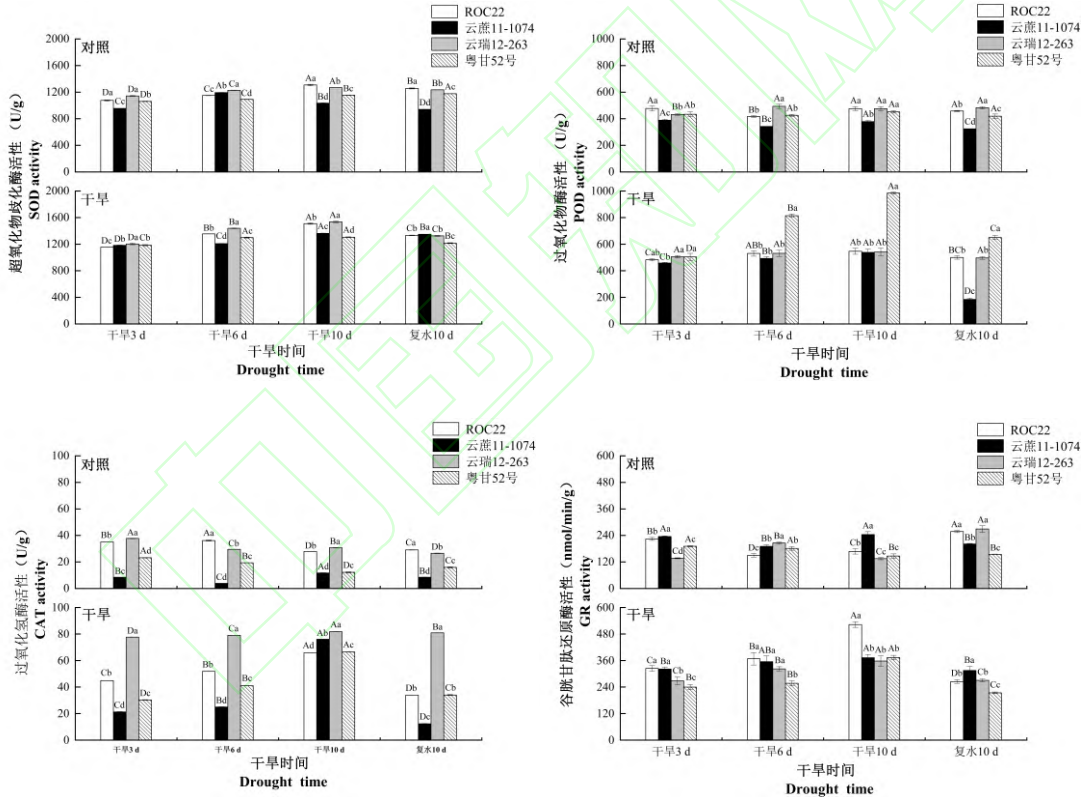


图 4 干旱胁迫对甘蔗抗氧化酶活性的影响

Fig.4 Effect of drought stress on antioxidant enzyme activity of sugarcane

2.4 干旱胁迫对甘蔗渗透物质的影响

由图 5 可以看出，干旱胁迫下 4 个甘蔗品种的脯氨酸含量随胁迫时间显著升高，在干旱 10 d 时显著高于其它处理。粤甘 52 号的脯氨酸含量在干旱 10 d 时为 139.70 μg/g，显著高于其它品种。ROC22 的脯氨酸

含量在干旱胁迫 4 个处理中均为最低。复水后，脯氨酸含量均显著下降。4 个品种的丙二醛含量都在干旱 10 d 时显著高于其它处理，其中云蔗 11-1036 显著高于其它品种。4 个品种的 MDA 含量均在复水后显著降低。云瑞 12-263 在干旱 6、10 d 和复水 10 d 处理中 MDA 含量最低，分别为 29.00、34.38 和 21.96 nmol/g。ROC22、云蔗 11-1036 和云瑞 12-263 的可溶性糖含量在干旱 10 d 时显著高于其它处理，粤甘 52 号在干旱 6 d 时显著高于其它处理。干旱 10 d 时，云瑞 12-263 的可溶性糖含量为 24.59 mg/g，显著高于其它品种。各品种可溶性蛋白含量在干旱 6 d 时达到最高，其中粤甘 52 号最高为 34.89 mg/g。在干旱 10 d 时，ROC22、云蔗 11-1074 和云瑞 12-263 可溶性蛋白含量为 4 个处理中的最低值，复水后则有所回升。

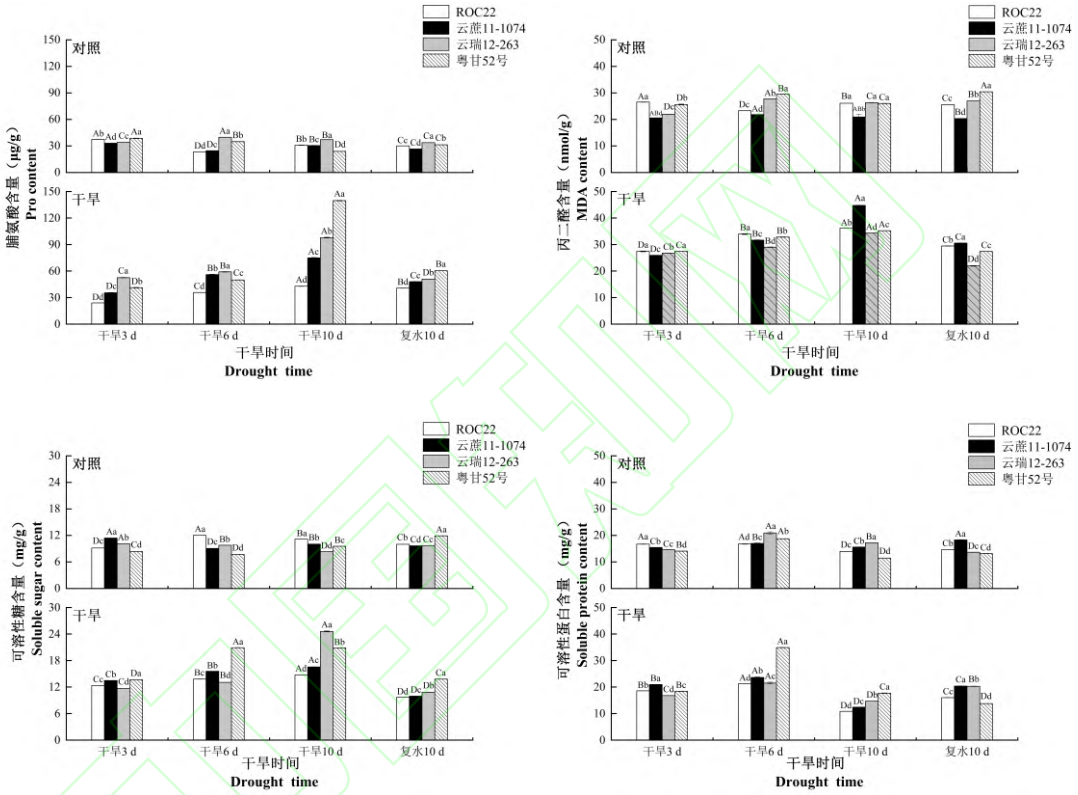


图 5 干旱胁迫对甘蔗渗透物质的影响

Fig.5 Effect of drought stress on penetrating substance of sugarcane

2.5 干旱胁迫下四个品种抗旱性综合评价

由表 4 可以看出各品种在干旱胁迫下的各项抗旱指标隶属函数值各不相同，通过平均隶属函数计算后得出各品种的抗旱性排序，结果为云瑞 12-263>ROC22>云蔗 11-1074>粤甘 52 号。

表 4 不同甘蔗品种伸长期抗旱性评价

Table 4 Evaluation of drought resistance of different sugarcane varieties during elongation period

指标	隶属函数值 Membership function value			
Index	新台糖 22 号	云蔗 11-1074	云瑞 12-263	粤甘 52 号
	ROC22	Yunzhe11-1074	Yunrui12-263	Yuegan52

绿叶率 Green leaf ratio	0.690571676	0.881733113	1	0
叶片含水率 Relative moisture content of leaves	0.873044514	0.937375471	1	0
净光合速率 Pn	0.723033791	0.396443854	1	0
气孔导度 Gs	0.021625767	0.224079755	1	0
蒸腾速率 Tr	0.024071527	0	1	0.323933975
胞间 CO_2 浓度 Ci	0.932678822	1	0.869565217	0
Fv/*Fo*	0.423218925	0	1	0.024340216
Fv/*Fm*	0.660070671	0.313074205	1	0
叶绿素值（SPAD） Chlorophyll value	0.742247434	0.63263101	1	0
超氧化物歧化酶 SOD	0.885920311	0.247340919	1	0
过氧化物酶 POD	0.017927893	0	0.006301961	1
过氧化氢酶 CAT	0	0.63874108	1	0.039285735
谷胱甘肽还原酶 GR	1	0.091709624	0	0.096558807
脯氨酸 Pro	0.089136768	0.233318646	0	1
丙二醛 MDA	0.966509463	0.918557641	0	1
可溶性糖	0	0.144550587	0.096805846	1

Soluble sugar 可溶性蛋白 Soluble protein	0.655936665	1	0	0.703628781
平均隶属函数值 Average affiliation function value	0.512117307	0.450562112	0.645451354	0.305161619
排名 Rank	2	3	1	4

3 讨　论

3.1 干旱胁迫对甘蔗农艺性状的影响

干旱胁迫下甘蔗的正常生长会受到抑制，形态上主要表现为绿叶数大幅度减少导致甘蔗生长变慢，茎径变小。蔗株在干旱胁迫下保持较多的绿叶数，则该品种抗旱性越强[7]。本研究表明，粤甘 52 号叶片形态受影响最为严重，绿叶率受损较高，云瑞 12-263 受影响较小。叶片相对含水率表现了叶片的水分情况，本研究发现 4 个品种甘蔗叶片含水率在遭受干旱胁迫初期不会有较大的改变，经历严重干旱后粤甘 52 号的叶片含水率降低较大。叶片中细胞在经历胁迫时能够有效的保持水分，然而当细胞因干旱而受到破坏时，抗旱性较差的品种由于细胞膜结构的损害，所以不能及时吸水造成叶片水分含量降低。株高作为甘蔗产量形成的关键因素之一，干旱胁迫对植株生长速率造成影响使甘蔗节间伸长受阻，造成甘蔗减产[31 – 32]。本研究中，干旱胁迫下株高生长速率减慢，云瑞 12-263 和 ROC22 受影响较小，其降低幅度较低，粤甘 52 号和云蔗 11-1074 受影响较大，与前人研究结果相一致。

3.2 干旱胁迫对甘蔗光合作用的影响

光合作用在干旱胁迫时降低的原因分为气孔限制和非气孔限制。若 CO_2 进入叶片受阻是由气孔导度降低引起光合速率降低的，属于气孔限制。胞间 CO_2 浓度升高，气孔导度降低，则说明光合速率的下降是因为光合色素量减少，叶绿体结构被破坏等非气孔原因引起的，属于非气孔限制。本试验中，随着干旱胁迫时间的增加，4 个甘蔗品种的叶片气孔导度、蒸腾速率明显降低，随着胞间 CO_2 的升高，气孔导度随之降低，说明造成光合作用降低的原因是非气孔限制，叶片进行光合作用的部位受到了破坏。在干旱胁迫初期，叶片蒸腾速率快速降低，是由于甘蔗感受到干旱胁迫，限制了气孔开度，减少水分流失。ROC22 和云瑞 12-263 在干旱前期气孔导度快速降低，限制了蒸腾作用，对干旱胁迫有着较快的响应。干旱严重时蒸腾速率的下降已经不明显，则说明气孔对应对干旱的调节已经起不到重要作用。有研究表明，耐旱性较强的品种在干旱胁迫中期叶绿素含量降低速度更小[15]，本研究中云瑞 12-263 降低速度最小，粤甘 52 号降低速度最大。ROC22 在正常浇水和干旱胁迫下均能保持较高的光合能力；云瑞 12-263 正常浇水时光合能力较差，

但干旱胁迫时光合能力最强；粤甘 52 号在正常浇水时光合能力最高，但干旱胁迫下其光合作用下降最严重。有研究发现甘蔗在苗期受旱后叶绿素荧光参数下降显著[33]。本研究分析了伸长期干旱胁迫下 4 个甘蔗品种叶片光合机构对干旱胁迫的响应。本试验结果表明，随着水分胁迫时间的延长，4 个品种的 F_v/F_m 和 F_v/F_o 均显著下降，ROC22 和云瑞 12-263 的降幅小于云蔗 11-1074 和粤甘 52 号。ROC22 水分胁迫下蔗叶 F_v/F_m 和 F_v/F_o 的动态变化因品种抗旱性能不同而有不同的模式，这与前人的研究相一致[18]。

3.3 干旱胁迫对甘蔗生理变化的影响

干旱胁迫会引起植物叶绿体、线粒体和过氧化物酶体中的 ROS 浓度升高到损害水平[34]。ROS 是有氧代谢不可避免的副产物，损害植物细胞的正常功能，ROS 的合成非常普遍，胞内和胞外都有产生部位[35]。本研究结果表明，随着干旱胁迫的持续，4 个品种的抗氧化酶活性均不断升高。ROC22 和云瑞 12-263 的抗氧化酶活性高于云蔗 11-1074 和粤甘 52 号，较高的抗氧化酶活性有助于减轻干旱带来的伤害。MDA 反映了细胞膜遭受破坏的程度，云蔗 11-1074 的 MDA 含量较高，说明其细胞膜遭破坏程度较为严重。干旱胁迫导致各品种渗透物质含量提升，如脯氨酸、可溶性糖和可溶性蛋白，可溶性糖是植物进行正常生理代谢的重要物质之一。有研究发现干旱条件下植物通过自身糖类的代谢以增强细胞的保水能力，使植物能够在逆境中更好生存[7]。云瑞 12-263 在遭受严重胁迫时，其可溶性糖含量在 4 个品种中含量最高，有利于植物抗旱。植物在失水时会产生可溶性蛋白进行保护，本研究发现，随着干旱胁迫的进行，在干旱 6d 时 4 个品种的可溶性蛋白达到最高，在干旱 10d 时除粤甘 52 号外其它 3 个品种可溶性蛋白含量为最低值，其中粤甘 52 号下降幅度最大而云瑞 12-263 下降幅度最小，说明胁迫导致蛋白酶活性迅速提高，加速了蛋白质的水解。

4 结　论

本研究表明，4 个甘蔗品种的抗旱性排序为云瑞 12-263＞ROC22＞云蔗 11-1074＞粤甘 52 号。ROC22 作为主栽品种，其光合作用能力、应对干旱的反应速度以及受干旱胁迫后的恢复能力均表现较好。较 ROC22 而言，云瑞 12-263 具有最强的抗旱能力，在干旱胁迫下光合作用能力强，具有较高的抗氧化酶活性，但是在正常供水条件下绿叶率以及光合作用较差。粤甘 52 号属于高光效甘蔗品种，绿叶率高、光合作用强且生长速度快，在干旱条件下却易受到很大影响严重损失光合及生长能力；云蔗 11-1074 在正常及干旱胁迫下，光合作用和生长能力均未有较好表现。因此，在进行抗旱育种时，干旱胁迫下甘蔗的光合能力应值得重点研究。

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植物铁素吸收机制研究进展

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摘 要 铁是植物生长发育过程中必不可少的矿质营养元素。虽然土壤中的铁元素总量丰富，但主要以不溶性的氧化铁和氢氧化铁的形式存在。为了从土壤中获得足够的铁元素，植物在长期的进化过程中，形成了两种不同的铁吸收策略，即依赖于 Fe^{3+}还原为 Fe^{2+}进行吸收的机制Ⅰ和依赖于 Fe^{3+}螯合而吸收的机制Ⅱ。通过查阅文献，发现植物存在一种新的铁吸收机制—基于微生物-植物互作的铁素吸收机制，但目前还未见到对其详细介绍的文献综述。因此总结阐述了对植物铁素吸收的两种机制以及微生物-植物互作铁素吸收的新机制，并对植物铁吸收机制未来发展方向进行展望，为植物栽培生产领域提供新思路。

关键词 植物铁素吸收；机制Ⅰ；机制Ⅱ；微生物-植物互作

中图分类号 S158.3；Q945 **文献标识码** A **DOI:** 10.12008/j.issn.1009-2196.2022.05.005

Advances on the Mechanism of Iron Absorption in Plants

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Abstract Iron is an essential mineral nutrient for plant growth and development. Although total Fe is abundant in soil, it exists mainly with the form of insoluble Fe oxide and Fe hydroxide. In order to obtain sufficient Fe from the soil, plants have developed two different Fe uptake strategies in the long period of evolution, mainly iron absorption strategy I which rely on the reduction of Fe^{3+} to Fe^{2+} and strategy II which rely on Fe^{3+} chelation. The literature review revealed that a new Fe uptake mechanism based on microbial-plant interactions exists in plants, but a detailed literature review on it has not yet been seen. Therefore, this paper analyzed two mechanisms of iron uptake in plants and a new mechanism of iron uptake by microbial-plant interactions, looked forward the future development of plant iron uptake mechanisms and provided new ideas for the field of plant cultivation and production.

Keywords plant iron absorption; strategy I; strategy II; microbial and plant interaction

铁元素在动植物细胞呼吸和金属蛋白的催化反应等过程中发挥重要作用，是重要的电子传递体，参与多种生物化学反应过程，具有重要的生理生化功能[1-2]。植物失绿症是典型的缺铁症状之一，缺铁会导致植物叶绿素合成减少，光合速率降低，严重缺铁时叶绿素合成停止，新叶变黄，生物量大幅度下降[3-5]。铁是动物及人体必需微量元素中含量最高的一种，在体内参与氧的运送、交换和组织呼吸等重要生理过程。人体缺铁是世界上严重的营养缺乏症之一，由缺铁而导致的缺铁性贫血发病率高、影响人群广泛，严重影响着人们的身体健康[6-7]。

铁是地壳中的第四大元素，在土壤中含量丰富，但是铁在土壤（尤其是碱性土壤）中主要以不溶性的氧化铁和氢氧化铁的形式存在，游离铁的总浓度一般不高于 10^{-15} mol/L，不能满足植物正常生长发育所需的铁浓度（10^{-9} 至 10^{-4} mol/L），导致植物缺铁现象普遍存在，从而影响作物产量和品质[8-9]。全球 1/3 的耕地是石灰质土壤，铁在这种碱性土壤中的溶解度更低，植物的缺铁现象非常严重[10-11]。为了从土壤中获得足够的铁，植物在长期的进化过程中，形成了 2 种不同的铁吸收策略[12-13]：一种是依赖于 Fe^{3+}还原为 Fe^{2+}进行吸收的机制Ⅰ，双子叶植物和非禾本科单子叶植物

收稿日期 2022-02-19；修回日期 2022-03-09

基金项目 云南省基础研究计划项目（No.202101AS070001）。

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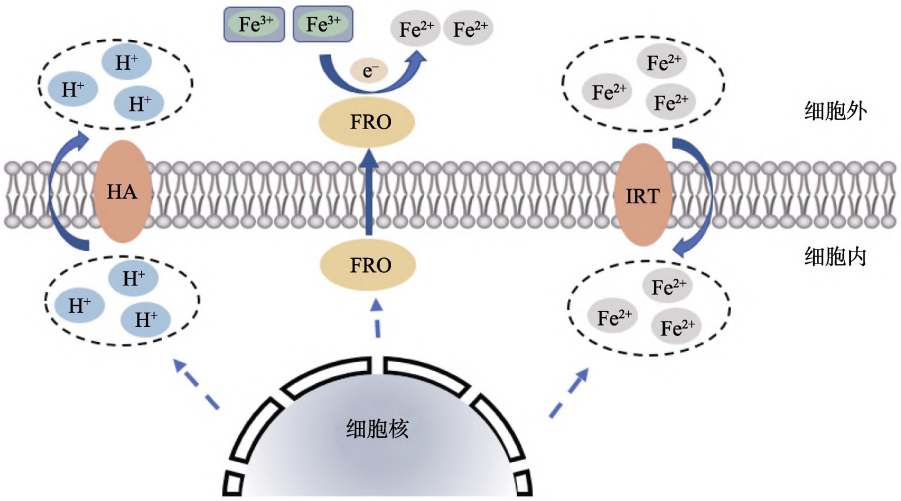
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主要以机制Ⅰ方式进行铁元素的摄取；另一种是依赖于 Fe^{3+}螯合而吸收的机制Ⅱ，它是禾本科单子叶植物特有的铁吸收机制。但是在最近的研究中，发现了一种植物铁吸收的新机制—微生物-植物互作吸收铁素的新机制[14-15]。目前对植物铁吸收、转运和利用的分子机制已经有了大量的文献报道[16-21]，但对于植物铁吸收新机制—微生物-植物互作机制还未见系统的综述。本文主要对植物铁吸收机制Ⅰ、机制Ⅱ和微生物-植物互作机制进行总结阐述，并对植物铁吸收机制未来发展方向进行展望。

1　依赖于 Fe^{3+}还原为 Fe^{2+}进行吸收的机制Ⅰ

土壤中的铁元素主要以 Fe^{3+}的形式存在，难以直接被植物吸收利用。因此，植物在长期进化过程中形成了将 Fe^{3+}还原成可被吸收的 Fe^{2+}的还原吸收机制，主要存在非禾本科植物中，包括双子叶植物和非禾本科单子叶植物，也被称为机制Ⅰ。该机制主要由 3 个部分组成（图 1）：（1）H^+-ATPase 泵系统，在该系统中植物通过向根际周围分泌 H^+以降低土壤 pH，从而增加土壤中 Fe^{3+}的可溶性，H^+-ATP 基因家族在拟南芥中有 12 个成员，其中 *AtAHA2*、*AtAHA3*、*AtAHA4*、和 *AtAHA7* 在缺铁条件下被诱导表达，可以主导质子跨膜运输并将其释放到根际周围，迅速将根际土壤 pH 降低到 3 或更低[8,22-23]；（2）Fe^{3+}还原系统，铁螯合还原酶（Ferric-chelate Reductase, FRO）是一个在植物根表皮细胞表达且受缺铁强诱导的铁还原蛋白，主要作用是将 Fe^{3+}还原成 Fe^{2+}[24-25]；（3）Fe^{2+}的转运系统，还原后的 Fe^{2+}通过位于根表皮的铁转运蛋白（Iron-regulated Transporter 1, IRT1）转运到细胞内，再由其他转运蛋白输送到各个细胞器和器官中利用[26-27]。AtIRT1 属于 ZIP 转运体家族（Zinc-regulated Transporter, ZRT; IRT-like Protein），是具有金属结构域的完整膜蛋白，参与铁、锌调节的一类转运体，并迅速响应缺铁条件被诱导表达，进而增强铁吸收转运能力[28-29]。



HA.H^+-ATPase；FRO.铁螯合还原酶；IRT.铁转运蛋白

图 1　植物铁吸收机制Ⅰ

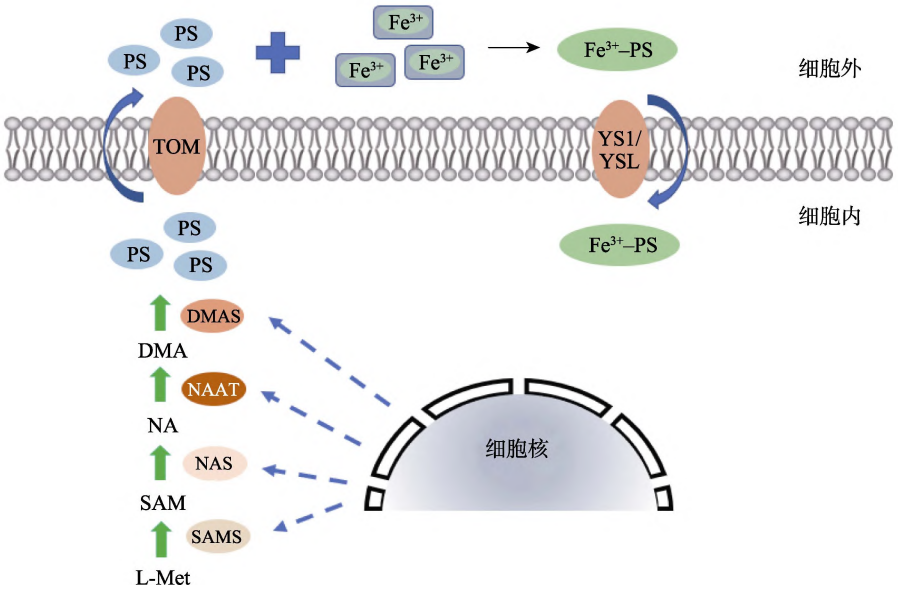
2　依赖于 Fe^{3+}螯合吸收的机制Ⅱ

禾本科植物，例如水稻（*Oryza sativa* L.）、玉米（*Zea mays* L.）、小麦（*Triticum aestivum* L.）、大麦（*Hordeum vulgare* L.）等，主要通过螯合 Fe^{3+}进行吸收的机制（机制Ⅱ）吸收土壤中的铁元素。植物感受到缺铁后，能够合成一类特殊物质—植物铁载体（Phytosiderophores, PS），并且将其分泌到根际土壤中，PS 对 Fe^{3+}具有非常高的亲和力，能够高效螯合土壤中的 Fe^{3+}形成 Fe^{3+}-PS 螯合物，然后通过 YS1/YSL（Yellow stripe like transporter）家族转运体转运到植物根细胞，以供植物吸收利用[30-32]（图 2）。麦根酸类物质（Mugineic acid, MA）是植物最常见的铁载体，目前已经初步阐明了麦根酸的合成过程。*L*-甲硫氨酸（*L*-Methionine, *L*-Met）是麦根酸合成的前体，首先在 *S*-腺苷基甲硫氨酸合成酶（Sadenosylmethionine synthase,

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SAMS）催化下生成 *S*-腺苷基甲硫蛋氨酸（S-adenosyl-L-methionine, SAM），后由烟胺合成酶（Nicotianamine synthase, NAS）将 SAM 三聚化生成烟胺（Nicotianamine, NA），NA 再经烟胺氨基转移酶（Nicotianamine aminotransferase, NAAT）催化生成麦根酸的 3'-酮基中间产物，随后由脱氧麦根酸合成酶（2'-Deoxymugineic acid synthase, DMAS）催化生成 2'-脱氧麦根酸（2'-Deoxymugineic acid, DMA）；DMA 是第一个合成的麦根酸，它进一步转化为麦根酸（MA）、阿凡酸（Avenic acid, AVA）、羟基麦根酸（3-Hydroxymugineic acid, HMA）、3-表-羟基麦根酸（3-Epi-hydroxy-mugineic acid, ep-iHMA）等麦根酸类植物铁载体[33-35]。根系细胞内形成的 MAs 被输出转运蛋白 OsTOM1 和 OsTOM2（Transporter of mugineic acid family phytosiderophores）运输到细胞外螯合游离的 Fe^{3+}形成 Fe^{3+}-MAs 螯合物并被植物吸收利用[36-37]。

与大多数禾本科植物不同，水稻长期生长在淹水环境中，土壤偏酸具有很好的还原条件，Fe^{2+}相对充足，为了更高效地吸收铁，除了利用 Mas 螯合 Fe^{3+}吸收铁供给机体利用外，水稻还进化出了针对 Fe^{2+}的铁吸收机制（机制Ⅰ），即利用 Fe^{2+}转运蛋白 OsIRT1 和 OsIRT2 直接吸收土壤中的 Fe^{2+}[38-39]。



TOM.麦根酸家族植物铁载体转运体；YSL.黄斑类转运体；*L*-Met.*L*-甲硫氨酸；SAM.*S*-腺苷基甲硫蛋氨酸；NA.烟胺；DMA.2'-脱氧麦根酸；PS.植物铁载体。

图 2　植物铁吸收机制Ⅱ

3　基于微生物-植物互作的铁素吸收机制

3.1　微生物铁载体合成及其调控

微生物在缺铁或低铁条件下会产生一些低分子量（500~1 000 Da）的有机化合物，称为铁载体（siderophores）[40]。研究发现，几乎所有经过培养的微生物都能产生铁载体，目前已知的铁载体大约有 500 种[8]。根据其结构特征和配体类型，细菌铁载体主要分为 4 类：儿茶酸类（Phenol catecholates）、氧肟酸类（hydroxamates）、羟基羧酸盐类（Carboxylate）和嗜铁素类（Pyoverdines）；真菌铁载体主要分为 3 类：红酵母酸类（Rhodotorulic acid）、高铁色素类（Ferrichromes）和镰孢氨酸类（Fusarinines）[41]。铁载体通常由非核糖体肽合成酶（NRPSs）或聚酮合成酶（PKS）域合成，少量铁载体也由不依赖于 NRPSs 和 PKS 的途径产生。通过 NRPSs 合成铁载体是最常见的形式，由多酶组装线组成，其中氨基酸、羧酸和羟基酸组成多肽前体分子，随后被 NRPSs 或其他酶修饰，形成最终的铁载体。铁载体的分泌是一个能量驱动的过程，并由流出泵介导，铁载体分泌后与环境中的 Fe^{3+}螯合，形成 Fe^{3+}-Siderophores 螯合物进入细胞[42-44]。

革兰氏阴性菌和革兰氏阳性菌对载铁螯合物的摄取不同。在革兰氏阴性细菌中，常见的机制

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是通过外膜上的受体特异性识别载铁螯合物，结合配体后，受体发生构象变化，将载铁螯合物转移到细胞质中；革兰氏阳性细菌不需要外膜受体，跨细胞膜的 ATP 结合转运蛋白直接结合并导入载铁螯合物[42]。铁载体如何释放铁的机制尚不清楚，在某些情况下，铁载体可以通过特定的回收机制进行回收，或者通过水解释放铁[43]。为维持细胞内的铁稳态，铁载体的合成、释放和摄取机制受到严格的调控。最常见的关键调节因子是 Fur（铁摄取调节因子），它是铁载体合成的转录抑制因子。当细胞内铁充足时，Fur 结合细胞质中的 Fe^{2+}形成复合物，然后结合铁载体合成基因或调控基因的启动子区域以阻断其转录；当细胞缺铁时，Fur 解除对铁载体合成基因和调控基因表达的抑制，诱导铁载体合成[43,45-47]。

3.2　微生物促进植物根系铁吸收

由于铁载体可以在不同物种细胞间分享，所以铁载体被认为是一种公共物质，可以通过互惠互利的合作形式同时供给生产者和接受者（其它物种）。植物的铁载体受体蛋白能识别某些微生物产生的铁载体，那么这类铁载体就作为公共物质被植物利用，形成了微生物-植物间的共生促进作用[48-49]。该类微生物受到缺铁胁迫后，合成铁载体并扩散至根际周围，铁载体与 Fe^{3+}螯合，形成 Fe^{3+}-Siderophores 螯合物。Fe^{3+}-Siderophores 螯合物一部分被微生物自身吸收，进入细胞之后还原成 Fe^{2+}，供微生物生长发育使用；另一部分被植物吸收利用（同机制 Ⅱ）（图 3）。Crowley 等[50-52]研究表明，某些微生物螯合化合物的产生增加了铁在根际的溶解度，从而增加了植物获取铁的能力，细菌和真菌的铁载体和其他螯合代谢产物被认为是根际植物可利用铁的重要来源。Masalha 等[53]研究发现，在正常条件（非无菌条件黄土土壤）下培养的玉米和向日葵生长良好，根系中铁的浓度较高；相比之下，无菌条件（灭菌黄土土壤）下生长的玉米和向日葵生长缓慢，而且缺铁严重，表明植物在无菌条件下生长时，缺乏微生物铁载体导致植物缺铁加剧。因此，微生物铁载体被认为是植物的有效铁源。还有研究发现，甘蔗和黑麦草根内的大肠杆菌分泌铁载体，从而促进了甘蔗和黑麦草的生长[54]；从泰国水稻根中分离出的内生链霉菌 GMKU3100 产生的铁载体能促进水稻和绿豆生长，显著提高根茎生物量和长度[55]。

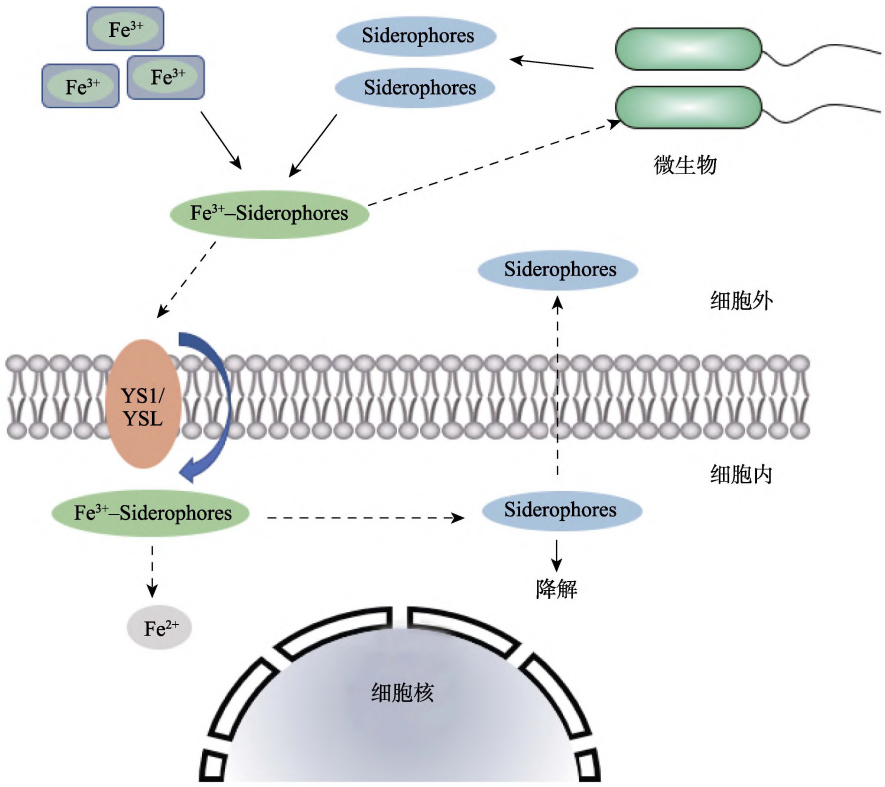


图 3　微生物-植物互作机制

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在病原菌侵染植物过程中，植物细胞周围坚硬的细胞壁限制了病原菌在细胞内的繁殖，但病原菌可以分泌大量的细胞壁降解酶和效应蛋白，损伤植物细胞并从中获得营养物质，包括从寄主植物中获取铁元素以用于自身的生长繁殖[56-58]。最近研究发现，一个细菌效应蛋白 AvrRps4 可以通过增加寄主质外体中铁的积累来促进病原菌的生长；另一方面，植物免疫系统严密调控着体内铁的稳态，限制病菌获取大量的铁元素，从而抑制病原菌的侵染[59]。植物除了可以利用微生物分泌的铁载体满足自身生长发育外，还可以通过调节体内铁稳态，限制病原菌的铁吸收，抑制病原菌的侵染。

4　展望

铁是每一个生命体的细胞中所必需的重要元素，植物位于食物链的底端，对于铁元素在生态系统中的循环至关重要，但是铁在碱性土壤中主要以不溶性的氧化铁和氢氧化铁的形式所存在，植物为了获得足够的铁以维持正常的生长发育，进化出了一系列铁吸收机制，在之前的研究中将植物对铁的吸收机制分为 2 种：非禾本科植物铁吸收机制（机制Ⅰ）和禾本科植物铁吸收机制（机制Ⅱ）。植物并非独立生活，而是长期与环境中的微生物尤其是土壤根围微生物互作，共同进化。研究发现，微生物铁载体也是植物的有效铁源之一，微生物铁载体的合成机制已经有大量文献报道，但是微生物分泌的铁载体如何被植物利用的分子机制还未见报道。植物主要以某一种吸收方式为主，伴随着其它吸收方式作为辅助，非禾本科植物在生长发育过程中，采用机制Ⅰ铁还原策略释放质子和铁还原酶还原土壤中的 Fe^{3+}，Fe^{3+} 还原成 Fe^{2+}后通过铁转运蛋白进入植物体内以供其正常生长发育；当土壤中的还原性铁不足以支持植物正常生长发育后，推测植物会感知缺铁并传递信号至根际的微生物中，诱导微生物进行群落调整和富集，加强铁载体的分泌，增强土壤中铁螯合物浓度，为植物生长发育提供一种外源性铁源。禾本科植物通过机制Ⅱ，自身合成并释放麦根酸类物质螯合土壤中的 Fe^{3+}形成螯合物，再通过特定运载蛋白转运至植物体内供植物生长发育；当自身分泌的麦根酸类物质不足以螯合形成足够的铁螯合物时，可能同样可以通过诱导根际微生物变化以提供额外铁源。部分植物（如水稻），同时存在机制Ⅰ和机制Ⅱ的铁素吸收方式，在生长发育过程中，首先采用机制Ⅰ通过铁转运蛋白 Fe^{2+}吸收铁，当土壤中的 Fe^{2+}不足以支持植物正常生长发育后，产生缺铁信号诱导机制Ⅱ相关通路，分泌铁载体螯合 Fe^{3+}通过特定运载蛋白转运至植物体内供植物生长发育，或与根际微生物互作以吸收铁。因此，植物与根际微生物协同共生吸收铁素在自然界中具有普遍性，通过螯合物形式吸收铁素可能是生命体早期原始的吸收形式。

植物铁吸收方式除了文中所述的 3 种途径外，还可能存在其它的铁吸收机制，如吞噬机制，吞噬机制主要涉及铁转运蛋白 *NRAMP* 基因家族和植物乙烯信号转导的核心组分—*EIN2* 基因。*NRAMP* 编码的蛋白结构与已知的同源性原核和真核运输系统相似，但这种功能还尚未揭示；植物乙烯信号转导的核心组分-*EIN2* 基因编码一种膜完整蛋白且与 NRAMP 家族成员蛋白有 21%的相似性，因此推测 EIN2 可能也是金属离子转运蛋白。土壤中铁素主要以非离子态铁形式存在时，离子态铁不足，植物通过机制Ⅰ、机制Ⅱ、微生物-植物互作机制摄取的铁素不足以支持其正常生长发育，由此，植物进化出类似细胞内吞的方式对含铁素的大分子物质进行摄取，再将内吞小体中的亚铁离子释放出来，然后再转入细胞质中，以供植物吸收利用。

迄今为止，已有发现并证明了多种调控途径参与植物体内铁素平衡的调节。如 *OsIDEF1* 和 *OsIDEF2* 是 2 个受缺铁诱导的转录因子，通过识别缺铁响应顺式元件激活下游铁吸收、转运基因[60-64]；bHLH 家族转录因子也在拟南芥、水稻等植物的铁离子吸收和转运中发挥重要作用，其中 *AtbHLH38*、*AtbHLH39*、*AtbHLH100*、*AtbHLH101*、*OsIRO2*、*OsbHLH60*、*OsbHLH156* 正向调控下游基因对铁离子的吸收和转运[61,65-68]，而 *OsIRO3*、*OsbHLH133* 负向调控下游铁吸收、转运基因[68-70]。目前的研究主要集中在植物铁离子吸收和转运的基因上[71-73]，对于微生物铁载体促进植物铁吸收分子机制的研究还未取得实质性进展。微生物铁载体除了可以直接应用在植物栽培生产领域外，研究微生物铁载体与植物互作机

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制也为物种间协同进化提供新的研究方向，促进和拓展了生物学和生态学的研究范畴。

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基于六个高代回交群体玉米叶形结构相关性状的杂种优势研究

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摘　要：本研究基于项目组前期构建的三角形杂种优势新模式，从 Suwan 1 群、Reid 群和非 Reid 群中选择 3 个最具有代表性的自交系 Y 46、Y 107 和 MON 2，构建了六个高代回交群体，分析各群体叶长、叶宽和叶夹角等叶形结构相关性状的遗传差异及杂种优势。结果表明，六个高代回交群体的叶长、叶宽及叶夹角均存在显著差异，其中 Y 46 与 Y 107 组配的高代回交群体叶形结构相关性状均显著高于 Y 46 与 MON 2 和 Y 107 与 MON 2 组配的群体，且 Y 46 与 MON 2 组配的正反交群体间各性状差异均较大。Y 107 与 MON 2 组配的高代回交群体其叶形结构相关性状的杂种优势较强，Y 46 与 MON 2 群体杂种优势较弱，Y 46 与 Y 107 群体杂种优势介于二者之间。在三角形杂种优势模式中，Reid×非 Reid 杂种优势较强，Suwan 1×Reid 杂种优势次之，Suwan 1×非 Reid 杂种优势较弱。研究表明，Reid×非 Reid 是培育理想株型新品种的重要杂种优势模式，建议在高密度品种选育中优先选用。

关键词： 玉米；高代回交群体；叶形结构相关性状；杂种优势
DOI：10.16590/j.cnki.1001－4705.2022.07.009
中图分类号： S 513　　**文献标志码：** A　　**文章编号：** 1001-4705(2022)07-0009-07

Investigation into the Heterosis of Leaf Morphological Traits of Using Six Advanced Generation Backcross Populations in Maize

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Abstract: Through a new model of triangular heterosis based on the preliminary construction of the project team, this study selected the representative inbred lines of three heterosis groups including Y 46, Y 107 and MON 2 from the Group Suwan 1, Group Reid and Group Non-Reid to construct six advanced generation backcross populations (namely $BC_1F_{7:8}$) and investigate into the genetic difference and heterosis of leaf morphological traits including leaf length (LL), leaf width (LW) and leaf angle (LA). The results showed that there were significant differences in LL, LW and LA traits among six $BC_1F_{7:8}$ populations. The leaf morphological traits of $BC_1F_{7:8}$ populations of Y 46 to Y 107 were higher than those of Y 46 to MON 2 and Y 107 to MON 2. What's more, there were relatively big difference in each trait between crossing and reciprocal crossing populations combined by Y 46 to MON 2. As for the heterosis, the leaf morphological traits of the Y 107 to MON 2 populations was strongest, and the $BC_1F_{7:8}$ populations derived from Y 46 and MON 2 was inferior. The combination Y 46 to Y107 populations were between the above two. This means that the heterosis of leaf morphological traits of Reid

收稿日期：2022-01-14
基金项目：国家自然科学基金项目(31661143004 和 31060192)；主要粮食作物分子设计育种项目(2016 YFD 0101803)；云南大学研究生科研创新基金项目(201915)。
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to Non-Reid was strongest, Suwan 1 to Reid ranked at the middle, and Suwan 1 to Non-Reid was inferior in the triangular heterosis group pattern. Therefore, the heterosis pattern of Reid to Non-Reid should be the principal choice to cross for screening ideotype maize hybrids with resistance to high-density in breeding program.

Key words: *Zea mays* L.; $BC_1F_{7:8}$ population; leaf morphological traits; heterosis

杂种优势是生物界的一种普遍现象,指两个遗传组成不同的亲本杂交所产生的杂交种,在生长势、抗逆性、适应性和产量等方面均超过其双亲的现象。玉米(*Zea mays* L.)是最早利用杂种优势的作物。20 世纪初期,杂种优势在美国玉米育种和生产中受到普遍关注并被广泛应用,使玉米产量得到大幅度提升,此后杂种优势逐步在其他作物中发掘并利用[1]。美国最早根据亲缘关系和系谱法将玉米种质划分为 Reid 和 Lancaster 两个杂种优势群,此后发展为温带地区最主要的杂种优势群和杂种优势模式[2]。墨西哥和中南美等热带、亚热带地区则采用以 Tuxpeno 和 ETO 为代表的杂种优势群和杂种优势模式[3]。我国 20 世纪末期对玉米杂种优势群和杂种优势模式的划分进行了大量的研究,明确了主要玉米种质的杂种优势群,其中温带玉米种质主要被划分为五大类群,包括 Reid、Lancaster、塘四平头、旅大红骨和 P 群[4-5],而热带亚热带玉米种质则划分为 Suwan 1、Reid 和非 Reid 三大类群,并据此提出三角形杂种优势新模式[6-8]。Fan 等[6]研究发现,正反交组合对产量性状的配合力及杂种优势群划分有重要影响。唐海涛等[9]研究表明,正反交对玉米穗粗、穗位高以及穗下茎节强度等性状有重要决定作用。奥妮等[10]发现,菊花正反交组合间叶片宽度、株高以及节间长等性状均存在显著差异。说明正反交对杂种优势表现有重要影响。宋方威等[11]利用高代回交群体研究正反交效应,但此类研究较少,目前正反交对热带、亚热带玉米杂种优势的影响尚不清楚。

杂种优势不仅体现在玉米产量及产量相关性状上,玉米的叶片大小及分布也存在杂种优势效应。Meyer 等[12]对 429 个重组自交系(Recombinant Inbred Lines,RIL)的农艺性状进行杂种优势分析,发现 RIL 叶面积的中亲优势效应为 58%~143%。司书丽等[13]用 80 个玉米组合及其亲本自交系进行株型性状的杂种优势研究,结果表明,株高、叶夹角及叶面积等大多数性状普遍存在杂种优势效应。郭宝健等[14]比较 Mo 17×B 73 及亲本苗期的叶长和叶宽,发现杂交种的中亲优势均达到显著或极显著水平。上述研究表明,玉米叶夹角、叶长、叶宽和叶面积等叶形结构相关性状间有显著的杂种优势效应。

目前,玉米杂种优势研究大多以温带玉米的 F_1 为材料,通过一般配合力和特殊配合力进行杂种优势群划分和利用,然而 F_1 为分离世代,通常根据表现型选择的优良性状不能在后代中稳定表达,因此研究结果对连续多代自交系选育的指导价值有限。此外,未见有关热带、亚热带玉米为材料的不同杂种优势模式间叶形结构相关性状差异性比较的报道。本研究以三角形杂种优势代表自交系 Y 46、Y 107 和 MON 2 为材料,构建六个高代回交群体($BC_1F_{7:8}$),利用其叶长、叶宽和叶夹角等性状的中亲优势和超亲优势表现情况来比较杂种优势强弱,明确玉米理想株型的杂种优势模式,为下一步高产玉米新品种选育提供理论依据。

1 材料与方法

1.1 试验材料

以热带种质 Y 46(来源于 Suwan 1 群)、温带种质 Y 107(来源于 Reid 群)和亚热带种质 MON 2(来源于非 Reid 群)等玉米自交系为试验材料。将各优良自交系相互杂交组配 3 个 F_1 杂交群体,继而连续自交形成 3 个 RIL-$F_{7:8}$ 群体。分别从 Y 46 与 Y 107 组配的 RIL-$F_{7:8}$ 群体中选择 253 个和 150 个 RIL 系与双亲回交组配,得到两个高代回交群体($BC_1F_{7:8}$)Y 46×Y 107/Y 46(正交)和 Y 46×Y 107/Y 107(反交)。分别从 Y 46 与 MON 2 组配的 RIL-$F_{7:8}$ 群体中选择 209 个和 124 个 RIL 系与双亲回交组配,得到两个高代回交群体($BC_1F_{7:8}$)Y 46×MON 2/Y 46(正交)和 Y 46×MON 2/MON 2(反交)。分别从 Y 107 与 MON 2 组配的 RIL-$F_{7:8}$ 群体中选择 197 个和 159 个 RIL 系与双亲回交组配,得到两个高代回交群体($BC_1F_{7:8}$)Y 107×MON 2/Y 107(正交)和 Y 107×MON 2/MON 2(反交)。

1.2 试验方法与统计分析

将六个高代回交群体分别种植于云南省昆明市(25°02′N,120°45′E;海拔 1 960 m)和砚山县(23°19′N,104°45′E;海拔 1 520 m)。试验采用随机区组设计,设置 2 次重复。每个小区长 2.5 m,株距 25 cm,密集种植 10 株,施肥、除草、除虫按田间标准管理方法进行。待玉米花期结束后 10 d 从每行中连续选择 5 株,

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并参照 Zhao 等[15]的方法测定其穗三叶的叶长(叶基部至叶顶端的长度)、叶宽(叶片中部展开的宽度)和叶夹角(叶片与茎秆的锐角角度)等 3 个叶形结构相关性状。

利用 Excel、SPSS 统计软件对叶形结构相关性状数据进行基本统计量和方差分析。中亲优势和超亲优势计算公式[16]如下：

中亲优势(%)=[($BC_1F_{7:8}$－MP)/MP]×100%；

超亲优势(%)=[($BC_1F_{7:8}$－HP)/HP]×100%，

式中，$BC_1F_{7:8}$、MP 和 HP 分别代表高代回交群体、双亲和高值亲本的叶形结构相关性状的平均值。

2　结果与分析

2.1　高代回交群体叶形结构相关性状的差异性比较

在两个不同环境条件下，对 Y 46、Y 107 和 MON 2 组配的六个高代回交群体的叶形结构相关性状进行比较，结果(表 1)表明，昆明点各群体的叶长、叶宽和叶夹角等 3 个性状平均值均高于砚山点，而砚山点各性状的变异系数均高于昆明点，说明环境对玉米叶形结构相关性状有显著影响。在昆明点，Y 46×Y 107/Y 107 群体的叶长、叶宽和叶夹角最大，分别为 73.6 cm、9.4 cm 和 30.4°，正反交组合间差异较小，砚山点的结果趋势与昆明点基本一致。Y 46×MON 2/MON 2 群体的叶长、叶宽和叶夹角最小，分别为 63.9 cm、9.2 cm 和 27.4°，正反交组合间差异较大，砚山点的结果与昆明点基本相同，说明不同群体间叶长、叶宽和叶夹角等差异显著，可通过遗传育种方法改良玉米叶形结构相关性状。进一步研究发现，六个高代回交群体叶形结构相关性状的偏度和峰度均基本介于－1.0～1.0 之间，符合正态分布，下一步可结合基因型数据进行玉米叶形结构相关性状的 QTL 定位分析。

2.2　高代回交群体叶形结构相关性状的方差分析

2.2.1　不同试点叶形结构相关性状的方差分析

利用方差分析比较不同试点对六个高代回交群体叶形结构相关性状的影响，结果(表 2)表明，在两试点中，叶长和叶宽的差异均达极显著水平，除了 Y 46×MON 2/MON 2 和 Y 107×MON 2/Y 107 群体叶夹角差异不显著外，其他群体叶夹角差异均达极显著水平。说明在 3 个叶形结构相关性状中，环境对叶长和叶宽的影响最大，而叶夹角则因材料而异。因此，在玉米理想株型选择中，叶夹角的遗传改良可能较叶长和叶宽更有效。

表 1　高代回交群体叶形结构相关性状的基本统计量

Table 1　The basic stastics volume of relative traits for leaf morphological traits among $BC_1F_{7:8}$ populations

群体	参数	昆明			砚山		
		叶长(cm)	叶宽(cm)	叶夹角(°)	叶长(cm)	叶宽(cm)	叶夹角(°)
1	最大值	85.9	11.4	38.6	86.4	17.6	49.0
	最小值	59.0	6.9	24.1	36.3	4.6	18.2
	均值±标准误	73.1±0.2	9.0±0.1	29.7±0.1	60.2±0.3	7.7±0.1	28.5±0.2
	变异系数(%)	6.2	7.8	7.7	11.6	15.6	17.2
2	最大值	87.7	11.1	38.1	78.9	12.3	61.0
	最小值	59.3	6.8	23.8	36.8	4.8	17.5
	均值±标准误	73.6±0.3	9.4±0.1	30.4±0.1	61.4±0.5	8.2±0.1	27.4±0.4
	变异系数(%)	6.7	8.5	8.6	12.5	13.4	21.2
3	最大值	87.5	12.1	36.9	74.2	19.2	55.9
	最小值	54.9	6.5	15.9	31.4	5.6	14.7
	均值±标准误	71.4±0.3	9.7±0.1	26.0±0.2	57.0±0.3	8.4±0.1	27.7±0.3
	变异系数(%)	9.2	7.2	12.7	12.6	13.1	22.7
4	最大值	124.3	16.9	39.1	78.5	12.7	58.8
	最小值	50.0	6.6	19.9	32.8	4.6	15.0
	均值±标准误	63.9±0.5	9.2±0.1	27.4±0.2	53.9±0.5	7.5±0.1	27.3±0.5
	变异系数(%)	12.2	13.0	10.2	14.7	13.3	24.9
5	最大值	78.7	12.0	38.6	76.9	12.1	42.0
	最小值	52	7.6	21.5	33.8	5.0	17.4
	均值±标准误	68.2±0.2	9.7±0.1	28.3±0.1	53.3±0.3	7.8±0.1	28.0±0.2
	变异系数(%)	6.0	7.1	7.8	12.2	11.5	15.0
6	最大值	85.7	16.4	35.6	83.0	11.1	41.2
	最小值	54.7	7.3	22.4	43.1	6.4	17.3
	均值±标准误	69.5±0.2	9.8±0.1	29.0±0.1	61.0±0.3	8.6±0.1	27.8±0.2
	变异系数(%)	6.0	8.2	9.0	8.9	8.1	14.4

注：群体 1：Y 46×Y 107/Y 46；群体 2：Y 46×Y 107/Y 107；群体 3：Y 46×MON 2/Y 46；群体 4：Y 46×MON 2/MON 2；群体 5：Y 107×MON 2/Y 107；群体 6：Y 107×MON 2/MON 2。下同。

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表 2 不同试点间叶形结构相关性状的方差分析
Table 2 ANOVA analysis of leaf morphological traits of different trial sites

群体	参数	叶长	叶宽	叶夹角
1	平方和	42 095	425	387
	均方	42 095	425	387
	自由度	1	1	1
	误差	35	1	15
	F	1 213**	427**	26**
2	平方和	19 220	289	2 958
	均方	19 220	289	2 958
	自由度	1	1	1
	误差	43	4	160
	F	452**	76**	18**
3	平方和	44 955	366	666
	均方	44 955	366	666
	自由度	1	1	1
	误差	48.0	0.8	25.0
	F	944**	432**	27**
4	平方和	11 426	363	3
	均方	11 426	363	3
	自由度	1	1	1
	误差	62.0	1.2	26.0
	F	184.0**	292.0**	0.1
5	平方和	42 405	751	15
	均方	42 405	751	15
	自由度	1	1	1
	误差	30.0	0.6	11.0
	F	492**	347**	20**
6	平方和	11 456	214	229
	均方	11 456	214	229
	自由度	1	1	1
	误差	23.0	0.6	11.0
	F	492**	347**	20**

注:“**”,“*”分别表示 0.01,0.05 显著水平。下同。

2.2.2 重复间叶形结构相关性状的方差分析

对六个高代回交群体不同重复间的叶长、叶宽和叶夹角等叶形结构相关性状进行方差分析(表 3)。由表 3 可知,除了 Y 107×MON 2/Y 107 群体外,其他群体大部分性状在不同重复间差异不显著。Y 46×Y 107/Y 46 和 Y 46×MON 2/Y 46 群体除叶长差异达极显著水平外,叶宽和叶夹角差异均不显著;Y 46×MON 2/MON 2 和 Y 107×MON 2/MON 2 群体除叶夹角差异达极显著水平外,叶长和叶宽差异均不显著;Y 46×Y 107/Y 107 群体在不同重复间差异均不显著。大部分高代回交群体的叶形结构相关性状在不同重复间差异不显著,说明试验地肥力均匀;后续叶形结构相关性状的杂种优势研究取两重复间的平均值进行分析。

2.2.3 高代回交群体内叶形结构相关性状的方差分析

对六个高代回交群体内重组系间叶形结构相关性状进行方差分析,结果(表 4)表明,在六个高代回交群体内,Y 107×MON 2/MON 2 群体所有叶形结构相关性状差异达极显著水平,而 Y 46×Y 107/Y 107 群体的所有性状差异均未达显著水平,说明 Y 107×MON 2/MON 2 群体的遗传差异较大,Y 46×Y 107/Y 107 群体的遗传差异较小。Y 46×Y 107/Y 46 群体的叶宽和叶夹角、Y 46×MON 2/Y 46 群体的叶夹角、Y 46×MON 2/MON 2 群体的叶长和叶夹角差异均达极显著水平。Y 46×Y 107/Y 46 群体的叶长、Y 46×MON 2/Y 46 群体的叶长和叶宽以及 Y 107×MON 2/Y 107 群体的叶夹角差异均达显著水平。Y 46×MON 2/MON 2 群体的叶宽以及 Y 107×MON 2/Y 107群体的叶长和叶宽差异不显著。说明这些重组系间叶形结构相关性状的遗传差异较大,进一步研究发现,差异最大的是叶夹角,说明通过育种选择较小的叶夹角是改良玉米株型的重要途径。这也可能是叶夹角成为近年来高密度玉米遗传研究热点的重要原因之一。

表 3 重复间叶形结构相关性状的方差分析
Table 3 ANOVA analysis of leaf morphological traits in among repeats

群体	参数	叶长	叶宽	叶夹角
1	平方和	1 645	5.5	36
	均方	1 645	5.5	36
	自由度	1	1	1
	误差	74	74	15
	F	22**	4.0	2.4
2	平方和	508	6	534
	均方	508	6	534
	自由度	1	1	1
	误差	77	4	165
	F	7	1	3
3	平方和	1 809	7.8	110
	均方	1 809	7.8	110
	自由度	1	1	1
	误差	97	1	25
	F	19.0**	6.2	4.3
4	平方和	508	6	534
	均方	508	6	534
	自由度	1	1	1
	误差	77	4	165
	F	7	1	3
5	平方和	3 425	42	504
	均方	3 425	42	504
	自由度	1	1	1
	误差	81	1.6	11
	F	424.0**	26.7**	47.4**
6	平方和	508	6	534
	均方	508	6	534
	自由度	1	1	1
	误差	77	4	165
	F	7	1	3

综上所述,这六个高代回交群体在不同试点中,其叶形结构相关性状差异均达极显著水平,而在不同重复间差异则不显著,群体内重组系间大部分叶形结构相关性状差异达显著水平。说明玉米的叶形结构相关性状主要由基因型决定,但同时受环境的影响。因此,

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表 4　高代回交群体叶形结构相关性状的方差分析
Table 4　ANOVA analysis of leaf morphological structure traits among $BC_1F_{7:8}$ populations

群体	参数	叶长	叶宽	叶夹角
1	平方和	17 873	440	6 284
	均方	67	440	23
	自由度	268	268	268
	误差	79	1	12
	F	1.2*	1.6**	1.9**
2	平方和	11 097	519	19 666
	均方	71	3	125
	自由度	157	157	157
	误差	81	5	182
	F	0.9	0.7	0.7
3	平方和	26 458	344	12 958
	均方	115.0	1.5	56.0
	自由度	231	231	231
	误差	93	1	15
	F	1.2*	1.3*	3.8**
4	平方和	17 230	307	5 705
	均方	129	2	43
	自由度	134	134	134
	误差	69	2	19
	F	1.9**	1.2	2.3**
5	平方和	8 068	183	2 762
	均方	39.0	0.9	13.0
	自由度	207	207	207
	误差	102	1.9	11
	F	0.4	0.5	1.3*
6	平方和	7 883	167	2 522
	均方	47.5	1.0	15.0
	自由度	166	166	166
	误差	39.0	0.9	10.0
	F	1.2**	1.1**	1.5**

在玉米育种进程中建议将这些叶形结构相关性状，特别是叶夹角作为重点改良对象，进而从生理学角度提高玉米产量潜力。

2.3　高代回交群体叶形结构相关性状的杂种优势研究

利用杂种优势表现筛选强优势组合是获得理想株型及实现经济效益的重要途径之一。由表 5 可以看出，在昆明点 Y 46 与 Y 107组配的正交群体中，叶长、叶宽和叶夹角的中亲优势、超亲优势均为正向杂种优势效应，其反交群体的叶长、叶宽和叶夹角的中亲优势也表现为正向优势效应。砚山点正交群体的叶长和叶宽，以及其反交群体的叶长、叶宽和叶夹角的中亲优势都表现为正向杂种优势效应，超亲优势均为负向杂种优势效应。在两试点中，Y 46 与 MON 2 组配的高代回交群体均表现出负向杂种优势效应。Y 107 与 MON 2 组配的高代回交群体，昆明点均表现为正向杂种优势效应，砚山点除了叶夹角的杂种优势呈负向优势效应外，叶长和叶宽都表现为正向杂种优势效应。说明大部分群体的叶形结构相关性状表现为正向杂种优势效应，但由于研究群体为 RIL 重组系组配的回交群体，杂种优势减弱。因此，大部分性状呈负向超亲优势。

在昆明点 Y 107 与 MON 2 组配的反交群体的叶长、叶宽和叶夹角的中亲优势分别为 13.3%、12.8%和 8.6%，均大于正交群体，超亲优势均小于正交群体。总体而言，反交群体的杂种优势比正交强，砚山点与昆明点杂种优势分析结果基本相同。Y 46 与 MON 2 组配的正交群体的叶宽和叶夹角的中亲优势分别为 −2.0%和 −8.3%，超亲优势分别为 −7.5%和 −9.1%，均大于反交群体，而叶长的中亲优势和超亲优势均小于反交群体，由此可表明在该杂交组合中正交群体的杂种优势强于反交群体，两试点杂种优势结果趋势一致。Y 46 与Y 107组配的正交群体的叶长、叶宽和叶夹角的中亲优势分别为 11.0%、13.5%和 19.8%，超亲优势分别为1.3%、2.5%和 2.7%，显著高于其反交群体的叶长、叶宽和叶夹角的中亲优势和超亲优势，表明在 Y 46 与Y 107组配的高代回交群体中，正交群体的杂种优势大于反交，砚山点与昆明点杂种优势结果趋势相反，其中 Y 46×Y 107/Y 107 群体的杂种优势均小于 Y 107 与 MON 2 正反交群体。因此，

表 5　高代回交群体叶形结构相关性状的杂种优势表现情况
Table 5　Heterosis performance of leaf morphological traits in six $BC_1F_{7:8}$ populations

单位：%

群体	杂种优势	昆明			砚山		
		叶长	叶宽	叶夹角	叶长	叶宽	叶夹角
1	中亲优势	11.0	13.5	19.8	13.6	3.1	−4.0
	超亲优势	1.3	2.5	2.7	−0.8	−8.8	−12.3
2	中亲优势	6.5	10.1	6.9	11.4	7.3	3.6
	超亲优势	−5.1	−5.7	−3.3	−9.4	−9.8	−9.6
3	中亲优势	−7.5	−2.0	−8.3	−0.5	−9.2	−10.9
	超亲优势	−21.1	−7.5	−9.1	−7.5	−16.0	−12.6
4	中亲优势	−6.3	−9.6	−10.3	−9.7	−11.2	−15.5
	超亲优势	−9.7	−19.0	−13.9	−11.4	−21.3	−17.6
5	中亲优势	9.2	7.0	5.8	16.7	3.2	−2.8
	超亲优势	5.8	6.3	2.8	6.0	2.9	−9.0
6	中亲优势	13.3	12.8	8.6	26.7	0.9	−2.9
	超亲优势	1.1	4.6	0.4	20.7	0.5	−7.8

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Y 46 与Y 107群体杂种优势介于 Y 107 与 MON 2 群体和 Y 46 与MON 2群体二者之间。

基于叶长、叶宽和叶夹角等叶形结构相关性状的杂种优势分析,各群体杂种优势从强到弱的总体趋势为:Y 107 与 MON 2 正反交群体>Y 46 与Y 107正反交群体>Y 46 与 MON 2 正反交群体,说明在三角形杂种优势模式中 Reid×非 Reid 杂种优势最强,Suwan 1×Reid 杂种优势次之,Suwan 1×非 Reid 杂种优势较弱。

3 讨 论

玉米叶形结构相关性状是衡量玉米株型紧凑程度的重要形态指标。合理的叶长、叶宽和叶夹角均有利于改善叶片的空间排列和几何构型,直接影响光和 CO_2 在冠层内的分布及群体的光能利用率,进而影响植株生长发育和生理特性。研究表明,叶形结构由多个微效基因共同调控的数量遗传性状,受环境影响较大。本研究中,Y 46 与 MON 2 正反交群体间叶长、叶宽和叶夹角的遗传差异以及变异系数较大,表明正反交对这 3 个性状有一定的影响,且遗传改良潜力较大,进一步证实在杂交育种过程中,可以通过遗传学方法增加叶长和叶宽,减小叶夹角,进而改良玉米株型。此外,玉米叶长、叶宽及叶夹角等叶形结构相关性状遗传特点因材料和环境差异明显不同[17-19],本研究针对不同温热玉米自交系组配的高代回交群体进行分析,发现其叶长、叶宽和叶夹角主要受基因调控,同时也受环境条件的影响。

正反交组合性状的遗传差异主要由组配杂交组合的两个亲本自交系所决定。Dermail 等[20]发现,正反交影响着杂种优势效应,可以提高玉米产量。郭海林等[21]发现,结缕草的高度、叶长、叶宽、节间直径受母体遗传效应的影响。朱娇等[22]发现,大红花蝴蝶兰后代的花色、花径与母本相似。上述研究表明,后代表型性状受母性遗传效应的影响。本研究表明,正反交群体间叶长、叶宽及叶夹角性状存在显著差异,尤其是Y 46与 Y 107 正反交群体性状杂种优势差异较大。因此,在杂交育种中,正确选择母本对杂交组合的叶形结构相关性状有重要决定作用,进而影响玉米产量。

杂种优势大小取决于亲本遗传组成和遗传差异,遗传差异越大杂种优势越强,目前大部分研究主要集中于根据自交系产量性状划分杂种优势群[23-24]。Cai 等[25]对玉米光合作用相关性状以及关键酶进行配合力测定,发现 Suwan 1 与 Reid 和非 Reid 间的光合作用相关性状的杂种优势有显著差异,目前还未见对不同杂种优势模式间群体叶形结构相关性状的杂种优势研究的相关报道。本研究首次利用六个高代回交群体叶长、叶宽及叶夹角的中亲优势、超亲优势来分析 Suwan 1、Reid 和非 Reid 三个类群间的杂种优势,发现 Reid×非 Reid 杂种优势最强,Suwan 1×Reid 次之,Suwan 1×非 Reid 较弱。本研究显示,亚热带与温带种质间遗传组成及背景差异引起 Reid×非 Reid 杂种模式叶形结构相关性状呈正向杂种优势。Suwan 1×非 Reid 杂种模式的叶形结构相关性状均表现出负向杂种优势,可能是热带、亚热带种质间存在的基因交流程度不同导致。因此,利用玉米叶形结构相关性状以及其生理指标的杂种优势分析确定杂种优势模式,从生理学角度来提高玉米产量可以打破以往通过产量相关性状杂种优势来划分强优势组合的常规性,有效地增强了目标性状改良的针对性,为玉米新品种选育提供了理论基础。

4 结 论

通过对 Y 46(Suwan 1 群)、Y 107(Reid 群)和 MON 2(非 Reid 群)三个自交系所组配的六个高代回交群体的叶长、叶宽和叶夹角等叶形结构相关性状进行遗传差异和杂种优势分析,发现在三角形杂种优势模式中,Reid×非 Reid 杂种优势较强,Suwan 1×Reid 杂种优势次之,Suwan 1×非 Reid 杂种优势较弱。因此,可优先选用 Reid×非 Reid 作为培育理想株型新品种的重要杂种优势模式。

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 DOI:10.3969/j.issn.1006-8082.2020.04.009 中国稻米 2020,26(4):40-43

不同海拔地区多年生稻稻米品质分析

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摘 要:利用长雄野生稻(*Oryza Longistaminata*)地下茎无性繁殖特性培育多年生稻已获成功。为明确多年生稻在不同海拔地区种植的稻米品质的差异,于2018年在海拔550 m(景洪)、955 m(孟连)和1 250 m(勐海)种植3个多年生稻品种(PR23、PR25和PR107),收获后进行稻米品质测定和分析。结果表明,PR23在景洪和孟连的整精米率符合国家标准优质稻谷1级和2级,在勐海的垩白度达到国家标准优质稻谷3级,在景洪、孟连和勐海的直链淀粉含量均符合国家标准优质稻谷1级;PR25在景洪和孟连的整精米率达到国家标准优质稻谷2级和1级,在勐海的垩白度达到国家标准优质稻谷3级,在景洪、孟连和勐海的直链淀粉含量均符合国家标准优质稻谷1级;PR107在景洪和孟连的整精米率符合国家标准优质稻谷2级和1级,在景洪、孟连和勐海的垩白度符合国家标准优质稻谷2级、2级和1级,在景洪、孟连和勐海的直链淀粉含量均符合国家标准优质稻谷1级。

关键词:多年生稻;稻米品质;海拔

中图分类号:S511 **文献标识码**:A **文章编号**:1006-8082(2020)04-0040-04

水稻(*Oryza sativa*. L)是全球最重要的粮食作物之一[1]。但一年生水稻每年都需买种、育秧、移栽、犁田、耙田等生产环节,种植成本高、劳动强度大,且长期翻耕犁田导致水土流失严重,生态环境恶化[2]。近年来,人们日益关注土地利用率高、环境友好,同时有助于提高粮食产量的多年生作物的培育及应用[3-5]。因此,部分研究人员提出发展多年生稻,在水稻生产中实现节本高效、省时省工的同时保护农田环境的设想[6-7]。多年生稻是指种植一次可以连续收获多年的稻作生产方式,从第2年起水稻生产不再需要买种、育秧、犁田、耙田等环节,极大地减少了劳动力投入,降低了劳动强度,是一项轻简化的稻作生产技术[8-9]。近年来,云南大学利用长雄野生稻地下茎无性繁殖特性培育多年生稻获得成功[8-10],多年生稻品种PR23(多年生稻23)已经通过审定(审定编号:滇审稻2018033号)。最近又培育了多年生稻PR25(多年生稻25)和PR107(云大107),这些品种外观品质、蒸煮品质和加工品质较好,受到广大农民和稻米加工者的喜爱[10]。然而,稻米品质受品种遗传特性和环境条件影响较大[7],不同海拔地区与稻米品质有密切关系,其中受气温和土壤肥力影响较大[11]。例如,灌浆结实期高温会导致稻米垩白度增加、透明度变差、整精米率下降、蒸煮食味品质变劣等[12-16]。分析多年生稻在不同海拔地区种植的稻米品质,对其适宜生产区域划分,加快生产应用具有重要意义。

1 材料与方法

1.1 试验材料

本试验以PR23、PR25和PR107等3个多年生稻品种为材料。PR23和PR25系由“RD23/长雄野生稻”杂交组合中选育而来,PR107系由“滇瑞449//RD23/长雄野生稻”杂交组合中选育。

1.2 试验设计

试验于2018年在云南省3个不同海拔地区进行(景洪,海拔550 m;孟连,海拔955 m;勐海,海拔1 250 m)。PR23、PR25和PR107分别于7月23日、7月4日、6月28日播种,3叶1心期移栽,各试验田肥力水平一致,种植面积为667 m^2。田间栽培管理技术均相同:(1)栽插规格20 cm×20 cm,每hm^2插0.11万丛,每丛1~2苗;(2)N:P_2O_5:K_2O=2:1:2,其中,氮肥按基肥:分蘖肥:穗肥:粒肥=5:2:2:1分4次施;磷肥全部基施;钾肥按基肥:穗肥=1:1分2次施。氮磷钾肥用量及种类:纯N 195 kg/hm^2、P_2O_5 97.5 kg/ hm^2、K_2O 195 kg/hm^2,氮肥、磷肥、钾肥分别为尿素(46%)、过磷酸钙(12%)和氯化钾(60%)。(3)水分管理参照张琳[17]的方法,即“浅水分蘖,够苗晒田,有水壮苞,干湿交替壮籽,后期晒田活根促苗”。

收稿日期:2020-01-15

基金项目:云南省科技计划重大专项(2019ZG013);全国农业专业学位研究生教育指导委员会重点课题(2019-NYZD-25-5);云南省科技计划青年项目(2018FD006)

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表 1 不同海拔地区多年生稻 PR23 稻米品质比较及变异系数

海拔	加工品质			外观品质			蒸煮品质	
(m)	出糙率(%)	精米率(%)	整精米率(%)	粒长(mm)	垩白度(%)	垩白粒率(%)	直链淀粉含量(%)	胶稠度(mm)
550(景洪)	81.6	73.0	67.7	5.0	8.3	40.0	13.9	75.0
955(孟连)	80.5	73.8	66.9	4.9	12.3	63.0	13.5	70.0
1 250(勐海)	82.0	72.6	35.5	4.8	5.2	34.0	13.2	75.0
均值	81.37	73.13	56.70	4.90	8.60	45.67	13.53	73.33
标准差	0.78	0.61	18.36	0.10	3.56	15.31	0.35	2.89
变异系数(%)	0.95	0.84	32.39	2.04	41.39	33.52	2.59	3.94

表 2 不同海拔地区多年生稻 PR25 稻米品质比较及变异系数

海拔	加工品质			外观品质			蒸煮品质	
(m)	出糙率(%)	精米率(%)	整精米率(%)	粒长(mm)	垩白度(%)	垩白粒率(%)	直链淀粉含量(%)	胶稠度(mm)
550(景洪)	81.7	74.5	61.0	5.0	8.5	32.0	13.2	73.0
955(孟连)	81.4	72.7	67.6	4.9	10.6	58.0	13.2	70.0
1 250(勐海)	82.3	74.2	40.2	4.8	5.6	40.0	14.5	80.0
均值	81.80	73.80	56.27	4.90	8.23	43.33	13.63	74.33
标准差	0.46	0.96	14.30	0.10	2.51	13.32	0.75	5.13
变异系数(%)	0.56	1.31	25.41	2.04	30.49	30.73	5.51	6.90

1.3 测定项目及方法

成熟时收取适量稻谷置于网袋中晒干，室温储藏 3 个月后送至农业农村部(武汉)食品质量监督检验测试中心检测,测定稻米加工品质(糙米率、精米率、整精米率)、外观品质(垩白粒率、垩白度、粒长等)和蒸煮品质(直链淀粉含量、胶稠度)等指标。

1.4 数据分析

试验数据采用 Excel 进行统计分析。

2 结果与分析

2.1 不同海拔地区 PR23 稻米品质

从表 1 可见,PR23 的出糙率随海拔升高先降低后增加,在海拔 1 250 m 处(勐海)最高为 82.0%;精米率随海拔升高呈先增加后降低的趋势，在海拔 955 m 处(孟连)最高为 73.8%;整精米率随海拔升高而降低,在海拔 550 m 处(景洪)最高为 67.7%。在海拔 550 m(景洪)至海拔 1 250 m(勐海)范围内,PR23 的出糙率和精米率变异系数较小,说明受海拔高度的影响较小;整精米率变异系数较大,说明其受海拔高度的影响较大。

从表 1 可见,PR23 粒长随海拔升高而下降，但变幅和变异系数均较小,说明受海拔高度影响小;垩白度和垩白粒率随海拔的升高先增加后降低,在海拔 1250 m 处(勐海)最低,分别为 5.2%和 34.0%;在海拔 550 m(景洪)至海拔 1 250 m(勐海)间,垩白度和垩白粒率变异系数较大,受海拔高度影响大。

从表 1 可见,PR23 直链淀粉含量随海拔升高而降低,在海拔 1 250 m 处(勐海)最低为 13.2%;胶稠度随海拔升高先降低后升高,在海拔 550 m 处(景洪)和海拔 1 250 m 处(勐海)均为 75.0 mm;在海拔 550 m(景洪)至海拔 1 250 m(勐海)间,PR23 直链淀粉含量和胶稠度变异系数较小,受海拔高度的影响小 。

综合来看，PR23 在景洪(海拔 550 m)和孟连(海拔 955 m) 的整精米率达到国家标准优质稻谷(GB/T 17891-2017,下简称“国优”)1 级和 2 级,在勐海(1 250 m)的垩白度达到国优 3 级,在三地的直链淀粉含量均符合国优 1 级。

2.2 不同海拔地区 PR25 稻米品质

从表 2 可见,PR25 的出糙率随海拔升高先降低后增加,在海拔 1 250 m 处(勐海)最高,为 82.3%;精米率随海拔升高先降低后增加,在海拔 550 m 处(景洪)最高,为 74.5%;整精米率随海拔升高先增加后降低,在海拔 955 m 处(孟连)最高为 67.6%。在海拔 550 m(景洪)至海拔 1 250 m(勐海)间,PR25 的出糙率和精米率变异系数较小,受海拔高度影响小;整精米率变异系数较大,受海拔高度的影响大。

从表 2 可见,PR25 粒长随海拔升高而下降，且变异系数较小,说明受海拔影响小;垩白度和垩白粒率均随着海拔的升高先增加后降低，分别在海拔 1 250 m(勐海)和海拔 550 m(景洪)较低,平均值分别为 7.05%和 36.00%;但垩白度和垩白粒率在不同海拔地区的变异系数大,受海拔高度影响大。

从表 2 可见,PR25 直链淀粉含量随海拔的升高而增加,在海拔 550 m 处(景洪)和海拔 955 m 处(孟连)较低为 13.20%； 胶稠度随海拔的升高先降低后增加,

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表 3 不同海拔地区多年生稻 PR107 稻米品质比较及变异系数

海拔	加工品质			外观品质			蒸煮品质	
(m)	出糙率(%)	精米率(%)	整精米率(%)	粒长(mm)	垩白度(%)	垩白粒率(%)	直链淀粉含量(%)	胶稠度(mm)
550(景洪)	77.5	68.4	55.8	7.6	4.9	15.0	13.8	76.0
955(孟连)	79.3	70.0	56.8	7.3	4.8	28.0	14.9	65.0
1 250(勐海)	77.9	69.3	36.6	7.5	1.0	5.0	13.7	75.0
均值	78.23	69.23	49.73	7.47	3.57	16.00	14.13	72.00
标准差	0.95	0.80	11.38	0.15	2.22	11.53	0.67	6.08
变异系数(%)	1.21	1.16	22.89	2.05	62.34	72.08	4.71	8.45

表 4 多年生稻在不同海拔地区稻米品质表现分级

海拔	整精米率(%)			垩白度(%)			直链淀粉含量(%)		
(m)	PR23	PR25	PR107	PR23	PR25	PR107	PR23	PR25	PR107
550(景洪)	一级	二级	二级	普通	普通	二级	一级	一级	一级
955(孟连)	二级	一级	一级	普通	普通	二级	一级	一级	一级
1 250(勐海)	普通	普通	普通	三级	三级	一级	一级	一级	一级

在海拔 550 m 处(景洪)和 1 250 m 处(勐海)较高;在海拔 550 m(景洪)至海拔 1 250 m(勐海)区域间,直链淀粉含量和胶稠度变异系数较小,说明蒸煮品质受海拔高度影响较小。

综合来看,在海拔 550 m 至海拔 1 250 m 之间,PR25 在景洪(550 m)和孟连(955 m)整精米率符合国优 2 级和 1 级,在勐海(1 250 m)垩白度符合国优 3 级,在三地的直链淀粉含量均符合国优 1 级。

2.3 不同海拔地区 PR107 稻米品质

从表 3 可见,PR107 的出糙率、精米率和整精米率均随海拔升高先增加后降低,在海拔 955 m 处(孟连)最高,分别为 79.3%、70.0%和 56.8%;在海拔 550 m(景洪)至海拔 1 250 m(勐海)间,PR107 出糙率和精米率的变异系数较小,整精米率变异系数较大,说明出糙率和精米率受海拔影响较小,而整精米率受海拔高度影响较大。

从表 3 可见,PR107 粒长随海拔升高先降低后增加,且其变化幅度和变异系数较小,说明 PR107 粒长受海拔高度影响较小;随海拔的升高 PR107 垩白度和垩白粒率先增加后降低,在海拔 1 250 m 处(勐海)最低分别为 1.00%和 5.00%;垩白度和垩白粒率变异系数较大,说明外观品质不稳定,受海拔高度影响较大。

从表 3 可见,PR107 直链淀粉含量随海拔的升高先增加后下降,在海拔 1 250 m 处(勐海)最低为 13.7%;胶稠度随海拔的升高先降低后增加,在海拔 550 m 处(景洪)和 1 250 m 处(勐海)较高。

综合来看,PR107 稻米加工品质和蒸煮品质表现较为稳定,外观品质在海拔 1 250 m 处(勐海)表现较优。PR107 在景洪(550 m)和孟连(955 m)的整精米率符合国优 2 级和 1 级,在景洪(550 m)、孟连(955 m)和勐海(1 250 m)的垩白度符合国优 2 级、2 级和 1 级,直链淀粉含量均符合国优1 级。

3 讨论与结论

不同海拔差异是影响稻米品质的重要因素之一。在加工(碾磨)品质方面,前人研究表明,稻米的出糙率和精米率受海拔高度影响较小[18–19],而整精米率受海拔影响较大,随海拔升高,整精米率明显增加[19–20]。这与本研究结果一致。本研究表明,在海拔 550~1 250 m 区域,多年生稻稻米的出糙率和精米率受海拔影响较小,但不同海拔区域对整精米率影响较大,在海拔 550~955 m 的低海拔地区,整精米率较高,均达到了国优 2 级以上(表 4),说明在低海拔地区(550 ~955 m)种植多年生稻其稻米加工品质更优。

在外观品质方面,前人研究表明,海拔高度对稻米垩白的影响较大,随海拔升高垩白粒率明显下降[18,21–22]。本研究表明,在海拔 550~1 250 m 区域,随海拔升高多年生稻稻米垩白度和垩白粒率呈先增加后降低的趋势。这与张现伟等[23]的研究结果一致。本研究中,不同海拔区域多年生稻稻米垩白度和垩白粒率变异系数较大,说明多年生稻外观品质受海拔高度影响大,但在中海拔 1 250 m 区域多年生稻稻米垩白度达到了国优 3 级以上(表 4)。说明多年生稻在此区域种植外观品质更优。

在蒸煮(食味)品质方面,苏振喜等[19]认为,直链淀粉含量受海拔的影响较小,其差异主要来自品种间的差异,而海拔梯度对胶稠度的影响较大。黄宗洪等[21]研究表明,杂交稻两优 363 的直链淀粉含量有随海拔升高而增加的趋势。本研究结果与苏振喜等[19]研究结果一致,不同海拔区域(550~1 250 m)对多年生稻稻米直

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链淀粉含量影响较小；胶稠度随海拔的升高先降低后增加,但不同海拔区域胶稠度的变异系数较小。结果表明,多年生稻稻米的蒸煮品质表现较为稳定,且直链淀粉含量在参试区域均达到国优1级(表4)。

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Quality Analysis of Perennial Rice in Different Altitude Regions

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Abstract: Perennial rice which is breeding by the clonal propagation characteristic via rhizome of *Oryza longistaminata* has been successful. In order to clarify the differences in perennial rice quality at different altitudes, 3 perennial rice varieties PR23, PR25 and PR107 were applied at 3 different altitudes of 550 m (Jinghong), 955 m (Menglian) and 1 250 m (Menghai) in 2018. The results showed that the head rice rate of PR23 in Jinghong and Menglian reached the first and second grade of national standard for high quality rice, respectively. But it's chalkiness degree in Menghai reached the third grade of national standard for high quality rice, and the amylose content in Jinghong, Menglian and Menghai met the first grade of national standard for high quality rice. The head rice rate of PR25 in Jinghong and Menglian reached the second and first grade of national standard for high-quality rice, respectively. It's chalkiness degree in Menghai reached the third grade of national standard for high quality rice, respectively, the amylose content in Jinghong, Menglian and Menghai met the first grade of national standard for high quality rice. The head rice rate of PR107 in Jinghong and Menglian reached the second and first grade of national standard for high quality rice, respectively. It's chalkiness degree in Jinghong, Menglian and Menghai met the second, second and first grade of national standard for high quality rice, respectively, and the amylose content in Jinghong, Menglian and Menghai met the first grade of national standard for high quality rice.

Key words: perennial rice; rice quality; altitude

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环境与植物激素对马铃薯块茎休眠与萌发的影响研究

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摘要：马铃薯是重要的经济作物，其块茎休眠期的长短一定程度上影响马铃薯的经济效益，而芽的萌发则是打破休眠的标志。目前，由于马铃薯块茎休眠及芽萌发机制尚不明确，严重阻碍马铃薯的种薯生产以及块茎的加工利用。因此，研究马铃薯块茎休眠和芽的萌发机制具有重要的经济价值。综述了植物激素和环境因素对马铃薯块茎休眠及芽萌发的影响，以期为马铃薯的贮藏保鲜和加工利用等提供参考。

关键词：马铃薯；块茎；休眠；萌发；环境因素；植物激素
DOI:10.19462/j.cnki.1671-895x.2022.07.031

马铃薯（*Solanum tuberosum* L.）是世界上最重要的粮食作物之一。因其特有的营养价值、经济价值、药用及保健价值和工业价值，对保障粮食生产安全和提高人民生活水平具有极其重要的作用。休眠是马铃薯块茎发育的特殊生理阶段，使块茎能够在不利条件下作为营养生殖器官保存，较长的休眠

基金项目：云南省自然科学基金（202101AT070434）
通信作者：邓纲

良品种广泛推广。

2.2.2 主观因素上 一是种质资源鉴定力度不够。由于缺乏精准的鉴定平台和种质资源研究项目支持，资源优势尚未转化为品种优势。二是先进育种技术欠缺。花生以常规育种为主，缺少高效的基因编辑、批量标记辅助选择和分子设计育种等现代定向育种技术。三是科研力量分散。品种选育以科研单位和高校为主，由于缺乏国家项目的统一支持，育种力量分散，单兵作战多。

3 下一步措施建议

3.1 加强技术创新攻关 在关键技术研发上开展重点攻关，加强花生重要性状功能基因克隆和专利保护，研发和建立高效的花生基因编辑技术，利用花生基因组学研究成果，加强花生分子设计育种研发，打造分子育种平台。聚焦育种方向，围绕高产早熟、高油、高油酸、耐盐碱、宜机化等目标，从国家和地方两个层面，针对普遍性和区域性问题，组织开展良种联合攻关。同时，加强抗逆、优质、适宜机械化检测平台建设，保障品种鉴定的可靠性。

3.2 严格品种登记管理 收紧品种登记，严把品种质量关，运用DNA指纹技术，加强同质化品种甄别，严格规范品种重要性状鉴定，分类细化品种特性用途，引领花生育种主流方向。同时分区域细化品种登记标准，提高品种入市门槛，倒逼行业聚焦靶心发力，促进突破性品种选育。

3.3 加强品种筛选与推广 推进政府部门、育种单位、种子企业、加工单位合作，促进优质专用品种的筛选与推广，构建全国花生品种展示示范网络，在不同生态区开展不同种植模式的优良品种及配套生产技术展示示范，组织各地开展优良品种高效生产擂台赛，创造品种和生产技术高产记录。大力推进订单化生产，推介和引导种植户优选品种和技术，促进优质高产品种应用。

3.4 推进品种商业化运作 搭建平台，建立机制，推进企业与科研单位联合，加大育种社会化投入，探索新品种选育与推广的商业化运作方式，推动形成商业化和公益性优势互补的花生种业体系。

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（收稿日期：2022-04-15）

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期有利于马铃薯的贮藏保鲜、加工利用，但同时也会造成马铃薯产量降低[1]。在马铃薯块茎的休眠期，芽的生长会受到明显抑制[2]。休眠时间的长短受到环境、生理和激素等影响[3]。而芽的萌发则标志着休眠期被打破，可用于相应的种薯生产等环节。如何做到人为控制马铃薯块茎休眠及芽萌发对马铃薯产业具有重要的经济价值，也是目前面临的一大挑战。

1 马铃薯块茎休眠及芽萌发的特征

马铃薯块茎达到生理成熟后，会进入很长一段时间的深度休眠状态。在此时种植马铃薯，即使是在理想的萌发条件下芽也不会萌发[4]。从生理学的角度来说，马铃薯块茎休眠期是指从匍匐茎顶端膨大开始到块茎发芽生长的一段时期，但人们习惯将块茎开始收获至块茎顶芽萌发结束的时期作为马铃薯块茎的休眠期[3]。处于休眠过程中的块茎仍保持着生命活动，维持着最基础的生理功能，只有经过一定的贮藏后休眠才会被解除，块茎才能进一步发芽生长[5]。休眠时期一般被划分为相对休眠、生理休眠和环境休眠。因各阶段间不存在明显的生理标志而导致休眠期不易被区分。对休眠时期的划分一般根据休眠是否可以被逆转来判断，相对休眠和环境休眠可受外界因子的影响被打破，而生理休眠则不会或很难被逆转[6]。

2 外界环境对马铃薯块茎休眠与萌发的影响

休眠时期的长短主要与品种的特性有关，同时也会受到环境、栽培措施和贮藏条件等因素的影响[3]。不同品种马铃薯的休眠期存在一定的差异，晚熟品种一般具有较短的休眠期，而早熟品种则相反。除此之外，休眠期时间还取决于马铃薯生长的土壤和气候等条件、块茎成熟度、贮藏条件以及块茎的完整度等。一般来说，栽培过程中适宜温度（15~21℃）范围内的高温、足够发育的水分和良好的土壤肥力会缩短块茎的生理休眠期；未成熟比成熟的块茎有着更长的休眠期[3]；在低温、潮湿条件下贮藏则有利于延长块茎的休眠期[4]。

2.1 温度对马铃薯块茎休眠和萌发的影响 一系列研究表明，马铃薯在收获后，块茎的休眠时间与贮藏温度有关。通常情况下，在持续高温条件下，休眠期会明显地缩短[4]。而随着学者们不断深入地研究，进一步发现不同的温度处理对马铃薯休眠期有显著的影响。Wurr等[7]通过分析3个马铃薯品种（Desiree、Vanessa、Home Guard）在不同贮藏温度（2~8℃、0~10℃、6~15℃）条件下休眠期的表现发现，马铃薯块茎在2~8℃条件下连续贮藏14d，块茎的萌发会受到明显抑制，而恢复到6~15℃时，休眠被打破，与对照组相比，休眠期缩短14d；而一直在10℃条件下贮藏的块茎则没有芽生长现象，同时揭示了经冷处理、常规温度条件下贮藏后，发芽的数量与品种密切相关。Van Ittersum等[8]对不同品种（Desiree、Diamant、Draga、Jaerla）的马铃薯进行热预处理，即在28℃条件下连续贮藏20d后恢复到18℃条件贮藏，与持续贮藏在18℃条件下相比，经热预处理的马铃薯块茎休眠期分别缩短了14d、29d、18d和15d；进一步分析发现，持续贮藏在28℃条件下也可以缩短马铃薯块茎的休眠期，分别缩短了49d、13d、48d和51d，与热预处理相比，Desiree、Draga、Jaerla品种块茎的休眠期明显缩短。

2.2 湿度与光照对马铃薯块茎休眠和萌发的影响 湿度在一定程度上会影响马铃薯块茎的休眠，关于湿度与马铃薯块茎休眠间的研究相对较少，主要是因为环境范围内湿度的变化对马铃薯块茎水分的影响不显著。然而，这种影响对块茎质量有重要的影响，尤其是新鲜的马铃薯[9]。同时高湿度有利于马铃薯块茎伤口的快速愈合，从而影响马铃薯的完整度，促进块茎芽的萌发，有利于打破休眠；相对湿度处于90%以下，可防止块茎重量下降过多；相对湿度处于38%~66%之间，块茎则开始发芽[10]。

有研究表明，短日照条件栽培的马铃薯比长日照条件栽培的马铃薯具有更短的休眠期[11]。在田间条件下，光周期对马铃薯块茎休眠的影响很难确定，所观察到的影响可能更多的与块茎形成时间或成熟度有关，而与休眠本身无关，通过对马铃薯（Desiree）处于休眠状态下的块茎进行不同的光照处理发现，光强对块茎休眠具有一定的影响。短日照条件下，与较低光强（5.3μmol/m^2·s PPF）的处理相比，最高光强（106μmol/m^2·s PPF）的处理则可以显著缩短马铃薯块茎的休眠期。同时发现块茎休眠期与光周期有关，与完全暴露在光照8h/d相比，完全黑暗处理条件下的马铃薯块茎休眠期明显缩短[6]。

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2.3 大气条件对马铃薯块茎休眠和萌发的影响

块茎休眠的打破和芽的萌发过程中伴随着高浓度的 CO_2 和 O_2 等条件，但两者之间具体的生理机制尚不明确[9]。Burton[12]研究发现马铃薯块茎处于1%~2%的 CO_2 含量下有利于打破休眠。后续的研究表明，随着块茎的老化，打破休眠和维持芽生长所需的 O_2 含量会显著增加（从2%增加到23%），同时提出了“最适厌氧可逆代谢”的生长抑制机制。Thornton[13]研究表明，马铃薯块茎在25℃条件下连续施用40%~60%的 CO_2 和20%的 O_2 3~7d，可有效打破休眠，同时还发现高浓度（20%~80%）的 O_2 能增强这种效果。Coleman等[14]发现对马铃薯块茎进行特定的 CO_2/O_2 气体混合物处理可缩短休眠期。其中有两种处理对所有马铃薯品种均适用，分别为低 O_2 含量（<10%）/10%~60% CO_2 含量以及高 CO_2 含量（60%）/O_2 含量（40%）。而最有效的方法则是对马铃薯的块茎进行20% CO_2 与40% O_2 或60% CO_2 与18%~20% O_2 处理，在此基础上引入乙烯可以进一步缩短马铃薯块茎的休眠期。

3 植物激素对马铃薯块茎休眠和萌发的影响

植物激素是影响马铃薯块茎休眠和芽萌发的重要因素，目前主要通过使用植物激素对块茎进行化学处理来提前解除休眠，但具体作用机制仍需进一步研究。前人研究表明，只有当一些特定的激素处于相对平衡状态，才可以促进或维持某一生理状态。在马铃薯块茎休眠过程中，植物激素会有明显的变化（表1），块茎休眠主要受生长促进剂和抑制剂的相对浓度调控，并提出了“抑制因子/促进因子平衡调节休眠”的学说来解释[3]。

表1 马铃薯块茎休眠过程中主要内源激素的含量变化

休眠时期	含量				
	IAA	GA	CTK	ABA	ETH
休眠初期	低水平	低水平	低水平	低水平	高水平
休眠深期	高水平	低水平	低水平	高水平	低水平
休眠释放期（萌发前）	低水平	高水平	高水平	低水平	低水平

IAA：植物生长素；GA：赤霉素；CTK：细胞分裂素；ABA：脱落酸；ETH：乙烯

3.1 生长素对马铃薯块茎休眠和萌发的影响 植物生长素（IAA）是影响马铃薯块茎休眠的主要激素之一。马铃薯块茎中内源IAA的含量会随着休眠程度的不同而产生相应的变化。在块茎形成过程的初期，内源IAA含量升高；随着块茎进一步发育，IAA含量会持续下降[15]。目前，IAA控制马铃薯块茎休眠和芽萌发的作用机制尚不明确。Hemberg[16]对马铃薯块茎进行生物测定发现，块茎休眠期内源IAA含量较低，伴随着早期芽的生长IAA含量明显增加。Sukhova等[17]进一步发现，马铃薯块茎中自由态IAA含量从休眠初期到休眠结束后没有显著变化，但总的IAA含量在减少，推测是结合态IAA含量降低所导致。Sorce等[18]分析了马铃薯块茎芽萌发前IAA的含量，发现萌发芽中自由态的IAA含量显著增加，进一步通过免疫组织化学方法分析块茎组织和芽中IAA分布发现，生长素有利于打破休眠，在发芽初期可促进芽的分化和生长。同时也发现高浓度IAA或合成的生长素对芽萌发有一定的抑制作用，通过施用不同IAA浓度梯度观察芽的萌发情况，发现高浓度IAA（1000mg/L）完全抑制芽的萌发，100mg/L IAA处理下抑制不明显，而低浓度IAA（<10mg/L）可刺激芽的萌发，且当IAA浓度为1mg/L时刺激芽萌发的效果最佳[16]。但也有研究表明，IAA会抑制马铃薯块茎腋芽的发生以及匍匐茎的伸长，IAA在抑制匍匐茎伸长的同时会促进无柄块茎的形成和块茎的膨大[19]。但IAA浓度高于5μmol/L时，会抑制块茎的形成，这可能是高浓度IAA含量影响了乙烯（ETH）的形成[20]。综上所述，内源IAA控制马铃薯块茎休眠的假设并没有得到有力证据的支撑，却证实了IAA和其他内源性生长素在马铃薯解除休眠后芽生长萌发过程中发挥作用。因此，一定浓度范围的IAA是芽生长所必需的，但不是芽生长萌发的启动因子。

3.2 赤霉素对马铃薯块茎休眠和萌发的影响 赤霉素（GA）是一类可以促进马铃薯块茎打破休眠及芽生长萌发的植物激素，在马铃薯块茎的生长发育过程中，GA通过细胞分裂及细胞骨架、光周期诱导块茎形成时信号的传递和碳水化合物的合成等过程来调控马铃薯块茎的形成和发育[21]。

GA对马铃薯块茎休眠的打破具有重要作用。低浓度GA可加速休眠打破和块茎芽的萌发进程[4]，而短日照条件下GA（浓度≥100mL/L）对马铃薯块茎的形成具有抑制或延迟块茎形成的作用[22]。Suttle[23]通过研究马铃薯块茎中 GA_1、GA_{19}

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和 GA_{20} 的含量发现，块茎中 GA_{19} 和 GA_{20} 的含量在发芽旺盛时期最高，且此时 GA 总含量增加。同时有研究表明发现，GA_{19} 对非休眠期块茎的发芽生长有一定的促进作用。以上结果表明，内源性 GA 对块茎休眠的打破与芽的生长萌发具有一定的调控作用。Xu 等[24]通过对诱导条件（8% 蔗糖 +GA 0.01~0.10μmol/L）和非诱导条件（1% 蔗糖；8% 蔗糖 +$GA_{4/7}$）下马铃薯块茎或匍匐茎中 GA 的含量进行比较发现，在匍匐茎膨大形成块茎的过程中，两者 GA 的含量均显著下降，在块茎中维持较低水平。进一步分析发现，非诱导条件（1% 蔗糖）下 GA 的含量较诱导条件（8% 蔗糖）下高，说明马铃薯块茎的形成需要 GA 的含量低于一个临界水平（1μmol/L）。内源 GA 类似化合物在块茎休眠期含量较低而在芽生长前增加，表明用 GA 处理休眠期的块茎可打破其休眠状态[25]。尽管 GA 在马铃薯块茎休眠调控中的作用一直存在争议，但通过外源施用、生物合成基因的异位表达以及人工增加块茎 GA 含量等手段都能够促进块茎打破休眠。

3.3 细胞分裂素对马铃薯块茎休眠和萌发的影响 细胞分裂素（CTK）是马铃薯块茎休眠及芽生长萌发过程中的调节因子，能够刺激植物细胞分裂、增强植物组织的吸收能力，进而促进块茎打破休眠和芽的生长萌发[26]。对 CTK 活性和含量的相关研究表明，当块茎处于深度休眠期时，CTK 含量较低，而在发芽前和出苗期前含量会增加且达到最高值。块茎休眠解除后，块茎和芽中 CTK 总含量进一步增加[17]。目前为止，细胞分裂素调控块茎休眠和发芽的作用机制仍不清楚。

细胞分裂素存在于马铃薯块茎的所有部位，在休眠时期，细胞分裂素均匀分布在顶端、外侧和节间组织。在块茎打破休眠的过程中，CTK 能够刺激块茎进行细胞分裂，解除细胞周期 G_1/S 期的阻滞，最终导致休眠被打破，相关的研究表明，D 型细胞分裂素参与细胞周期 G_1/S 期的阻滞的去除[27]，一定程度上印证了 CTK 对块茎的解除休眠作用。据报道，块茎休眠的打破往往伴随着根尖芽以及相邻组织中游离细胞分裂素水平的迅速增加，且细胞分裂素在根尖组织顶端优势存在的情况下就会维持高水平的表达；而当顶端优势被克服，侧芽及邻近组织的细胞分裂素水平与根尖区域的细胞分裂素表达水平相当，表明细胞分裂素苷被运输到块茎的分生区，并被水解成自由碱基，在块茎的分生区和芽中明显地转化为贮藏形式（特别是玉米素糖苷）[28]。研究证明，马铃薯块茎中同时含有反式玉米素家族的细胞分裂素和顺式玉米素衍生物，外源细胞分裂素（顺式和反式玉米素）不仅可以有效地打破块茎休眠，还能引起内源游离顺式玉米素含量的增加，而顺式玉米素被认为是马铃薯块茎休眠的潜在调控因子[29]。此外，马铃薯块茎对细胞分裂素的敏感性随着休眠程度的进一步加深而逐渐增加。尽管收获后或收获后早期的马铃薯块茎对外施的细胞分裂素不敏感，但其内源性细胞分裂素含量会随着细胞分裂素的外施而增加[27]，认为是外源性的细胞分裂素被休眠的块茎迅速代谢从而导致休眠被打破[30]。此外，休眠块茎对细胞分裂素表达水平随着时间的推移而不断下降，同时细胞分裂素代谢水平并没有明显的变化，表明细胞分裂素的感知、信号传导途径等受休眠状态的影响[29]。这些研究结果进一步表明，内源性细胞分裂素是马铃薯块茎中天然的终止休眠因子。

3.4 乙烯对马铃薯块茎休眠和萌发的影响 植物激素乙烯（ETH）是一种有效的生长调节剂，能够影响植物的生长、分化和衰老等进程[31]。一系列关于乙烯与马铃薯块茎发芽间的研究表明，乙烯有利于块茎休眠的启动和维持，因此有必要对乙烯影响休眠的作用机制进行相应的研究和阐述。研究发现，乙烯在维持休眠的第 1 阶段表现最为活跃，内源乙烯的含量在离体培养的块茎进入休眠初期时达到最高，随后迅速下降。用乙烯拮抗剂、硝酸银和降冰片二烯等可以使块茎休眠被打破，表现为块茎提前发芽，而这个过程可以被乙烯处理逆转，且在乙烯的作用下，休眠初期具有一定的稳定性[6]。与施用乙烯相比，休眠块茎经乙烯发生剂 2- 氯乙基膦酸处理后，不仅提高了组织中乙烯的含量，而且促进了 ABA 的生物合成，最终表现为马铃薯块茎休眠期的延长。因此，乙烯被认为对块茎休眠有调控作用[3]。但也有学者提出不同的观点，Suttle[32] 利用乙烯合成抑制剂氨基乙氧基乙烯基甘氨酸（AVG）和 NG 同时处理微型薯时，发现乙烯含量没有增加，但微型薯提前解除休眠，而外源乙烯或者乙烯合成激活剂氨基环丙烷羧酸（ACC）处理的微型薯

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并没有提前解除休眠，认为内源乙烯没有参与诱导块茎休眠解除。Rylska 等[33]用不同浓度的乙烯处理马铃薯块茎，发现休眠块茎在浓度为 2μL/L 的乙烯处理 72h，块茎发芽的效果最好，且环氧乙烷的处理效果比乙烯更好。用乙烯处理马铃薯休眠块茎超过 72h，则会抑制块茎发芽；对于已解除休眠期的块茎，用乙烯长期处理，也会抑制芽的伸长。但在马铃薯块茎中检测到的乙烯浓度很低（小于 0.1μL/L），认为内源乙烯并不是芽的抑制剂。Prange 等[34]用乙烯持续处理商品化贮藏的块茎，通过分析马铃薯块茎中还原糖的含量发现，虽然马铃薯块茎中还原糖含量有所增加，但芽的生长萌发仍被抑制；且内源乙烯的表达水平只在发芽期才略有增加，在此之前乙烯的含量达不到萌芽的条件，因此乙烯不能作为芽的内源抑制剂。

3.5 脱落酸对马铃薯块茎休眠和萌发的影响 脱落酸（ABA）是导致和维持块茎休眠的主要植物激素之一。ABA 首次在马铃薯块茎中 β- 抑制性复合物中被检测，发现能够抑制块茎茎尖生长，在块茎休眠时含量最高，随着休眠的解除，茎尖开始生长时，含量迅速下降，被认为可以调控块茎的休眠[35]。杨柏云等[1]研究发现，芽眼和块茎组织中 ABA 含量在休眠开始时均增加，在深度休眠期最高，在休眠结束时急剧下降，块茎中 ABA 含量在打破休眠后也随之降低，表明 ABA 可以调控马铃薯块茎的休眠。

研究证实外源 ABA 会对马铃薯块茎芽的生长产生暂时性、剂量依赖性的抑制作用[31]。Suttle 等[36]通过系统研究了内源 ABA 以及 ABA 生物合成抑制剂（氟醚酮）在块茎休眠中的作用后发现，马铃薯块茎经氟醚酮处理后 ABA 的合成被有效抑制，表现为块茎提前发芽。而外源施用 ABA 则使块茎中 ABA 恢复到对照水平，相应地观察到早熟芽被抑制的现象。此外，对休眠的马铃薯块茎外源施用 ABA 抑制剂也会观察到 ABA 含量下降以及早芽率的增加。综上所述，马铃薯块茎休眠的诱导和维持都需要 ABA 的持续生物合成。近年来，块茎在生理休眠期间 ABA 含量的变化与某些 ABA 生物合成和分解基因之间的时间相关性已有报道，然而由于长时间的自然休眠过程使得阐述这些现象变得复杂。

4 展望

如何做到人为调控马铃薯块茎的休眠期以及芽的萌发，从而发挥马铃薯最大的经济价值是国内外学者所面临的重大科学问题。在马铃薯块茎休眠的调控中，外界环境因素和内源植物激素的特性和作用已经取得了一部分的进展。然而，关于植物激素在马铃薯块茎生理和休眠等过程中的调控机制，还需要进一步的研究。目前通过控制光照、温度、湿度等外界环境因素虽然可以控制马铃薯块茎的休眠期，一定程度上提高马铃薯的产量和品质。在此基础上，对内源植物激素间互相调控机制的阐述必将使得马铃薯产业得到进一步的发展。目前通过基因工程已确定马铃薯休眠过程中一些与调控激素相关的基因和蛋白质等物质，为更好地解释块茎休眠和芽萌发调控机制奠定了基础。

已知的内源植物激素间的调控及互作机制仍需要进一步探索，而其他内源植物激素在块茎休眠过程中的作用也在不断地被认知，如油菜素内酯等。为解决马铃薯休眠机制阐述这个问题，深入了解各激素特别是赤霉素和乙烯在块茎休眠和萌发中的作用机制尤为必要；而各植物内源激素间的平衡及相互调控机制以及其他内源激素在休眠和萌发中产生的影响和调控途径的相关研究则可以进一步加速对马铃薯块茎休眠机制的认识。最后，可利用基因工程等对马铃薯休眠机制进行更深层次的阐述。相信在不久的将来，随着马铃薯休眠及芽萌发机制的不断清晰，马铃薯的经济价值可以得到充分的利用。

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(收稿日期: 2022-04-18)

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 DOI:10.3969/j.issn.1006-8082.2020.04.008 中国稻米 2020,26(4):35-39

多年生稻云大107产量潜力分析

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摘 要:利用长雄野生稻地下茎无性繁殖特性培育具有多年生性的栽培稻品种已经获得成功。云大107是通过"滇瑞449//RD23/长雄野生稻"杂交选育出来的具有多年生性的优质稻品种。为明确云大107的产量潜力,进而为优化其栽培技术提供科学依据,笔者用云南主栽的优质稻品种滇屯502、滇瑞449、文稻5作对照,于2018年在云南景洪进行田间试验,调查分析了各品种生育期、干物质积累、光合特性、产量及产量潜力等性状。结果表明,云大107产量可达564.52 kg/667 m^2,较滇屯502、文稻5和滇瑞449分别增产25.94%、5.41%和2.93%,其中与滇屯502相比差异达显著;从产量构成看,云大107每穗粒数、千粒重均比对照文稻5、滇屯502和滇瑞449高,粒多粒大是其高产的原因;同时,云大107剑叶净光合速率分别比滇屯502、滇瑞449和文稻5增加4.45%、4.65%和16.83%,差异显著。因此,挖掘云大107产量潜力的主要途径是提高其每穗粒数,同时提高齐穗后剑叶光合能力,以积累较多光合产物,进而提高成熟期生物产量。

关键词:多年生稻;干物质;光合特性;产量;产量潜力

中图分类号:S511.042 **文献标识码**:A **文章编号**:1006-8082(2020)04-0035-05

近年来,云南大学利用长雄野生稻地下茎无性繁殖特性培育的多年生稻获得了成功[1-3],多年生稻品种多年生稻23已经通过了云南省审定(审定编号:滇审稻2018033号)。多年生稻是指种植一次就可以连续收获多年的稻作生产方式,从第2年起水稻生产不再需要种子、育秧、犁田耙田、栽秧等环节,不仅减少了劳动力投入和生产成本,也大大降低了劳动强度,是一项轻简化的稻作生产技术。最近,云南农业大学又从"滇瑞449//RD23/长雄野生稻"杂交组合中选育出了具有多年生性的优质稻品种云大107。为了明确云大107的产量潜力,为其栽培技术优化提供依据,我们用云南主栽的优质稻品种滇屯502、滇瑞449、文稻5作对照,于2018年在云南景洪进行了田间比较试验,以期为云大107的推广与栽培技术的优化提供详实数据和理论依据。

1 材料与方法

1.1 供试材料与供试地点

供试材料为滇屯502、文稻5、滇瑞449、云大107。试验于2018年在云南景洪市嘎洒镇试验基地进行(海拔550 m)。试验地土壤基础肥力:pH值5.05,有机质含量34.0 g/kg,全氮2.1 g/kg,碱解氮155.60 mg/kg,速效磷7.58 mg/kg,速效钾139.10 mg/kg。

1.2 试验设计

采用随机区组设计,3次重复,小区面积20 m^2(长×宽=4 m×5 m)。种植方式和施肥措施与当地生产水平类似。7月23日播种,8月17日移栽,移栽行株距20 cm×20 cm,每667 m^{2}插1.67万丛,每丛1~2苗;氮肥、磷肥、钾肥分别选用尿素(46%)、过磷酸钙(12%)和氯化钾(60%),纯N用量13 kg/667 m^2,P_2O_5 6.5 kg/667 m^2,K_2O 13 kg/667 m^2。其中,氮肥按基肥:分蘖肥:穗肥:粒肥=5:2:2:1分4次施,磷肥全部作基施,钾肥按基肥:穗肥=1:1分2次施。水分管理和病虫害防治参照水稻高产管理措施进行。

1.3 测定项目及方法

1.3.1 生育期

记载播种期、成熟期,并计算最终的全生期天数。

1.3.2 干物质积累

分别在齐穗期和成熟期取样,每小区取5丛,洗净泥沙,测定茎蘖数,然后剪去根。齐穗期将样品分成茎、叶、穗3部分;成熟期将样品分成稻草、枝梗、实粒、秕粒4部分。样品整理好及时放入烘箱105℃杀青30 min,后调温度至75℃烘干至恒质量,分别测定干物质

收稿日期:2020-02-25

基金项目:国家重点研发计划"七大农作物育种"专项(2017YFD0100201);云南省科技重大专项"生物种业和农产品精深加工"(2019ZD013)

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表1 不同品种产量及产量构成表现

品种	有效穗数（万/667 m²）	每穗粒数（粒）	结实率（%）	千粒重（g）	理论产量（kg/667 m²）	实际产量（kg/667 m²）
滇屯502	17.23 ab	127.96 b	78.03 a	29.32 a	500.73 a	448.25 b
文稻5	18.89 ab	120.88 b	83.80 a	29.69 a	566.72 a	535.57 a
滇瑞449	22.78 a	120.07 b	82.35 a	25.23 b	569.34 a	548.46 a
云大107	16.23 b	138.82 a	82.38 a	30.80 a	570.59 a	564.52 a

同列数据后不同小写字母表示各品种间差异在0.05水平显著。下同。

表2 不同品种生育期表现

品种名称	始穗期（月-日）	移栽期（月-日）	齐穗期（月-日）	成熟期（月-日）	播始历期（d）	始穗-成熟（d）	全生育期（d）
滇屯502	10-22	08-17	10-30	12-03	91	42	133
文稻5	10-04	08-17	10-13	11-18	73	45	118
滇瑞449	10-25	08-17	11-03	12-06	94	42	136
云大107	10-20	08-17	10-26	11-29	89	40	129

量。

1.3.3 净光合速率和叶绿素含量

在齐穗期，用LI-6400XT便携式光合测定仪梭形叶室测定剑叶净光合速率(Pn)，每个小区测3株，测定时间选择晴朗天气的上午9:00—11:00；同时将测定净光合速率(Pn)剑叶对应做好标记取样放入封口袋中，带回实验室测其叶绿素含量。叶绿素含量的测定参考张其德[14]的方法。每次测定重复3次，取平均值。

1.3.4 产量与产量构成

采用5点取样法进行取样调查，调查有效穗数、穗粒数、结实率、千粒重等性状，计算理论产量。同时每个小区取5 m²进行实割测产，计算实际产量。

1.4 数据分析

试验数据采用Excel和SPSS 23.0软件进行处理和分析。

2 结果与分析

2.1 产量及产量构成

从表1可见，实际产量和理论产量均表现为云大107>滇瑞449>文稻5>滇屯502，其中实际产量云大107为564.52 kg/667 m²，较滇屯502、文稻5和滇瑞449分别增加25.94%、5.41%和2.93%，与滇屯502相比产量差异显著。从产量构成因素看，云大107每穗粒数为138.82粒，显著多于滇瑞449、文稻5和滇屯502，分别提高8.49%、14.84%和15.62%；云大107有效穗数16.23万/667 m²，较滇屯502、文稻5和云大107分别减少5.80%、14.08%和28.75%，其中与滇瑞449差异显著；云大107千粒重30.8 g，显著高于滇瑞449；各品种之间结实率差异不显著。可见，云大107的产量比云南主栽优质稻品种略高，其高产的原因主要在于有较多的穗粒数和较高的千粒重。

2.2 生育期

从表2可见，全生育期表现为滇瑞449>滇屯502>云大107>文稻5，云大107较亲本滇瑞449缩短7 d，较滇屯502缩短4 d，比文稻5长11 d。品种间生育期差异主要表现在播始历期，为3~21 d，而始穗至成熟期差异不明显。说明云大107的生育期适合云南优质稻生产的生育期需求。

2.3 干物质积累

从表3可见，云大107成熟期干物质量为1 984.8 g/m²，比文稻5、滇屯502、滇瑞449分别增加28.87%、19.94%、11.44%，其中与文稻5差异显著；齐穗期干物质量为1 305.4 g/m²，比滇瑞449显著增加308.7 g/m²，而与滇屯502、文稻5差异不显著；云大107收获指数为0.55，与滇瑞449、滇屯502以及文稻5差异不显著；云大107齐穗至成熟期生长速率为20.0 g/(m²·d)，显著高于滇屯502和文稻5。这4个参试品种的产量与成熟期干物质量和生长速率呈显著正相关（$r=0.775^{*}$，$r=0.778^{*}$）（表4）。总的来看，云大107在成熟期及齐穗期的干物质量均大于其他优质稻品种，收获指数和生长速率亦较高，具有适应云南优质稻区种植的潜力。

2.4 光合特性

2.4.1 叶绿素含量

从图1可见，云大107的叶绿素a、类胡萝卜素和叶绿素a+b小于滇屯502，但均大于文稻5和滇瑞449。表明云大107在云南优质稻主栽区具有较高的光合特性，利于通过优化栽培措施提高产量。

2.4.2 净光合速率

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表 3　不同品种干物质积累、生长速率、收获指数差异

品种	齐穗期干物质量 (g/m²)	成熟期干物质量 (g/m²)	齐穗期-成熟期生长速率 [g/(m²·d)]	收获指数 (%)
滇屯 502	1 331.6 a	1 654.8 ab	9.8 c	0.48 a
文稻 5	1 063.3 bc	1 540.1 b	13.2 bc	0.59 a
滇瑞 449	996.7 c	1 781.0 ab	23.8 a	0.51 b
云大 107	1 305.4 ab	1 984.8 a	20.0 ab	0.55 ab

表 4　不同品种干物质积累、生长速率、收获指数相关性分析

项目	齐穗期干物质量	成熟期干物质量	齐穗-成熟期生长速率	收获指数	产量
齐穗期干物质量	1				
成熟期干物质量	0.403	1			
生长速率	-0.360	0.706*	1		
收获指数	-0.195	-0.103	-0.005	1	
产量	-0.041	0.601*	0.615*	0.464	1

*表示在 P<0.05 条件下相关性显著，**表示在 P<0.01 条件下相关性显著。下同。

表 5　不同品种净光合速率比较

品种	净光合速率 [μmol/(m²·s)]	气孔导度 [mol/(m²·s)]	胞间 CO_2 浓度 (μmol/mol)	蒸腾速率 [μmol/(m²·s)]
滇屯 502	25.19 b	0.52 b	261.97 ab	8.73 a
文稻 5	22.52 c	0.50 ab	267.39 ab	8.16 b
滇瑞 449	25.14 b	0.48 b	252.24 c	7.92 b
云大 107	26.31 a	0.61 a	275.11 a	4.86 c

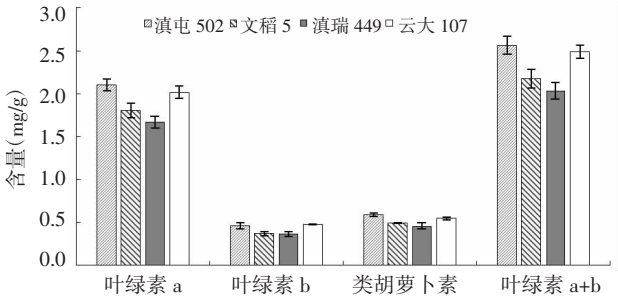


图 1　云南主栽优质稻不同品种叶绿素含量差异

从表 5 可见，云大 107 齐穗期剑叶净光合速率为 26.31 μmol/(m²·s)，显著高于滇屯 502、滇瑞 449 和文稻 5，分别增加 4.45%、4.65%和 16.83%；而气孔导度是 0.61 mol/(m²·s)，显著高于滇屯 502、滇瑞 449，与文稻 5 持平；胞间 CO_2 浓度为 275.11 μmol/mol，和文稻 5、滇屯 502 以及滇瑞 449 持平；蒸腾速率 4.86 μmol/(m²·s)，显著小于文稻 5、滇屯 502 和滇瑞 449。云大 107 的净光合速率与叶绿素 a、叶绿素 b、叶绿素 a+b 和类胡萝卜素呈极显著正相关性（相关系数分别为 0.880**、0.906**、0.908**、0.895**）（表 6）。从光合特性和净光合速率可以看出，云大 107 具有高产的光合潜力。

3　讨论与结论

3.1　产量与产量构成关系

在产量构成要素中，有效穗数、每穗实粒数是衡量库容量大小的主要指标，千粒重反应的则是库容的充实程度。研究表明，在高产水平下产量构成要素中，千粒重与产量的偏相关系数最大，每穗实粒数次之[5]，所以说提高库容量是获得高产的重要途径，而库的充实程度是获得高产的保证。研究证明，增加穗粒数和千粒重是获得高产的直接原因，尤其在高产条件下关系更为密切[6]。本研究中，实际产量和理论产量均表现为云大 107>滇瑞 449>文稻 5>滇屯 502，云大 107 的每穗粒数显著多于滇屯 502、文稻 5 和滇瑞 449，而千粒重也高于滇屯 502、文稻 5 和滇瑞 449。与其他品种相比，云大 107 产量潜力优势主要体现在穗大、粒重上，通过调控栽培措施可以进一步协调产量构成四要素的关系，进而挖掘云大 107 产量潜力。

3.2　产量与干物质积累、收获指数关系

水稻各器官干物质的积累是产量形成的基础[7]。产量潜力提高更多地是依靠增加生物产量和协调生物产量与收获指数的关系[8]。高产高效栽培和超高产栽培可以显著提高水稻干物质总积累量，特别是齐穗至成熟阶段的干物质积累量，使产量随干物质的增加而同步增加[9]。云大 107 成熟期的干物质积累量最大，这也是云大 107 产量高于其他优质稻的主要原因之一。

3.3　剑叶净光合速率、叶绿素含量与产量、干物质积

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表 6 不同品种剑叶净光合速率和叶绿素含量的相关性分析

项目	叶绿素 a	叶绿素 b	类胡萝卜素	叶绿素 a+b	净光合速率
叶绿素 a	1				
叶绿素 b	0.896**	1			
类胡萝卜素	0.945**	0.870**	1		
叶绿素 a+b	0.994**	0.937**	0.942**	1	
净光合速率	0.774**	0.820**	0.825**	0.801**	1

累的关系

产量变化主要取决于抽穗至成熟期光合生产能力[10]，而光合生产能力又主要取决于净光合速率和蒸腾速率的快慢。云大 107 净光合速率显著高于滇屯 502、滇瑞 449 和文稻 5，气孔导度和胞间 CO_2 浓度显著大于或略高于其他 3 个云南主栽的优质稻；蒸腾速率却显著小于文稻 5、滇屯 502 和滇瑞 449。可见，云大 107 具有较高的光合潜力，这也是其产量高于其他优质稻品种的主要原因之一。

光合生产能力取决于植物体叶片的叶色，水稻叶色的深浅是由体内叶绿素含量的多寡决定的，叶绿素在光合作用的光能吸收、传递和转换中起着关键作用[11]。叶绿素含量与净光合速率呈显著相关性，并且直接影响产量[12]，叶绿素含量高，有助于获取更多太阳辐射量，也利于光合产物的形成和积累，提高植物的光能利用率。本研究表明，净光合速率与叶绿素 a、叶绿素 b、叶绿素 a+b 和类胡萝卜素呈极显著正相关性，云大 107 除了叶绿素 a、类胡萝卜素和叶绿素 a+b 小于滇屯 502 外，其他各指标均大于文稻 5 和滇瑞 449。

云大 107 较云南主栽其他优质稻产量高的主要原因是粒多粒大，同时生长后期具有较高的光合产物生产能力，保证成熟期有较高干物质积累。因此，提高包括多年生稻云大 107 在内的云南优质稻产量及挖掘产量潜力的主要途径是提高每穗粒数，同时提高齐穗后剑叶光合能力，以积累较多光合产物，进而提高成熟期生物产量。

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Yield Potential Analysis of Perennial Rice Yunda 107

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Abstract: Perennial rice has been successfully bred by the clonal propagation characteristic via rhizome of *Oryza longistaminata*, can survive for many years. Yunda 107 is a perennial rice variety with high quality, selected through the cross of 'Dianrui449//RD23/Oryza longistaminata'. In order to explore the yield potential of Yunda 107, an experiment was conducted in 2018 using high quality rice varieties Diantun 502, Dianrui 449 and Wendao 5 as controls. In the experiment, we investigated the traits such as growth period,

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dry matter accumulation, photosynthetic characteristics, yield and yield potential of each tested variety. The results showed that, the yield of Yunda 107 can reach 564.52 kg/667 m^2, which increase by 25.94%, 5.41% and 2.93% compared with Diantun 502, Wendao 5 and Dianrui 449, respectively, and the difference is significant compared with Diantun 502. In terms of yield composition, the number of grains per panicle and the weight per thousand grains of Yunda 107 were higher than those of Wendao 5, Diantun 502 and Dianrui 449. The large number and size of grains were the main reason for its higher yield. At the same time, the net photosynthetic rate of flag leaves of Yunda 107 increased by 4.45%, 4.65% and 16.83%, compared with Diantun 502, Dianrui 449 and Wendao 5, respectively, with significant differences. Therefore, the main way to tap the yield potential of Yunda 107 is to increase the number of grains per panicle and the photosynthetic capacity of flag leaves after full heading, so as to accumulate more photosynthetic products, thereby increasing the biological yield at mature stage.

Key words: perennial rice; dry matter; photosynthetic characteristics; yield; yield potential.

·综合信息·

至2020年农业农村部认定的133个超级稻品种

2020年6月8日,农业农村部认定11个超级稻品种,退出10个超级稻品种冠名,至此农业农村部共认定(不含退出)133个超级稻品种,具体列于表1。另附认定而退出冠名的超级稻品种。

表1 至2020年农业农村部认定的133个超级稻品种

类型	品种名称	认定年份	育种单位	类型	品种名称	认定年份	育种单位
粳型常规稻(26)	吉粳88	2005	吉林省农科院水稻所	籼型三系杂交稻(49)	中浙优1号	2005	中国水稻研究所
	桂农占	2006	广东省农科院水稻所		Ⅱ优明86	2005	福建省三明市农科所
	龙粳21	2009	黑龙江省农科院水稻研究所		Ⅱ优602	2005	四川省农科院水稻所
	辽星1号	2007	辽宁省农科院稻作所		天优998	2005	广东省农科院水稻所
	楚粳27	2007	云南省楚雄州农科所		Q优6号	2006	重庆中一种业有限公司
	沈农9816	2011	沈阳农业大学		珞优8号	2009	武汉大学生科院
	连粳7号	2012	连云港市农业科学研究院		五优308	2010	广东省农业科学院水稻研究所
	龙粳31号	2013	黑龙江省农业科学院佳木斯水稻研究所等		五丰优T025	2010	江西农业大学
	松粳15号	2013	黑龙江省农业科学院五常水稻研究所		天优3301	2010	福建省农业科学院生物技术研究所等
	镇稻11号	2013	江苏丘陵地区镇江农业科学研究所		特优582	2011	广西农业科学院水稻研究所
	扬粳4227	2013	江苏里下河地区农业科学研究所		德香4103	2012	四川省农业科学院水稻高粱研究所
	宁粳4号	2013	南京农业大学农学院		天优华占	2012	中国水稻研究所等
	龙粳39	2014	黑龙江省农业科学院佳木斯水稻研究所等		宜优673	2012	福建省农科院水稻所
	连稻1号	2014	佳木斯市莲粳种业有限公司等		深优9516	2012	清华大学深圳研究生院
	长白25号	2014	吉林省农业科学院水稻研究所		天优3618	2013	广东省农业科学院水稻研究所
	南粳5055	2014	江苏省农业科学院粮食作物研究所		天优华占	2013	中国水稻研究所等
	武运粳27号	2014	江苏(武进)水稻研究所等		中9优8012	2013	中国水稻研究所
	扬育粳2号	2015	江苏盐城市盐都区农业科学研究所		H优518	2013	湖南农业大学等
	南粳9108	2015	江苏省农业科学院粮食作物研究所		五丰优615	2014	广东省农业科学院水稻研究所
	镇稻18号	2015	江苏丰源种业有限公司等		盛泰优722	2014	湖南洞庭高科种业股份有限公司等
	吉粳511	2016	吉林省农业科学院水稻所		内5优8015	2014	中国水稻研究所
	南粳52	2016	江苏省优质水稻工程技术研究中心等		荣优225	2014	江西省农业科学院水稻研究所等
	南粳0212	2017	江苏省农业科学院粮食作物研究所等		F优498	2014	四川农业大学水稻研究所等
	楚粳37号	2017	楚雄州农业科学研究推广所		宜香优2115	2015	四川省绿丹种业有限责任公司等
	宁粳7号	2018	南京农业大学		深优1029	2015	江西现代种业股份有限公司
	苏垦118	2020	江苏省农业科学院粮食作物研究所		德优4727	2016	四川省农业科学院水稻高粱研究所
籼型常规稻(8)	玉香油占	2007	广东省农科院水稻所		丰田优553	2016	广西农业科学院水稻研究所
	中嘉早17	2010	中国水稻研究所等		五优662	2016	江西惠农种业有限公司等
	合美占	2010	广东省农业科学院水稻研究所		吉优225	2016	江西省农业科学院水稻所等
	中早35	2012	中国水稻研究所		五丰优286	2016	江西现代种业股份有限公司
	金农丝苗	2012	广东省农科院水稻所		五优航1573	2016	江西省超级水稻研究发展中心等
	中早39	2013	中国水稻研究所		宜香4245	2017	宜宾市农业科学院
	华航31号	2015	华南农业大学植物航天育种研究中心		吉丰优1002	2017	广东省农业科学院水稻研究所等
	中组143	2020	中国水稻研究所		五优116	2017	广东省现代农业集团有限公司等

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生态学报
Acta Ecologica Sinica
ISSN 1000-0933,CN 11-2031/Q

《生态学报》网络首发论文

题目：云龙天池国家级保护区云南松种群年龄结构及动态分析
作者：张锦堂，潘志立，田云海，侯佳蓝，彭明春，段禾祥，李云芳，李永萍
收稿日期：2021-05-08
网络首发日期：2022-07-12
引用格式：张锦堂，潘志立，田云海，侯佳蓝，彭明春，段禾祥，李云芳，李永萍．云龙天池国家级保护区云南松种群年龄结构及动态分析[J/OL]．生态学报．https://kns.cnki.net/kcms/detail/11.2031.Q.20220711.1244.032.html

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网络首发时间：2022-07-12 15:59:12
网络首发地址：https://kns.cnki.net/kcms/detail/11.2031.Q.20220711.1244.032.html

第 42 卷第 22 期 生 态 学 报 Vol.42,No.22
2022 年 11 月 ACTA ECOLOGICA SINICA Nov.,2022

DOI: 10.5846/stxb202105081208

张锦堂, 潘志立, 田云海, 侯佳蓝, 彭明春, 段禾祥, 李云芳, 李永萍.云龙天池国家级保护区云南松种群年龄结构及动态分析.生态学报,2022, 42(22): - .

Zhang J T, Pan Z L, Tian Y H, Hou J L, Peng M C, Duan H X, Li Y F, Li Y P.Age structure and dynamics of *Pinus yunnanensis* population in Yunlong Tianchi Nature Reserve.Acta Ecologica Sinica,2022,42(22)

云龙天池国家级保护区云南松种群年龄结构及动态分析

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摘要:云南松是中国西南地区的特有树种。云南松林是暖温性针叶林的典型代表,主要分布于滇中高原。云南松种群是否可以自我更新,将影响滇中地区的植被动态。明晰未受干扰的云南松种群的结构和动态是研究云南松林可持续发展的重要基础。云南省云龙天池自然保护区内的云南松林是较为原始的森林。本研究以云龙天池国家级自然保护区云南松天然种群为研究对象,分析了云南松天然种群静态生命表、存活曲线、生存函数曲线和谱分析。结果表明:云龙天池国家级自然保护区的云南松年龄结构为倒 J 型,种群年龄结构数量变化动态指数 V_{pi} 为 28.29%,存在外部干扰时侯的种群年龄结构动态指数 V'_{pi} 为 1.77%,种群动态指数 V_{pi} 和 V'_{pi} 都大于 0,种群在外部环境干扰下承担的风险概率 P_{max} 为 6.25%,以上结果表明云南松种群为增长型种群结构,具有一定的抗干扰能力。云南松种群的存活曲线为 Deevey-Ⅱ型,生存分析发现生存率、累计死亡率、死亡密度和危险率在Ⅰ—Ⅶ龄级(胸径<35 cm)变化较大,Ⅶ—XVI 龄级(胸径 35—80 cm)趋于稳定;谱分析结果表明第Ⅲ龄级(胸径 10—15 cm)是影响种群数量的主要阶段。以上结果说明云南松种群的低龄级个体数量波动大,个体死亡较多,高龄级衰退,建议自然保护区内的天然云南松幼龄林和中龄林可以采取抚育间伐措施来促进其种群的生长发育和更新。

关键词:云南松;静态生命表;龄级结构;存活曲线;生存分析;谱分析

Age structure and dynamics of *Pinus yunnanensis* population in Yunlong Tianchi Nature Reserve

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Abstract: *Pinus yunnanensis* is an endemic tree species in Southwest China. *P. yunnanensis* forest is a typical representative of warm coniferous forest, mainly distributed in the central Yunnan plateau. Whether *P. yunnanensis* populations can self-renew or not will affect the vegetation dynamics in central Yunnan. Clarifying the structure and dynamics of undisturbed *P. yunnanensis* populations is an important basis for studying the sustainable development of *P. yunnanensis* forests. The

基金项目:云南省教育厅科学研究基金项目(2020Y0017);国家自然科学基金项目(31660118);中国植被志(针叶林卷)编研

收稿日期:2021-05-08

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P. yunnanensis forest in the Yunlong Tianchi Nature Reserve in Yunnan Province is a relatively pristine forest without human interference. In this study, the static life table, survival curve, survival function curve and spectral analysis of *P. yunnanensis* natural populations were analyzed in Yunlong Tianchi Nature Reserve. The results demonstrated that the age structure of *P. yunnanensis* in Yunlong Tianchi National Nature Reserve was inverted J—type, the dynamic index of population age structure change in quantity V_{pi} was 28.29%, the dynamic index of population age structure in the presence of external disturbance V'_{pi} was 1.77%, the population dynamic index V_{pi} and V'_{pi} were both greater than 0, and the probability of the population assuming risk under external disturbance was P_{max} 6.25%. The above results indicated that the *P. yunnanensis* population is a growing population structure with some resistance to disturbance. Furthermore, the *P. yunnanensis* population had a survival curve tending to the type of Deevey-II, and the survival analysis found that the survival rate, cumulative mortality rate, mortality density and hazard rate varied greatly from Ⅰ to Ⅶ age classes (diameter at breast height<35 cm), and stabilized from Ⅶ to XVI age classes (diameter at breast height 35—80 cm). The spectral analysis exhibited that the population dynamics of *P. yunnanensis* followed one major trend, and the Ⅲ age class (diameter at breast height 10—15 cm) was the main stage affecting the population size. The above results reveal that the number of individuals in the lower age classes of *P. yunnanensis* populations in the Yunlong Tianchi Nature Reserve fluctuates greatly, with more individuals dying and the upper age classes declining. It is suggested that the young and middle—aged natural *P. yunnanensis* forests in the Yunlong Tianchi Nature Reserve can be nurtured and intercut to promote their population growth, development and renewal. Based on all these results, the population of *P. yunnanensis* was found to remain sustainable in the long term, although it fluctuates in the middle and lower ages.

Key Words: *Pinus yunnanensis*; static life table; age class structure; survival curve; survival analysis; spectral analysis

云南松(*Pinus yunnanensis*)是松科(Pinaceae)松属的裸子植物,是我国西南地区特有的树种,也是西南地区荒山造林的先锋树种,具有重要的经济、生态和社会效益[1—3]。云南松林是暖温性针叶林的典型代表,在滇中高原分布极广。现存的云南松天然林大都是在地带性亚热带半湿润常绿阔叶林遭受破坏后,在迹地上更新起来的天然次生林,人工林较少[4]。云南松林是否会被常绿阔叶林所替代是群落演替研究的核心问题。明晰云南松天然林在无人为干扰的自然状态下的种群结构和动态是探究云南松天然林能否实现自我更新的关键,对探究该地区的森林群落的演替具有重要意义。

目前已有较多的研究关注云南松的种群结构。王磊等[5]的综述认为云南松天然林群落结构单一,退化的云南松种群缺乏大径级个体,出现低龄化特征。还有研究也认为云南松林下无小苗、小树不能实现种群更新和维系,是衰退的种群,将会被常绿阔叶林所替代[6—7]。而黄小波等[8]和 Tang 等[9]的研究都认为云南松种群是能够自我更新的种群。何斌等[10]对黔西北地区云南松种群结构及动态进行了研究,但黔西北地区位于云南松分布的东界,其研究结果不能确切全面反映云南松种群的发展趋势。因此,有必要对未受干扰自然状态下的云南松种群的结构和动态进行研究,从而明晰云南松天然林的发展趋势。

云南云龙天池国家级自然保护区位于大理州云龙县,是云南松种质资源保护基地。研究认为该保护区内的云南松林是目前留存下来较为原始的云南松森林群落[9],区内分布有树龄 100 年以上的云南松个体。该保护区为研究未受人为干扰下云南松的种群结构和动态提供了最佳的试验场所。

本文以云龙天池国家级自然保护区云南松天然种群为研究对象,采用静态生命表、存活曲线、生存分析和谱分析等方法研究云南松种群结构和动态特征,旨在阐明云南松天然林的种群发展动态,为研究该地区的云南松群落可持续发展提供依据。

1 材料与方法

1.1 研究区概况

调查区域位于云南省大理州云龙天池国家级自然保护区(25°49′48″—26°14′16″N,99°11′36″—99°20′34″E)

(以下简称“云龙天池保护区”)。全区干湿季分明,年均气温 13.2℃左右,年均降水量为 815.5 mm 左右,海拔为 2100.0—3638.9 m,土壤以棕壤为主。该保护区内的云南松林还伴生少量的其他乔木和灌木,如:华山松(*Pinus armandii*)、锐齿槲栎(*Quercus aliena*)、大白杜鹃(*Rhododendron decorum*)、南烛(*Vaccinium bracteatum*)、水红木(*Viburnum cylindricum*)、银木荷(*Schima argentea*)、山杨(*Populus davidiana*)、散毛樱桃(*Cerasus patentipila*)、乌鸦果(*Vaccinium fragile*)、西南栒子(*Cotoneaster franchetii*)、金丝桃(*Hypericum monogynum*)、野拔子(*Elsholtzia rugulosa*)、小檗(*Berberis amurensis*)等,草本植物主要有草血竭(*Polygonum paleaceum*)、剪股颖(*Agrostis clavata*)、一把伞南星(*Arisaema erubescens*)、苞草(*Arthraxon hispidus*)、藏象牙参(*Roscoea tibetica*)、大花夏枯草(*Prunella grandiflora*)、大戟(*Euphorbia pekinensis*)、云南兔儿风(*Ainsliaea yunnanensis*)、狼毒(*Stellera chamaejasme*)、瓦韦(*Lepisorus thunbergianus*)、木蓝(*Indigofera tinctoria*)等。

1.2　样地设置与种群调查

对云龙天池保护区进行踏查后,选择具有代表性的云南松中龄林、近熟林、成熟林和过熟林,分别布设 1 个 20 m × 50 m 的样方。记录每个样方的经纬度、海拔、坡度、坡向、坡位、坡面等信息,4 个样方的基本情况见表 1。对每个样方内的树高 > 1.3 m 的云南松,记录其胸径(diameter at breast height,DBH)、树高;对于树高 <1.3 m的云南松,划分为幼苗(5 cm≤H<50 cm)和幼树(50 cm≤H<130 cm)分别记录株数、树高[9]。

表 1　云南松林样方的基本情况

Table 1　Basic information of *P. yunnanensis* forest plots

样方 Quadrat	面积 Plot area	海拔/m Elevation	坡向/(°) Aspect	坡度/(°) Slope	坡位 Slope position	坡面 Slope surface	北纬 Northern latitude	东经 East longitude	郁闭度 Canopy density
中龄林 Middle-aged stand	20 m × 50 m	2577	西南 243	21	中	凸	25°52′20.820″	99 °17′13.680″	0.80
近熟林 Near-mature stand	20 m × 50 m	2530	东南 152	36	下	凸	25°52′9.012″	99°16′21.468″	0.80
成熟林 Mature stand	20 m × 50 m	2464	东 107	7	下	平	25°50′56.634″	99°16′20.021″	0.75
过熟林 Over-mature stand	20 m × 50 m	2649	西北 313	22	中	平	25°52′34.907″	99°16′40.847″	0.80

1.3　龄级划分及种群年龄结构动态量化

本研究用种群胸径代替年龄,绘制种群年龄结构图。胸径级的划分标准参考 Tang 等[9],每间隔 5 cm 为 1 级,即 DBH<5 cm(包括树高小于 1.3 m 的植株),为第Ⅰ龄级;5 cm≤DBH<10 cm,为第Ⅱ龄级;依此类推,75 cm≤DBH<80 cm,为第 XVI 龄级,共 16 级。以龄级作为横坐标,样方中活立木的株数作为纵坐标,绘制云南松的龄级结构图。

为定量描述云南松种群动态特征,参考“植物种群与群落结构动态量化分析方法研究”[11]。其中涉及到的参数及其意义如下:V_n表示从其 n 到 n+1 龄级间的个体数量变化动态,V_{pi}是整个种群年龄结构的数量变化动态指数,V'_{pi}是考虑未来的外部环境干扰的整个种群年龄结构的数量变化动态指数,P 是种群在外部环境干扰下承担的风险概率。

1.4　静态生命表的编制及种群的生存分析

静态生命表的编制参考“珍稀濒危植物长苞铁杉种群生命表分析”[12],龄级划分同 1.3。表中参数主要有:a_x是 x 龄级内树木现存个体数;l_x是标准化存活个体数(一般最小龄级标准化成 1000);d_x、q_x分别是相邻龄级间隔期内树木标准化死亡数和相应的死亡率;L_x是从 x 进入 x+1 龄级间隔期间还存活的树木个体数(区间寿命);T_x是 x 龄级到超过 x 龄级的树木个体总数(总寿命);e_x是进入 x 龄级后树木个体的平均期望寿命(生命期望);K_x是各年龄组致死力,即消失率;S_x为存活率。

在编制静态生命表的过程中,当出现种群的死亡率为负值的情况时采用分段匀滑技术对种群个体的存活

数据进行处理[13],x 龄级现有的存活数 a_x 经过匀滑修正处理后可以得到 a_x^* 值。绘制云南松的存活曲线,横坐标为与径级对应的龄级,纵坐标为 $\ln l_x$。分别采用指数函数 $N_x=N_0e^{-bx}$ 和幂函数 $N_x=N_0x^{-b}$ 模型检验云南松种群的存活曲线类型[12, 14—15]。

计算云南松的种群生存率($S(i)$)、累计死亡率($F(i)$)、死亡密度($f(t_i)$)、危险率($\lambda(t_i)$),并绘制生存率、累计死亡率、死亡密度和危险率曲线[16—18],公式如下:

$$S(i)=S_1\times S_2\times\cdots\times S_i;$$

$$F(i)=1-S(i);$$

$$f(t_i)=(S(i-1)-S(i))/h_i;$$

$$\lambda(t_i)=2(1-S(i))/[h_i(1+S(i))]$$

式中,S_i 为存活率,h_i 为龄级宽度,t_i 表示第 i 龄级。

1.5 谱分析

谱分析是傅里叶级数(Fourier series)的展开式,Fourier 证明过复杂的周期现象可以由不同振幅和相应的谐波组成[19—21],写成正弦波形式为:

$$N_t=A_0+\sum_{k=1}^{p}A_k\sin(A_kt+\theta_k)$$

式中,N_t 为 t 时刻种群的大小;A_0 为周期变化的平均值;A_k 为各谐波的振幅($k=1,2,3,\cdots,p$),A_k 的大小标志着各周期作用的大小差别;A_1 为基波的振幅,$A_2,A_3,\cdots,A_p$ 为谐波的振幅,$p=n/2$ 是谐波的个数,n 为时间系列总长度(龄级总数);ω_k 和 θ_k 分别是谐波频率和相角;T 为正弦波的基本周期(时间系列 t 的最长周期);每个谐波的周期分别是基本周期的 1/2,1/3,…,1/p。

在编制生命表时,云南松种群共划分为 16 个龄级,所以谐波的个数 $p=n/2=8$。将静态生命表的龄级作为谱分析的级差,谱分析方法涉及到的参数的计算参照"阔叶红松林红松种群动态的谱分析"[19]。

1.6 数据分析

使用 Microsoft Excel 2010 完成数据的统计整理、制图,使用 SPSS 25 完成模型拟合及统计检验。

2 结果与分析

2.1 年龄结构及动态量化

云南松种群年龄结构为增长型(倒 J 型)(图 1),随着龄级的增大,云南松个体数逐渐减少。第Ⅰ—Ⅶ龄级个体占调查总数的 91.79%,第Ⅱ龄级和Ⅲ龄级的云南松数目最多,分别占所有云南松数目的 33.05% 和 23.37%。第Ⅳ龄级(46 株)和第Ⅴ龄级(45 株)、第Ⅵ龄级(24 株)和第Ⅶ龄级(23 株)的云南松数目几乎相同。第Ⅸ—XVI 龄级的云南松数目较少,仅占 6.11%。

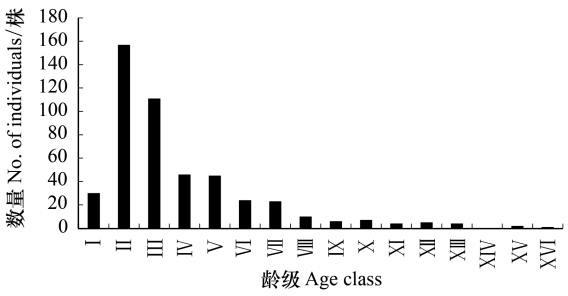


图 1 云南松种群龄级结构

Fig.1 Size class structure of *P. yunnanensis* population

Ⅰ:DBH(胸径,diameter at breast height)<5 cm;Ⅱ:5 cm≤DBH<10 cm;Ⅲ:10 cm≤DBH<15 cm;Ⅳ:15 cm≤DBH<20 cm;Ⅴ:20 cm≤DBH<25 cm;Ⅵ:25 cm≤DBH<30 cm;Ⅶ:30 cm≤DBH<35 cm;Ⅷ:35 cm≤DBH<40 cm;Ⅸ:40 cm≤DBH<45 cm;Ⅹ:45 cm≤DBH<50 cm;Ⅺ:50 cm≤DBH<55 cm;Ⅻ:55 cm≤DBH<60 cm;XIII:60 cm≤DBH<65 cm;XIV:65 cm≤DBH<70 cm;XV:70 cm≤DBH<75 cm;XVI:75 cm≤DBH<80 cm

种群数量变化动态显示(表 2),云南松种群动态指数 V_1、V_9、V_{11} 和 V_{14} 均小于 0,表明云南松在第Ⅰ—Ⅱ龄级、第Ⅸ—Ⅹ龄级、第Ⅺ—Ⅻ龄级、第 XIV—XV 龄级间个体数量减少;V_2—V_8、V_{10}、V_{12}、V_{13} 和 V_{15} 均大于 0,表明Ⅱ—Ⅸ龄级、第Ⅹ—Ⅺ龄级、第 XIII—XIV 龄级以及第 XVI—XV 龄级云南松个体数量呈现增长的结构动态特征。云南松整个种群年龄结构的数量变化动态指数 V_{pi} 为 28.29%,种群呈现增长的年龄结构数量动态关系,考

虑随机干扰情况下的动态指数 V'_{pi} 为 1.77%,V_{pi} 和 V'_{pi} 都大于 0,表明云南松种群为增长型种群结构。P_{max} 为 6.25%表明云南松种群具有一定的抗干扰能力。

表 2　云南松种群动态变化指数

Table 2　Dynamic indices of *P. yunnanensis* population

龄级 Age class	种群动态指数 Dynamic index	动态指数值/% Dynamic index value	龄级 Age class	种群动态指数 Dynamic index	动态指数值/% Dynamic index value
Ⅰ	V_1	-80.89	Ⅹ	V_{10}	42.86
Ⅱ	V_2	29.30	Ⅺ	V_{11}	-20.00
Ⅲ	V_3	58.56	Ⅻ	V_{12}	20.00
Ⅳ	V_4	2.17	XIII	V_{13}	100.00
Ⅴ	V_5	46.67	XIV	V_{14}	-100.00
Ⅵ	V_6	4.17	XV	V_{15}	50.00
Ⅶ	V_7	56.52		V_{pi}	28.29
Ⅷ	V_8	40.00		V'_{pi}	1.77
Ⅸ	V_9	-14.29		P_{max}	6.25

V_1—V_{15}:相邻龄级数量动态变化指数 quantitative dynamics index of adjacent age class;V_{pi}:种群数量动态变化指数 quantitative dynamics index of population;V'_{pi}:受外界干扰时种群数量动态变化指数 quantitative dynamics index of population by external interference;P_{max}:随机干扰风险概率极大值 the maximum of probability in random disturbance

2.2　静态生命表

随龄级的增加,云南松匀滑处理后的存活数(a_x^*)和标准化存活数(l_x)逐渐减小(表 3)。云南松种群的

表 3　云南松种群静态生命表

Table 3　Static life table of *P. yunnanensis* population

龄级 Age class	胸径/cm Diameter at breast height	组中值/cm Mid-value of class	a_x	a_x^*	l_x	$\ln l_x$	d_x	q_x	L_x	T_x	e_x	K_x	S_x
Ⅰ	0—5	2.5	30	141	1000	6.908	298	0.298	851	2911	2.911	0.354	0.702
Ⅱ	5—10	7.5	157	99	702	6.554	298	0.424	553	2060	2.934	0.552	0.576
Ⅲ	10—15	12.5	111	57	404	6.002	78	0.193	365	1507	3.728	0.214	0.807
Ⅳ	15—20	17.5	46	46	326	5.788	7	0.022	323	1142	3.500	0.022	0.978
Ⅴ	20—25	22.5	45	45	319	5.766	149	0.467	245	819	2.567	0.629	0.533
Ⅵ	25—30	27.5	24	24	170	5.137	7	0.042	167	574	3.375	0.043	0.958
Ⅶ	30—35	32.5	23	23	163	5.094	92	0.565	117	408	2.500	0.833	0.435
Ⅷ	35—40	37.5	10	10	71	4.262	14	0.200	64	291	4.100	0.223	0.800
Ⅸ	40—45	42.5	6	8	57	4.038	7	0.125	53	227	4.000	0.134	0.875
Ⅹ	45—50	47.5	7	7	50	3.905	7	0.143	46	174	3.500	0.154	0.857
Ⅺ	50—55	52.5	4	6	43	3.751	7	0.167	39	128	3.000	0.182	0.833
Ⅻ	55—60	57.5	5	5	35	3.568	7	0.200	32	89	2.500	0.223	0.800
XIII	60—65	62.5	4	4	28	3.345	7	0.250	25	57	2.000	0.288	0.750
XIV	65—70	67.5	0	3	21	3.058	7	0.333	18	32	1.500	0.405	0.667
XV	70—75	72.5	2	2	14	2.652	7	0.500	11	14	1.000	0.693	0.500
XVI	75—80	77.5	1	1	7	1.959	7	1.000	4	4	0.500	1.959	—

a_x:x 龄级内树木现存个体数 individual number of age class x;a_x^*:匀滑处理后的存活数 the correction value of a_x;l_x:标准化存活个体数 standardized survivors number;$\ln l_x$:标准化存活个体数的自然对数 the natural logarithm of l_x;d_x:相邻龄级间隔期内树木标准化死亡数 the standardized number of death individuals from age class x to age class $x+1$;q_x:相邻龄级间隔期内树木死亡率 mortality from age class x to age class $x+1$;L_x:从 x 进入 $x+1$ 龄级间隔期间还存活的树木个体数 the number of surviving individuals from age class x to age class $x+1$;T_x:x 龄级到超过 x 龄级的树木个体总数 the total individuals number of age class x and age class elder than x;e_x:进入 x 龄级后树木个体的生命期望 life expectancy of individuals in the age class x;K_x:消失率 vanish rate;S_x:存活率 survival rate

死亡率(q_x)和消失率(K_x)反映了该种群随龄级增加的动态特征。死亡率和消失率在第Ⅰ—Ⅷ龄级存在较大的波动性,第Ⅱ、第Ⅴ、第Ⅶ龄级均出现了峰值。死亡率和消失率从第Ⅸ龄级开始,随着龄级的增加逐渐增长,在第 XVI 龄级处出现最大值。生命期望(e_x)反映 x 龄级内植株个体的平均生存能力,云南松种群生命期望最高的 3 个龄级依次为第Ⅵ、第Ⅸ和第Ⅲ龄级,从第Ⅷ—XVI 龄级的生命期望逐渐降低,第 XVI 龄级生命期望值最低。存活率(S_x)在第Ⅱ、第Ⅴ和第Ⅶ龄级较低,分别为 0.576、0.533 和 0.435,在第Ⅸ—XV 龄级期间,存活率随着龄级的增大逐渐减小。

2.3 存活曲线

云南松种群存活曲线呈现随龄级增大而下降的趋势(图 2)。第Ⅴ—Ⅵ龄级及Ⅶ—Ⅷ龄级下降幅度最大,仅有 13.33%的云南松能从第Ⅴ龄级穿过该环境筛到达第Ⅷ龄级。通过第Ⅷ龄级之后,云南松种群存活曲线趋于平缓。

存活曲线拟合模型结果显示,指数函数的 F 值(264.547)及相关系数 R(0.975)均大于幂函数的 F 值(48.326)及相关系数 R(0.881)(表 4)。因此,云龙天池保护区的云南松种群更趋向于 Deevey-Ⅱ型(对角线型)。Deevey-Ⅱ型有三个亚型(B_1,B_2和 B_3亚型),结合图 2 可以看出,该存活曲线属于其中的 B_1亚型,表明各龄级的存活数围绕对角线产生波动,并且各个龄级的存活数相差较大。

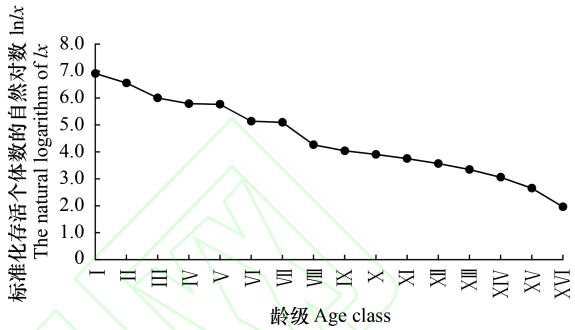


图 2 云南松种群存活曲线

Fig.2 Survival curve of *P. yunnanensis* population

表 4 存活曲线拟合模型

Table 4 Survival curve fitting model

存活曲线 Survival curve	拟合模型 Fitting model	拟合结果 Fitting result	F	R
Deevey-Ⅱ	$N_x = N_0 e^{-bx}$	$N_x = 7.823e^{-0.072x}$	264.547	0.975
Deevey-Ⅲ	$N_x = N_0 x^{-b}$	$N_x = 8.997x^{-0.391}$	48.326	0.881

N_x:匀滑后 x 龄级内存活数 the correction value of a_x;N_0:种群形成初期的个体存活数 number of surviving individuals in the early formation stage of population

2.4 生存分析

云南松的生存率随龄级增大而降低,累计死亡率随龄级增大逐渐增大(图 3)。在Ⅰ—Ⅶ龄级,种群生存率和累计死亡率变化较大,Ⅶ—XVI 龄级生存率和死亡率趋于稳定(图 3)。第Ⅰ龄级的生存率为 0.702,第Ⅶ龄级迅速降低至 0.071;第Ⅰ龄级的累计死亡率为 0.298,到第Ⅶ龄级迅速增加为 0.929。第Ⅶ—XVI 龄级云南松种群的生存率和累计死亡率的增减变缓,大量个体由于到达生理年龄而死亡,种群呈现衰退特征,说明该阶段云南松种群进入生理衰老阶段。

由图 4 可知,云南松种群的死亡密度在第Ⅰ—Ⅶ龄级存在波动,之后较平稳降低。危险率呈递增状态,第Ⅰ—Ⅶ龄级增长较快,第Ⅷ—XVI 龄级增长缓慢。综上,云南松种群第Ⅰ—Ⅶ龄级生命状态不稳定,死亡可能性较大,种群个体数量减少,第Ⅷ—XVI 龄级表现出衰退的特点。这与静态生命表、存活曲线的分析结果是相符的。

2.5 谱分析

谱分析结果显示,振幅值呈现逐渐减小的趋势,其中基波 A_1最大,说明云南松种群的具有明显的大周期(表 5)。基本的周期长度为种群本身所固有,表明云南松种群数量动态变化主要受生物学特征所控制。

此外,云南松种群除了受基波的影响外,也表现出了其中迭加了明显的小周期波动,即 A_6和 A_8。A_6对应的周期为基本周期的 1/6,胸径为 5 × 16/6 = 13.3 cm,对应第Ⅲ龄级;A_8对应的周期为基本周期的 1/8,胸径为

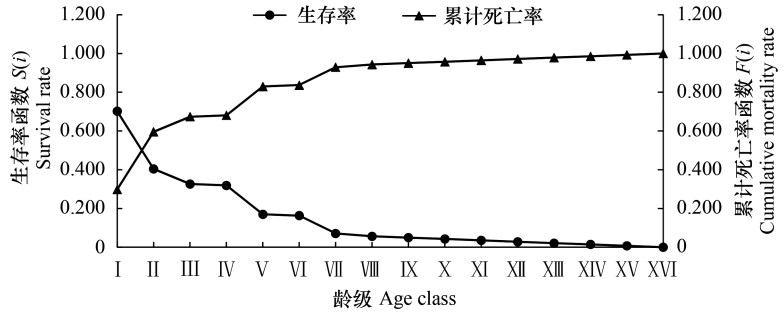


图 3　云南松种群生存率函数 $S(i)$ 和累计死亡率函数 $F(i)$ 曲线

Fig.3　Survival rate $S(i)$ and cumulative mortality rate $F(i)$ functional curve of *P. yunnanensis* population

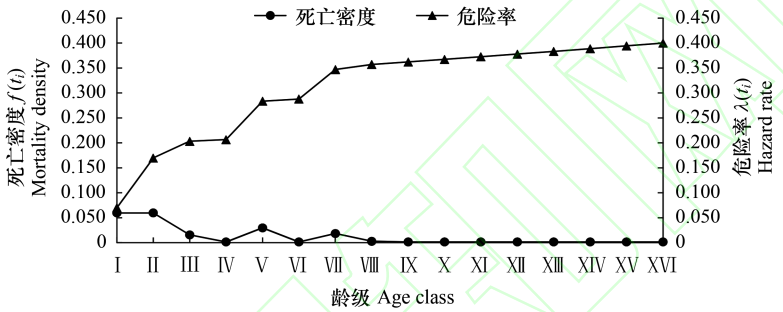


图 4　云南松种群死亡密度 $f(t_i)$ 和危险率 $\lambda(t_i)$ 曲线

Fig.4　Mortality density $f(t_i)$ and hazard rate $\lambda(t_i)$ functional curve of *P. yunnanensis* population

5 × 16/8 = 10 cm,也对应第Ⅲ龄级。可见,第Ⅲ龄级(胸径 10—15 cm)是影响云南松种群波动的重要阶段。

表 5　云南松种群的周期性波动

Table 5　Periodic fluctuation of *P. yunnanensis* population

谐波 Harmonic	A_1	A_2	A_3	A_4	A_5	A_6	A_7	A_8
振幅值 Amplitude value	1.613	0.836	0.552	0.205	0.147	0.341	0.209	0.291

A_1:基波振幅 fundamental amplitude;A_2—A_8:各谐波振幅 amplitude of each harmonic

3　讨论

云龙天池自然保护区云南松种群的年龄结构表明其是增长型种群。种群的年龄结构可以作为判断种群是否衰退的依据。一般认为,具有间歇型和倒 J 型的种群更新良好[22—23]。本研究中云南松种群胸径级结构属于倒 J 型的种群,这与黄小波等[8]和 Tang 等[9]的研究结果一致。云南松种群数量动态指数 V_{pi} 和 V'_{pi} 大于 0,也表明该云南松种群属于增长型种群。调查中发现云南松林下幼苗较少,但这不能说明种群是衰退的。种源和更新方式是影响种群持续的主要因素。云南松进入成熟期后每年能够产生大量的种子,不缺少种源[24]。云南松可以通过林窗、林下实现更新,也能在火烧后迅速长出幼苗[4, 25]。因此,云南松是能持续更新的种群。另外,尽管本研究的样方中存在有常绿阔叶树种,如银木荷(*S. argentea*),但是银木荷在样方中的幼树仅仅 30 株,树高均在 20 m 以下,无论数量质量都远低于云南松。可见,云南松种群年龄结构为增长型,更新方式多样,种源充足,短期内不会出现衰退。

云龙天池自然保护区云南松种群也表现出低龄级的个体数量波动大,死亡率高的特点。结合静态生命表、存活曲线和生存分析函数也表明云南松种群在第Ⅰ—Ⅶ龄级有较为明显的波动。通过谱分析进一步确

认,低龄级的胸径 10—15 cm 云南松个体之间存在强烈的竞争。之所以出现这样的现象是因为云南松中幼林密度较大且处于生长旺盛的阶段,对资源与空间的需求较大[26]。云南松是喜光树种,光照是其竞争的主要资源。金振洲等[4]认为,随着云南松的生长及林分的郁闭,缺乏光照会使得长势较差的个体枯死。云南松幼龄林的数量波动是种群通过自疏剔除冗余的过程,也是种群维系的重要方式[27]。

云南松林是暖温性针叶林的典型代表,主要分布于滇中高原山地。本研究选择在云南云龙天池自然保护区进行,该保护区的保护对象是云南松种群,为进行云南松种群结构研究提供了典型试验场所。对长寿命的木本植物的种群数量动态研究主要以“空间推时间”的方法,编制静态生命表进行[13, 28—32]。树木静态生命表的编制需要提供树木的确切年龄,生长锥钻取胸高部位的树芯和 WinDENDRO 年轮分析系统分析树轮[33]是常用的测定树木的准确年龄的方法。但是这些方法操作复杂,在野外调查中制约了工作效率提高。树木的年龄和胸径、地径及树高存在正相关关系[34],实际工作中常常用胸径、地径及树高代替树木年龄[35]。Tang 等[9]对该保护区内云南松的年龄和胸径进行拟合后发现该区域云南松的年龄和胸径存在显著的线性相关关系。因此,本研究用云南松的胸径代替年龄进行种群年龄结构的研究是可行的。

4 结论

云南云龙天池自然保护区云南松种群是能够自我更新的种群,种群动态呈现出低龄级个体数量波动大,高龄级衰退的特点。建议在云南松林的幼龄林和中龄林适当采取抚育间伐措施促进其种群的生长发育及更新,维持种群的稳定性。

致谢:感谢云南云龙天池国家级自然保护区管护局在研究工作中的支持以及管护局工作人员杨桂天在野外调查采样中予以的帮助。

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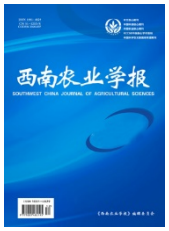
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西南农业学报
Southwest China Journal of Agricultural Sciences
ISSN 1001-4829,CN 51-1213/S

《西南农业学报》网络首发论文

题目：基于 SSR 标记的花菜类作物遗传多样性及群体结构分析
作者：卢俊浩，张婷，张鹏，木万福，杨龙，张建华，黄晓蓉，寸婕，管俊娇
收稿日期：2021-07-05
网络首发日期：2022-07-22
引用格式：卢俊浩，张婷，张鹏，木万福，杨龙，张建华，黄晓蓉，寸婕，管俊娇．基于 SSR 标记的花菜类作物遗传多样性及群体结构分析[J/OL]．西南农业学报．https://kns.cnki.net/kcms/detail/51.1213.S.20220721.1135.056.html



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网络首发时间：2022-07-22 11:44:45
网络首发地址：https://kns.cnki.net/kcms/detail/51.1213.S.20220721.1135.056.html

投稿日期：2021－07－05

基金项目：云南省科技人才和平台计划（2017HB087）；云南省科技重大专项（2019ZG001）

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基于 SSR 标记的花菜类作物遗传多样性及群体结构分析

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摘　要：【目的】为了解花菜类种质的遗传基础，分析种质群体的亲缘关系，为花菜育种提供参考。【方法】选用 22 对 SSR 引物，利用毛细管电泳荧光检测技术对 96 份花菜类种质材料进行遗传多样性分析，根据 Nei’s 遗传距离和非加权配对法（UPGMA）进行聚类分析以及以 Structure 软件的混合模型聚类法推断材料构成群体的遗传结构。【结果】22 对引物在 96 份材料中共扩增出 91 个等位位点，平均每对引物为 4.136 个。从 22 对引物中筛选出了 10 对条带清晰、稳定性好的多态性引物，可用于花菜类作物种质资源的鉴定和研究。根据花球性状，将材料分为青花菜（24 份）以及花椰菜（72 份）2 个组群，经多态性分析，2 个组群的观察等位基因数值（*Na*）分别为 2.727 和 3.091；有效等位基因数（Ne）分别为 1.910 和 1.841；Shannon′s 指数（*I*）分别为 0.675 和 0.667，说明这 2 个组群的遗传多样性均不够丰富。聚类分析也将 96 份材料分为 2 个组群，第Ⅰ组为 24 份青花菜材料，包括 1 份西兰苔杂交种和 23 份青花菜杂交种；第Ⅱ组为 72 份花椰菜材料，包括 29 份花椰菜自交系、36 份花椰菜杂交种和 7 份花椰菜主栽品种。花椰菜自交系被分到了 4 个亚群，但在各亚群中材料之间的遗传距离较小，杂交组配时，选用不同亚群的自交系进行组配，以获得更大的杂交优势。群体结构分析中，当 K=2 时$\triangle K$ 出现明显的峰值，最大值为 1658.48，为最佳群体组群数，与聚类分析结果基本一致。且 96 份材料的 Q 值均大于 0.6，说明该花菜资源的遗传结构较为单一。【结论】所参试材料种质群体内遗传多样性不够丰富，遗传背景单一，亟需重视对遗传背景差异大、亲缘关系远的种质资源的发掘利用，加强种质材料共享和交流，促进花菜种业发展。

关键词：花菜；SSR 标记；遗传多样性；群体结构

中图分类号：S635　　文献标识码：A

Analysis of genetic diversity and population structure of cauliflower crops based on SSR markers

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Abstract:【Objective】This study aimed to understand the genetic basis of cauliflower germplasm and analyze the genetic relationship of cauliflower germplasm, and provide reference for cauliflower breeding.【Method】 22 pairs of SSR primers were selected, and the genetic diversity of 96 cauliflower germplasm materials was analyzed by capillary electrophoresis fluorescence detection technology. Furthermore, cluster analysis model was performed according to the Nei's genetic distance and unweighted pairing method (UPGMA), the genetic structure of population material was inferred by the mixed model clustering method of Structure software. 【Result】A total of 91 alleles were amplified from 22 primer pairs in 96 materials with an average of 4.136 alleles per primer pair. 10 pairs of polymorphic primers with clear bands and good stability were screened from 22 pairs of primers and used to identify and investigate the cauliflower germplasm resources. In light of the characters of flower head, the materials were divided into two groups: broccoli (24 samples) and cauliflower (72 samples). Furthermore, the polymorphism analysis showed that the observed allele values (*Na*) of the two groups were 2.727 and 3.091, respectively, and the effective alleles numbers (*Ne*) were 1.910 and 1.841, respectively, indicating that the genetic diversity of those two groups was not rich enough. According to the cluster analysis, 96 cauliflower materials were divided into two subgroups. The Ⅰ subgroup consisted of 24 broccoli materials, including 1 broccoli moss hybrid and 23 broccoli hybrids. Meanwhile, the Ⅱ subgroup consisted of 72 cauliflower materials, including 29 cauliflower inbred lines, 36 cauliflower hybrids, and 7 cauliflower main varieties. The cauliflower inbred lines were divided into four groups, however, the genetic diversity was narrowed in each group material. Hence, the inbred lines of different groups should be selected for gaining the greater heterosis in the term of cross combination. In the group structure analysis, when the *K* value equalled 2, $\triangle K$ showed an obvious peak with maximum value of 1658.48, which was the optimal number of groups, which was basically consistent with the

cluster analysis. Moreover, the Q values of 96 materials were all more than 0.6, indicating that the genetic structure of the cauliflower resource was relatively simple. 【Conclusion】The genetic diversity of the tested materials in the germplasm population was not rich enough, and the genetic background was simple. Therefore, it was necessary to pay attention to the exploration and utilization of germplasm resources with different genetic backgrounds and distant relationships, and strengthen the sharing and exchange of germplasm materials for promoting the development of the cauliflower seed industry.

Key words: *Brassica oleracea*；SSR molecular marker; Genetic diversity; Population structure

【研究意义】花菜类作物包括青花菜（var. *italic* Plenck）、花椰菜（var. *botrytis* L.）和芥蓝（var. *alboglabra*）、结球甘蓝（var. *capitata* L.）等，由甘蓝野生种经过自然和人工的双重选择形成的不同的栽培类型[1]。该类作物起源于欧洲的地中海沿岸地区，19 世纪起中国从国外引进品种，经多年驯化栽培，已育出若干地方特色品种[2]。由于花椰菜和青花菜含有丰富的维生素、矿物质、粗纤维等营养物质及独特的保健功能，多年来倍受消费者的青睐，其栽培面积不断加大，在蔬菜市场上占有越来越重要的地位[3]。种质资源是遗传育种的基础。尽管目前中国是世界上最大的花椰菜和青花菜的生产国和消费国，但是拥有的种质资源非常匮乏，优良新品种的选育进程有一定程度滞缓[4]，亟需加强花菜类作物品种资源和材料的共享、交流和评价，重视对遗传背景差异大、亲缘关系远的种质资源的发掘利用，对促进花菜类作物种业的发展有重要指导意义。

【前人研究进展】前人利用不同分子标记技术和不同来源结构的种质材料群体，导致对花菜类蔬菜的分析结果一致性不高，如姚雪琴等[5]利用 SRAP 技术分析花椰菜遗传多样性及与其近缘种亲缘关系，得到花椰菜和宝塔花菜亲缘关系最近，青花菜次之，芥蓝最远；熟性是影响花椰菜品种资源类群的主要因素。刘运霞[6]等利用形态学标记和分子标记相结合的方法，将 78 份花椰菜材料按照熟性和花球性状进行了分类，为花椰菜育种提供理论依据。【本研究切入点】SSR 分子标记技术是一种具有操作简单、重复性好、位点特异稳定性强，并且为共显性遗传，能在近缘种属之间通用等优点的 DNA 分子标记技术，已被广泛应用于玉米[7]、水稻[8]、马铃薯[9]、小麦[10]等大田作物的遗传多样性及群体结构的研究，使新品种选育效率有较大提高。目前国内花菜种质资源匮乏，遗传多样性狭窄，主栽品种依赖进口，亟需加快优良新品种的选育进程。【拟解决的关键问题】本研究采用 SSR 标记对 96 份花菜类作物种质材料的遗传多样性和群体结构进行分析，以期揭示花菜品种间的遗传差异和亲缘关系，为提高优异种质资源利用效率和杂交亲本的选配提供参考。

1 材料与方法

1.1 试验材料

试验材料选取花椰菜、青花菜品种材料共 96 份（表 1）。其中，花椰菜材料有 72 份，包括 7 份主栽品种材料（NO.30、NO.47、NO.48、NO.49、NO.63、NO.67、NO.69）、29 份松花菜亲本自交系和 36 份松花菜杂交一代材料。青花菜材料有 24 份。

表 1　96 份供试材料基本信息

Table 1 The basic information of 96 materials

编号 NO.	材料名称 Varieties	材料类型 Type	材料性质 Properties	编号 NO.	材料名称 Varieties	材料类型 Type	材料性质 Properties
1	W131	松花菜	杂交一代	49	亚非 100	松花菜	杂交一代
2	1519	松花菜	杂交一代	50	贝绿美	西兰苔	杂交一代
3	ms1750	白花菜	自交系	51	锦翠 2 号	青花菜	杂交一代
4	C18232	松花菜	自交系	52	亚松 20010	松花菜	杂交一代
5	18115	青花菜	杂交一代	53	亚松 20002	松花菜	杂交一代
6	C18222	松花菜	自交系	54	亚松 20003	松花菜	杂交一代
7	C18203	松花菜	自交系	55	翡翠 11 号	青花菜	杂交一代
8	1518	松花菜	杂交一代	56	绿辉 6 号	青花菜	杂交一代
9	C18208	松花菜	自交系	57	MKS-1307	青花菜	杂交一代
10	1808	松花菜	杂交一代	58	NHYX 耐寒优秀	青花菜	杂交一代
11	MS048-1-2XC18222	松花菜	自交系	59	TRN 三月鲜	青花菜	杂交一代
12	C18243	松花菜	自交系	60	时田绿雄 90	青花菜	杂交一代
13	C18245	松花菜	自交系	61	福蝶	青花菜	杂交一代
14	C18190	松花菜	自交系	62	浙青 75	青花菜	杂交一代
15	B17183	松花菜	自交系	63	祥云 90	松花菜	杂交一代
16	C18210	松花菜	自交系	64	时田美青	青花菜	杂交一代
17	C18269	松花菜	自交系	65	圣尼斯 强汉	青花菜	杂交一代
18	C18246	松花菜	自交系	66	雅翠 91	青花菜	杂交一代
19	女神	青花菜	杂交一代	67	松 s100	松花菜	杂交一代
20	MS042-1-2XC18211	松花菜	自交系	68	NS 女神	青花菜	杂交一代
21	C1713-1 无蜡质	松花菜	自交系	69	YF75	松花菜	杂交一代
22	C1713-1 有蜡质	松花菜	自交系	70	亚松 20004	松花菜	杂交一代
23	MS048-1-2	松花菜	自交系	71	亚松 20005	松花菜	杂交一代
24	C18247	松花菜	自交系	72	亚松 20006	松花菜	杂交一代

25	C18205	松花菜	自交系	73	亚松 20007	松花菜	杂交一代
26	B1750	紧花菜	自交系	74	亚松 20008	松花菜	杂交一代
27	C18220	松花菜	自交系	75	亚松 20009	松花菜	杂交一代
28	C1713-2 无蜡质	松花菜	自交系	76	松 LZZ JB-S-020	松花菜	杂交一代
29	C18199	松花菜	自交系	77	松 LZZ2008	松花菜	杂交一代
30	亚菲 75	松花菜	杂交一代	78	松 LZZ JB026	松花菜	杂交一代
31	C18225	松花菜	自交系	79	松 1907	松花菜	杂交一代
32	C18218	松花菜	自交系	80	松 1942	松花菜	杂交一代
33	C18336	松花菜	自交系	81	松 2166	松花菜	杂交一代
34	1402	松花菜	杂交一代	82	松 2169	松花菜	杂交一代
35	C1713-3	松花菜	自交系	83	松 LZZ 2046	松花菜	杂交一代
36	C18225	松花菜	自交系	84	松 LZZ 2090	松花菜	杂交一代
37	W133	松花菜	杂交一代	85	松 LZZ 2076	松花菜	杂交一代
38	C18520	松花菜	自交系	86	亚松 19001	松花菜	杂交一代
39	坂田喜鹊	青花菜	杂交一代	87	亚松 16001	松花菜	杂交一代
40	台绿 3 号	青花菜	杂交一代	88	亚松 15006	松花菜	杂交一代
41	17-2305 泷井	青花菜	杂交一代	89	松 1947	松花菜	杂交一代
42	美之奥 扭扭	青花菜	杂交一代	90	松 1821	松花菜	杂交一代
43	绿辉 7 号	青花菜	杂交一代	91	松 2170	松花菜	杂交一代
44	冷翠	青花菜	杂交一代	92	松 1963	松花菜	杂交一代
45	浙青 80	青花菜	杂交一代	93	松 LZZ JB042	松花菜	杂交一代
46	耐寒优秀	青花菜	杂交一代	94	松 LZZ 923	松花菜	杂交一代
47	强雪	白花菜	杂交一代	95	松 2167	松花菜	杂交一代
48	庆扬 90	松花菜	杂交一代	96	松 LZZ JB059	松花菜	杂交一代

1.2 DNA 的提取

采用改良 CTAB 法[11]提取植株幼嫩叶片总 DNA，分别用琼脂糖凝胶电泳和紫外分光光度计进行 DNA 纯度、质量和浓度检测，稀释 DNA 浓度至 20~30 ng/μL，作为 SSR 电泳的模板。

1.3 引物的设计和筛选

依据前人研究公布的甘蓝类微卫星遗传标记[12－16]，经过初筛，在每条染色体上均匀选择 1~3 对，最终共筛选 22 对 SSR 荧光标记引物（表 2）用于遗传多样性检测。引物由上海擎科生物有限公司提供。

表 2　22 对 SSR 引物基本信息

Table 2 Primers used for SSR analysis

序号 NO.	引物名称 Primer	染色体 Chromosome	正向引物序列（5′–3′） Forward primer sequence（5′–3′）	反向引物序列（5′–3′） Reverse primer sequence（5′–3′）
1	HCSSR1	–	TTCTGACACGGTGACACCTC	AGACGATAACCGAGTGGTGG
2	BoSF2564	C9	TTGCTTTTGCTTCTGGGTTT	TGTTTGTGTCCGAAATCACG
3	BoE607	C1	TCTATTCACAACGATTCAACTAAC	CGGTACGGCTGGCTCTT
4	BoE718	C3	CAAGAAACGGACGTGGTGAAAG	TCTCGCGTATGGGGCTGTCT
5	BoE450	C4	TCTCGCCATGGCTGATAAG	TCGGGGCGTTGATTCTCGTCTCT
6	BoE699	C5	TCCCCACCCCCAAAAAGAGA	AACGAGCCATCCGAAGAAGAGG
7	BoE761	C6	CATTCAGCGACTTCCTTCAAACTT	GGCGCACTTCTTCCCCTGTA
8	BoE723	C7	CGTTGAGGCCGAGAGTGAGAG	ATGGACGCCGGAAATGAGAA
9	BoE134	C8	CTCTTATTTCTTGTAGGGCTTTTA	CCGTTGGAGATGACTGACTG
10	BoE051	C9	GAGTCTTCGTCTTCTTCTTCC	AGTCGCCATTATTAACACCTCTA
11	BoGMS1322	C1	CTCTCCAATCCTTCTTCTCAC	CCACCTTCTCCACTAATAACC
12	BoGMS1119	C2	ACTGGAGTTTCAATTGGATG	CATCATCTTCAGCACTAGCA
13	BoGMS2431	C3	CAACCACCGACTCAAATACAGA	GCACCTGCAATCTCCATACATA
14	BoGMS0501	C4	ATGATGAGTTTGCTCGTTAGG	AAATCCTTCCTCCTTTCAC
15	BoGMS1004	C5	ATGTATCTCAGCAGCCCACAA	CAATCACAACAGACGTACATGC
16	BoGMS0941	C6	GTTGAAGAAACTAAGGAGGAAA	GAACGACAGCGAAGAGAG
17	BoGMS1530	C6	TTTGGGCAGGTCATAAATAG	GAGATTAGATTCCCTCACC
18	BoGMS1009	C7	CGAAACCAGGATAAGTCA	CAATGCTTCTGTATGCGTC
19	BoGMS0808	C8	GTCTCCTCCACCATTATCTTT	GACCTCGTGTTTCCTTGA
20	BoGMS0624	C9	AAGACGAAGTCAAGTCAAGGT	CGTATCATCCAGAGTATCCAG
21	Ol11G11	–	GTTGCGGCGAAACAGAGAAG	GAGTAGGCGATCAAACCGAG
22	Na10D03	–	ATGATTTGCCTTGAAATGCC	GATGAAACAATAACCTGAGACACAC

1.4 PCR体系与毛细管电泳荧光检测

PCR 总反应体系为 10 μL，包括 2×mix 混合液 5 μL、10 mol/L 正向引物 0.3 μL、10mol/L 反向引物 0.3 μL、样品 DNA 1 μL、超纯水 3.4 μL。PCR 扩增程序为 94 ℃预变性 5 min；94 ℃变性 45 s，55 ℃退火 45 s，72 ℃延伸 1 min，循环 30 次，72 ℃下延伸 10 min，4 ℃保存。

扩增产物在毛细管荧光电泳系统 AB3730XLDNA 分析仪（Applied Biosystems，USA）上检测。用 GeneMapper Ver.3.7（Applied Biosystems，USA）对原始数据进行统计和校正，读出每个样品各位点的等位变异片段大小数据。

1.5 数据统计与分析

利用软件 Popgen 32 和软件 PowerMarker 3.25 计算对应引物的观察等位基因数（*Na*），有效位点数（*Ne*）、Shannon′s 信息指数（*I*）、观测杂合度（*Ho*）、期望杂合度（*He*）、多态性指数（*PIC*）、基因多样性（*GD*）和 Nei's 遗传距离，并按非加权配对法（UPGMA）进行聚类分析。分析结果使用在线软件 iTOL（https://itol.embl.de/）进行优化。以软件 Structure 2.3.4 推断材料构成群体的遗传结构，采用混合模型设置 *K* 值范围为 1~10，每个 K 值运算重复 10 次，将 MCMC（Markov Chain Monte Carlo）设置 10000 次迭代，初始 burn‐in 次数为 50000 次，计算 *Q* 值。参考 Evanno 等[17]的统计模型进行分析计算$\triangle K$，由$\triangle K$来确定最优群体数 *K*。

2 结果与分析

2.1 SSR标记多态性分析

毛细管电泳检测结果显示，22 对 SSR 引物在 96 份材料的 DNA 样品中共扩增出 91 个等位位点，平均每对引物为 4.136 个位点（表 3）。96 份材料的群体有效等位基因数（*Ne*）变异范围在 1.043~3.489，平均为 2.246；Shannon′s 信息指数（*I*）和基因多样性（*GD*）的变异范围分别为 0.101~1.720 和 0.041~0.766，平均值分别为 0.909 和 0.496；观测杂合度（*Ho*）和期望杂合度（*He*）的变异范围分别为 0~0.573 和 0.041~0.766，平均值分别为 0.278 和 0.496；22 对引物扩增产物的多态性信息含量（*PIC*）值变异范围在 0.040~0.735，平均值为 0.443，其中 *PIC* 值大于 0.5 的高多态性引物有 10 对，表明该 10 对引物在 96 份材料群体中的扩增多态性较高，可用于花菜类种质资源的鉴定和研究。

表 3　22 对 SSR 标记引物遗传信息统计

Table 3 Genetic information statistics of 22 pairs of SSR Markers

引物 Primers	观察等位基因数 *Na*	有效等位基因数 *Ne*	Shannon′s 指数 *I*	观测杂合度 *Ho*	期望杂合度 *He*	基因多样性 *GD*	多态性信息含量 *PIC*
BoE051	3	1.359	0.502	0.177	0.264	0.264	0.242
BoE450	3	1.721	0.649	0.021	0.419	0.419	0.340
BoGMS0624	7	3.450	1.441	0.573	0.710	0.710	0.670
BoGMS1004	5	1.659	0.740	0.094	0.397	0.397	0.352

BoE607	3	2.738	1.046	0.458	0.635	0.635	0.557
BoE723	3	2.341	0.968	0.198	0.573	0.573	0.510
BoGMS1009	6	2.277	1.146	0.188	0.561	0.561	0.526
BoGMS2431	4	2.336	1.018	0.333	0.572	0.572	0.513
BoE134	2	1.546	0.538	0.000	0.353	0.353	0.291
BoE718	6	1.953	1.030	0.135	0.488	0.488	0.463
BoGMS050	5	2.061	1.072	0.365	0.515	0.515	0.490
BoGMS0808	5	3.489	1.417	0.396	0.713	0.713	0.674
BoE761	4	2.968	1.232	0.442	0.663	0.663	0.615
BoGMS1119	3	1.318	0.475	0.156	0.241	0.241	0.225
BoGMS1530	10	4.272	1.720	0.398	0.766	0.766	0.735
BoSF2564	4	2.264	0.920	0.365	0.558	0.558	0.468
BoE699	2	1.043	0.101	0.000	0.041	0.041	0.040
BoGMS0941	5	2.853	1.224	0.552	0.650	0.650	0.588
BoGMS1322	4	1.245	0.387	0.219	0.197	0.197	0.181
HCSSR1	2	1.999	0.693	0.146	0.500	0.500	0.375
Na10D03	2	1.863	0.656	0.417	0.463	0.463	0.356
OI11G11	3	2.657	1.027	0.490	0.624	0.624	0.544
Mean	4.136	2.246	0.909	0.278	0.496	0.496	0.443

2.2 群体的遗传多样性分析

根据 96 份种质材料的花球性状，将其分为 2 个组群，即组群 1：青花菜和西兰苔（24 份）和组群 2：花椰菜（72 份）。对 2 个组群 SSR 标记多态性进行分析，结果显示（表 4），组群 1 和组群 2 的观察等位基因数值（*Na*）分别为 2.727 和 3.091；有效等位基因数（*Ne*）分别为 1.910 和 1.841；Shannon′s 指数（*I*）分别为 0.675 和 0.667。表明经过高强度的人工选择，花菜类作物 2 个组群的遗传多样性均不够丰富。

表 4 群体材料的遗传多样性

Table 4 Genetic diversity among the two populations

群体 Populations	观察等位基因数 *Na*	有效等位基因数 *Ne*	Shannon′s 指数 *I*
青花菜 Broccoli	2.727	1.910	0.675
花椰菜 Cauliflower	3.091	1.841	0.667

2.3 聚类分析

利用软件 PowerMarker 计算 Nei's 遗传距离，按非加权配对法（UPGMA）进行聚类分析。结果显示全部材料可划分为 2 个组群（图 1），第Ⅰ组为 1 份西兰苔材料和 23 份青花菜材料。西兰苔来源于青花菜与芥蓝的杂交后代，亲缘关系较近，被聚到同一组内。其中"女神"（NO.19）与"NS 女神"（NO.68）遗传距离最小仅为 0.0080，为不同来源的同一品种；其次是"翡翠 11 号"（NO.55）和"雅翠 91"（NO.66）遗传距离为 0.0130；"贝绿美"（NO.50）和"祥云 90"（NO.63）的遗传距离最大，为 0.6896。

第Ⅱ组包括全部的花椰菜材料。作为亲本的 29 份自交系分别被聚到了 4 类亚群，第 1 类仅 1 份材料 NO.17，第 2 类中有 11 份材料，第 3 类中有 4 份，第 4 类中有 7 份。在各类中的自交系材料彼此之间的遗传距离较小，如 NO.21 与 NO.22 遗传距为 0.0418，为同一个品系分出来的不同材料，仅 1 个性状上存在差异，表现为一个叶片有蜡质，一个无蜡质。杂交亲本配组时，用不同类中的材料进行杂交，可获得杂种优势。材料 NO.17 与其他 28 份材料的亲缘关系较远，利用这个材料与其他材料杂交，更有可能获得突出的杂种优势。

综上，该 96 份花菜种质资源总体遗传多样性较低，但部分材料存在较大的遗传多样性，为供试材料在杂交亲本选配利用方面提供了参考。

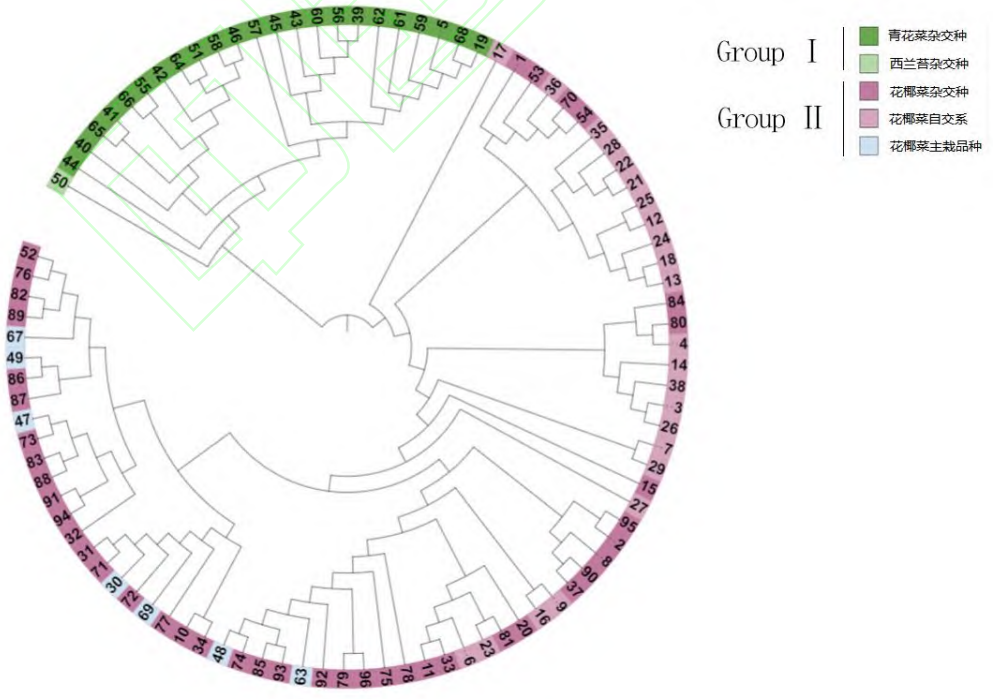


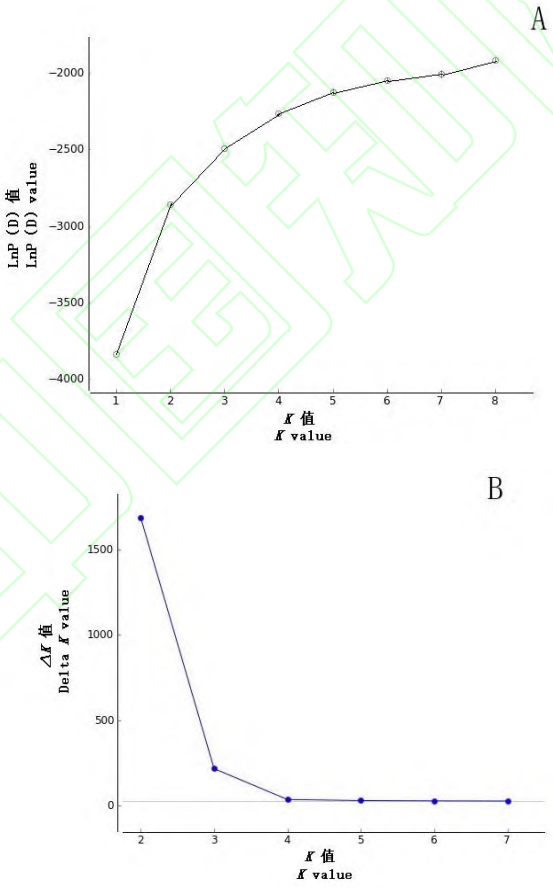
图 1　96 份材料聚类分析图

Fig. 1 Cluster analysis of 96 materials

2.4 群体遗传结构分析

利用均匀分布在花菜基因组 9 条染色体上的 22 对 SSR 标记引物对 96 份种质材料进行群体遗传结构分析。由图 2–A 和图 2–B 可以看出，随着 *K* 值的增加，LnP（D）值也持续增加，当 *K*=2 时，⊿*K* 出现明显的峰值，最大值为 1658.48，表明可以将 96 份花菜资源划分为 2 个组群（图 3）：第 1 组群（图 3 红色部分）共 24 份，包括 23 份青花菜杂交一代品种和 1 份西兰苔材料；第 2 组群（图 3 绿色部分）共 72 份，为全部的花椰菜材料。

群体百分比 Q 值越大，说明该资源材料遗传背景相对比较单一，否则认为该资源材料遗传背景相对较复杂[18]。群体结构的分析结果显示，96 份花菜材料的 Q 值均大于 0.6，说明该花菜资源的遗传结构较为单一，可以清楚的划分到不同组群中。群体结构分析结果与聚类分析的结果基本一致。



A：*K* 值与 LnP（D）的趋势变化图；B：*K* 值与⊿*K* 的趋势变化图

A: Trend diagram of *K* value with LnP (D); B: Trend of *K* value with ⊿*K*

图 2　*K* 值与 LnP（D）和⊿*K* 趋势变化图

Fig.2 The distribution of *K*-values with LnP（D）and *K* values with ⊿*K*

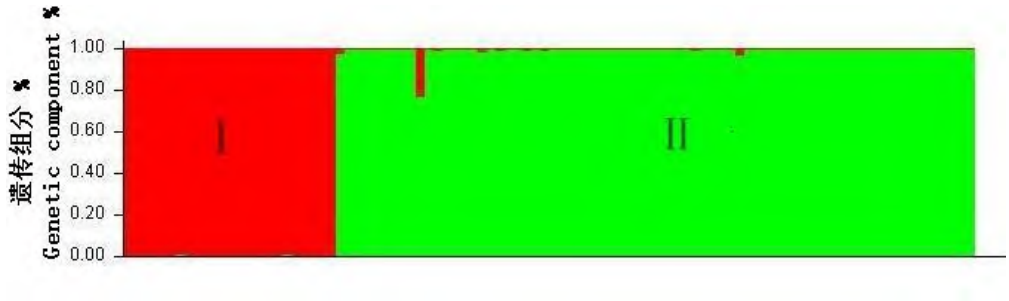


图 3　96 份材料遗传结构分析

Fig. 3 Genetic structure analysis of 96 materials

3 讨　论

3.1 SSR标记评价

随着分子标记技术的发展，分子标记技术在花菜类作物中得到应用，其中 SSR 标记是应用较多的技术之一[19]。本试验毛细管电泳检测结果显示，22 对引物在 96 份材料中共扩增出 91 个等位位点，平均每对引物为 4.136 个；22 对引物扩增产物的 *PIC* 值平均值为 0.443，其中大于 0.5 的高多态性引物有 10 对。一方面表明毛细管电泳检测的灵敏度较高，另一方面表明了所选引物在 96 份材料群体中的扩增多态性较高，可用于花菜类种质资源的鉴定和研究。前人的研究中，SSR 标记技术在玉米、水稻中的实际应用价值也得到了验证[20－21]。SSR 标记技术具有操作简便、多态性高、成本较低[22]，在种质遗传多样性研究中有广阔的应用前景。

3.2 不同种质材料的遗传多样性

种质资源是进行新品种选育的重要因素，遗传基础过于狭窄往往会制约种质创新。本研究中，西兰苔贝绿美（NO.50）来源于青花菜与芥蓝的杂交后代，与青花菜杂交种遗传距离更近，聚到青花菜组内。花椰菜杂交种“亚松 20007”（NO.73）、“亚松 20008”（NO.74）、“亚松 19001”（NO.86）分别与主栽品种“强雪”（NO.47）、“庆扬 90”（NO.48）、“亚非 100”（NO.49）之间的遗传距离较小，亲缘关系较近，相互渗透，具有相似的遗传背景。目前大部分的品种都由个别自交系培育而来，加上在人为选择和环境不适宜导致所选育出的品种遗传相似度较高，品种遗传多样性低，遗传差异小[23]。盛小光[15]等的研究表明，花椰菜和青花菜被清晰地分到了两个极端，两者之间没有交叉，群体内的遗传变异丰度低，是经过长期高强度的人工选择后，形成了相对较为独立的栽培类型；刘春晴[24]等将青花菜、松花菜、

紧花菜和芥蓝 145 份材料，按亲缘关系分成了 4 个类群，类群内品种间的遗传多样性较低，与本研究结果一致。因此，在今后的研究中，需要扩大群体的范围，尽可能挑选遗传背景不同、亲缘关系相对较远的材料作为亲本，提高杂种优势，选育出优良创新品种。也可以利用 SSR 标记构建花菜类作物种质资源的 DNA 指纹图谱，以更高效地区分该类作物地品系或品种，对杂交种纯度鉴定和品种保护等方面具有重要意义。

3.3 遗传距离聚类和Structure模型聚类结果比较

种质遗传聚类方法有多种，包括遗传遗传距离法、相似系数法、模型聚类法等，选择不同的方法也会导致分析结果不同[25]。本研究通过 Nei’s 遗传距离按非加权配对法（UPGMA）将 96 份花菜材料划分为 2 个组群，分别包括 24 份青花菜和 72 份花椰菜材料，从聚类分析图上来看，花椰菜和青花菜被清晰地分到了两个组群，进一步说明了这两个组群是相对较为独立的栽培类型。同时基于 Structure 模型的群体遗传结构分析也将这些材料分为两个组群。这与前人[15,23]的研究结果一致。

研究发现，Structure 软件的群体结构分析可以避免人为因素对类群划分的影响，进而对材料群体的遗传结构进行校正，有利于研究种质间的遗传关系，将类群划分更加细致[26]。而结合聚类分析和 Structure 模型聚类法能更准确地确定多份种质间的亲缘关系。

4 结　论

本研究选用 22 对 SSR 引物，利用毛细管电泳荧光检测技术对 96 份青花菜、花椰菜材料的遗传多样性和群体结构进行了分析，96 份种质材料群体内遗传多样性不丰富，根据遗传距离并按 UPGMA 的方法聚类，可以将 96 份材料划分为花椰菜和青花菜 2 个组群，与 Structure 模型群体遗传结构分析结果一致。花椰菜自交系被分到了 4 个亚群，但在各亚群中材料之间的遗传距离较小，杂交组配时，可以选用不同亚群的自交系进行组配，以获得更大的杂交优势。本试验为青花菜、花椰菜在杂交亲本选配、育种研究等方面提供了参考。

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责任编辑：赵丹丹

云南大学学报(自然科学版), 2022, 44(0):1~8 DOI: 10.7540/j.ynu.20210526
Journal of Yunnan University: Natural Sciences Edition

硼和镁元素缺乏对工业大麻生长及大麻二酚(CBD)含量的影响

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摘要: 硼和镁对植物的正常生长和次生代谢物合成具有重要作用. 为明确硼和镁元素缺乏对工业大麻生物量和叶片中大麻二酚(CBD)质量分数的影响, 本研究采用砂培盆栽的方法, 以云麻 7 号和晋麻 1 号为材料, 在苗期进行缺硼和缺镁培养 4 周后观测植株的形态特征、生物量以及叶片中的 CBD 质量分数等指标. 结果表明, 缺硼导致工业大麻植株新叶卷曲发黄, 随后生长点坏死, 植株生长停滞; 缺硼培养 4 周后, 株高、茎粗、叶干重分别较对照低 35.2%、12.5%、11.1%, 叶中的 CBD 质量分数较对照低 55.0%. 镁缺乏首先是老叶出现脉间失绿症状, 随后逐渐向新叶扩展; 缺镁培养 4 周后, 株高、茎粗、叶干重分别较对照低 9.3%、12.5%、13.9%, 叶中的 CBD 质量分数与对照没有显著差别. 本研究结果可为诊断工业大麻硼和镁元素缺乏, 通过优化硼和镁元素管理提高 CBD 产量提供依据.

关键词: 工业大麻; 硼; 镁; 生物量; CBD 质量分数
中图分类号: S563.3 **文献标志码:** A **文章编号:** 0258−7971(2022)00−0001−08

工业大麻(*Cannabis sativa* L.)是植株中的致幻成分四氢大麻酚(THC)质量分数低于 0.3%、不具备制毒价值的大麻品种类型, 因其茎秆中含有高品质纤维, 可用于纺织、造纸、制作复合材料等. 工业大麻曾在我国广泛种植, 是我国历史上最为重要的纤维作物, 素有“国纺源头, 万年衣祖”之称[1]. 近年来, 工业大麻花叶中所特有的非致幻性大麻素受到广泛关注, 其中大麻二酚(CBD)可用于治疗焦虑、抑郁、癫痫等神经类疾病, 在抗肿瘤、神经系统保护、免疫调剂和抗炎抗氧化等方面具有巨大的医疗和保健价值[2-3]. 因此, 以收获花叶提取 CBD 为主要目的的花叶用工业大麻种植迅速发展, 成为云南高原特色农业产业的新亮点.

硼和镁是植物生长所必需的元素. 硼参与细胞壁的合成, 影响植株生殖生长及碳水化合物的合成、转运、代谢等, 从而影响作物的产量和次生代谢物含量[4]. Sugier 等[5] 通过叶面喷施 $Na_2B_8O_{13}·4H_2O$ 显著提高了菊科植物山车金(*Arnica montana* L.)花叶产量及花叶中的精油的含量. Yu 等[6] 通过浇灌含硼砂的水溶液提高了东北龙胆(*Gentiana manshurica* Kitag.)的生物量和药用成分龙胆苦甙的含量. 镁是叶绿素的中心离子, 是很多酶的活化剂, 参与能量代谢, 对植物生物量的积累也有重要作用[7]. 杜玉霞等[8] 研究表明缺镁会导致柠檬叶片中的叶绿素含量和光合能力下降, 抑制植株生长. 王丹等[9] 发现施用 $MgSO_4·7H_2O$ 可显著影响中药材植物艾纳香叶片中的激素水平, 提高生物量以及药用成分 *l*-龙脑的含量.

Cockson 等[10] 研究了硼和镁元素缺乏对大麻植株表型的影响, 发现硼元素缺乏会导致植株干物重显著下降, 但目前尚未有研究明确硼和镁元素对 CBD 质量分数的影响. 鉴于此, 本研究采用砂培试验研究硼、镁元素缺乏对工业大麻生长和叶片中 CBD 质量分数的影响, 以期为花叶用工业大麻种植提供硼、镁元素管理依据.

收稿日期: 2021-11-08; **接受日期:** 2022-03-29; **网络出版日期:** 2022-06-14
基金项目: 云南大学引进人才科研启动经费(C176220100026); 现代农业产业技术体系建设专项资金(CARS-16-E15).
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1 材料与方法

1.1 试验材料 以云麻 7 号和晋麻 1 号为材料,在云南大学农学院试验温室开展盆栽试验. 云麻 7 号由云南省农业科学院选育,是目前云南省花叶用工业大麻栽培的主要品种,其在云南地区的生育期为 210 d 左右,属晚熟型品种. 晋麻 1 号由山西省农业科学院选育,是山西地区主要推广的籽粒和纤维兼用型品种,其在山西地区的生育期为 116 d 左右,属早熟型品种. 试验用盆为 280 mm 口径、210 mm 高带有托盘的圆形塑料花盆,基质为用去离子水冲洗干净的石英砂.

1.2 试验处理 每盆装入 5 kg 石英砂. 每盆播种 15 粒饱满均匀的种子,出苗之后每隔 3 d 浇 300 mL 营养液,营养液的养分质量分数为改良 Hoagland 完全营养液(表 1)的一半. 当植株长到第 3 对真叶时间苗,每盆留长势均匀的幼苗 7 株. 间苗后分别进行缺硼和缺镁处理,缺硼处理为在完全营养液中不添加 HB_3O_3,缺镁处理为不添加 $MgSO_4·7H_2O$,缺失的 SO_4^{2-}通过 Na_2SO_4 补充,对照浇完全营养液,每个处理设置 3 个重复. 每隔 3 d 浇 300 mL 营养液,培养 4 周后收获,此时对照植株的第 6 对真叶展开.

表 1 改良 Hoagland 完全营养液及缺硼和缺镁营养液配方

Tab. 1 The composition of modified Hoagland, boron-deficient and magnesium-deficient nutrient solutions

成分	完全/(mg/L)	缺硼/(mg/L)	缺镁/(mg/L)
KNO_3	759	759	759
$Ca(NO_3)_2·4H_2O$	945	945	945
$MgSO_4·7H_2O$	493	493	0
KH_2PO_4	204	204	204
$FeSO_4·7H_2O$	13.9	13.9	13.9
EDTA-Na_2	18.65	18.65	18.65
H_3BO_3	2.86	0	2.86
$MnCl_2·4H_2O$	1.81	1.81	1.81
$ZnSO_4·7H_2O$	0.22	0.22	0.22
$CuSO_4·5H_2O$	0.08	0.08	0.08
$(NH_4)_6Mo_7O_{24}·4H_2O$	0.02	0.02	0.02
Na_2SO_4	0	0	284

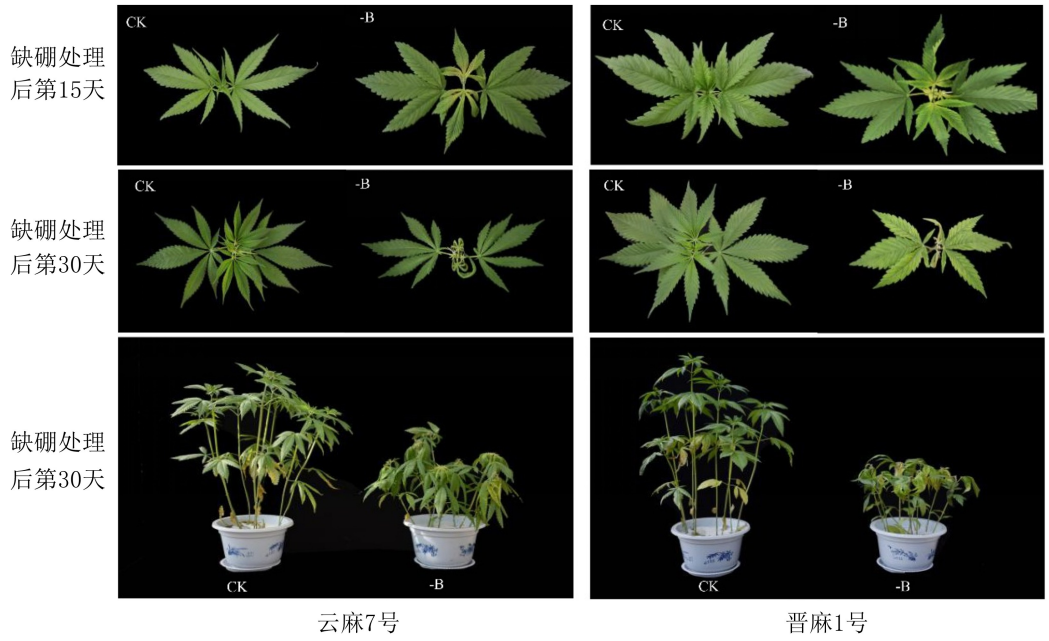
1.3 指标测定 收获时,一次性拔出所有植株,测量每株株高和茎基部直径,之后按盆将植株分离为根、茎、叶,108 ℃ 杀青 20 min,80 ℃ 烘干,称重. 烘干后的叶片用于测定硼和镁质量分数以及 CBD 和 THC 质量分数. 硼和镁质量分数的测定使用电感耦合等离子体质谱法(ICP-MS)、参照 GB 5009.268—2016《食品安全国家标准 食品中多元素的测定》程序进行. CBD 和 THC 含量的测定参照 NY/T 3252.1—2018《工业大麻种子 第一部分:品种》使用液相色谱法,其中提取液改为甲醇,流动相 B 改为纯水.

1.4 数据处理 使用 MS Excel 2016 对收集的数据进行整理、计算和绘图. 采用 SPSS 25.0 对数据进行统计分析,试验中品种和缺素对生物量及大麻素含量的影响采用双因素方差分析,若方差分析显示处理间存在显著差异,则采用 Duncan 法进行多重比较(P<0.05).

2 结果与分析

2.1 硼缺乏对工业大麻生长及叶中大麻素质量分数的影响 缺硼处理 2 周后,植株新生小叶出现卷曲和失绿症状. 4 周后,植株生长点坏死,株高增长停滞(图 1). 处理 4 周后收获所有植株,此时云麻 7 号和晋麻 1 号植株叶片中的硼质量比分别为 0.83、0.97 mg/kg,是完全营养液培养条件下的 7.7%、8.5%(图 2). 缺硼处理导致植株的平均株高、茎粗和单株茎干重显著降低(P < 0.05),分别较对照低 35%、13% 和 19%,但对根干重、叶干重以及根冠比的影响不显著(P > 0.05). 硼缺乏对根、茎、叶干重以及根冠比的影响在品种间差异不显著(表 2). 缺硼处理植株叶片中的 CBD 质量分数和 THC 质量分数均低于对照,云麻 7 号缺硼处理植株叶片中的 CBD 质量分数较对照低 50%,THC 质量分数较对照低 61%,晋麻 1 号缺硼处理植株叶片中的 CBD 质量分数较对照低 69%,THC 质量分数较对照低 9%(图 2).

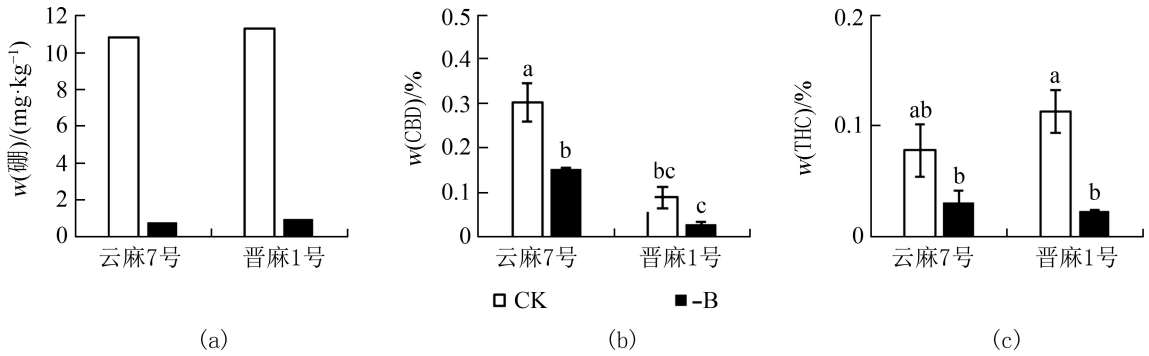
2.2 镁缺乏对工业大麻生长及叶中大麻素质量分数的影响 缺镁处理 2 周后,植株表现出老叶脉间失绿症状. 4 周后,老叶脉间失绿程度加深,部分变黄枯死,新叶表现出失绿症状(图 3). 缺镁处理 4 周后,植株叶片中的镁质量比仅为完全营养液培养条件下的 10.1%(云麻 7 号,928 mg/kg)和 17.1%(晋麻 1 号,1 660 mg/kg)(图 4). 与对照相比,缺镁处理植株的茎粗、根干重和茎干重分别低 13%,



CK 为对照；-B 为缺硼处理.

图 1　缺硼处理对不同工业大麻品种叶片和植株形态的影响

Fig. 1　Effect of boron-deficiency on the leaf and plant morphology of different hemp cultivars



（a）中测定的样品为三个重复的混合样；CK 为对照；-B 为缺硼处理；同一图内条柱上方不同的字母表示数值间存在显著差异（P< 0.05）.

图 2　缺硼处理对不同工业大麻品种叶片中硼质量比和 CBD、THC 质量分数的影响

Fig. 2　Effect of boron-deficiency on the leaf boron, CBD and THC concentration of different hemp cultivars

29% 和 35%, 达到了显著水平(P < 0.05), 但株高、叶干重和根冠比没有显著差异(P > 0.05). 镁缺乏对上述指标的影响在品种间差异不显著(表 3). 缺镁处理植株叶片中的 CBD 质量分数和 THC 质量分数与对照相比没有显著差异(P > 0.05)(图 4).

3　讨论

3.1　缺硼对工业大麻产量与大麻素质量分数的影响　硼是植物生长所必需的营养元素, 缺硼培养 2 周后工业大麻植株即表现出明显的症状, 4 周后植株叶片中的硼含量显著下降, 植株的生长点坏死, 茎和叶发育畸形. 这一症状与 Cockson 等[10] 对毒品大麻植株的观测一致, 也与红麻等植物对硼缺乏的响应类似[11].

在缺硼条件下培养工业大麻幼苗 4 周后, 植株的茎和叶干重分别较对照低 19% (P = 0.002) 和 11%(P = 0.069). 缺硼导致植株干物质积累量降低在针对棉花、油菜等作物的研究中得到广泛证实[12], 其原因可能有两方面: 一是叶片发育畸形、生长点坏死导致光合面积减小(图 1), 二是光合系统发育异常导致单位叶面积的光合速率降低. 已有研究表明, 硼参与细胞壁的形成, 对细胞分裂和伸

表 2 缺硼处理对不同工业大麻品种生物量的影响

Tab. 2 Effect of boron-deficiency on the biomass of different hemp cultivars

统计项		干物质量/(g/株)			根冠比	茎粗/mm	株高/cm
		根	茎	叶			
处理	CK	2.4 ± 0.16 a	6.2 ± 0.25 a	7.2 ± 0.29 a	0.18 ± 0.01 a	4.0 ± 0.22a	47.5 ± 1.60 a
	-B	2.1 ± 0.20 a	5.0 ± 0.11 b	6.4 ± 0.45 a	0.18 ± 0.01 a	3.5 ± 0.05 b	30.8 ± 0.77 b
品种	云麻7号	2.6 ± 0.14 a	5.5 ± 0.27 a	7.5 ± 0.34 a	0.20 ± 0.01 a	3.9 ± 0.23 a	37.6 ± 3.05 b
	晋麻1号	2.0 ± 0.15 b	5.7 ± 0.39 a	6.2 ± 0.25 b	0.17 ± 0.01 a	3.6 ± 0.09 a	40.7 ± 4.58 a
*P*值	处理	0.090	0.002	0.069	0.935	0.024	0.000
	品种	0.011	0.500	0.008	0.066	0.067	0.029
	处理×品种	0.122	0.373	0.577	0.216	0.038	0.017

表中数据为平均值 ± 标准差;同一指标后不同字母代表处理间和品种间差异显著(P<0.05);CK为对照;-B为缺硼处理.



CK 为对照;-Mg 为缺镁处理.

图 3 缺镁处理对不同工业大麻品种叶片和植株形态的影响

Fig. 3 The effect of magnesium-deficiency on the leaf and plant morphology of different hemp cultivars

长以及叶绿体的发育有较大影响,因而缺硼会抑制叶面积扩大,降低叶绿素含量,进而导致光合能力下降以及干物质积累量降低[12-13]. 除了对干物质积累的影响外,硼缺乏还会影响植物体内的酚类物质代谢[4]. 本研究缺硼条件下叶片中的 CBD 质量分数和 THC 质量分数显著低于对照(图 2),这可能是因为 CBD 和 THC 的积累与细胞壁形成有关. Mahlberg 等[14] 和 Livingston 等[15] 研究发现,CBD 和 THC 等大麻素的合成主要是在叶片和苞叶表面腺体的圆片形细胞膜上,合成的大麻素贮存在由细胞壁包裹形成的腔室内. 因此,硼缺乏引起的细胞壁发育异常可导致 CBD 和 THC 的积累量下降. 类似的,Hajlboland 等[16] 研究发现茶树缺硼会导致与细胞壁结合的酚类物质含量下降.

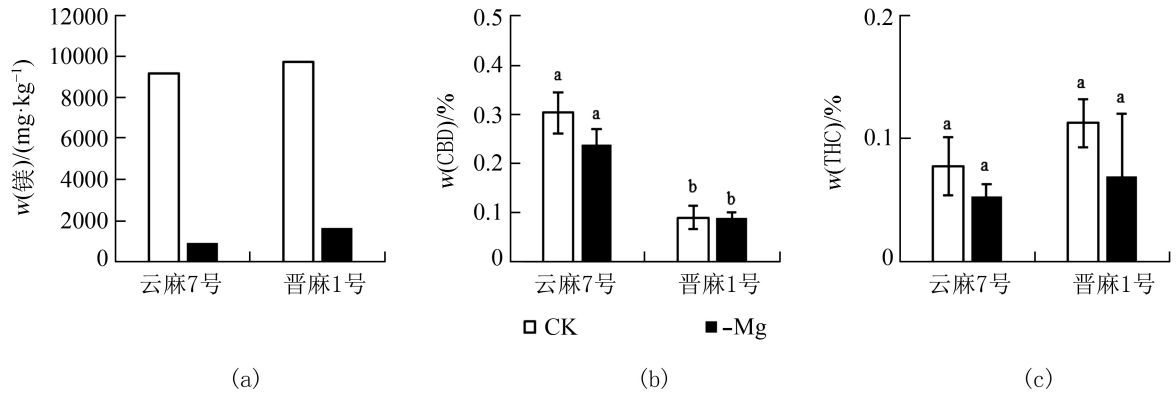
硼缺乏会显著抑制工业大麻生长,降低叶片中的 CBD 质量分数,进而影响种植效益,因此明确工业大麻对硼的需求量对提高工业大麻产量具有重要意义. 本研究表现出缺硼症状植株叶片中的硼质

表 3　镁缺乏对工业大麻不同品种生物量的影响

Tab. 3　Effect of magnesium deficiency on the biomass of different hemp cultivars

统计项		干物质量/（g/株）			根冠比	茎粗/mm	株高/cm
		根	茎	叶			
处理	CK	2.4 ± 0.16 a	6.2 ± 0.25 a	7.2 ± 0.29 a	0.18 ± 0.01 a	4.0 ± 0.22 a	47.5 ± 1.60 a
	-Mg	1.7 ± 0.21 b	4.0 ± 0.50 b	6.2 ± 0.59 a	0.17 ± 0.01 a	3.5 ± 0.12 b	43.1 ± 3.02 a
品种	云麻7号	2.1 ± 0.25 a	4.7 ± 0.62 a	7.3 ± 0.32 a	0.18 ± 0.01 a	3.9 ± 0.25 a	41.0 ± 1.63 b
	晋麻1号	2.0 ± 0.24 a	5.5 ± 0.61 a	6.1 ± 0.56 a	0.17 ± 0.01 a	3.6 ± 0.14 a	49.6 ± 1.93 a
*P*值	处理	0.038	0.002	0.133	0.389	0.026	0.080
	品种	0.696	0.132	0.104	0.651	0.169	0.005
	处理×品种	0.617	0.451	0.958	0.789	0.053	0.380

表中数据为平均值 ± 标准差; 同一指标后不同字母代表处理间和品种间差异显著(P<0.05); CK为对照; -Mg为缺镁处理.



（a）中为测定的样品为 3 个重复的混合样；CK 为对照，-B 为缺硼处理；同一图内条柱上方不同的字母表示数值间存在显著差异（P< 0.05）.

图 4　缺镁处理对不同工业大麻品种叶片中镁质量比和 CBD、THC 质量分数的影响

Fig. 4　The effect of magnesium-deficiency on the leaf magnesium, CBD and THC concentration of different hemp cultivars

量比小于 1.0 mg/kg, 正常生长植株叶片中的硼质量比在 2.3−11.3 mg/kg 之间, 但 Cockson 等[10] 针对毒品大麻的研究显示, 当植株叶片中的硼质量比为 58.6 mg/kg 也能正常生长. 因此, 有必要进一步开展研究明确工业大麻对硼的需求范围.

3.2　缺镁对用工业大麻产量与大麻素质量分数的影响　镁是植物生长所必需的营养元素, 在植物体内的移动性强, 因而缺镁培养工业大麻植株的老叶先出现脉间失绿症状, 随着缺素程度的加深, 新叶也表现出脉间失绿症状, 部分老叶枯死凋落. 本研究中工业大麻植株对镁元素缺乏的响应与 Cockson 等[10] 对毒品大麻植株的观测一致, 也与草莓、香蕉等植物的响应类似[17-18].

在缺镁条件下培养 4 周后, 工业大麻植株的根干重和茎干重显著低于对照, 叶干重是对照的 86%. 缺镁导致工业大麻植株干物质积累量减少可能是因为叶片的光合作用受阻. 镁是叶绿素的重要组成部分, 也参与光合产物由源向库的转运, 对叶片光合系统的正常进行具有重要作用[19-20]. 唐磊等[20] 研究发现缺镁导致茶树光合器官破坏, 光合能力降低, 从而抑制茶树生物量的积累; 杜玉霞等[21] 研究表明在低镁胁迫时柠檬的光合作用受到抑制, 限制了植株生长. 镁元素也会对植株次生代谢物积累产生影响, 例如王丹等[22] 研究发现外源镁显著提高了艾纳香中的 *l*-龙脑含量(2-茨醇), 但本研究试验结果表明镁缺乏对工业大麻中的 CBD 含量和 THC 含量影响不显著, 因此镁可能不参与 CBD 和 THC 合成过程.

硼缺乏会显著降低工业大麻植株的生物产量, 因此工业大麻种植中需要满足植株对镁的最低需

求. 水稻、玉米、小麦等作物叶片中的临界镁质量比在 1 000 ~ 2 000 mg/kg 左右, 低于这一质量比即会表现出缺素症状[23]. 在 Cockson 等[10] 针对毒品大麻的研究中, 植株表现出缺镁症状时的叶片镁质量比为 1 200 mg/kg, 正常生长植株的镁质量比为 6 100 mg/kg. 本研究中植株表现出缺镁症状时的叶片镁质量比(900 ~ 1 700 mg/kg)与 Cockson 等的研究结果相似, 但正常生长植株的镁质量比(9 200 ~ 9 800 mg/kg)偏高. 因此, 进一步研究品种类型对镁需求的差异, 可为工业大麻种植过程中的镁元素管理提供依据.

4 结论

硼和镁元素缺乏皆会抑制工业大麻植株生长, 降低植株生物量和花叶产量, 镁元素缺乏不会导致花叶中的 CBD 和 THC 质量分数降低, 但硼元素缺乏会导致花叶中的 CBD 质量分数和 THC 质量分数降低.

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Effect of boron and magnesium deficiency on the hemp (*Cannabis Sativa* L.) growth and cannabidiol concentration

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Abstract: Boron (B) and magnesium (Mg) play a vital role in plant growth and secondary metabolites synthesis. To understand the effect of B and Mg deficiency on the biomass accumulation and leaf cannabinoid (CBD) concentration of hemp (*Cannabis sativa* L.), sand cultured seedlings of Yunma #7 and Jinma #1 were irrigated with nutrient solution deficient in B or Mg for four weeks. Plant morphology, biomass accumulation and CBD concentration in the leaves were determined. Under B deficient condition, the plants developed curly and yellowish new leaves, followed by shoot-tip necrosis and height stagnation. Four weeks after B deficient treatment, the plant height, stem diameter, and leaf dry weight were lower than those of control treatment (CK) by 35.2%, 12.5% and 11.1%, respectively, the CBD concentration in leaves was lower than that of CK by 55.0%. Under Mg deficient treatment, the plants showed firstly interveinal chlorosis on the lower leaves, then the symptom gradually appeared on the upper leaves. Four weeks after Mg deficient treatment, the plant height, stem diameter, and leaf dry weight were lower than those of CK by 9.3%, 12.5% and 13.9%, respectively,, the concentration of CBD in the leaves did not differ significantly from that of CK. These results could support the diagnosis of B and Mg deficiency in hemp plants, and the increase of CBD yield through optimizing B and Mg management.

Key words: hemp (*Cannabis Sativa* L.)**;** boron**;** magnesium**;** biomass yield**;** CBD concentration



云南大学2020年省级、校级优秀博士、硕士学位论文评审结果公示

发布时间：2020-12-31 21:13:37.0

根据云南省学位委员会办公室《关于开展2020年优秀博士、硕士学位论文评选工作的通知》文件要求及云南大学校级优秀博士、硕士学位论文评选工作安排，云南大学2020 年度云南省优秀博士、硕士学位论文评选共124人申报，其中申报博士、学术硕士和专业硕士优秀学位论文的人数分别为24人，74人和26人。经学校组织各学科专家评审，拟推荐博士学位论文18篇、学术硕士学位论文17篇，专业硕士学位论文17篇为省级优秀学位论文，博士学位论文6篇、学术硕士学位论文57篇，专业硕士学位论文9篇，为校级优秀学位论文，现将评审结果予以公示，公示期为 2020 年 12 月 31日—2021 年 1 月 4 日。凡对评审结果存有异议者，可于公示期内与研究生质量管理办公室联系。联系方式：0871-65033585，联系人：王老师。

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2.云南大学 2020年校级优秀博士、硕士学位论文评审结果汇总表

云南大学2020年省级、校级
http://www.grs.ynu.edu.cn/info/1051/3546.htm
全国入秋进程图来了
百度 谷歌 网址大全 360搜索 游戏中心 Links

专业型硕士学位论文

序号	作者姓名	授予学位日期	专业学位类别名称	专业领域名称	论 文 题 目	导师姓名	学位论文层次	评审结果
1	常敬	2018年6月	0251金融	025100金融	我国机电产品出口的政策性金融需求研究	黄宁	硕士	省级优秀论文
2	王晓波	2018年6月	0252应用统计	025200应用统计	K个大维协方差矩阵的齐性检验问题	李会琼	硕士	省级优秀论文
3	袁欢	2018年6月	0257审计	025700审计	审计意见对关联交易的影响--来自2003年-2016年A股上市公司的证据	徐琰超	硕士	省级优秀论文
4	张翼	2018年6月	0351法律	035102法律（法学）	民事公益诉讼中调解适用研究	周麒	硕士	省级优秀论文
5	李忠豪	2018年6月	0352社会工作	035200社会工作	提升流动儿童同伴交往能力的社会工作实践研究-以昆明市F社区流动儿童为例	高万红	硕士	省级优秀论文
6	谢飞	2018年6月	0451教育	045101教育管理	特殊学校学生需求调查研究—以云南省H中等专业学校为例	董云川	硕士	省级优秀论文
7	严勇	2018年6月	0552翻译	055201英语笔译	《神圣的柳树：——一个越南家族四代人的沉浮》选译（英译汉）及其翻译研究报告	徐志英	硕士	省级优秀论文
8	童领	2018年6月	0852材料科学与工程	085204材料工程	硅碳及锗碳复合材料的锂离子电池负极材料研究	杨宇	硕士	省级优秀论文
9	陈怡真	2018年6月	0810信息与通信工程	085208电子与通信工程	云南重彩画的风格转移	普园媛	硕士	省级优秀论文
10	曹子成	2018年6月	0812计算机科学与技术	085211计算机技术	基于广义二肽组成特征融合表达的细胞穿膜肽预测算法研究	王顺芳	硕士	省级优秀论文
11	王连鹏	2018年6月	0852工程	085213建筑与土木工程	石屏县景观格局演变及其生态效应研究	徐坚	硕士	省级优秀论文
12	田然	2018年6月	0852工程	085229环境工程	青藏高原南部枪勇浅冰芯可溶性化学记录与过去环境重建	田立德	硕士	省级优秀论文
13	梁贤	2018年6月	0852工程	085229环境工程	抗性细菌 Pseudomonas sp. DC-B3对水中Cu2+, Cd2+, Cr6+的吸附转化特性及初步机理研究	常军军	硕士	省级优秀论文
14	王荻	2018年6月	0852工程	085229环境工程	青藏高原南部羊卓雍措流域降水稳定同位素与大尺度大气环流关系研究	田立德	硕士	省级优秀论文
15	孙鑫俐	2018年6月	0852工程	085238生物工程	蛋白质乙酰化调控Bacillus nematocida B16杀线虫初探	张克勤	硕士	省级优秀论文
16	李世钰	2018年6月	0951农业	095102园艺	长期施用有机肥对设施菜地土壤费力及氮矿化的影响	何飞飞	硕士	省级优秀论文
17	白杨	2018年6月	1255图书情报与档案管理	125500图书情报	基于区块链的图书馆数字源版权保护研究	张禹	硕士	省级优秀论文

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Q/GXSXM

保山隆阳区果馨麝香猫咖啡厂企业标准

Q/GXSXM　001-2021

企业标准信息公共服务平台
公开　2021年03月29日 13点03分

猫屎咖啡生产技术操作规程

企业标准信息公共服务平台
公开　2021年03月29日 13点03分

云南大学资源植物研究院

保山隆阳区果馨麝香猫咖啡厂

云南省农业科学院热带亚热带经济作物研究所

发布

2021 年 3 月 23 日

前言

本标准按照 GB/T 1.1-2020《标准化工作导则第 1 部分：标准化文件的结构和起草规则》给出的规则起草。

本标准起草单位：云南大学资源植物研究院、保山隆阳区果馨麝香猫咖啡厂、云南省农业科学院热带亚热带经济作物研究所

本标准主要起草人：李　贝、何飞飞、郭文魁、黄家雄、高　晶

本标准由保山隆阳区果馨麝香猫咖啡厂负责解释。

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公开　2021年03月29日 13点03分

企业标准信息公共服务平台
公开　2021年03月29日 13点03分

猫屎咖啡生产技术操作规程

1 范围

本标准规定了猫屎咖啡的术语定义、原料要求、加工条件、工艺流程、质量管理、产品标志、包装材料、贮存运输等相关要求。

本标准适用于猫屎咖啡的生产加工过程。

2 规范性引用文件

下列文件对本标准的应用是必不可少的。凡是注日期的引用文件，仅注日期的版本适用于本标准。凡是不注日期的引用文件，其最新版本（包括所有的修改单）适用于本标准。

GB 191 包装贮运图标标志

GB 2762 食品安全国家标准 食品中污染物限量

GB 2763 食品安全国家标准 食品中农药最大残留限量

GB 5009.3 食品安全国家标准 食品中水分的测定

GB 5009.4 食品安全国家标准 食品中灰分的测定

GB 5009.139 食品安全国家标准 饮料中咖啡因的测定

GB 5749 生活饮用水卫生标准

GB 7718 预包装食品标签通则

GB 9683 复合食品包装袋卫生标准

GB 13078 饲料卫生标准

GB/T 15033 生咖啡 嗅觉和肉眼以及杂质和缺陷的测定

GB/T 18007 咖啡及其制品术语

GB 18596 畜禽养殖业污染物排放标准

GB/T 18635 动物防疫 基本术语

GB/T 19525.2 畜禽场环境质量评估准则

NY/T 388 畜禽场环境质量标准

NY/T 604 生咖啡

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NY/T 605 焙炒咖啡

NY/T 1518 袋装生咖啡 取样

NY/T 1167 畜禽场环境质量及卫生控制规范

NY/T 1168 畜禽粪便无害化处理技术规范

NY/T 1169 畜禽场环境污染控制技术规范

NY/T 2554 生咖啡 贮存和运输导则

LY/T 1563 陆生野生动物（兽类）饲养场通用技术条件

LY/T 2359 陆生野生动物疫源疫病监测技术规范

LY/T 2360 陆生野生动物疫病危害性等级划分

LY/T 2410 陆生野生动物保护及相关产业分类导则

LY/T 2504 野生动物饲养

ISO 4150 生咖啡 粒度分析 手工和机械筛分

3 术语定义

猫屎咖啡：特指用灵猫科果子狸（*Paguma larvata* Swinhoe）食用咖啡鲜果后排出体外的咖啡豆加工制成的咖啡产品。

4 房屋笼舍要求

4.1 饲养环境条件

果子狸为林缘兽类，夜行性动物，喜欢在黄昏、夜间和日出前活动，要求饲养环境空气清新、安静，无噪音影响。

4.2 房屋建筑要求

房屋建筑规模根据养殖数量而定，一般高 3～4m，宽 6～8m，一般包括饲料处理区、消毒区、饲养区三个部分，用砖瓦等坚固材料建成；饲养区室内分为 2 列，中间为过道，宽 1.4m，每个笼舍占地面积 9m^2，笼舍间用砖隔断，中间预留果子狸进出通道；内室以水泥地面为宜，便于冲洗。

4.3 笼舍建设要求

笼舍门宽 0.9m，高 1.8m，用镀锌铁网焊接而成；过道除门外，用砖砌高 1.3m，其余部分及顶部用镀锌铁网，网孔不大于 4 平方厘米，以防止动物逃跑和保证安

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全。笼内用砖砌成小室和暗室，笼舍内要设置栖架，供果子狸攀跳和栖息。

4.4 安防监控设施

动物饲养区、笼舍应有安防监控设施，监控范围应全覆盖。

4.5 环境卫生设施

有动物粪便、动物尸体或其附属物无害化处理设施，易于识别，能防止动物接近，不能对周围环境造成污染和危害。

5 加工工艺流程

咖啡鲜果→清洗除杂→鲜果喂食→肠道发酵→收集咖啡豆→干燥→脱壳→分级→烘焙→包装→猫屎咖啡→入库或销售。

5.1 鲜果成熟度

咖啡鲜果必须充分成熟，果实软滑多汁，果汁含糖量达 18%以上；果实必须新鲜，当天采摘当天喂食。

5.2 鲜果清洗除杂

咖啡鲜果采摘回来后要用清水清洗除杂，清除干果、瘪果、未熟果、枝、叶、灰尘等杂质，然后晾干表面水分才能喂食。

5.3 咖啡鲜果投食

一般在黄昏时喂食，每只猫投食量为鲜果 2.5kg，用以保证果子狸可以从中挑选处足够多成熟饱满的鲜果进食。

5.4 收集咖啡豆

果子狸食用咖啡豆后在体内消化发酵约 8～12 小时，第 2 天 6 点左右排出咖啡豆，此时即收集咖啡豆，收集咖啡豆务必小心，不要弄散咖啡豆。

5.5 咖啡豆干燥

将捡拾的咖啡豆在晾晒架上进行干燥，一般干燥时间约 7～10 天，当咖啡豆水分含量达 12%时即可入库保存或进入下一步加工环节。

5.6 脱壳与分级

为确保咖啡豆新鲜度，一般需要销售或烘焙时再进行脱壳。咖啡利用咖啡脱壳机对咖啡豆进行脱壳分级，分级标准根据客户需求而定，一般分为三级，一级粒径≥6.30mm（16#筛及以上），二级粒径≥5.60mm（14#筛及以上），三级粒径

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＜5.60mm（14#筛以下），要求外来杂质≤0.3%，缺陷豆≤10%，水分≤12%。

5.7 咖啡豆烘焙

用咖啡豆专用烘焙机进行烘焙，每次烘焙量 3.00kg，当烘焙温度达 180℃时开始投料，采用中度烘焙，色度 55～65，也可根据客户需求确定烘焙度，一般烘焙时间约 12～15 分钟，当达到要求的烘焙度时即出料冷却。

5.8 产品包装

产品有猫屎咖啡生豆、焙炒豆、焙炒粉（挂耳咖啡）；生咖啡可根据客户需求进行包装，一般采用真空包装，每袋 1.00～5.00kg 不等；焙炒咖啡豆一般每袋 120g，采用内有锡铂的单向排气阀的牛皮纸袋包装；挂耳咖啡每袋 12g，每盒 6 袋，密封包装。

5.9 贮藏运输

贮藏和运输咖啡的环境和工具必须整洁，无异味，并且防潮防霉。

6 动物卫生与健康

6.1 清洁卫生

每周对饲养笼舍清洁 1 次，用清水清洗地板，并用医用消毒液进行消毒处理；清洁时间一般在下午 2～4 点气温较高时进行。

6.2 营养调剂

咖啡果实一般在 11 月至翌年 4 月成熟，此时可喂食和生产猫屎咖啡，但长期食用咖啡不能满足果子狸的营养需求，需要间隔 3 天喂食玉米粥、牛奶、鸡蛋、小鱼、水果等配合饲料；非生产季（5～10 月）也需要喂食玉米粥等配合饲料。

6.3 动物健康

便秘即大便干燥，可喂食香蕉或甘薯等果类进行预防；腹泻是对其威胁最大的一种病，可喂食土霉素等进行预防。

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Q/BJL

云南景兰热作科技有限责任公司企业标准

Q/BJL 001-2021

企业标准信息公共服务平台
公开 2021年04月20日 14点54分

咖啡果皮茶生产技术操作规程

企业标准信息公共服务平台
公开 2021年04月20日 14点54分

云南大学资源植物研究院

云南景兰热作科技有限责任公司

云南省农业科学院热带亚热带经济作物研究所

发布

2021 年 4 月 17 日

前言

本标准按照 GB/T 1.1－2020《标准化工作导则第 1 部分：标准化文件的结构和起草规则》给出的规则起草。

本标准起草单位：云南大学资源植物研究院、云南景兰热作科技有限责任公司、云南省农业科学院热带亚热带经济作物研究所

本标准主要起草人：张佳欣、黄伟、黄家雄、吕玉兰、何飞飞

本标准由云南景兰热作科技有限责任公司负责解释。

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咖啡果皮茶生产技术操作规程

1 范围

本标准规定了咖啡果皮茶的术语定义、原料要求、加工条件、工艺流程、质量管理、产品标志、包装材料、贮存运输等相关要求。

本标准适用于咖啡果皮茶的初加工过程。

2 规范性引用文件

下列文件对本标准的应用是必不可少的。凡是注日期的引用文件，仅注日期的版本适用于本标准。凡是不注日期的引用文件，其最新版本（包括所有的修改单）适用于本标准。

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GB 5009.4 食品安全国家标准 食品中灰分的测定

GB 5009.5 食品安全国家标准 食品中蛋白质的测定

GB 5009.6 食品安全国家标准 食品中脂肪的测定

GB 5009.7 食品安全国家标准 食品中还原糖的测定

GB 5009.139 食品安全国家标准 饮料中咖啡因的测定

GB 5749 生活饮用水卫生标准

GB 9683 复合食品包装袋卫生标准

GB 10789 饮料通则

GB/T 18007 咖啡及其制品术语

GB/T 30767 咖啡类饮料

JJF 1070 定量包装商品净含量计量检验规则

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3 术语定义

下列术语和定义适用于本文件。

3.1 咖啡果皮

咖啡果皮：指咖啡新鲜成熟果实的外果皮和中果皮混合物，因品种不同一般呈红色和黄色两种，是咖啡初加工过程中产生的重要副产物。

3.咖啡果皮茶

咖啡果皮茶：咖啡鲜果经过人工或机械脱皮产生的新鲜果皮再经过干燥、烘焙等工艺制成的饮料；根据干燥后的果皮是否经过烘培，分为生果皮茶（未经烘焙）和熟果皮茶（已经烘焙）。

4 加工场所要求

4.1 加工环境

加工场所要求应符合 GB 14881—2013 的规定加工环境和场地，远离污染源，无废水、废气、废渣和有异味污染物，确保空气清新。

4.2 加工用水

加工用水应符合 GB 5749—2006 的规定，确保加工所需的水质、水压和水量，供水设施卫生安全还应符合国家相关规定。

4.3 加工设备

包括供水设施、清洗池（盆、缸等）、脱皮机（手动、电动）、装果皮的盆（筛、箩、筐等）、晾晒架、烘焙机、包装袋等，这些器材必须清洁干净。

4.4 安保监控

加工场所应有安防监控设施，监控范围应全覆盖；主要用于监测、控制、记录设备的正常操作，如晾晒架、烘焙机等，应定期校准、维护。

4.5 环境卫生

应建立清洁消毒制度和清洁消毒用具管理制度，每天加工完毕必须对使用过的加工器具如脱皮机、盛装器具、加工场地等相关设施设备进行全面清洁。

4.6 原料要求

咖啡鲜果必须为健康成熟果实，果汁含糖量达 18%以上，采摘时不带果柄、

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枝叶等杂质，正常成熟果与干果、病果等劣质果分开盛装和分别加工。

5 加工工艺流程

咖啡鲜果→清洗除杂→优质鲜果→鲜果脱皮→果皮晾晒→微发酵→果皮筛分→烘焙→熟果皮茶→称量→包装→咖啡果皮茶→入库或销售。

5.1 鲜果成熟度

咖啡鲜果必须完全成熟，果实软滑多汁，鲜果汁含糖量达 18%以上。

5.2 鲜果清洗除杂

咖啡鲜果要用清水清洗除杂，清除干果、瘪果、未熟果、枝、叶、灰尘等杂质，直到水变清澈为止，以获得新鲜、干净、成熟、饱满的果实

5.3 鲜果脱皮

脱皮方式有机械脱皮和人工脱皮两种，人工脱皮则果皮完整、售价高，但加工效率低、成本高；机械脱皮则效率高、成本低，但果皮破碎不完整、售价低；采用机械脱皮时只能采用无水脱皮，否则影响甜度和品质。

5.4 果皮晾晒

将咖啡果皮置于晾晒架上均匀摊薄晾晒，晾晒时间 1～2 天，晾晒厚度不超过 2 厘米，每隔 2～3 小时均匀翻动一次，晾晒至水分含量 10～12%。

5.5 果皮微发酵

将晾晒后的果皮置于密封袋内微发酵，要求环境空气湿度 50～60%，温度 18～22℃，微发酵 3～4 天。

5.6 果皮筛分

用 14#号筛去除果皮碎末，保留完整均匀的果皮。

5.7 果皮烘焙

用咖啡烘焙机进行烘焙，烘焙温度为 100～180℃，烘焙时间为 3～5 分钟，含水率≤5%。

5.8 称量包装

一般熟果皮茶净重 100g，也可根据客户需求进行包装，净含量应符合 JJF 1070 的规定，包装应符合 GH/T 1070—2011 的规定。

5.9 入库销售

企业标准信息公共服务平台 公开 2021年04月20日 14点54分

生果皮含水量达 10～12%时即可入库或销售或烘焙，生果皮包装需用密封性好的食品级塑料袋包装，包装袋上应有产品标识牌，注明产地、品种、加工时间、脱皮方式、入库时间、净重、生产管理员签字、仓管员签字，贮藏期间严防回潮霉变；咖啡果皮茶（熟果皮）应该及时加工和销售，以确保其新鲜度。

6 贮藏及运输

6.1 贮藏

应符合 GB/T 30375—2013 的规定进行，要求环境清洁无异味和防湿。

6.2 运输

运输咖啡鲜果、鲜果皮、干果皮及咖啡果皮茶相关器具及环境必须整洁、干燥、无异味，并且防潮防霉。

企业标准信息公共服务平台
公开 2021年04月20日 14点54分
企业标准信息公共服务平台
公开 2021年04月20日 14点54分

CSSC 中国作物学会

荣誉证书

何 迷:

荣获2016年至2020年全国农科学子联合实践行动

优秀实践队员

特发此证，以资鼓励。

中国作物学会

二〇二一年四月

荣誉证书

李凌宏:

荣获2016年至2020年全国农科学子联合实践行动

优秀实践队员

特发此证，以资鼓励。

中国作物学会

二〇二一年四月

荣誉证书

王 坤:

荣获2016年至2020年全国农科学子联合实践行动

优秀实践队员

特发此证，以资鼓励。

中国作物学会

二〇二一年四月

CSSC 中国作物学会

荣誉证书

张树竹:

荣获2016年至2020年全国农科学子联合实践行动

优秀实践队员

特发此证，以资鼓励。

中国作物学会

二〇二一年四月

CSSC 中国作物学会

助力精准扶贫，聚力乡村振兴

在全国农科学子联合实践行动当中表现突出，成绩显著，被评为“2019年度全国农科学子联合实践优秀小队”。

小队成员：贺艳杰、杜梦影、柏　杰、袁亚男、陈　蓉、张树竹、袁　豪

指导老师：陈　蕊、黄立钰、李永萍

教育部植物生产类专业教学指导委员会
中国农业大学（代章）

中国作物学会

二〇一九年八月

CSSC 中国作物学会

荣誉证书

云南大学农学院赴河口暑期社会实践小队

在 2018 年参加由中国作物学会作物学人才培养与教育专业委员会主办的全国农科学子联合实践行动当中表现突出，成绩显著，被评为“2018 年度全国农科学子联合实践优秀小队”。

中国作物学会作物学人才培养与教育专业委员会（代章）

二〇一八年十一月

CSSC
中国作物学会

荣誉证书

云南大学农学院赴玉溪戛洒暑期社会实践小队

在 2018 年参加由中国作物学会作物学人才培养与教育专业委员会主办的全国农科学子联合实践行动当中表现突出，成绩显著，被评为“2018 年度全国农科学子联合实践优秀小队”。

中国作物学会作物学人才培养与教育专业委员会(代章)

二〇一八年十一月

CSSC 中国作物学会

荣誉证书

胡凤益:

荣获2016年至2020年全国农科学子联合实践行动

先进工作者

特发此证，以资鼓励。

中国作物学会

二〇二一年四月

荣誉证书

秦世雯:

荣获2016年至2020年全国农科学子联合实践行动

优秀指导教师

特发此证，以资鼓励。

中国作物学会

二〇二一年四月

荣誉证书

吴学尉:

荣获2016年至2020年全国农科学子联合实践行动

优秀指导教师

特发此证，以资鼓励。

中国作物学会

二〇二一年四月

CSSC
中国作物学会

黄立钰:

在全国农科学子联合实践行动当中表现突出，成绩显著，被评为“2019年度全国农科学子联合实践优秀指导教师”。

教育部植物生产类专业教学指导委员会
中国农业大学（代章）

中国作物学会

二〇一九年八月

CSSC
中国作物学会

李永萍：

在全国农科学子联合实践行动当中表现突出，成绩显著，被评为“2019年度全国农科学子联合实践优秀指导教师”。

教育部植物生产类专业教学指导委员会
中国农业大学（代章）

中国作物学会

二〇一九年八月

CSSC 中国作物学会

荣誉证书

秦世雯

在 2018 年参加由中国作物学会作物学人才培养与教育专业委员会主办的全国农科学子联合实践行动当中表现突出，成绩显著，被评为“2018 年度全国农科学子联合实践优秀指导教师”。

中国作物学会作物学人才培养与教育专业委员会（代章）

二〇一八年十一月

CSSC
中国作物学会

荣誉证书

何飞飞

在 2018 年参加由中国作物学会作物学人才培养与教育专业委员会主办的全国农科学子联合实践行动当中表现突出，成绩显著，被评为“2018 年度全国农科学子联合实践先进工作者”。

中国作物学会作物学人才培养与教育专业委员会（代章）

二〇一八年十一月

荣誉证书

CERTIFICATE OF HONOR

为表彰2021年全国农科研究生志愿者暑期实践活动优秀成果奖获得者，特颁发此证书。

成果名称：走进乡土乡村，致力乡村振兴

获 奖 者：李凌宏　张树竹　李禹甫　王蕊嘉

指导教师：何飞飞　黄光福　陈　蕊

完成单位：云南大学

获奖等级：二等奖

证书编号：NAGVSU-2021025

全国农业专业学位研究生教育
指导委员会秘书处

全国农科研究生志愿服务
联盟秘书处

二〇二一年十二月五日

昆明學院
KUNMING UNIVERSITY

荣誉证书

证书编号：NLC-MAE2021 YXAL021

吴学尉 胡凤益 刘海英 何飞飞 黄光福同志：

您撰写的《科技小院助推专业硕士研究生培养改革》，获2021年全国农林院校研究生教育管理研修班暨农林研究生教育助力乡村振兴学术年会优秀管理工作案例。

特颁此证！

中国学位与研究生教育学会农林学科工作委员会

全国农业专业学位研究生教育指导委员会秘书处

2021年5月21日

所属学科：农学
学科代码：210.50
项目编号：2019Y0030

云南省教育厅科学研究基金项目

任务合同书

V2019

项目名称：生物炭对咖啡红壤硝化作用的影响机制

项目单位：云南大学

负 责 人：樊帆

联系电话：18468239682

签订日期：2019 年 6 月 13 日

云南省教育厅　　制表

项 目 组 成 员 情 况

姓　名	性别	出生年月	职务职称	专　业	分　工	工作月数	签　名
何飞飞	女	1976.12	副教授		研究理论与技术指导		何飞飞
蔡嘉慧	女	1995.01	硕士生	园艺	负责具体研究工作		蔡嘉慧
李正涛	男	1996.11	硕士生	农艺与种业	负责具体研究工作		李正涛
李耕	男	1994.08	本科生	园艺	负责具体研究工作		李耕
沈龙	男	1997.06	本科生	园艺	负责具体研究工作		沈龙

所在单位科研管理部门意见

同 意

（盖章）
2019 年 6 月 18 日

云南省教育厅科技处意见

同意立项，请严格按研究目标、进度计划组织实施。

（盖章）
2019 年 6 月 20 日



立项证明

兹证明胡庆毅同学主持科研项目立项情况：

项目名称：多年生稻 PR23 香味性状的遗传定向改良。

项目类型：云南省教育厅科学研究基金研究生项目。

指导老师：黄立钰副教授。

项目主持人：胡庆毅。

项目成员：刘金涛、陈本佳、龚寓瑞、敖若寅、焦淑亮、李臻园、薛志峰。

联系电话：15198957615。

立项年度：2020 年。

资助金额：0.5 万元。

特此证明。

云南大学

2020 年 12 月 17 日

云南大学2021年教育厅科学研究基金教师类项目、研究生类项目立项评审结果公示

发布时间：2020-09-30 浏览次数：895

根据《云南省教育厅关于开展2021年度科学研究基金项目申报工作的通知》，经自由申报，科技处组织相关学科领域专家于2020年9月30日对资格审查通过的项目进行了评审，现将评审结果进行公示，如对评审结果有异议，请于2020年10月7日前以书面形式实名向科技处反映。

联系人：赵老师

电话：65031169

地址：呈贡校区明远楼335室

科技处

2020年9月30日

云南大学2021年教育厅科学研究基金教师类项目、研究生类项目立项评审结果

2021年度云南省教育厅科学研究基金项目申报立项名单				
序号	项目类别	课题名称	负责人	项目经费（万元）
1	教师类	宁芜梅山IOA矿床磁铁贫Ti机制及对矿床成因的指示	李婉婷	2
2	教师类	烟草青枯病与根际微生物多样性关系研究	牟宗敏	2
3	教师类	滇池铜绿微囊藻次生代谢物对滇池金线鲃胚胎的致畸性研究	李娇娇	2
4	教师类	基于线粒体COI基因序列的未定种金线鲃与文山金线鲃的分子鉴定	李春青	2
5	教师类	基于新媒体大数据的城市意象传播模式与传播机制研究	徐颖	2
6	教师类	最大团问题高性能并行算法研究	江华	2
7	教师类	基于区块链智能合约的标注数据共享技术研究	刘俊晖	2
8	教师类	跨组织业务流程中一种基于环境的公共流程建模方法研究	陈一通	2
9	教师类	人血清及卵泡液全氟化合物对体外受精结局的影响	王敏	2
10	教师类	超薄切片技术在磁性纳米材料中应用的研究	刘彦雄	2
11	教师类	三硫杂三聚茚盘状液晶分子的设计合成、自组装及光电性质研究	高红飞	2
12	教师类	靶向成对转录因子DNA结合区的二价肽的构建及与DNA的活性研究	汪萍	2
13	教师类	数字全息大景深调整衍射再现方法研究	何阿呷	2
14	教师类	工业大麻花芽性别分化关键基因筛	杨阳	2

		选		
15	教师类	基于细胞凋亡通路的靶向声学造影技术评价DVT静脉壁损伤的实验研究	李云燕	2
16	教师类	云贵高原糖尿病前期周围神经病早期诊断的神经电生理研究	程映秋	2
17	教师类	磁共振3D-SPGR技术对糖尿病视网膜病变患者视网膜氧张量水平变化临床研究	周敏洁	2
18	教师类	阿卡波糖介导的突触可塑性发生在治疗抑郁行为中的药物机理	杨会军	2
19	教师类	VTE-BLEED评分量表对静脉血栓栓塞症抗凝治疗出血风险预测评估	白敏	2
1	研究生类	量子点改性SnO2电子传输层构筑高校有机-无机杂化钙钛矿太阳能电池	赵荣俊	0.5
2	研究生类	生物质炭负载亚铜化合物的π络合选择性脱除燃料油中的氮、硫化合物	金鑫	0.5
3	研究生类	高性能CuO基甲醛气敏材料及性能研究	字包叶	0.5
4	研究生类	春末南亚高压的位置对大湄公河次区域降水的影响	杜骁睿	0.5
5	研究生类	基于GIS的昆明市区共享单车运维调度研究	秦红富	0.5
6	研究生类	基于GRACE重力数据研究青藏高原东北缘时变重力场特征	杨滢	0.5
7	研究生类	花岗岩中岩浆混合作用的识别——以薄竹山岩体为例	李美丽	0.5
8	研究生类	蛋白质组学分析揭示纳米银对鲤鱼鳃线粒体的毒性机制	向乾乾	0.5
9	研究生类	热带雨林土壤微生物多样性及多度对土壤呼吸的影响研究	陈德赟	0.5
10	研究生类	水电站建设对伊江流域（中国境内）城镇空间扩张影响研究	杨柳	0.5
11	研究生类	云南省干热河谷范围与土壤侵蚀特征分析	邓杨	0.5
12	研究生类	土壤胞外16S rDNA和ITS的类群特异性降解速率及其影响因素	李婷	0.5
13	研究生类	高黎贡山树轮记录的气候变化及其对亚洲低纬度火山喷发的响应	岳伟鹏	0.5
14	研究生类	基于耳石 Sr/Ca 值的怒江洄游鱼类习性研究	丁刘勇	0.5
15	研究生类	云南省边坡玄武岩干湿循环力学特性研究	刘述明	0.5
16	研究生类	基于演变及驱动机制的滇池流域景观格局变化预测研究	魏爱松	0.5
17	研究生类	云南省差异性高原山地城市紧凑度测算与优化研究	张越	0.5
18	研究生类	基于证据链的高效物联网收费稽查信誉机制研究	王基书	0.5



19	研究生类	基于智能合约和网络协议优化的高校传输、安全的区块链系统研究	吴子锋	0.5
20	研究生类	基于区块链的跨部门智能交通信息管理系统	蒋家昊	0.5
21	研究生类	一种基于视图的协同业务过程构建方法	汪煜祺	0.5
22	研究生类	基于深度学习模型加速的红外与可见光视频实时融合技术研究	丁斋生	0.5
23	研究生类	非编码 RNA 与疾病关联识别的智能计算技术研究	郭延哺	0.5
24	研究生类	孪生条件生成对抗网络的图像融合研究	李华光	0.5
25	研究生类	基于GAN的不平衡学习方法研究及其在生物信息学中的应用	武丽雯	0.5
26	研究生类	融合两级权重的深度多视角聚类	杜国王	0.5
27	研究生类	民族绘画情感计算的多模态分析方法融合及应用	张浩	0.5
28	研究生类	基于图注意力网络的多任务问题生成研究	袁理	0.5
29	研究生类	聚苯胺聚苯胺/粘土纳米管生物质5-羟甲基糠醛催化氧化性能的研究/粘土纳米管生物质5-羟	胡宸源	0.5
30	研究生类	C3N4@FePcs光催化降解水中双酚A的研究	邓鸣	0.5
31	研究生类	基于过氧化氢检测的活性氧自由基荧光探针的合成及性质研究	卢宇勋	0.5
32	研究生类	基于吡啶酰胺配体的配位聚合物的固态[2+2]环加成反应及应用研究	朱蓉蓉	0.5
33	研究生类	可见光介导的手性氧化吲哚合成研究	张阳	0.5
34	研究生类	基于Polya-Gamma_Gibbs抽样的教学测验中异常行为研究	秦川杨	0.5
35	研究生类	基于区块链技术的大数据精准推荐系统	陈慧媛	0.5
36	研究生类	拓扑绝缘体Bi2Te3的光电特性研究	周顺	0.5
37	研究生类	碳量子点用于肿瘤细胞标记及声光成像研究	柏 晗	0.5
38	研究生类	基于电极修饰提高有机场效应晶体管偏压稳定性的研究	杨振新	0.5
39	研究生类	基于量子点的固体芯—液体包层光流控波导及其应用研究	李东阳	0.5
40	研究生类	BRD4沉默与奥沙利铂联合对胃癌的生长及机制研究	杨鑫锐	0.5
41	研究生类	一株拟无枝酸菌烯二炔沉默基因簇的激活及其产物鉴定	宋志强	0.5
42	研究生类	双斑侧沟茧蜂病毒蛋白的big domain 调控寄主细胞hemichannel的研究	孟江慧	0.5
43	研究生类	植物源与微生物源生物制剂复配防	舒洁	0.5

		治根结线虫的探究		
44	研究生类	浣熊被试蛔虫的分子鉴定及其系统发育	曾红霞	0.5
45	研究生类	全球变化背景下中国杜鹃属植物代表种及濒危种适应区变化与预测	李坤翼	0.5
46	研究生类	多年生稻PR23香味性状的遗传定向改良	胡庆毅	0.5
47	研究生类	线虫抗菌肽基因scl家族功能研究	秦子弦	0.5
48	研究生类	少孢节丛孢外泌体对秀丽隐杆线虫运动神经调控机制的初探	周亮	0.5

上一条：云南省科技厅关于组织申报国家重点研发计划“政府间国际科技创新合作”等重点专项2021年度第一批项目的通知
下一条：关于发布组织器官再生修复的信息解码及有序调控重大研究计划2020年度项目指南的通告

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云南大学第十一届研究生科研创新项目验收结果公告

云南大学第十一届研究生科研创新项目研究期满，经项目负责人提出申请，各研究生培养单位评审，学校审核，现将验收结果公告如下：

项目编号	培养单位	项目名称	项目负责人	项目类型	验收结果
2019z001	文学院	唐宋时期传入云南大理白族地区佛教文献的收集整理研究	尹恒	重点项目	延期
2019z002	文学院	《九歌．山鬼》与祈雨风俗研究	张云	重点项目	延期
2019z003	法学院	农村留守儿童“委托监护”运行现状调查研究——以滇东北农村地区为研究视角	陈思静	重点项目	合格
2019z004	法学院	恢复性司法理念下社区矫正中的被害人参与制度研究	张敏	重点项目	合格
2019z005	法学院	新媒体与高校法学教学实践研究，以“云大法之声”微信公众号为例	王一鸣	重点项目	延期
2019z006	经济学院	基于供应链金融的云南省边境小微企业融资困境及对策研究	赵文婷	重点项目	延期
2019z007	经济学院	审计全覆盖视角下精准扶贫及脱贫绩效的审计评价体系构建及运用——以云南省西盟县为例	滕丽娜	重点项目	合格
2019z008	经济学院	“一带一路”倡议下中国与沿线国家金融合作对贸易转型的影响及对策研究	刘林龙	重点项目	优秀
2019z012	经济学院	原油政策规制对宏观经济的稳定效应研究	范文睿	重点项目	合格
2019z013	经济学院	新能源电力政策的“挤出效应”研究	王蕊	重点项目	合格
2019z009	历史与档案学院	论元代中央与地方财政关系——以“羡余”为中心的考察	孙朋朋	重点项目	优秀
2019z010	历史与档案学院	乡风文明治理背景下云南少数民族乡规民约档案开发研究	张伟	重点项目	优秀
2019z011	历史与档案学院	过分利得税征管研究（1939-1948）	赵珍理	重点项目	合格
2019z014	民族学与社会学学院	香蕉的流动：“一带一路”下中缅跨境种植合作的民族志研究	陈滢至	重点项目	合格

2019z015	民族学与社会学学院	流动的社会——缅甸藏人村落的民族志研究	杨猛	重点项目	合格
2019z023	民族学与社会学学院	反脆弱视域下云南民族民间工艺品传承创新研究	张茜	重点项目	延期
2019z016	马克思主义学院	“一带一路”背景下云南边境自贸试验区国家文化形象建设研究	王勇	重点项目	合格
2019z017	新闻学院	乡村精英的新媒体实践与日常权威建构	赵亚净	重点项目	优秀
2019z018	新闻学院	“新城移民”日常生活中社会交往的民族志考察	陈宸	重点项目	合格
2019z019	外国语学院	密松项目搁置后的缅甸民意走向	方渝	重点项目	优秀
2019z020	外国语学院	福柯身体理论视野下的非裔美国文学研究	赵燕	重点项目	合格
2019z021	艺术与设计学院	独龙族审美观念探微—以独龙族“卡雀哇”节为例	马国贤	重点项目	延期
2019z022	艺术与设计学院	大理巍山与周城扎染异同研究	郑丽君	重点项目	合格
2019z024	国际关系研究院	印度“能源贫困”问题研究	张帅	重点项目	合格
2019z025	体育学院	“一带一路”背景下留学生民族传统体育耦合平台构建研究	冯睿森	重点项目	合格
2019z026	职业与继续教育学院	云南省高职院校专业设置与区域经济适应性研究	王艳芬	重点项目	优秀
2019z027	政府管理学院	中缅边境非传统安全问题治理研究——以芒市为例	张骁	重点项目	合格
2019z028	政府管理学院	基于 PHESC 的云南省长期护理保险研究——以安宁新村社区为例	洪润琴	重点项目	合格
2019z029	工商管理与旅游管理学院	不同领办主体农民合作社多维减贫效应研究	何梦丹	重点项目	合格
2019z030	工商管理与旅游管理学院	旅游经济孤岛效应的形成机理及动力机制研究	普蔚	重点项目	合格
2019001	文学院	木心的世界性美学思想研究	李元奇	一般项目	延期
2019002	文学院	宋元两代“和杜诗”研究	罗荣福	一般项目	合格
2019003	文学院	“江南三部曲”的叙事艺术及其接受效应研究	田彤彤	一般项目	合格
2019004	文学院	普米、彝、纳西丧葬仪式中《指路经》口头叙事的特征分析	奚利	一般项目	优秀

2019005	文学院	新媒体时代白族大本曲的保护与传承研究	杨识余	一般项目	合格
2019006	文学院	语境对泰国留学生汉语塞擦音产出的影响研究	万龄蔓	一般项目	延期
2019007	文学院	输入加工和 3P 教学法对比研究——以对越南留学生的把字句教学为例	杨柏丽	一般项目	合格
2019008	文学院	石黑一雄的国际化写作研究	冯琪雯	一般项目	优秀
2019009	文学院	杨慎述滇文学文献整理研究	邓歌尔	一般项目	合格
2019010	文学院	彝汉文化的交融与重构：云南楚雄地方女神“姑奶奶”信仰的文化阐释	王倩倩	一般项目	合格
2019011	法学院	云南自贸区营商环境的法治建设：逻辑理路与路径展开	孙涛	一般项目	合格
2019012	法学院	中国高新技术企业对外投资法律风险控制——以华为 5G 技术面临的困境为例	李欣然	一般项目	合格
2019013	法学院	股权让与担保纠纷裁判逻辑的实证研究	王玉婷	一般项目	优秀
2019014	法学院	“校闹”处理机制研究——以大学生突发性事件为视角	毕美琪	一般项目	合格
2019015	法学院	澜湄区域执法合作机制研究——以云南边境“三非”人员跨境犯罪为切入点	王晓云	一般项目	合格
2019016	法学院	我国自然人破产制度探讨——基于全国首例自然人破产案例的分析与思考	周宇	一般项目	合格
2019017	法学院	人工智能背景下视频换脸技术的法律困境——“基于‘ZAO’协议的深入探讨”	李朝瑞	一般项目	合格
2019018	法学院	举报行为可诉性实证研究——兼谈最高人民法院[2013]行他字第 14 号复函案例	陈龙	一般项目	优秀
2019019	法学院	“互联网+护理服务”纠纷中第三方平台的民事责任问题研究	李佳玮	一般项目	延期
2019020	法学院	住宅改建城市民宿的法律问题研究:以呈贡区个案调研为视角	任婷	一般项目	合格
2019021	法学院	医疗保险反欺诈的法律对策研究——以昆明市为例	罗燕秋	一般项目	合格
2019022	经济学院	混合所有制改革程度与国企过度负债——政府治理及政府隐	陈博宇	一般项目	合格

		性担保的影响			
2019023	经济学院	5G 背景下智慧金融对云南省旅游业发展的影响研究	任子飞	一般项目	合格
2019024	经济学院	农业高质量可持续发展内生动力研究——基于技术进步、制度变迁与经济绩效互动关系视角	普友少	一般项目	优秀
2019025	经济学院	数字经济推动劳动转型的政治经济学研究	梁倩倩	一般项目	优秀
2019026	经济学院	积累的社会结构理论的演进路径：从戈登到奥哈拉	杨伯乐	一般项目	合格
2019027	经济学院	数字经济对贵州经济高质量发展的作用机制研究	唐若良	一般项目	合格
2019028	经济学院	边疆少数民族地区农村居民养老模式研究——以云南省大理白族自治州云龙县为例	徐晓堃	一般项目	合格
2019029	经济学院	云南易地扶贫搬迁政策跟踪审计问题及改进研究	李昕怡	一般项目	合格
2019030	经济学院	大理洱海治污达标审计指标体系的构建研究	周铭	一般项目	合格
2019031	经济学院	金融产品和服务创新助力云南省乡村振兴战略研究	翟九媛	一般项目	合格
2019032	经济学院	财政扶贫资金绩效审计评价体系构建及评价研究——以怒江傈僳族自治州福贡县为例	施海艺	一般项目	合格
2019044	经济学院	婴儿死亡率下降的生育效应影响经济增长的实证研究	袁君丽	一般项目	合格
2019045	经济学院	人口老龄化对服务业优化升级的区域异质性效应研究	宋晓莹	一般项目	优秀
2019046	经济学院	中日在大湄公河次区域第三方市场合作模式的探讨	戎雪	一般项目	合格
2019047	经济学院	高管性别结构对海外中国企业性别工资差距的影响机制研究	安然	一般项目	优秀
2019048	经济学院	创新驱动背景下解决农业人口“能力贫困” 问题研究	梁伟	一般项目	优秀
2019053	经济学院	特色小镇建设能实现旅游扶贫目标吗？	郝琪	一般项目	合格
2019033	历史与档案学院	李拂一与傣泰民族研究	杨童	一般项目	合格
2019034	历史与档案学院	病历数字档案馆建设模式及路径选择研究	何志丽	一般项目	延期
2019035	历史与档案学院	20 世纪以来云南橡胶的引种及其环境变迁	杜香玉	一般项目	优秀

2019036	历史与档案学院	从军事征讨到屯田守土——爨焚军与蒙元的西南边疆治理	张述友	一般项目	合格
2019037	历史与档案学院	云南罗婺彝族凤氏家族文书的整理与研究	万小玥	一般项目	合格
2019038	历史与档案学院	试论“禹贡全图”上的黑水	孔庆贤	一般项目	优秀
2019039	历史与档案学院	报刊中的水利问题：民国时期云南官方水利治理研究	谢太芳	一般项目	合格
2019040	历史与档案学院	以需求为导向的公共图书馆儿童分级阅读服务研究——以昆明市的图书馆为例	王君	一般项目	合格
2019041	历史与档案学院	从《东方杂志》看清末民初时期边疆观念变革	邓旭	一般项目	延期
2019042	历史与档案学院	明代征伐礼研究	刘协邦	一般项目	合格
2019043	历史与档案学院	云南少数民族地区基层博物馆发展现状与提升路径研究	郭秋菊	一般项目	延期
2019049	民族学与社会学学院	我国智慧养老模式的探究与对策研究	郑伊然	一般项目	合格
2019052	民族学与社会学学院	流动人口家庭化迁移的空间演变及影响因素研究	黄小丽	一般项目	合格
2019054	民族学与社会学学院	藏传佛教在欧洲的传播研究——以比利时为中心的考察	高云松	一般项目	合格
2019055	民族学与社会学学院	一个村庄的厕所革命：两种知识体系的冲突、调适与融合	唐烨	一般项目	合格
2019056	民族学与社会学学院	道路建设与云南边疆民族地区“中心化”过程的人类学研究	方伟洁	一般项目	合格
2019057	民族学与社会学学院	共商、共建与互惠：民族社区参与白马雪山保护区共管的模式与机制研究	张辉	一般项目	优秀
2019058	民族学与社会学学院	影像史学视域下云南少数民族题材电影中少数民族形象研究	刘金泉	一般项目	优秀
2019059	民族学与社会学学院	促进农村精神障碍康复者社区参与的行动研究	师艺萌	一般项目	延期
2019060	民族学与社会学学院	灾害、知识与应对:行动回应视角下三亚疍民的灾害人类学研究	朱德贤	一般项目	合格
2019061	民族学与社会学学院	普米族“拈达则”封山仪式及其山岳生态文化研究	徐双燕	一般项目	不合格
2019062	民族学与社会学学院	疾病与治愈：佤族治疗仪式的研究	张靖嗣	一般项目	合格
2019063	民族学与社会学学院	民国时期红河瓦渣土司与土民互诉案件的档案整理与研究	徐帆	一般项目	合格

2019064	民族学与社会学学院	中缅边境农村地区跨界移民劳工的组织社会学研究	杨天	一般项目	优秀
2019095	民族学与社会学学院	共生理论视角下艺术群体参与石林乡村文化振兴的机制建构	张俊祥	一般项目	合格
2019096	民族学与社会学学院	文化认同视域下建水紫陶的图式甄选与内容生产	杨小灵	一般项目	合格
2019065	马克思主义学院	“一带一路”倡议下中国与尼泊尔合作的进展与挑战	黑然	一般项目	合格
2019066	马克思主义学院	隐性教育在高校马克思主义基本理论教育中的应用研究	郭焕焕	一般项目	合格
2019067	马克思主义学院	习近平民族团结思想视域下大理郑家庄村民族团结示范村建设现状研究	周玉婷	一般项目	合格
2019068	马克思主义学院	边疆少数民族地区大学生铸牢中华民族共同体意识路径探析	张瑜敏	一般项目	合格
2019069	马克思主义学院	莫迪执政以来印度共产党（马）的发展态势及前景研究	胡月	一般项目	合格
2019070	新闻学院	微信“树洞”群互动与大学生抑郁症状研究	刘滔	一般项目	合格
2019071	新闻学院	环境传播与环保议题的形成及变迁——对景洪污水处理环保议题的个案分析	李鑫阳	一般项目	合格
2019072	新闻学院	影视剧中的云南想象与云南旅游形象建构研究	宇晴	一般项目	合格
2019073	新闻学院	大学生网络“翻墙”行为现状分析及解读——以昆明在校大学生为例	李嘉琦	一般项目	优秀
2019074	新闻学院	社会建构论视野下县级融媒体中心建设研究	罗晓欢	一般项目	合格
2019075	新闻学院	跨越数字鸿沟：家庭中新媒体技术反哺的影响因素研究	刘宝琦	一般项目	合格
2019076	新闻学院	环境传播视域下“垃圾分类”议题的媒体建构	荣韵	一般项目	合格
2019077	外国语学院	“一带一路”背景下留学生的EMI课堂学习困境及策略——以云南大学为例	董娟	一般项目	延期
2019078	外国语学院	《愚弄鸦族》中历史事件再现及重塑研究	华玉	一般项目	合格
2019079	外国语学院	现代美国文学中的跨文化互动研究： “垮掉的一代”文学作品透视	唐娇娇	一般项目	合格
2019080	外国语学院	印度罗摩故事对缅甸文化的影响	李雨曈	一般项目	合格

2019081	外国语学院	缅甸主流报刊对“中缅经济走廊”的舆情报道走向研究	刘文宇	一般项目	合格
2019082	外国语学院	从“炉边谈话”看莫迪的强人政治	周颖娉	一般项目	延期
2019083	外国语学院	“一带一路”汉法双语语料库建设	赖灿杰	一般项目	优秀
2019084	艺术与设计学院	玉溪窑青花瓷非遗活态化传承模式研究	谢凌峰	一般项目	合格
2019085	艺术与设计学院	摩梭人婚俗仪式音乐文化研究	王梧旬	一般项目	合格
2019086	艺术与设计学院	计算机辅助设计应用于保山苗族草木染服饰文创产品开发	王加军	一般项目	合格
2019087	艺术与设计学院	哈尼族传统民居空间分析与再设计——以云南元阳梯田村寨为例	李慧	一般项目	优秀
2019088	艺术与设计学院	以丽江金山乡为例的农村传统公共文化空间与乡村振兴研究	龙思圻	一般项目	合格
2019089	艺术与设计学院	影视艺术中的视觉污染治理研究	郄欣萌	一般项目	优秀
2019090	艺术与设计学院	少数民族传统面料应用于现代服饰的创意设计研究	崔馨婷	一般项目	合格
2019091	高等教育研究院	学生视角下的云南大学学分制改革研究	尧媛媛	一般项目	合格
2019092	高等教育研究院	跨学科视角下云南大学青年志愿者参与云南省社会治理研究	张娇	一般项目	合格
2019093	高等教育研究院	校地合作培训背景下基层进修教师专业发展追踪研究	杨丽芳	一般项目	优秀
2019094	高等教育研究院	云南大学南亚、东南亚留学生教育满意度研究	赵基琳	一般项目	合格
2019097	汉语国际教育学院	孟加拉留学生汉语双字调语音实验研究	康永超	一般项目	优秀
2019098	汉语国际教育学院	云南省跨境民族留学生的汉语认同与学习动机相关性研究	陈梦秋	一般项目	合格
2019099	国际关系研究院	土耳其政治文化：历史传统、转型和新发展	李岩	一般项目	合格
2019100	国际关系研究院	中印在缅甸地缘影响力的比较分析	马晓东	一般项目	合格
2019101	国际关系研究院	南非本土知识保护与利用国家政策的建构：历史、现状及趋势	王达	一般项目	合格
2019102	国际关系研究院	“一带一路”下的“债务陷阱”风险产生、问题与应对研究	马天放	一般项目	优秀
2019103	体育学院	滇西边境山区学生健康生活方式与行为素养研究	骆兴楠	一般项目	合格

2019104	体育学院	自我决定理论视角下云南省大学生体质健康问题的干预研究	王雷	一般项目	延期
2019105	体育学院	有氧运动促进强制戒毒人员身心健康的研究	田飞	一般项目	延期
2019106	体育学院	智慧城市视域下云南省全民健身服务人力资源平台的开发与设计研究	徐力达	一般项目	优秀
2019107	职业与继续教育学院	虚拟现实技术在英语词汇教学中的应用研究	刘嘉珮	一般项目	合格
2019108	职业与继续教育学院	脱贫攻坚时期云南省职业教育对接精准扶贫的 SWOT 以及对策分析	黄春艳	一般项目	合格
2019109	职业与继续教育学院	基于知识地图的个性化学习系统的开发与应用	雷宁	一般项目	优秀
2019110	职业与继续教育学院	普职沟通视域下职业教育启蒙的实践路径研究	毛婷婷	一般项目	合格
2019111	昌新国际艺术学院	昆明市晋宁区非物质文化遗产“乌铜走银”制作研究与保护发展	匡双杰	一般项目	优秀
2019112	昌新国际艺术学院	云南阿昌族传统服饰视觉符号设计创新研究	罗茜	一般项目	延期
2019113	政府管理学院	德宏傣族服饰伦理研究	胡晓	一般项目	合格
2019114	政府管理学院	基于结构方程模型下的昆明市营商环境满意度及其影响因素研究	徐佳铭	一般项目	延期
2019115	政府管理学院	基于 SWOT-PEST 的云南省数字经济战略发展研究	李鹏丽	一般项目	合格
2019116	政府管理学院	一以贯之：朱子“理一分殊”的理论纲脉	刘一尘	一般项目	合格
2019117	政府管理学院	阳明“心之本体”意象诠释	宋宇婷	一般项目	优秀
2019118	政府管理学院	“三权分置”视域下耕地闲置治理研究——以云南省 L 村为例	谢怡	一般项目	合格
2019119	政府管理学院	乡村振兴战略下云南边境村社治理困境及路径选择	潘淳	一般项目	合格
2019120	政府管理学院	云南边境治理中的外事界务员机制研究	侯红霞	一般项目	优秀
2019121	政府管理学院	昆明市金星社区少数民族流动人口服务管理体系研究	李青霖	一般项目	合格
2019122	政府管理学院	以云南省为例的价值共创视角下边境旅游扶贫多元主体协同机制研究	王翠玲	一般项目	合格

2019123	政府管理学院	中华民族共同体建设的普洱经验研究—基于 PEST 模型下的组态分析	佘欣	一般项目	延期
2019124	工商管理与旅游管理学院	居民旅游感知对旅游支持行为的影响异质性及调控	葛绪锋	一般项目	合格
2019125	工商管理与旅游管理学院	云南省能源消费结构与大气保护动态关系管理研究	汤瑞丰	一般项目	合格
2019126	工商管理与旅游管理学院	云南健康旅游目的地建设评价体系与路径模式研究	汪洋周颖	一般项目	合格
2019127	工商管理与旅游管理学院	云南省边境旅游系统协调发展与失衡调控研究	张琴悦	一般项目	合格
2019128	工商管理与旅游管理学院	中国人力资本结构与产业结构的耦合协调发展研究	柳瑞	一般项目	合格
2019129	工商管理与旅游管理学院	云南省康养旅游产业发展政策协调多维实证研究	李娇	一般项目	延期
2019050	工商管理与旅游管理学院	高质量增长约束下测度我国木本生物燃料产业发展的研究	张智晗	一般项目	合格
2019051	工商管理与旅游管理学院	云南省交通基础设施对旅游业的空间溢出效应研究——以公路、铁路为例	刘婷	一般项目	合格
2019130	工商管理与旅游管理学院	产业融合视角下的云南养老旅游发展策略及实现路径研究	王梓	一般项目	延期
2019131	建筑与规划学院	城镇化对城市绿地生态效益的影响及对策研究——以昆明市县域为例	王玉阁	一般项目	延期
2019z031	地球科学学院	基于 GIS 的泸水市滑坡灾害风险性评价	袁静	重点项目	合格
2019z032	地球科学学院	能源消费加热效应对我国华北夏季高温的影响研究	宋欣	重点项目	合格
2019z042	地球科学学院	黔西滇东晚二叠世—早三叠世大孢子植物群研究	隋群	重点项目	合格
2019z046	地球科学学院	南海东北部冷泉活动演化历史研究——以 GMGS2-08 钻孔为例	邓义楠	重点项目	优秀
2019z059	地球科学学院	云南省缓步动物门在不同海拔的分布及适应性研究	房艳秋	重点项目	延期
2019z033	建筑与规划学院	基于切比雪夫多项式的梁式桥振动传感器优化布置研究	莫相龙	重点项目	延期
2019z034	建筑与规划学院	滇中城市群空间结构演变与环境效应研究	汪琳	重点项目	延期
2019z035	物理与天文学院	阻抗磁层中脉冲星高能辐射特性研究	杨雄帮	重点项目	合格
2019z036	物理与天文学院	基于碳量子点的有机农残多菌灵的荧光检测	杨悦	重点项目	优秀

2019z037	物理与天文学院	广角切伦科夫荧光望远镜光电探测器性能研究	鲁睿	重点项目	优秀
2019z060	物理与天文学院	相互作用暗能量模型下暗物质晕性质的研究	刘赟	重点项目	延期
2019z038	信息学院	基于视觉感知的实时目标检测算法研究	张超	重点项目	合格
2019z039	信息学院	造影超声各次谐波的自适应分离方法研究	韩素雅	重点项目	合格
2019z040	软件学院	基于复杂网络共演化博弈动力学研究	胡蝶	重点项目	优秀
2019z041	软件学院	时空演化下的群智数据智慧交通研究	杨兵	重点项目	优秀
2019z043	数学与统计学院	平面上带直线的多约束斯坦纳树问题	王文成	重点项目	合格
2019z044	数学与统计学院	多线性 PageRank 向量的唯一性及其求解研究	王笑笑	重点项目	优秀
2019z045	资源植物研究院	云南大学呈贡校区常见昆虫资源调查与利用	许振邦	重点项目	延期
2019z047	化学科学与工程学院	金钗石斛化学成分诱导其内生菌 Diaporthe sp.代谢活性新颖化合物的研究	胡明	重点项目	合格
2019z048	化学科学与工程学院	开发新型 MOR 材料从工业废水中高效去除金属氰化物	陈慕涵	重点项目	合格
2019z049	生命科学学院	人类单个密码子的偏好性对基因表达影响的研究	陈顺楷	重点项目	延期
2019z050	生命科学学院	藏羚羊群体基因组学和保护遗传学研究	匡卫民	重点项目	优秀
2019z051	生态学与环境学院	竹叶水分光合对干旱的响应、干旱后的恢复及生理生态适应策略	王婷	重点项目	优秀
2019z052	生态学与环境学院	地上和地下兰花菌根真菌网络对宿主兰花分布和丰富度的影响	李太强	重点项目	优秀
2019z053	材料与能源学院	基于 CdS 量子点的铜离子荧光检测研究	赵荣俊	重点项目	合格
2019z054	材料与能源学院	硫酸钾活化生物质碳制备富氧多孔碳超级电容器电极材料及其性能研究	洪萍	重点项目	合格
2019z055	材料与能源学院	In_2O_3纳米材料的改性及气敏性能研究	周仕强	重点项目	合格
2019z056	医学院	灰树花多糖抗阿尔兹海默症作用及其机制研究	白瑶	重点项目	优秀

2019z057	国际河流与生态安全研究院	元江干热河谷区退耕地土壤净氮转化过程与机制	李雅文	重点项目	合格
2019z058	国际河流与生态安全研究院	青藏高原濒危植物气候避难所识别	刘锋	重点项目	合格
2019132	地球科学学院	初夏南亚高压对前期北太平洋海温年际变异的响应及其物理机制	杨宇	一般项目	合格
2019133	地球科学学院	基于 GIS 的云南边境沿线地缘环境信息提取与建模	蔡敬芝	一般项目	合格
2019134	地球科学学院	云南典型喀斯特关键带植被时空变化特征及自然与人为因子解析	余岚	一般项目	优秀
2019135	地球科学学院	中缅边境地区不透水面动态变化研究--以瑞丽市为例	吴博闻	一般项目	合格
2019136	地球科学学院	基于 RS 和 GIS 的瑞丽市退耕还林生态效益评价研究	刘师旖	一般项目	合格
2019137	地球科学学院	冬半年南支槽影响我国西南和东南地区降水对比分析	殷举玥	一般项目	合格
2019138	地球科学学院	基于天气学和气候学特征的南支槽新指数的构建	毛梦妮	一般项目	合格
2019167	地球科学学院	滇东黔西晚二叠世栉羊齿型植物分类学及其演化研究	周煜	一般项目	优秀
2019168	地球科学学院	基于硝酸盐氮、氧同位素技术示踪解析抚仙湖污染来源研究	姜宇	一般项目	合格
2019179	地球科学学院	云南景谷早中新世植物群多样性及古气候研究	宋艾	一般项目	合格
2019180	地球科学学院	新疆淖毛湖中侏罗世木化石分类学与古环境研究	缑旭东	一般项目	合格
2019181	地球科学学院	青藏高原东南缘新生代盆地演化：碳酸盐矿物 U-Pb 年代学约束	刘高政	一般项目	合格
2019182	地球科学学院	贡觉盆地新生代沉积物源示踪及其对云南高原构造地貌演化的指示	边紫璇	一般项目	合格
2019228	地球科学学院	寒武纪早期小石坝生物群珊瑚研究	杨小宇	一般项目	优秀
2019238	地球科学学院	云南省昆明市晋宁地区中侏罗统恐龙足迹化石研究	李洪清	一般项目	合格
2019139	建筑与规划学院	国土空间视角下的高原山地民族聚落空间格局研究	单欣	一般项目	优秀
2019140	建筑与规划学院	SMA 弹簧-三段式变摩擦复合阻尼器设计与分析	刘超	一般项目	合格

2019141	建筑与规划学院	云南传统村落民居群体建筑震害预测研究	杨成峰	一般项目	延期
2019142	建筑与规划学院	非共沸工质分液冷凝有机闪蒸循环系统研究	袁志鹏	一般项目	优秀
2019143	建筑与规划学院	盾构隧道穿越既有建筑物桩基础托换承载机理研究	陆子豪	一般项目	合格
2019144	建筑与规划学院	云南省异地扶贫搬迁项目房屋质量综合评价及质量成本优化研究	周振	一般项目	延期
2019145	物理与天文学院	高温超导材料 YBCO 的光电特性研究	周顺	一般项目	合格
2019146	物理与天文学院	高熵合金的结构特征及变形机理的研究	巩龙	一般项目	合格
2019147	物理与天文学院	多色掺杂碳量子点的制备及其作为荧光探针的光学性能研究	唐菊	一般项目	优秀
2019148	物理与天文学院	微型光流控激光器及其在单层蛋白质分子检测中的应用研究	李东阳	一般项目	合格
2019149	物理与天文学院	有机无机钙钛矿石墨烯复合材料的制备及其光电性能的研究	褚景豫	一般项目	合格
2019150	物理与天文学院	一种新型自组装多孔 CO_3O_4/TiO_2 微球的制备及甲醛气敏性能研究	荣茜	一般项目	合格
2019234	物理与天文学院	星系内禀指向的相关性对弱引力透镜信号峰值统计的影响	张天宇	一般项目	延期
2019235	物理与天文学院	基于 LAMOST M 矮星样本精确测定太阳邻域分子云距离	沈涵	一般项目	延期
2019236	物理与天文学院	利用 HDBScan 对 YSO 区域进行多尺度结构分析	周吉璇	一般项目	延期
2019237	物理与天文学院	利用 Mephisto 颜色直接证认天琴座 RR 型变星并对其物理性质的研究	王涛	一般项目	延期
2019151	信息学院	无接触人体行为识别研究与跌倒检测应用	蒙倩霞	一般项目	合格
2019152	信息学院	面向药物-靶标发现的图神经网络构建、推理与应用	郭延哺	一般项目	优秀
2019153	信息学院	基于深度学习的联合服装及妆容时尚提升	阳秋霞	一般项目	合格
2019154	信息学院	基于深度学习的青少年骨骼年龄自动监测方法研究	王嘉庆	一般项目	合格
2019155	信息学院	开放世界假设下基于贝叶斯网的知识图谱实体相关性发现方法与应用研究	李杰	一般项目	合格

2019156	信息学院	基于深度矩阵分解的多视角聚类	杜国王	一般项目	合格
2019157	信息学院	基于定制文本情感分析的云南旅游大数据应用研究	张有	一般项目	优秀
2019158	信息学院	基于遗传混成算法的多层次软件运行行为挖掘方法	汤雅惠	一般项目	优秀
2019159	信息学院	云南民族绘画情感感知计算与可视化分析	张浩	一般项目	合格
2019160	信息学院	适用于5G基于ISGW缝隙耦合馈电的圆极化电磁偶极子天线研究	周利平	一般项目	合格
2019161	软件学院	行为语义保持的过程模型化简及验证	黄月	一般项目	优秀
2019162	软件学院	针对帕金森病患活动识别的可穿戴智能技术研究	彭西阳	一般项目	合格
2019163	软件学院	基于GPU的智能索引树k-NN查询框架研究	王淑娟	一般项目	合格
2019164	软件学院	基于循环生成对抗网络的图像彩色化方法研究	黄珊珊	一般项目	合格
2019165	软件学院	基于复杂网络位置属性的节点重要性研究	景铭	一般项目	合格
2019166	软件学院	卷积神经网络在蛋白质二级结构预测的研究	郭淋	一般项目	合格
2019169	数学与统计学院	分数阶微分方程的概周期型解研究	胡兴	一般项目	合格
2019170	数学与统计学院	时标上脉冲四元数值神经网络的动力学研究	黄晓丽	一般项目	合格
2019171	数学与统计学院	多项分布和泊松--逆高斯分布的三臂实验的设计和分析	陈慧媛	一般项目	优秀
2019172	数学与统计学院	带有上下界限制的指派问题	胡郭军	一般项目	合格
2019173	数学与统计学院	带有时间窗口的限制性路增广问题	蔡力健	一般项目	合格
2019174	资源植物研究院	黄毛草莓（Fragaria nilgerrensis）同源四倍体转录组学研究	卢玠	一般项目	延期
2019175	资源植物研究院	大白菜紫色酸性磷酸酶基因家族（PAPs）与低磷响应的关联及分子标记开发	蔡永芳	一般项目	延期
2019176	资源植物研究院	香蕉球茎组织淀粉积累对镰刀菌酸毒性抑制的机制研究	张晶	一般项目	优秀
2019177	资源植物研究院	长雄野生稻和多年生稻内生菌的分离鉴定及其对水稻病害的	龚禹瑞	一般项目	优秀

		拮抗研究			
2019178	资源植物研究院	工业大麻磷胁迫转录组学分析	李璇	一般项目	延期
2019183	化学科学与工程学院	基于硼酸基质的光致响应有机室温磷光探索和研究	程艺	一般项目	合格
2019184	化学科学与工程学院	基于 MOFs 微观结构调控强化去除水体中低浓度磷的研究	李声剑	一般项目	优秀
2019185	化学科学与工程学院	基于荧光碳点和纳米金的多信号可视化检测方法的构建及其应用	吴素仪	一般项目	合格
2019186	化学科学与工程学院	抗临床分离耐药菌的纳米镓复合材料的合成	廖文慧	一般项目	延期
2019187	化学科学与工程学院	滇重楼内生真菌及其代谢产物的研究	胡俊涛	一般项目	合格
2019188	化学科学与工程学院	基于 OSMAC 策略下的一株松茸内生菌（Trichoderma sp.）的次级代谢产物研究	王雪银	一般项目	合格
2019189	化学科学与工程学院	用于铂离子固相萃取的新型离子印迹聚合物研究	金朝帆	一般项目	合格
2019190	化学科学与工程学院	木紫珠二萜化合物抑制炎症小体的活性及作用机制初探	王棋	一般项目	合格
2019191	化学科学与工程学院	喹啉类有机配体的稀土 MOFs 的发光传感研究	范文雯	一般项目	优秀
2019192	化学科学与工程学院	一步氢解乙酰丙酸制γ-戊内酯单原子催化剂的创制	陆耀威	一般项目	优秀
2019193	化学科学与工程学院	固定床反应器中催化剂对生物质原位催化重整制备合成天然气的特性研究	张旭	一般项目	合格
2019194	生命科学学院	基于叶绿体基因的 7 种卷柏比较基因组学研究	赵镜	一般项目	合格
2019195	生命科学学院	紫茎泽兰叶内生炭疽真菌（Colletochichum spp.)对经济作物的致病风险评估和机理研究	杨爱玲	一般项目	延期
2019196	生命科学学院	基于 RAD-seq 技术探究云岭黑山羊与努比山羊重要经济性状形成的遗传基础	胡芳	一般项目	合格
2019197	生命科学学院	根结线虫二龄幼虫微生物侵染的分子机制	何忠看	一般项目	合格
2019198	生命科学学院	大肠杆菌膜蛋白 OmpG 在抗生素处理时对细菌的保护机制	李媛园	一般项目	优秀

2019199	生命科学学院	DOCK1 参加核仁完整性维持和有丝分裂的研究	李娟	一般项目	合格
2019200	生命科学学院	caspase-1 介导的紫杉醇诱发乳腺癌细胞焦亡	曹珈琪	一般项目	合格
2019201	生命科学学院	蒿甲醚对肺癌细胞内 Connexin43 及其转录因子作用机制的研究	石梦姿	一般项目	延期
2019202	生命科学学院	丛枝菌根介导不同品种玉米重金属积累的差异性响应特征及机理研究	殷泽鹏	一般项目	合格
2019203	生态学与环境学院	利用转录组探讨象牙参泌蜜的分子调控机制	王雯婧	一般项目	合格
2019204	生态学与环境学院	卷柏科异形孢子的演化历史及其适应性意义	叶紫瑜	一般项目	优秀
2019205	生态学与环境学院	猕猴桃不同倍性植株沿海拔替代分布的潜在生理机制	刘彩艳	一般项目	合格
2019206	生态学与环境学院	结合系统发育学的石栎属果实形态和类型进化研究	覃元艺	一般项目	延期
2019207	生态学与环境学院	西双版纳傣族对庭院外来植物认知研究	徐蕾	一般项目	合格
2019208	生态学与环境学院	梯级大坝库区水环境梯度与鱼类群落分布格局的关系研究	陈美玲	一般项目	合格
2019209	生态学与环境学院	铅镉胁迫下栽叶酸膜的生理特性和遗传分化研究	杨化菊	一般项目	合格
2019210	材料与能源学院	钙钛矿 $LiCuNb_3O_9$陶瓷材料的 B 位调控制备与巨介电性质研究	高丹丹	一般项目	合格
2019211	材料与能源学院	液态金属复合低温碳电极构筑高效率柔性钙钛矿光伏器件	赵建红	一般项目	合格
2019212	材料与能源学院	纳米结构 CeO_2基复合材料的制备及其 CO 催化氧化性能研究	张旭	一般项目	合格
2019213	材料与能源学院	三苯胺空穴传输材料合成及其在钙钛矿太阳能电池中的应用	欧阳美	一般项目	合格
2019214	材料与能源学院	纳米一维SnO_2与二维 NiO 异质结构设计、材料复合及气体敏感性能研究	李成	一般项目	合格
2019215	材料与能源学院	全固态钠离子电池 NZSPO/PEO 固体电解质构筑及界面性能研究	刘书铭	一般项目	合格
2019229	材料与能源学院	基于机器学习的钙钛矿和锂电池材料的性能筛选预测技术的研究	赵轩	一般项目	合格
2019230	材料与能源学院	石墨烯复合纳米碳黑颗粒与硼酸钴纳米片三维材料的电催化	倪子涛	一般项目	合格

		性能研究			
2019231	材料与能源学院	生物模板碳材料用于太阳能下污水净化处理	敖亚丽	一般项目	合格
2019232	材料与能源学院	基于硅金字塔阵列的石墨烯量子点杂化异质结太阳能电池的研究	周维杰	一般项目	合格
2019233	材料与能源学院	多孔 Si/C 基纳米复合材料的结构调控及脱嵌 Li 性能研究	戴瑞杰	一般项目	合格
2019216	材料与能源学院	高性能甲醛敏感一维 SnO2 阵列/rGO 复合材料的设计合成及气敏机制研究	赵燕平	一般项目	合格
2019217	医学院	细胞坏死在草酸钙晶体诱导肾脏损伤中的作用机理研究	杜心平	一般项目	合格
2019218	医学院	灵芝多糖对抑郁症小鼠的治疗作用及其机制研究	肖玉焕	一般项目	优秀
2019219	医学院	FXR 受体调控炎症小体和神经突触可塑性的抗抑郁分子机制研究	李浩然	一般项目	合格
2019220	医学院	BDNF 信号联动 IL-1β “双向”效应失稳与阿尔茨海默病神经元损伤的实验研究	时子旭	一般项目	合格
2019221	医学院	抑制 MUC5B 逆转非小细胞肺癌顺铂耐药的分子机制研究	李蔚源	一般项目	合格
2019222	国际河流与生态安全研究院	干旱胁迫和复水作用对不同生长时长生物膜吸附及释放重金属的影响机制	杨远昊	一般项目	延期
2019223	国际河流与生态安全研究院	基于不同气候变化情景的元江流域水文干旱变化趋势预估	刘春雨	一般项目	合格
2019224	国际河流与生态安全研究院	云南省自然保护区保护优先性评估	王一婷	一般项目	合格
2019225	国际河流与生态安全研究院	近 38 年来喀喇昆仑山南北坡积雪物候差异及其对气候的响应研究	易颖	一般项目	合格
2019226	国际河流与生态安全研究院	基于深度学习的表碛覆盖型冰川表面特征识别方法研究	谢福明	一般项目	优秀
2019227	国际河流与生态安全研究院	跨境河流洄游鱼类迁徙习性及保护策略研究	丁刘勇	一般项目	优秀

研究生院·学科建设办

2020 年 10 月 22 日

云南大学第十二届研究生科研创新项目拟立项名单

序号	培养单位	项目名称	项目分类	项目类型	项目负责人
2020Z01	工商管理与旅游管理学院	频繁并购重组风险与审计师风险管理策略研究	人文社科	重点项目	宋玉禄
2020Z02	工商管理与旅游管理学院	社会资本对乡村旅游企业社会责任行为的影响机制	人文社科	重点项目	葛绪锋
2020Z03	工商管理与旅游管理学院	众创空间对创客创新绩效的机制研究及对策提升	人文社科	重点项目	陈雅惠
2020Z04	外国语学院	佛本生故事对缅甸文化的影响	人文社科	重点项目	罗佳丽
2020Z05	外国语学院	云南边境地区应急语言能力的构建研究	人文社科	重点项目	倪志娟
2020Z06	昌新国际艺术学院	南北书风融合下云南碑刻书法及其文化价值研究	人文社科	重点项目	董泽衡
2020Z07	昌新国际艺术学院	昆明市晋宁区晋城镇非物质文化遗产 “小独龙舞”的记述与传承保护	人文社科	重点项目	霍彦玮
2020Z08	法学院	民法典时代滇池湖滨生态环境修复责任适用问题及对策研究	人文社科	重点项目	龚璇
2020Z09	法学院	挑战与重塑：德宏州管理跨境来华务工人员经验与法律规制研究	人文社科	重点项目	周晓光
2020Z10	法学院	疫情防控常态化背景下不可抗力与合同解除适用研究	人文社科	重点项目	周雅婷
2020Z11	法学院	基层人民调解研究——以S市D区基层组织人民调解为样本的分析	人文社科	重点项目	牟奕霖
2020Z12	法学院	组织守法视角下的产险企业合规治理研究	人文社科	重点项目	邹舟
2020Z13	国际关系研究院	印太战略背景下东南亚安全新态势与 “海上丝绸之路”建设研究	人文社科	重点项目	赵祺
2020Z14	汉语国际教育学院	留学生来华前后对中国印象的对比研究 ——以云南大学为例	人文社科	重点项目	赵丹妮
2020Z15	汉语国际教育学院	基于交互理论的对外汉语线上教学互动模式研究	人文社科	重点项目	袁睿廷
2020Z16	历史与档案学院	基于档案馆网站平台的云南少数民族档案信息资源多向度开发研究	人文社科	重点项目	邱志鹏
2020Z17	历史与档案学院	清代前期城市税收管理研究	人文社科	重点项目	王雷
2020Z18	历史与档案学院	法律视角下的宋代疫病研究	人文社科	重点项目	蹇雪
2020Z19	历史与档案学院	互动与交流：唐宋转型视域下的“丝路”整体性研究	人文社科	重点项目	张欣
2020Z20	历史与档案学院	多民族国家建构视野下中国古代西南边疆与内地政治交往研究	人文社科	重点项目	黄丰富
2020Z21	民族学与社会学学院	市场嵌入过程中S村当代代际生计变迁研究	人文社科	重点项目	高源
2020Z22	民族学与社会学学院	“大滇西旅游环线建设”战略下松赞酒店的地域文化呈现及经验研究	人文社科	重点项目	邬迪
2020Z23	民族学与社会学学院	“给你梦想的西双版纳”：关于告庄西双景区的旅游想象与主客互动研究	人文社科	重点项目	杨琪
2020Z24	文学院	罗兰·巴特“能指解放”思想研究	人文社科	重点项目	于子然
2020Z25	政府管理学院	边疆民族政府旅游扶贫政策效益研究 —以西盟县为例	人文社科	重点项目	龙琴霞
2020Z26	政府管理学院	基于SLA-TOE模型的云南民族边疆地区 “脱贫防返”研究	人文社科	重点项目	刘昀煜
2020Z27	政府管理学院	铸牢中华民族共同体意识的政治符号机制研究	人文社科	重点项目	刘春呈
2020Z28	职业与继续教育学院	基于数据驱动的大学现代教育技术课程精准教学模式设计与应用研究	人文社科	重点项目	江孟雪

2020Z29	经济学院	国际国内双循环背景下我国西部地区经济增长级 “成昆渝经济区”的构建探究	人文社科	重点项目	林彩云
2020Z30	经济学院	“后疫情”时代我国产业链韧性研究——投入产出网络传染视角	人文社科	重点项目	侯威
2020Z31	经济学院	云南省光伏扶贫项目的绩效和能源环境效益研究	人文社科	重点项目	刘亚庆
2020Z32	经济学院	大股东减持、企业金融化和实体经济发展	人文社科	重点项目	方紫意
2020Z33	经济学院	“双循环”格局下滇中新区和昆明市产业分工的机制与路径研究	人文社科	重点项目	戴祎楠
2020Z34	经济学院	云南省金融脆弱性评价指标构建及其与经济增长的动态关系研究	人文社科	重点项目	宋淮海
2020Z35	艺术与设计学院	摩梭人葬俗仪式音乐文化研究	人文社科	重点项目	王梧旬
2020Z36	艺术与设计学院	近代以来石林彝族服饰的审美变迁研究	人文社科	重点项目	唐俊
2020Z37	新闻学院	“数字地方感”：短视频中的人地连结	人文社科	重点项目	黄博
2020Z38	新闻学院	网络直播观看的角色互构与文化互动研究	人文社科	重点项目	甘庆超
2020Z39	体育学院	云南大学学生健康体适能状况的Meta分析及促进研究	人文社科	重点项目	孟令飞
2020Z40	高等教育研究院	后疫情时代在线课堂评价体系研究	人文社科	重点项目	黄盼盼
2020001	工商管理与旅游管理学院	组织生态视角下企业绿色创新行为诱发机理与激励研究	人文社科	一般项目	窦垚
2020002	工商管理与旅游管理学院	基于LDA模型的西南地区旅游扶贫模式的演进和创新	人文社科	一般项目	兰洪超
2020003	工商管理与旅游管理学院	风险容忍度与大学生就业满意度研究——家庭就业观念的调节作用	人文社科	一般项目	李仕灵
2020004	工商管理与旅游管理学院	非接触经济下的品牌感知实现机理研究	人文社科	一般项目	李娇
2020005	工商管理与旅游管理学院	乡村振兴背景下企业家精神与农村经济创新发展的耦合机制研究	人文社科	一般项目	钟雨芮
2020006	工商管理与旅游管理学院	控股股东股权质押、机构投资者异质性对企业创新投资的影响研究	人文社科	一般项目	赵东倩
2020007	工商管理与旅游管理学院	促进还是抑制 中国人口老龄化对产业结构升级的影响研究	人文社科	一般项目	周大辉
2020008	工商管理与旅游管理学院	基于网络文本分析的云南省健康旅游目的地形象传播研究	人文社科	一般项目	郑婷
2020009	工商管理与旅游管理学院	普洱茶地理标志品牌建设研究	人文社科	一般项目	李刚
2020010	工商管理与旅游管理学院	大滇西旅游环线旅游品牌的共建共享机制研究	人文社科	一般项目	张婧怡
2020011	工商管理与旅游管理学院	长江经济带数字经济发展对绿色全要素生产率的影响研究——基于空间杜宾模型	人文社科	一般项目	杜昱辰
2020012	外国语学院	女性主义叙事学视阈下欧茨小说叙事策略研究	人文社科	一般项目	曾兰云
2020013	外国语学院	日本对东帝汶民主共和国的政府开发援助	人文社科	一般项目	汪诗琦
2020014	外国语学院	“一带一路”背景下缅甸国内冲突对中国在缅投资的影响及对策研究	人文社科	一般项目	李阳行
2020015	外国语学院	文化图式理论视阈下《茶马古道》文化负载词的英译研究	人文社科	一般项目	李玥月
2020016	外国语学院	《面纱》女主人公自我成长的列维纳斯式解读	人文社科	一般项目	廖恒
2020017	外国语学院	新冠肺炎疫情下泰国外籍劳工面临的挑战及其对策研究	人文社科	一般项目	孙国丽
2020018	外国语学院	诗歌美学视域下20世纪30年代中越“新诗”创作比较研究	人文社科	一般项目	黄伟

2020019	外国语学院	“气盛言宜”：翻译美学视角下韩愈散文语言风格之再现——基于不同法译文本比较	人文社科	一般项目	赵忻
2020020	昌新国际艺术学院	脱贫攻坚视角下云南省禄劝县彝苗童声合唱团文化宣传歌曲创作	人文社科	一般项目	赵雪皓
2020021	昌新国际艺术学院	脱贫攻坚任务下哈尼族聚落特色乡土建筑“蘑菇房”保护及发展研究	人文社科	一般项目	姚绍松
2020022	昌新国际艺术学院	云南民间传统工艺现代化探索-建水紫陶色泥配制多样性实验研究	人文社科	一般项目	关晓杰
2020023	昌新国际艺术学院	叶枝镇同乐村傈僳族传统文化保护区木构建筑的测绘研究与保护发展	人文社科	一般项目	陈思
2020024	昌新国际艺术学院	篆铸艺——刻字在昆明的地区的发展研究与保护	人文社科	一般项目	罗梦珍
2020025	昌新国际艺术学院	基于扶贫设计视域下斑铜文创产品设计与应用	人文社科	一般项目	韩婧
2020026	法学院	刑事庭审被告人对质权运行现状实证研究 —以50个庭审样本为依据	人文社科	一般项目	蔡东恒
2020027	法学院	边民互市民事法律问题研究：以云南河口调研为例	人文社科	一般项目	杨虎
2020028	法学院	中缅界碑两侧边境村的法律生活与社会控制 ——以马洛村（中）与外马洛（缅）为例	人文社科	一般项目	鲍蓓
2020029	法学院	从疫情中的法律适用思考危害公共安全罪中其他危险方法的认定	人文社科	一般项目	李亚倩
2020030	法学院	宋元司法中亲属问题研究	人文社科	一般项目	王鹤
2020031	法学院	基层法律援助运行现状调查研究 ——以滇东北农村离婚女性权益保护为视角	人文社科	一般项目	黄昇
2020032	法学院	乡村振兴战略背景下农村宅基地“三权分置”政策运行现状实证研究	人文社科	一般项目	蓝资颖
2020033	法学院	边境地区跨界民族公共卫生服务援助（供给）研究	人文社科	一般项目	宋克敏
2020034	法学院	性别平等视角下的性侵法律问题研究	人文社科	一般项目	唐亚康
2020035	法学院	认罪认罚从宽制度中证据开示问题研究	人文社科	一般项目	王敬业
2020036	法学院	“和气”视阈下的扶贫政策与低保制度衔接问题实证研究	人文社科	一般项目	王晓玲
2020037	法学院	壮族法制文化融入家事案件多元化纠纷解决机制实证研究	人文社科	一般项目	吴兢
2020038	法学院	《民法典》背景下空间权制度研究	人文社科	一般项目	李兆能
2020039	法学院	高空抛物致损的侵权责任研究-——以《民法典》第1254条的实践适用为视角	人文社科	一般项目	代迪
2020040	法学院	高校性骚扰的预防与救济制度研究	人文社科	一般项目	李婷
2020041	法学院	校园性骚扰的法律认定与处理 —基于新加坡的经验	人文社科	一般项目	刘英伟
2020042	法学院	道歉理论视角下的非诉讼医疗纠纷解决机制研究	人文社科	一般项目	王康宁
2020043	法学院	旧城改造中的纠纷类型化及其多元解决机制研究——基于Y省G区800个拆迁户的实证研究	人文社科	一般项目	李顺彩
2020044	法学院	农副产品电商交易中非强制登记主体的市场规制问题研究	人文社科	一般项目	宋欣恬
2020045	法学院	街头牌坊文化的法律规制与保护 ——以昆明市昆阳街道文化巷为例	人文社科	一般项目	刘明
2020046	国际关系研究院	自主与多元：越南的多边主义外交战略	人文社科	一般项目	李根
2020047	国际关系研究院	结构性暴力中的“被迫献祭”：撒哈拉以南非洲“巫童”问题研究	人文社科	一般项目	黄世顺
2020048	国际关系研究院	“印太战略”视域下印泰安全合作及中国的策略研究	人文社科	一般项目	林汉东

2020049	国际关系研究院	孟加拉国对中国“一带一路”倡议的认知与反应	人文社科	一般项目	王玉浩
2020050	国际关系研究院	越南历史教科书中的中国形象	人文社科	一般项目	张晗
2020051	国际关系研究院	加快推进中国与湄公河国家跨境经济合作区建设的路径和对策研究	人文社科	一般项目	王欢欢
2020052	汉语国际教育学院	基于中韩活字印刷术起源的留学生中华文化课教学研究	人文社科	一般项目	李杨
2020053	汉语国际教育学院	跨语言母语者汉语声调产出声学实验研究	人文社科	一般项目	李瑾皓
2020054	汉语国际教育学院	云南大学留学生汉语学术写作调查及其问题研究	人文社科	一般项目	拜梦娇
2020055	汉语国际教育学院	基于“5W”理论的中国文化传播分析——以“歪果仁研究协会”短视频为例	人文社科	一般项目	黄维
2020056	汉语国际教育学院	云南大学留学生学术适应情况调查及问题研究	人文社科	一般项目	代碧瑶
2020057	汉语国际教育学院	云南地域文化在对外汉语教学中的导入研究	人文社科	一般项目	马雪梅
2020058	建筑与规划学院	贫困山区农户生计变迁的时空格局及影响因素研究	人文社科	一般项目	祝庆鹏
2020059	建筑与规划学院	基于“三生融合”理念的云南省城市空间布局及优化策略研究	人文社科	一般项目	万统帅
2020060	历史与档案学院	农村留守儿童阅读现状研究	人文社科	一般项目	严孟娟
2020061	历史与档案学院	抗战记忆视角下西南联大文献资源知识地图构建研究	人文社科	一般项目	蔡锐
2020062	历史与档案学院	WSR视角下个人数字存档行为影响因素研究	人文社科	一般项目	钟志龙
2020063	历史与档案学院	新冠疫情背景下网络问答社区用户信息需求及情感动因研究	人文社科	一般项目	支茵
2020064	历史与档案学院	用户间交互行为对高校图书馆服务效能的影响研究	人文社科	一般项目	傅津
2020065	历史与档案学院	烧结粘土砖在作为文物保护与修复材料时的冻融循环损伤机理研究	人文社科	一般项目	刘斌
2020066	历史与档案学院	国家治理视阈下清代普洱府地区政治地理格局演进研究	人文社科	一般项目	高一鸣
2020067	历史与档案学院	方言学堂与云南近代教育的转型	人文社科	一般项目	邓宣
2020068	历史与档案学院	清政府对南海地区行政管辖的特点、嬗变及其历史意义	人文社科	一般项目	李铮
2020069	历史与档案学院	考古学视域下怒江州傈僳族与彝族基督教本土化对比研究	人文社科	一般项目	唐辰昊
2020070	历史与档案学院	民国时期云南地理研究成就的学术检视	人文社科	一般项目	林秋炀
2020071	历史与档案学院	民国时期滇东南盘江流域水利近代化及其影响探究	人文社科	一般项目	刘迪
2020072	历史与档案学院	抗日战争时期中国女性身体观念变迁与社会对女性身体规训研究	人文社科	一般项目	常璐
2020073	历史与档案学院	明清云南地区战象演变研究	人文社科	一般项目	李鹏飞
2020074	历史与档案学院	明代万历年间邓子龙戍滇行实考述	人文社科	一般项目	陈濡忘
2020075	历史与档案学院	人才培养与海疆建设——国立海疆学校研究	人文社科	一般项目	王倩
2020076	历史与档案学院	民国时期昆明疫病流行与公共卫生体系的构建研究（1912～1945）	人文社科	一般项目	张浩浩
2020077	历史与档案学院	宋代县主簿勾稽职能研究	人文社科	一般项目	朱倩倩
2020078	历史与档案学院	晚清中法越南勘界问题研究	人文社科	一般项目	张清涛

2020079	历史与档案学院	从并立走向融合：明代云龙州与五井盐课提举司关系演变研究	人文社科	一般项目	钟行
2020080	马克思主义学院	晚年恩格斯对科学社会主义的贡献及当代启示	人文社科	一般项目	卢俊岚
2020081	马克思主义学院	边疆少数民族地区大学生爱国主义教育路径探析	人文社科	一般项目	马　娇
2020082	马克思主义学院	英模精神在培育时代新人中的价值意蕴及路径选择	人文社科	一般项目	张　翠
2020083	马克思主义学院	习近平关于“以人民为中心”思想的发展研究	人文社科	一般项目	李佳闰
2020084	马克思主义学院	脱贫攻坚决胜期“第一书记”在驻村精准扶贫工作中的作用研究——以云南省鹤庆县为例	人文社科	一般项目	罗富平
2020085	马克思主义学院	马克思交往理论对铸牢中华民族共同体意识的启示	人文社科	一般项目	毕梦洁
2020086	马克思主义学院	“五育”并举视域下新时代大学生劳动教育研究	人文社科	一般项目	王　周
2020087	民族学与社会学学院	流动与阈限：对南亚东南亚在昆留学生的人类学研究	人文社科	一般项目	陈菲
2020088	民族学与社会学学院	藏传佛教中国化的地方性实践——以云南藏语系佛学院为例	人文社科	一般项目	陈经宇
2020089	民族学与社会学学院	价值共创理论视角下非遗传承主体与受众的互动关系研究	人文社科	一般项目	方达
2020090	民族学与社会学学院	国际通道与边界空间：中老“两国一城”跨境经济合作区的现代性建构	人文社科	一般项目	方伟洁
2020091	民族学与社会学学院	拉市坝民族团结进步示范村建设研究	人文社科	一般项目	和琴
2020092	民族学与社会学学院	互嵌共生：迪庆藏族自治州成立初期民族团结工作的实践研究	人文社科	一般项目	和淑清
2020093	民族学与社会学学院	新媒体对非遗文化重构的影响研究	人文社科	一般项目	和梓铃
2020094	民族学与社会学学院	社会工作促进随迁儿童城市融入的策略研究——基于生态系统理论视角	人文社科	一般项目	胡语嫣
2020095	民族学与社会学学院	孟加拉国乡村社会结构及其变迁的民族志研究	人文社科	一般项目	李兴军
2020096	民族学与社会学学院	消逝的草场：香格里拉牧民的历史记忆与环境认知	人文社科	一般项目	林露露
2020097	民族学与社会学学院	金沙江溜索影音档案中的社会文化变迁	人文社科	一般项目	卢俊糖
2020098	民族学与社会学学院	乡村振兴战略下云南省基层公共文化服务体系建设研究	人文社科	一般项目	罗婧
2020099	民族学与社会学学院	传统居民向民宿转变的空间生产研究	人文社科	一般项目	宋浩湘
2020100	民族学与社会学学院	新中国乡村社会变迁史研究：以珠江口疍民村落为例	人文社科	一般项目	谭任均
2020101	民族学与社会学学院	学生援助计划（SPA）在高校中的应用	人文社科	一般项目	汪思含
2020102	民族学与社会学学院	突围——县城民办学校推行教育信息化改革的逻辑思考	人文社科	一般项目	赵清歌
2020103	民族学与社会学学院	昆明千佛堂寺庙创收与自养调查研究	人文社科	一般项目	周密
2020104	文学院	国产动画对古代神话的解构与重构	人文社科	一般项目	杨思语
2020105	文学院	“热巴舞”在迪庆州藏族、纳西族和傈僳族中的传承现状研究	人文社科	一般项目	邓芝
2020106	文学院	服饰的个体记忆和生命史——新平花腰傣（傣雅）服饰的田野研究	人文社科	一般项目	龚颖
2020107	文学院	当代文学里的上海影像	人文社科	一般项目	李珈漫
2020108	文学院	论马原小说的创作转型	人文社科	一般项目	马艳娥

2020109	文学院	论建国初期诗歌创作的新指向和构想 （1949-1957）	人文社科	一般项目	房梦蝶
2020110	文学院	性别视野下的中国大陆耽美小说研究	人文社科	一般项目	那晓颖
2020111	文学院	“以耳记录”——艾芜《南行记》的听觉实践	人文社科	一般项目	罗文斌
2020112	政府管理学院	农村贫困化与空心化耦合发展的空间分异及影响因素研究——以镇雄县为例	人文社科	一般项目	马然
2020113	政府管理学院	“树洞”机制：高校学生心理健康管理的创新与挑战	人文社科	一般项目	罗淞麒
2020114	政府管理学院	基于RPD的高教类本科民办非企业单位评估研究 ——以云大滇池学院为例	人文社科	一般项目	李雄
2020115	政府管理学院	后脱贫时代下直过民族聚居区教育扶贫的进路与出路探析——以云南省三个典型聚居区为例	人文社科	一般项目	解燕林
2020116	政府管理学院	云南在次区域跨国城市网络中的功能定位和辐射能力考察	人文社科	一般项目	张鹭梅
2020117	政府管理学院	朱子《太极图说解》的意象诠释	人文社科	一般项目	王寒
2020118	政府管理学院	多民族和谐发展的“迪庆经验”调查研究	人文社科	一般项目	周兴妍
2020119	政府管理学院	近代中国的国民改造研究	人文社科	一般项目	陈媛艺
2020120	政府管理学院	边民补助机制的国民身份塑造作用研究	人文社科	一般项目	曹莉
2020121	政府管理学院	民族国家构建中边疆人口的“国民化”——基于德宏州的历史考察	人文社科	一般项目	常玲
2020122	政府管理学院	我国现代国家建构中的边境治理	人文社科	一般项目	刘华林
2020123	政府管理学院	基于 NVivo 质性分析下的地方政府 数据开放政策注意力配置	人文社科	一般项目	黄岚
2020124	职业与继续教育学院	高职院校工业机器人技术专业混合式教学效果评价指标体系构建	人文社科	一般项目	罗姝婧
2020125	职业与继续教育学院	三全育人改革背景下云南省中等职业院校课程思政建设研究-以云南省x中等职业院校为例	人文社科	一般项目	廖梦鲲
2020126	职业与继续教育学院	高职院校汽修检测与维修专业人才培养现代学徒制实践研究	人文社科	一般项目	刘婷婷
2020127	职业与继续教育学院	职业教育对非遗扶贫产业化支持的实践策略研究——以景谷县为例	人文社科	一般项目	常玲玲
2020128	职业与继续教育学院	基于智慧学习环境的深度学习模型构建研究	人文社科	一般项目	彭培环
2020129	经济学院	增长波动、社会能力与中国经济韧性	人文社科	一般项目	高策
2020130	经济学院	政府采购如何影响企业技术创新	人文社科	一般项目	杜鹏飞
2020131	经济学院	中缅金融合作与疫情常态化防控并行机制 ——以瑞丽开放口岸为例	人文社科	一般项目	蒋鑫
2020132	经济学院	中国 OFDI 是否改善了“一带一路”沿线东道国女性的就业与收入状况	人文社科	一般项目	白林鹭
2020133	经济学院	产业转型升级对农村相对贫困人口的收入效应研究	人文社科	一般项目	莫黎
2020134	经济学院	中美贸易摩擦对我国推进区域贸易自由化的影响及对策研究	人文社科	一般项目	刘欣
2020135	经济学院	政府补贴对企业创新影响---基于企业生命周期视角研究	人文社科	一般项目	蒲云洋
2020136	经济学院	照料资源可得性对我国老年健康城乡差异的影响研究	人文社科	一般项目	徐婧
2020137	经济学院	5G时代背景下信息通信技术对云南省投资便利化的影响研究	人文社科	一般项目	付彦清
2020138	经济学院	数字普惠金融对资本错配的纠正效应研究 ： 中国经验	人文社科	一般项目	肖洋

2020139	经济学院	基于区块链的数字人民币在跨境支付中的应用研究	人文社科	一般项目	朱峰琳
2020140	经济学院	实体企业金融化与全要素生产率：非线性效应存在吗——基于我国上市公司的经验研究	人文社科	一般项目	张悦
2020141	经济学院	金融集聚对产业结构升级的影响—基于技术创新的中介效应分析	人文社科	一般项目	李馨
2020142	经济学院	地方政府隐性担保对城投债信用风险的影响研究	人文社科	一般项目	黄美娇
2020143	经济学院	数字经济背景下我国数据要素价值研究	人文社科	一般项目	严鹏
2020144	经济学院	生命周期、专用性投资与企业价值	人文社科	一般项目	黄子建
2020145	经济学院	乡村振兴背景下农村产业融合促进减贫动力内生化问题研究	人文社科	一般项目	赵熙
2020146	经济学院	青春的空间流动与回归：大坡乡农村劳动力外出务工与返乡的调研	人文社科	一般项目	恭希言
2020147	经济学院	新冠疫情冲击的市场反应研究——基于事件研究法	人文社科	一般项目	马啸宇
2020148	经济学院	现金流波动性对房地产企业价值影响的实证分析——基于我国A股上市房地产企业的经验数据	人文社科	一般项目	张顺华
2020149	经济学院	人工智能时代“机器换人”就业效应研究——以东莞为例	人文社科	一般项目	林龙芳
2020150	经济学院	基于全球价值链的云南咖啡产业优化升级策略研究	人文社科	一般项目	刘丹
2020151	经济学院	新冠肺炎疫情应对策略与宏观经济表现：基于中、美、韩三国的比较研究	人文社科	一般项目	许学茜
2020152	艺术与设计学院	通海“妙善学”女子洞经音乐发展现状与传承研究	人文社科	一般项目	余沁芮
2020153	艺术与设计学院	人工智能语境下的艺术新实践研究	人文社科	一般项目	郗家玉
2020154	艺术与设计学院	四川长宁县：以竹编工艺带动乡村脱贫发展研究调查	人文社科	一般项目	尹耀连
2020155	艺术与设计学院	线性文化景观视角下昆明米轨的保护研究	人文社科	一般项目	李子莹
2020156	艺术与设计学院	云南“小水井”唱诗班合唱与传统苗族唱法的异同研究	人文社科	一般项目	董一麟
2020157	艺术与设计学院	以儿童绘本的形式延续云南白族甲马艺术的尝试	人文社科	一般项目	杜沁霜
2020158	艺术与设计学院	古建筑文化遗产的采集与测绘以云南大学“会泽院为例”	人文社科	一般项目	何春波
2020159	艺术与设计学院	白鹤滩水电站巧家移民安置区乡村文化的延续与重构探究	人文社科	一般项目	尹厚萌
2020160	新闻学院	新媒体视域下大学生面容焦虑及整容倾向研究	人文社科	一般项目	李孟
2020161	新闻学院	微博舆论中正当防卫案件的文本、结构与互动研究	人文社科	一般项目	陈嫣然
2020162	新闻学院	大学生社交焦虑与网络游戏沉迷研究--以昆明市呈贡大学生为例	人文社科	一般项目	王佳慧
2020163	新闻学院	后疫情时代大学生的风险感知研究	人文社科	一般项目	谢翔
2020164	新闻学院	乡村振兴视角下非遗的直播路径探究—以大理周城白族扎染为例	人文社科	一般项目	刘静
2020165	新闻学院	国际传播视域下国乐类短视频的跨文化传播策略研究	人文社科	一般项目	夏铱
2020166	新闻学院	健康传播与HPV疫苗接种态度与行为研究	人文社科	一般项目	李彤
2020167	体育学院	云南省高校体育思政与新媒体融合发展研究	人文社科	一般项目	华夏
2020168	体育学院	“体教融合”视域下学校体育促进大学生体质健康的实现路径	人文社科	一般项目	石晴晴

2020169	体育学院	“民族传统体育”课程思政构建研究——以云南大学为例	人文社科	一般项目	王新奇
2020170	体育学院	体教融合背景下云大附中“一校多品”建设现状与对策研究	人文社科	一般项目	武加文
2020171	高等教育研究院	云南大学少数民族大学生校园文化适应调查研究	人文社科	一般项目	袁惠
2020172	高等教育研究院	疫情背景下云南大学学生线上学习满意度调查分析	人文社科	一般项目	杨紫琪
2020Z41	材料与能源学院	CCTO/TiO_2/SiO_2复合体系电介质材料的制备与性能的研究	理工科	重点项目	郭勇
2020Z42	材料与能源学院	双活性中心调控少层共价有机骨架在锂离子电池中的应用	理工科	重点项目	赵根福
2020Z43	材料与能源学院	基于柔性基底的电纺$CdIn_2O_4$/PANI/Ti_3C_2 MXene 纳米复合材料的制备及氨气敏感特性研究	理工科	重点项目	田旭
2020Z44	地球科学学院	昆明市城市热岛效应研究	理工科	重点项目	传婷
2020Z45	地球科学学院	贵州贵定竹沟锗锌矿床中Ge的赋存状态研究	理工科	重点项目	杨智谋
2020Z46	国际河流与生态安全研究院	桉树引种区生态安全格局动态识别及其优化调控	理工科	重点项目	黄佩
2020Z47	国际河流与生态安全研究院	基于BREACH和HEC-RAS模型的冰湖溃坝过程重建、预测及洪水演进模拟	理工科	重点项目	祁苗苗
2020Z48	国际河流与生态安全研究院	基于人工智能的大型河流数据驱动水文模型研究及应用	理工科	重点项目	袁旭
2020Z49	化学科学与工程学院	生物炭基催化剂对小麦秸秆热解产物的影响及催化剂失活机理的探索	理工科	重点项目	鲁秋香
2020Z50	化学科学与工程学院	基于二维纳米材料的电化学免疫传感器的构建及疾病标志物的检测	理工科	重点项目	梁还
2020Z51	化学科学与工程学院	aza-bodipy类材料分子的合成与研究	理工科	重点项目	丁伟
2020Z52	化学科学与工程学院	咖啡壳改性水处理应用的研究	理工科	重点项目	康娟雪
2020Z53	建筑与规划学院	基于基因图谱的滇西北村落文化景观研究	理工科	重点项目	袁晓蝶
2020Z54	建筑与规划学院	基于椭球凸集模型的非概率桥梁结构评估理论及其应用	理工科	重点项目	尤忠义
2020Z55	软件学院	多模态关联图下的肺部病变诊断研究	理工科	重点项目	张兰
2020Z56	软件学院	基于迁移学习和活动识别的对帕金森患者病情日常评估量化研究	理工科	重点项目	王湘
2020Z57	生态与环境学院	以麻竹为例探究竹子夜间水分分配及其水分生态适应策略	理工科	重点项目	陈芳
2020Z58	生态与环境学院	基于无人机3D摄影技术退耕还林生态交错带尺度推移研究	理工科	重点项目	马雨欣
2020Z59	生命科学学院	表观遗传因子Ezh2调控膀胱上皮损伤修复的分子机制	理工科	重点项目	蒋 军
2020Z60	生命科学学院	反义寡核苷酸调控转录过程	理工科	重点项目	高旭
2020Z61	生命科学学院	钙结合蛋白S100A11促进肝细胞癌变的作用机理研究	理工科	重点项目	李成斌
2020Z62	生命科学学院	自噬基因 Epg5 在巨噬细胞炎性反应中的作用	理工科	重点项目	杨意
2020Z63	生命科学学院	大肠杆菌激活秀丽线虫免疫反应的机制	理工科	重点项目	何亚涛
2020Z64	生命科学学院	DGUOK（线粒体脱氧鸟苷激酶）调节NAD+生物合成机制研究	理工科	重点项目	桑磊
2020Z65	生命科学学院	YAP调控巨噬细胞极化的分子机制	理工科	重点项目	韩晖
2020Z66	数学与统计学院	最小Steiner线段树问题	理工科	重点项目	刘苏定

2020Z67	数学与统计学院	流形正则化非负张量Triple分解及其在视频图像处理中的应用	理工科	重点项目	吴封盛
2020Z68	数学与统计学院	多性状多位点遗传关联分析的统计方法研究及其应用	理工科	重点项目	贾楠
2020Z69	物理与天文学院	少层黑磷薄膜的太赫兹非线性光学特性研究	理工科	重点项目	王秋仪
2020Z70	物理与天文学院	Kinematics of the Galactic disk as revealed by the LAMOST red clump stars	理工科	重点项目	孙伟祥
2020Z71	信息学院	基于组件划分的多层次软件运行过程挖掘方法	理工科	重点项目	汤雅惠
2020Z72	信息学院	基于双极性超快超声的动脉粥样硬化早期特征提取	理工科	重点项目	何冰冰
2020Z73	信息学院	高通量生物测序数据建模的深度神经网络构建研究	理工科	重点项目	郭延哺
2020Z74	信息学院	复杂环境下多源图像研究	理工科	重点项目	刘琰煜
2020Z75	信息学院	基于EEG脑机接口的视觉情感语义分析研	理工科	重点项目	赵坤圆
2020Z76	信息学院	基于深度学习的退变性脊柱侧凸应力分布估计方法研究	理工科	重点项目	赵阳
2020Z77	信息学院	面向无监督学习的聚焦检测研究	理工科	重点项目	李华光
2020Z78	医学院	调控性细胞坏死在顺铂诱导的急性肾损伤中的作用	理工科	重点项目	刘昕蓓
2020Z79	资源植物研究院	矮杆基因*SD1*在水、陆稻群体矮化育种中的演化分析	理工科	重点项目	焦淑亮
2020Z80	资源植物研究院	长雄野生稻（Oryza longistaminata）水平基因转移的研究	理工科	重点项目	赵晋洁
2020173	材料与能源学院	高性能人体呼气疾病检测用氨气传感材料 WO_3/Mxene的研究	理工科	一般项目	姚丽佳
2020174	材料与能源学院	液态金属柔性印刷电极的制备和性能调控研究	理工科	一般项目	段良飞
2020175	材料与能源学院	纳米片自组装核桃状WO_3纳米材料的可控合成及对三乙胺气敏性能研究	理工科	一般项目	曾吉阳
2020176	材料与能源学院	基于ZnO量子点掺杂SnO_2电子传输层的高效、稳定钙钛矿太阳能电池研究	理工科	一般项目	赵荣俊
2020177	材料与能源学院	基于多孔纳米硅基印迹电化学传感器研究及重金属As(Ⅴ)的测定	理工科	一般项目	付开新
2020178	材料与能源学院	MOF驱动氧空位调控的多孔Bi2O3-X@C构筑及光催化降解四环素类抗生素机理研究	理工科	一般项目	邹肖肖
2020179	材料与能源学院	基于聚乙二醇探究聚合度对碳量子合成的影响	理工科	一般项目	姜俞翔
2020180	材料与能源学院	燃料电池双钙钛矿阳极材料原位脱溶对结构和电学性能的影响	理工科	一般项目	柯明蕙
2020181	材料与能源学院	气凝胶法可控合成SnO_2量子点及其气敏性能机制研究	理工科	一般项目	马逸轩
2020182	材料与能源学院	电子空穴诱发O3型富镍层状氧化物的阴离子氧化还原过程及机理研究	理工科	一般项目	王诗敏
2020183	地球科学学院	云南普雄风化壳型铌稀土矿床的稀土矿物学系统研究	理工科	一般项目	王敏
2020184	地球科学学院	云南省昭通市桃树井地区P-T期间古地磁与古环境研究	理工科	一般项目	马荣耀
2020185	地球科学学院	中南半岛生物质燃烧对我国西南地区气溶胶辐射强迫影响	理工科	一般项目	何舒璇
2020186	地球科学学院	基于MODIS数据的滇中高原农业干旱时空变化特征及驱动机制研究	理工科	一般项目	屈新星
2020187	地球科学学院	云南省水资源利用与城镇化水平的时空差异及协调耦合性研究	理工科	一般项目	张帆
2020188	地球科学学院	利用多源夜光遥感数据的数字乡村经济时空格局研究	理工科	一般项目	宋璐

2020189	地球科学学院	基于中介效应模型的杞麓湖流域土地利用变化对水质演变的影响分析	理工科	一般项目	胡智文
2020190	地球科学学院	飓风激发双频地脉动特征分析	理工科	一般项目	王嗣尹
2020191	地球科学学院	基于SCOOPS3D模型的怒江州滑坡灾害危害性评价	理工科	一般项目	施旖奇
2020192	国际河流与生态安全研究院	集成时序InSAR技术的公路红层软岩重大滑坡形变监测研究	理工科	一般项目	赵鑫
2020193	国际河流与生态安全研究院	植物富集重金属对土壤微生物群落及抗生素抗性基因的影响	理工科	一般项目	高金龙
2020194	国际河流与生态安全研究院	诺氟沙星对河蚬体内外硝化、反硝化生态功能的影响研究	理工科	一般项目	韦俊伶
2020195	国际河流与生态安全研究院	基于Google Earth 影像的红河流域干热河谷沟蚀调查及易蚀性分析	理工科	一般项目	成淑贤
2020196	国际河流与生态安全研究院	金沙江丽段河岸带人工林对土壤氮素和磷转化机制的影响	理工科	一般项目	陈瑞
2020197	国际河流与生态安全研究院	干旱胁迫和复水作用下生物膜对铅、锌耐受性及吸附性的影响	理工科	一般项目	谢珊珊
2020198	国际河流与生态安全研究院	侵蚀影响下植被恢复初期土壤氮素转化规律	理工科	一般项目	刘太聪
2020199	国际河流与生态安全研究院	联合多源数据的澜沧江-湄公河流域气象与水文干旱演变机制研究	理工科	一般项目	罗璇
2020200	国际河流与生态安全研究院	TRMM降水数据在高原寒区的偏差校正	理工科	一般项目	刘畅
2020201	国际河流与生态安全研究院	汞挥发菌株添加生物炭基人工湿地去除废水中的研究	理工科	一般项目	邓盛炯
2020202	国际河流与生态安全研究院	不同施肥模式对云南昭通退化草地土壤有机碳组分的影响	理工科	一般项目	钱钦栋
2020203	国际河流与生态安全研究院	阳宗海食物网中重金属的营养动力学研究和环境风险评价	理工科	一般项目	严辉
2020204	化学科学与工程学院	苯并噻二唑三嵌段两亲性液晶化合物的设计合成、自组装及应用研究	理工科	一般项目	常清
2020205	化学科学与工程学院	基于吡啶酰胺配体的配位聚合物的固态【2+2】环加成反应及应用研究	理工科	一般项目	朱蓉蓉
2020206	化学科学与工程学院	Arthrinium arundinis DJ-13代谢产物及活性研究	理工科	一般项目	舒燕
2020207	化学科学与工程学院	高性能混合型锂离子电容器的研究	理工科	一般项目	杨晓萍
2020208	化学科学与工程学院	新型催化不对称烯丙基化反应的发展及其在生物碱(+)-Deethylibophyllidine和(+)-Limaspermidine	理工科	一般项目	王珊
2020209	化学科学与工程学院	抗动脉粥样硬化的黄酮类化合物药物筛选	理工科	一般项目	方飞
2020210	化学科学与工程学院	宿主山药对一株山药内生真菌（Penicillium sp.）次级代谢产物的诱导研究	理工科	一般项目	赵统德
2020211	化学科学与工程学院	基于Cl--Cu2+催化信号放大和金纳米棒刻蚀的多巴胺可视化检测	理工科	一般项目	卢朝粉
2020212	化学科学与工程学院	几种新型金属有机树脂去除磷的性能研究	理工科	一般项目	罗国婷
2020213	化学科学与工程学院	基于双金属MOFs去除水体中砷（Ⅴ）的研究	理工科	一般项目	尹春
2020214	化学科学与工程学院	高能量密度生物燃料清洁合成中双贵金属纳米合金催化剂协同效应的研究	理工科	一般项目	王英浩
2020215	化学科学与工程学院	金属Pd催化的N原子的α位芳基化	理工科	一般项目	张欣
2020216	化学科学与工程学院	毛细管区带电泳法同时分离分析长春花中的4种生物碱	理工科	一般项目	易婷
2020217	化学科学与工程学院	官能化磁性纳米颗粒对污水中重金属离子的去除研究	理工科	一般项目	蒋雪
2020218	化学科学与工程学院	基于天然产物的新型p38α抑制剂筛选	理工科	一般项目	沈天择

2020219	化学科学与工程学院	磺酸基MOFs及其衍生物的质子传导性质研究	理工科	一般项目	赵黎嘉
2020220	化学科学与工程学院	阻燃型离子塑晶全固态电解质的制备与表征	理工科	一般项目	周贺
2020221	化学科学与工程学院	通过双Diels-Alder反应构筑具有曲面结构的多环芳烃分子	理工科	一般项目	肖明君
2020222	建筑与规划学院	国土空间规划背景下多灾山地区域乡村聚落演变及优化研究	理工科	一般项目	曹亚豪
2020223	建筑与规划学院	基于信息流的高原山地城市网络结构特征研究 —以云南省为例	理工科	一般项目	邓浩洋
2020224	建筑与规划学院	钢结构火灾评估及人员疏散可视化研究	理工科	一般项目	赵颖
2020225	建筑与规划学院	砂粒式环氧沥青混合料的配合比设计及性能研究	理工科	一般项目	陈飞
2020226	建筑与规划学院	云南大理白族新型社区建筑城镇适应性研究	理工科	一般项目	石若利
2020227	建筑与规划学院	有机涂层对混凝土抗氯离子侵蚀性能的试验及模拟研究	理工科	一般项目	陈敬男
2020228	软件学院	基于稀疏共享的多任务学习对阿兹海默疾病的预测研究	理工科	一般项目	毕高杉
2020229	软件学院	基于异构图神经网络的药物和疾病的关联预测方法	理工科	一般项目	王再霞
2020230	软件学院	基于非监督深度学习模型的多聚焦图融合方法研究	理工科	一般项目	周鼎
2020231	软件学院	基于深度神经网络的图像彩色化以及超分辨率方法研究	理工科	一般项目	冯佳男
2020232	软件学院	基于知识图谱的软件开发第三方库推荐研究	理工科	一般项目	赵静转
2020233	软件学院	基于知识图谱的认知推荐系统研究	理工科	一般项目	杨小波
2020234	软件学院	基于强化学习的温度感知多处理器任务调度	理工科	一般项目	张志远
2020235	软件学院	软件生态系统质量及健康性研究	理工科	一般项目	李柱东
2020236	软件学院	无领导近地空间机器人编队系统的机动控制方案研究	理工科	一般项目	王煊
2020237	生态与环境学院	基于梁王山五个象牙参属近缘种探究杂交带中物种界限维持机制	理工科	一般项目	陈虹帆
2020238	生态与环境学院	SUF1调控拟南芥与印度梨形孢共生的分子机制	理工科	一般项目	陈小洁
2020239	生态与环境学院	基于土地利用变化和景观破碎化的西南自然保护区保护成效评估	理工科	一般项目	冷仙
2020240	生态与环境学院	花柱运动与繁殖策略的成本—收益分析：以两种耧斗菜属近缘物种为例	理工科	一般项目	李会琼
2020241	生态与环境学院	云南松林火后土壤微生物群落结构变化的时空格局	理工科	一般项目	任丽娜
2020242	生态与环境学院	横断山南段气候树线检测及时空动态	理工科	一般项目	邹福燕
2020243	生态与环境学院	梯度增温对哀牢山常绿阔叶林土壤酶活性及酶化学计量的影响	理工科	一般项目	张淑婷
2020244	生态与环境学院	应用机器学习预测水华蓝藻的化学生态危害及其毒理机制	理工科	一般项目	字元燕
2020245	生态与环境学院	环境选择和扩散限制对土壤微生物群落构建的影响	理工科	一般项目	侯海燕
2020246	生命科学学院	黑腹果蝇中特异性盐受体的探究	理工科	一般项目	曲广男
2020247	生命科学学院	表观遗传因子EP300调控人羊膜分娩启动关键合酶PTGS-2的分子机制	理工科	一般项目	张箐
2020248	生命科学学院	一种预测 SARS-CoV-2 RBD-ACE2 结合能力的深度学习方法及其结合能力机理研究	理工科	一般项目	王玉霜

2020249	生命科学学院	鼯鼠和鼯猴滑翔能力适应的基因组趋同进化机制研究	理工科	一般项目	高珊珊
2020250	生命科学学院	IQCG参与细胞质动力蛋白功能的研究	理工科	一般项目	吴勇臻
2020251	生命科学学院	长链非编码RNA对自闭症小鼠的调控作用	理工科	一般项目	李竹
2020252	生命科学学院	铜绿微囊藻胞外分泌物对乳腺癌细胞 Hedgehog 通路的影响研究	理工科	一般项目	周辉
2020253	生命科学学院	解析肠道菌群如何影响小鼠对酒精的易感性	理工科	一般项目	武冰鑫
2020254	生命科学学院	通过调节Kupffer细胞的极化缓解药物诱导性肝损伤	理工科	一般项目	杨若雪
2020255	生命科学学院	壳梭霉素Fusicomycin 生物合成机制研究	理工科	一般项目	陈雪梅
2020256	生命科学学院	云南五种珍稀药食用牛肝菌的纯培养及其比较基因组分析	理工科	一般项目	安霜
2020257	生命科学学院	钙结合蛋白S100A11在肝癌中的作用机制研究	理工科	一般项目	吴慧银
2020258	生命科学学院	药物处理时大肠杆菌 YihE 激酶对磷酸转移酶系统的调控及对代谢的影响	理工科	一般项目	李春
2020259	生命科学学院	自噬基因 Epg5 在诺如病毒感染中的作用	理工科	一般项目	张进
2020260	生命科学学院	自噬基因Epg5在肠道簇细胞免疫反应中的作用	理工科	一般项目	杨霞
2020261	生命科学学院	细菌 capsule 调控宿主发育的机理	理工科	一般项目	刘慧敏
2020262	生命科学学院	秀丽线虫C49C8.1抗菌蛋白抵抗病原菌P. aeruginose感染的分子机制	理工科	一般项目	何用娟
2020263	生命科学学院	濒危植物大树杜鹃在酸性生长环境下耐受 Al3+毒害的分子机制	理工科	一般项目	周雄丽
2020264	生命科学学院	滇池中可培养稀有放线菌的多样性及其生态分布规律研究	理工科	一般项目	许灿海
2020265	生命科学学院	解脂耶氏酵母异源合成檀香醇的菌株构建研究	理工科	一般项目	李红梅
2020266	生命科学学院	一株交链孢菌产交链孢酚二聚体新化合物及其抗植物病原真菌的研究	理工科	一般项目	徐唐昌
2020267	生命科学学院	MTERF1 通过 AMPK-mTOR 通路调控结直肠癌发生的分子机制研究	理工科	一般项目	邹亚艳
2020268	生命科学学院	云南建水石漠化下土壤真菌多样性及其对植物抗干旱胁迫的影响	理工科	一般项目	郑华
2020269	生命科学学院	人工与野生冬虫夏草关键微生物群落比较及功能研究	理工科	一般项目	孙涛
2020270	生命科学学院	紫茎泽兰垂直传播和横向传播病原菌相互作用研究	理工科	一般项目	李锦鹏
2020271	生命科学学院	藏羚羊高海拔适应的肠道微生物组机制研究	理工科	一般项目	田晨
2020272	生命科学学院	保育植物马桑与复垦滇杨对尾矿区重金属污染的联合修复效应及机制	理工科	一般项目	李雪娇
2020273	生命科学学院	胚胎发育与肿瘤发生相关性研究	理工科	一般项目	李响
2020274	数学与统计学院	混合域上的概周期动力系统问题研究	理工科	一般项目	陈德素
2020275	数学与统计学院	分数阶微分方程边值问题的可解性及应用	理工科	一般项目	李金秋
2020276	数学与统计学院	基于区块链下的缺失数据探讨及客户画像研究	理工科	一般项目	陈慧媛
2020277	数学与统计学院	云南省旅游业统计机器学习模型研究及应用	理工科	一般项目	郝琦
2020278	数学与统计学院	基于地摊宝APP大学城周边顾客消费倾向分析与预测	理工科	一般项目	张思杰

2020279	数学与统计学院	区间删失数据下超高维数据的统计推断	理工科	一般项目	钱永静
2020280	数学与统计学院	疫情谣言识别的大数据统计机器学习模型研究	理工科	一般项目	莫荣良
2020281	数学与统计学院	函数型部分线性空间自回归模型的推断与应用	理工科	一般项目	季亚飞
2020282	数学与统计学院	隐私保护下分位数回归的统计推断问题	理工科	一般项目	罗君
2020283	数学与统计学院	非随机缺失机制下集群数据的统计分析及应用	理工科	一般项目	李丽华
2020284	数学与统计学院	带有缺失数据的单指标阈值模型的分位数回归	理工科	一般项目	汪晓岸
2020285	数学与统计学院	高维异质分类数据的类别自适应变量筛选	理工科	一般项目	田香妮
2020286	物理与天文学院	单层二硫化钼在近邻诱导作用下的谷霍尔效应	理工科	一般项目	刘建
2020287	物理与天文学院	钙钛矿的电化学生长机制及其模拟研究	理工科	一般项目	关琳琳
2020288	物理与天文学院	A Large catalogue of molecular clouds with accurate distances in the Milky Way	理工科	一般项目	郭贺龙
2020289	物理与天文学院	欠阻尼区域活性布朗粒子的熵随机共振研究	理工科	一般项目	岳伟浩
2020290	物理与天文学院	一种基于平衡探测雪崩光电二级管过剩噪声因子的测试系统	理工科	一般项目	李再波
2020291	物理与天文学院	上转化荧光碳点的制备及应用研究	理工科	一般项目	赵哲贤
2020292	物理与天文学院	重子物质效应对弱引力透镜统计量的影响	理工科	一般项目	赵安
2020293	信息学院	适用于5G的ISGW平行耦合线的耦合理论研究	理工科	一般项目	撒玉超
2020294	信息学院	云边协同环境中关注能耗的计算迁移及资源分配关键技术研究	理工科	一般项目	苏茜
2020295	信息学院	基于等离激元纳米粒子阵列的晶格共振及器件研究	理工科	一般项目	熊磊
2020296	信息学院	基于GAN的不平衡学习方法研究及其在蛋白质亚细胞定位中的应用	理工科	一般项目	武丽雯
2020297	信息学院	基于超声射频信号乳腺肿瘤计算机辅助诊断系统研究	理工科	一般项目	姚瑞晗
2020298	信息学院	基于色彩迁移弥补色差损失的红外可见光视频融合技术研究	理工科	一般项目	丁斋生
2020299	信息学院	多模态脑肿瘤MR图像的分割方法研究	理工科	一般项目	张婷婷
2020300	信息学院	基于深度学习的太阳射电爆发的分类研究	理工科	一般项目	程如如
2020301	信息学院	面向多轮对话自适应情感回复生成的聊天机器人研究	理工科	一般项目	孔峻
2020302	信息学院	基于云计算的便携超声肿瘤辅助诊断系统	理工科	一般项目	王致诚
2020303	信息学院	基于深度学习的频谱监测应用研究	理工科	一般项目	王晓燕
2020304	信息学院	5G毫米波高增益宽频带集成基片间隙波导腔体滤波天线阵列设计	理工科	一般项目	林秋华
2020305	信息学院	边缘计算场景下基于软件定义与高可用的智慧春城监控系统研究	理工科	一般项目	陈飞龙
2020306	信息学院	基于边缘智能的车联网应用研究	理工科	一般项目	杨超
2020307	信息学院	云南省土壤环境质量监测预警与数据信息化管理WebGIS平台	理工科	一般项目	徐文杰
2020308	信息学院	基于深度卷积神经网络的青少年骨龄自动识别方法及软件开发研究	理工科	一般项目	张帅

2020309	信息学院	基于机器学习的毫米波透镜天线选择研究	理工科	一般项目	卢松琴
2020310	信息学院	毫米波贴片天线阵与基于 ISGW的滤波功分器的集成设计	理工科	一般项目	陈吉鹏
2020311	信息学院	移动边缘计算环境下网约车内异常行为检测与视频分析	理工科	一般项目	袁也
2020312	信息学院	基于PG-WGAN的混合门控卷积图像修复	理工科	一般项目	陈杰
2020313	信息学院	基于时间变化的体内轻量型补片术后皱缩分析方法	理工科	一般项目	耿如霞
2020314	信息学院	基于条件概率的互耦合深度卷积神经网络的多聚焦图像融合	理工科	一般项目	王成超
2020315	信息学院	多级并置模式挖掘算法及应用研究	理工科	一般项目	刘新斌
2020316	信息学院	基于联邦学习架构的医疗导诊 Web 平台	理工科	一般项目	刀加旭
2020317	信息学院	基于动作捕获技术的云南民族舞蹈类文化数字化保护研究	理工科	一般项目	柳雁安
2020318	信息学院	基于生成对抗网络的非均匀纹理图像合成	理工科	一般项目	谢莎莎
2020319	医学院	灰树花多糖调节APP/PS1小鼠脑内自噬稳态发挥抗AD作用及分子机制	理工科	一般项目	陈瑶
2020320	资源植物研究院	不同休眠时期珠芽魔芋 NCED 基因表达分析	理工科	一般项目	王天喜
2020321	资源植物研究院	马铃薯Patatin糖基化关键基因鉴定与功能分析	理工科	一般项目	刘亚宁
2020322	资源植物研究院	长雄野生稻(Oryza longistaminata)稻纵卷叶螟抗性的分子定位研究	理工科	一般项目	王河原
2020323	资源植物研究院	干旱胁迫和复水作用对咖啡幼苗碳代谢的影响	理工科	一般项目	喻好好
2020324	资源植物研究院	大白菜胼胝质合成酶编码基因 *BrCalS*15的表达模式分析及功能验证	理工科	一般项目	江白雪
2020325	资源植物研究院	抗稻瘟病水稻资源中抗性同源基因分析研究	理工科	一般项目	管娟
2020326	资源植物研究院	水陆稻内生菌多样性比较分析及其抗旱相关微生物功能验证	理工科	一般项目	姬梦琳
2020327	资源植物研究院	基于机器学习算法的花卉自动分类和分级评价	理工科	一般项目	余明芳
2020328	资源植物研究院	灌木绣球与藤本绣球的体细胞杂交探究	理工科	一般项目	张艺泷
2020J01	历史与档案学院	经济认同在两德统一中的作用	人文社科	基础学科专项	段钧祎
2020J02	历史与档案学院	论宋代的印刷图书市场——以科举用书为中心的考察	人文社科	基础学科专项	李少伟
2020J03	历史与档案学院	《云南府猺獞峒蛮考》校注	人文社科	基础学科专项	郝静
2020J04	历史与档案学院	1937—1949年昆明城市下层社会群体研究	人文社科	基础学科专项	冀嘉伟
2020J05	历史与档案学院	知识史视角下的“大清万年一统”系地图研究	人文社科	基础学科专项	陈旭
2020J06	历史与档案学院	明永乐出兵安南与“郡县其地”研究	人文社科	基础学科专项	杨勇
2020J07	历史与档案学院	清以来滇南地区少数民族祷疾活动研究	人文社科	基础学科专项	王彤
2020J08	文学院	感性解放视域下波瓦的被压迫者诗学研究	人文社科	基础学科专项	张勰
2020J09	文学院	“新生代”华语女性导演电影情感结构研究	人文社科	基础学科专项	晏舒曼
2020J10	文学院	《七略》和《吕氏春秋》学术体系研究	人文社科	基础学科专项	朱笑玉

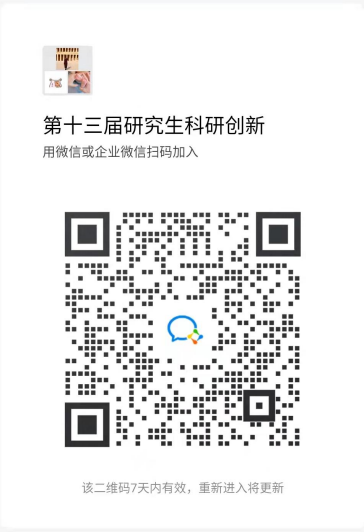
2020J11	政府管理学院	论谢林《先验唯心论体系》中的艺术直观	人文社科	基础学科专项	姚彦成
2020J12	政府管理学院	傣族关于人与森林关系的伦理研究	人文社科	基础学科专项	李春芝
2020J13	政府管理学院	《扶桑》的女性书写与性别平等研究	人文社科	基础学科专项	周祝伊
2020J14	政府管理学院	符号操纵下的现代消费社会研究	人文社科	基础学科专项	景敏
2020J15	化学科学与工程学院	铁皮石斛内生植物病原菌Nigrospaora oryzae与Collectotrichum gloeosporioides共培养的植物毒	理工科	基础学科专项	吴娅梅
2020J16	化学科学与工程学院	细菌胞外聚合物回收贵金属资源同时合成纳米电催化剂	理工科	基础学科专项	刘畅
2020J17	化学科学与工程学院	吲哚并环化合物的合成及活性研究	理工科	基础学科专项	张忠伟
2020J18	化学科学与工程学院	铜催化β，γ-不饱和环丁内酰胺的不对称炔丙基化串联环化反应	理工科	基础学科专项	任江涛
2020J19	化学科学与工程学院	可再生工程塑料单体的合成与金属催化剂的定向设计	理工科	基础学科专项	张浩
2020J20	化学科学与工程学院	四苯乙烯类分子亚铜配合物的发光性质及应用研究	理工科	基础学科专项	王秋凤
2020J21	化学科学与工程学院	基于多酸复合光催化剂制备及其光催化降解有机污染物机理探讨	理工科	基础学科专项	孙彭亮
2020J22	数学与统计学院	基于中国邮递员问题的充电站选址研究	理工科	基础学科专项	胡郭军
2020J23	数学与统计学院	二维相对论欧拉方程组若干问题的研究	理工科	基础学科专项	张梅梅
2020J24	数学与统计学院	时标上神经网络的Besicovitch概周期研究	理工科	基础学科专项	齐玮玮
2020J25	数学与统计学院	半在线带重排的等级调度问题	理工科	基础学科专项	赵姝
2020J26	物理与天文学院	基于p-NiO/SiO_2/n-ZnO垂直结构的紫外光电探测器的制备	理工科	基础学科专项	贾梦涵
2020J27	物理与天文学院	硅烯负载希贵金属单原子催化剂的理论设计与性能调控	理工科	基础学科专项	李文忠
2020J28	物理与天文学院	能量势垒与熵垒共同作用下的振动共振研究	理工科	基础学科专项	蒋嘉豪
2020X01	昌新国际艺术学院	云南大学景观命名及数字化演示		做云大主人：校园公益研究专项	樊静怡
2020X02	地球科学学院	基于Gephi可视化的云南大学呈贡校园可食性景观优化及文化旅游认同效应研究		做云大主人：校园公益研究专项	周美岐
2020X03	地球科学学院	云南大学无人机三维建模研究		做云大主人：校园公益研究专项	计培琨
2020X04	国际河流与生态安全研究院	铁碳微电解人工湿地深度净化云大污水站尾水的研究		做云大主人：校园公益研究专项	江明
2020X05	经济学院	“双微”时代云南大学微公益发展路径研究		做云大主人：校园公益研究专项	朱原娇
2020X06	新闻学院	云南大学在校女学生月经知识和态度调查		做云大主人：校园公益研究专项	施桂娟
2020X07	新闻学院	云南大学“垃圾分类”的知识传播与应用策略		做云大主人：校园公益研究专项	荣韵
2020X08	艺术与设计学院	云南大学校园公益文创产品设计		做云大主人：校园公益研究专项	宗蕊
2020X09	生态与环境学院	云南大学校园生物志与生物多样性格局研究及保护		做云大主人：校园公益研究专项	蒋萱
2020X10	历史与档案学院	云南大学历史博物馆现状调查及“双一流”视域下的建设发展研究		做云大主人：校园公益研究专项	邹坚

云南大学第十三届研究生科研创新项目拟立项名单公示

我校第十三届研究生科研创新项目评审现已结束，重点项目、一般项目均为定额申报，经学院评审并公示无异议后上报 556 项，校评审委员会形式复审后拟对 554 项进行立项。

现对新闻学院《媒介演变与少数民族女性生命历程的互动研究》等 554 项拟立项项目名单予以公示，若有异议，请于 2021 年 11 月 12 日 17:00 前以书面形式向研究生院反映，电话：65033585。若无异议，则公示期满自动生效。

公示期满获准立项的项目负责人请自动申请加入微信群：



项目编号	培养单位	项目名称	项目分类	项目类别	项目负责人	项目负责人培养层次	指导教师
2021Z001	新闻学院	媒介演变与少数民族女性生命历程的互动研究	人文社科	重点项目	钱浩	博士	孙信茹
2021Z002	法学院	清代冕宁司法档案中命案裁判的实践面相	人文社科	重点项目	马靖然	博士	胡兴东
2021Z003	工商管理与旅游管理学院	基于文本分析的城乡融合政策演进及协调研究	人文社科	重点项目	何梦丹	博士	秦德智
2021Z004	国际关系研究院	后疫情时代东盟“绿色恢复”进展及对策研究	人文社科	重点项目	屈婕	博士	毕世鸿
2021Z005	经济学院	易地扶贫搬迁户市民化进程中的自生能力缺口及其提升机制研究	人文社科	重点项目	蔡华龙	博士	陈瑛
2021Z006	历史与档案学院	宋代食盐消费研究—日常生活史视角的考察	人文社科	重点项目	李俊杰	博士	薛政超

2021Z007	马克思主义学院	21 世纪南亚共产党的新动向研究	人文社科	重点项目	王恩明	博士	袁 群
2021Z008	民族学与社会学学院	栖居视角下的居民生活与城市更新：建水古城的景观人类学研究	人文社科	重点项目	刘金泉	博士	桂榕
2021Z009	民族学与社会学学院	清代以来台湾少数民族命名制度演变研究	人文社科	重点项目	向青松	博士	马腾嶽
2021Z010	文学院	杜甫诗歌自然灾害书写研究	人文社科	重点项目	张云	博士	李道和
2021Z011	政府管理学院	云南边境地区野生动物生态补偿机制构建与政策研究	人文社科	重点项目	陈茜	博士	马桑
2021Z012	信息学院	图像视频编码及压缩感知研究	理工科	重点项目	王伟	博士	陈建华
2021Z013	生命科学学院	新星脂滴蛋白 LET-767 参与调控脂质稳态	理工科	重点项目	付林	博士	梁斌

2021Z014	生命科学学院	少孢节丛孢外泌体内含物影响自身增殖的机制探究	理工科	重点项目	周亮	博士	张克勤
2021Z015	物理与天文学院	估计约 67000 颗天琴座变星的测光金属丰度和距离及其应用	理工科	重点项目	李新意	博士	黄样
2021Z016	材料与能源学院	高性能富阳离子空位二元过渡金属硒化物电极材料的设计与合成	理工科	重点项目	卢清杰	博士	柳清菊
2021Z017	地球科学学院	青藏高原东南缘大气热源与西南雨季开始期关联的物理机制研究	理工科	重点项目	文大勇	博士	曹杰
2021Z018	国际河流与生态安全研究院	高海拔和高纬度典型地区冰川跃动的时空异质性及其驱动机制	理工科	重点项目	高永鹏	博士	刘时银
2021Z019	化学科学与工程学院	基于木脂素的抗风湿性关节炎先导化合物的发现及研究	理工科	重点项目	王梦如	博士	肖伟烈 李晓莉
2021Z020	化学科学与工程学院	豨莶草中抗 MRSA 活性天然产物及其作用机制研究	理工科	重点项目	周忠顺	博士	罗晓东

2021Z021	生态与环境学院	马来良姜2655基因及其同源基因调控花药异熟的分子机制	理工科	重点项目	黄奥丹	博士	李庆军
2021Z022	生态与环境学院	不同本地植物叶反馈和土反馈对紫茎泽兰早期生长的影响	理工科	重点项目	曾召英	博士	张汉波
2021Z023	医学院	一个高度近视大家族的新型PRSS56 c.C827T 变异的鉴定	理工科	重点项目	秦曼	博士	胡敏
2021Z024	历史与档案学院	深化档案对外交流合作：海外抗战档案文献联合开发研究	人文社科	重点项目	董慧囡	硕士	华林
2021Z025	法学院	我国“亲亲相隐”的历史沿革与现实困境——以德国法中亲属容隐制度之变迁为参照	人文社科	重点项目	王曰可	硕士	张剑源
2021Z026	法学院	社会性别视角下农村离婚诉讼中女性权益保护实证研究——以N县人民法院为例	人文社科	重点项目	郭敏	硕士	甘霆浩
2021Z027	法学院	农村城镇化下毒品犯罪控制策略研究——基于广东省博社村的治理经验	人文社科	重点项目	郭昌宁	硕士	李春

2021Z028	法学院	社会系统论视角下的基层公共卫生治理法治化问题研究	人文社科	重点项目	黄朝阳	硕士	徐清
2021Z029	法学院	民法典视域下生态破坏责任与环境修复问题研究——以昆明滇池违建拆除为例	人文社科	重点项目	李春灵	硕士	杨惟钦
2021Z030	法学院	数字法治政府建设问题研究——以云南省政务云平台为视角	人文社科	重点项目	阎静宇	硕士	杨临宏
2021Z031	工商管理与旅游管理学院	基于一部手机游云南的辐射南亚东南亚的智慧文旅支撑体系研究	人文社科	重点项目	李娇	硕士	邓伟升
2021Z032	工商管理与旅游管理学院	大滇西环线旅游高质量发展对居民幸福水平影响的差异化研究	人文社科	重点项目	张鸿	硕士	李云霞
2021Z033	工商管理与旅游管理学院	文旅融合背景下乡村旅游地农户生计转型及可持续发展研究——基于改进的 SLFT 框架	人文社科	重点项目	王昕	硕士	王克岭
2021Z034	工商管理与旅游管理学院	数字经济背景下企业数据资产化的会计治理路径研究	人文社科	重点项目	李欣蕊	硕士	王健康

2021Z035	昌新国际艺术学院	非物质文化遗产视域下“晋宁泥塑”的工艺传承与创新实践	人文社科	重点项目	王一涵	硕士	朱倩
2021Z036	昌新国际艺术学院	民族文化交融下云南丝绸之路书法嬗变及价值研究	人文社科	重点项目	毛鑫洋	硕士	唐楷之
2021Z037	高等教育研究院	云南大学学术型硕士研究生专业课程满意度及影响因素研究	人文社科	重点项目	吴成军	硕士	李敏
2021Z038	汉语国际教育学院	基于语料库的综合教材对比研究	人文社科	重点项目	谭正娇	硕士	刘克强
2021Z039	汉语国际教育学院	东盟贸易优势转化为中文传播效能的策略研究：本土员工语言要求调查	人文社科	重点项目	陈涵静	硕士	余江英
2021Z040	汉语国际教育学院	中文学术边缘化问题及治理措施：以 COVID-19 主题研究为例	人文社科	重点项目	望清华	硕士	余江英
2021Z041	经济学院	基于 STIRPAT 模型的中非出口多元化对非洲国家碳排放影响研究	人文社科	重点项目	王子明	硕士	刘洪波

2021Z042	经济学院	技术进步、产业结构优化与经济动态均衡—基于马克思两大部类再生产模型分析	人文社科	重点项目	魏宇航	硕士	王守义
2021Z043	经济学院	数字经济发展对我国金融资源配置效率的影响研究	人文社科	重点项目	张婷婷	硕士	李波
2021Z044	经济学院	跨境电商发展的制度需求分析——基于与国内电商发展巨大差距的经济事实	人文社科	重点项目	何天翠	硕士	黄宁
2021Z045	经济学院	劳动保护与企业出口国内附加值	人文社科	重点项目	张雨微	硕士	李波
2021Z046	经济学院	美国量化宽松货币政策对中国地方政府债务风险的溢出效应研究	人文社科	重点项目	谭宇宏	硕士	马子红
2021Z047	经济学院	普惠金融助力云南省乡村振兴的绩效研究	人文社科	重点项目	营增	硕士	刘志坚
2021Z048	历史与档案学院	思想史视野下的“三才一贯图”研究	人文社科	重点项目	刘洁	硕士	成一农

2021Z049	历史与档案学院	明清江南瘟疫流行、防治与医家群体	人文社科	重点项目	刘雅仙	硕士	耿金
2021Z050	历史与档案学院	全面抗战时期云南的公路走私	人文社科	重点项目	刘书好	硕士	齐春风
2021Z051	历史与档案学院	西南地区考古学中的羽人形象	人文社科	重点项目	段自渲	硕士	李昆声
2021Z052	马克思主义学院	新时代边疆少数民族青少年中华民族共同体意识培育——以临沧佤族为例	人文社科	重点项目	姜建宇	硕士	李维昌
2021Z053	民族学与社会学学院	非遗整体性保护的地方政府实践研究：以大理文化生态保护实验区为例	人文社科	重点项目	王卓臻	硕士	苏俊杰
2021Z054	民族学与社会学学院	从“焦土”到“沃土”：对麻栗坡天保“乡村振兴示范园”的个案研究	人文社科	重点项目	王燕	硕士	朱凌飞
2021Z055	民族学与社会学学院	漾濞县非遗学校教育与各民族青少年“三交”研究——基于主体间性视角	人文社科	重点项目	赵裕民	硕士	桂榕

2021Z056	民族学与社会学学院	滇西北永胜水源地保护与村庄秩序重构研究	人文社科	重点项目	钮泓宇	硕士	刘彦
2021Z057	民族学与社会学学院	中越边境猛硐蓝靛瑶村落传统文化资源保护与空间建构	人文社科	重点项目	蔡春静	硕士	邓玉函
2021Z058	体育学院	生物多样性背景下云南省户外体育活动提升大学生生态素养的实现路径研究	人文社科	重点项目	华夏	硕士	杨霞
2021Z059	外国语学院	文学地理学视域下的元明清时期中越邦交诗研究	人文社科	重点项目	何娟	硕士	于在照
2021Z060	外国语学院	缅甸政局变动以来东盟政治-安全共同体的对缅外交协调研究	人文社科	重点项目	张馨予	硕士	祝湘辉
2021Z061	外国语学院	中美博弈背景下的东盟安全机制讨论	人文社科	重点项目	刘星月	硕士	钟智翔
2021Z062	文学院	车王府藏目连戏研究	人文社科	重点项目	胡雪源	硕士	曾莹

2021Z063	文学院	“夜”的体验——论莫里斯·布朗肖的文学思想	人文社科	重点项目	李连杰	硕士	耿占春
2021Z064	新闻学院	人民日报新媒体矩阵对“建党100周年”主题报道的叙事策略研究	人文社科	重点项目	谭幸欢	硕士	林进桃
2021Z065	新闻学院	村史馆：乡土情境下文化空间的意义建构研究	人文社科	重点项目	宦宏明	硕士	杨星星
2021Z066	政府管理学院	云南边境国门社区治理体系的系统化构建研究	人文社科	重点项目	许姝钰	硕士	贺琳凯
2021Z067	政府管理学院	总体国家安全观下边疆韧性社区治理研究	人文社科	重点项目	岩温罕	硕士	罗强强
2021Z068	政府管理学院	政策过程视角下基层政府会议效能提升研究——以独山县下司镇为例	人文社科	重点项目	贺昌梅	硕士	邵宇
2021Z069	职业与继续教育学院	基于新时代质量观昆明市中职学校教师教学质量评价体系研究	人文社科	重点项目	吴鑫悦	硕士	王雯

2021Z070	职业与继续教育学院	基于 STEAM 教育理念的小学图形化编程教学设计研究	人文社科	重点项目	郑晴	硕士	李甦
2021Z071	生命科学学院	*eges-1* 错义突变调控基因表达稳定性和寿命	理工科	重点项目	代统	硕士	浦敏铁
2021Z072	信息学院	基于改进的 YOLO 网络红外和可见光融合图像的机器人目标识别检测	理工科	重点项目	郑皓元	硕士	杨志军
2021Z073	信息学院	基于数据增强的小样本文本分类的研究	理工科	重点项目	杨茂琴	硕士	周小兵
2021Z074	信息学院	微带线结构多频滤波器的设计	理工科	重点项目	罗万鸿	硕士	陈哲
2021Z075	信息学院	基于双鉴别器混合融合网络的多模态 MRI 图像合成算法	理工科	重点项目	韩永强	硕士	李海燕
2021Z076	信息学院	情感可控的多轮对话生成模型研究	理工科	重点项目	陈锐君	硕士	王津

2021Z077	信息学院	适应于 6G 太赫兹低交叉极化、高增益的 ISGW 滤波天线阵列的研究	理工科	重点项目	王彪	硕士	申东娅
2021Z078	信息学院	解释性东巴画内容的因果推断	理工科	重点项目	潘森垒	硕士	钱文华
2021Z079	信息学院	边缘计算场景中资源分配与最小功率信号覆盖问题的关键技术研究	理工科	重点项目	张庆辉	硕士	张学杰
2021Z080	建筑与规划学院	云南省生态安全预警时空演变及模拟研究	理工科	重点项目	薛梦柯	硕士	李志英
2021Z081	建筑与规划学院	迪庆深切峡谷地区乡村聚落的多尺度人居环境适应性研究	理工科	重点项目	郑良俊	硕士	徐坚
2021Z082	生命科学学院	嗜热杜邦菌中异戊烯基吲哚生物碱 Talathermophilins 生物合成机制	理工科	重点项目	勾江会	硕士	牛雪梅
2021Z083	生命科学学院	昭通野生蜜环菌（*Armillaria* sp.）多代伴栽天麻后发生退化的影响因素研究	理工科	重点项目	李玉	硕士	张汉波

2021Z084	生命科学学院	KDM6A 突变膀胱癌的精准治疗靶点筛选	理工科	重点项目	李璐	硕士	郭春明
2021Z085	生命科学学院	探究 DDX3X 在酒精性肝病中的分子机制理工科	理工科	重点项目	祝贤娥	硕士	单昭
2021Z086	生命科学学院	高原牦牛适应极端环境的胃肠道微生物组机制及胃功能分化机制的研究	理工科	重点项目	白丽艳	硕士	张志刚
2021Z087	生命科学学院	dguok 缺失导致小鼠不孕的机制研究	理工科	重点项目	董瑞	硕士	孙建伟
2021Z088	生命科学学院	EGF/EGFR-*yap1* 轴介导黑色素瘤 BRAF 抑制剂 Vemurafenib 抗药性引起的 STIM1 水平的上调的机制研究	理工科	重点项目	严程浩	硕士	孙建伟
2021Z089	数学与统计学院	航空机组智能公平排班问题	理工科	重点项目	邓彬	硕士	李伟东
2021Z090	数学与统计学院	“天眼”探物，走进西双版纳自然保护区	理工科	重点项目	刘香	硕士	赵越

2021Z091	数学与统计学院	“石榴”隔山海，山海皆可平	理工科	重点项目	卓庆丰	硕士	赵越
2021Z092	数学与统计学院	基于卷积神经网络的医学图像噪点识别及统计处理	理工科	重点项目	陈睿靖	硕士	李会琼
2021Z093	物理与天文学院	全固态近红外连续波可调谐双波长 Nd^{3+}:LiYF 激光器的研究	理工科	重点项目	何佳欣	硕士	吕彦飞
2021Z094	物理与天文学院	极限条件下飞秒时域和微纳尺寸分辨的磁光电输运系统的研发和应用	理工科	重点项目	张闰华	硕士	任杨
2021Z095	材料与能源学院	基于不同卤素有机小分子调控钙钛矿太阳能电池性能及载流子动力学研究	理工科	重点项目	吴太	硕士	华雍
2021Z096	材料与能源学院	聚精氨酸分子印迹电化学传感器对双酚 A 的高灵敏度检测特性研究	理工科	重点项目	赖亭润	硕士	王毓德
2021Z097	材料与能源学院	改变配位环境提升具有"Fe-N4"结构材料的 Zn-Air 电池性能	理工科	重点项目	梅至远	硕士	郭洪

2021Z098	地球科学学院	东南亚低纬高原非绝热加热年代际特征及其与周边气候系统的联系	理工科	重点项目	赫栋	硕士	曹杰
2021Z099	地球科学学院	川滇生态屏障关键区识别与“退化—修复”演变机制研究	理工科	重点项目	施馨雨	硕士	赵筱青
2021Z100	地球科学学院	那丹锑矿床中方解石矿物学和矿物化学研究	理工科	重点项目	彭旎	硕士	周家喜
2021Z101	国际河流与生态安全研究院	干热河谷区不同侵蚀程度植被恢复初期的限制因子研究	理工科	重点项目	刘文旭	硕士	荣丽
2021Z102	国际河流与生态安全研究院	基于机器学习的元江干热河谷沟蚀易发性分析	理工科	重点项目	曹文悦	硕士	董一帆
2021Z103	国际河流与生态安全研究院	元阳哈尼梯田生态系统文化服务空间分布及其影响因素研究	理工科	重点项目	杨璐营	硕士	李艳波
2021Z104	国际河流与生态安全研究院	基于耳石微化学的怒江云南鲱鲏洄游路线研究	理工科	重点项目	唐邦利	硕士	丁城志

2021Z105	国际河流与生态安全研究院	纳米二氧化钛对重金属镉在水-生物膜-底泥中迁移的影响研究	理工科	重点项目	朱时俊	硕士	骆霞
2021Z106	化学科学与工程学院	芒柄花素衍生物抗骨质疏松作用机制研究	理工科	重点项目	闫晓君	硕士	赵云丽
2021Z107	化学科学与工程学院	基于羧基 TPE 配体构筑的稀土配合物及其光学性质研究	理工科	重点项目	王澍雨	硕士	杜琳
2021Z108	化学科学与工程学院	高荷电分离异质结的迭代构筑与仿生膜的光化学合成	理工科	重点项目	张奇钊	硕士	方文浩
2021Z109	化学科学与工程学院	基于 PVDF 聚合物包容膜的构建及萃取金(Ⅰ)的研究	理工科	重点项目	朱贵萍	硕士	杨项军
2021Z110	化学科学与工程学院	一株云南特境放线菌次生代谢产物研究	理工科	重点项目	解天鹏	硕士	丁中涛
2021Z111	软件学院	无人机赋能的移动边缘计算中计算与缓存协作调度策略	理工科	重点项目	包聆言	硕士	赵明雄

2021Z112	软件学院	大规模自然语言释义识别研究	理工科	重点项目	杜鲲鹏	硕士	张璇
2021Z113	软件学院	基于多源域迁移学习的活动识别	理工科	重点项目	贾启	硕士	杨云
2021Z114	生态与环境学院	兰科植物种子-真菌共生萌发复合体的研制及在濒危物种保护上的应用	理工科	重点项目	杨华	硕士	高江云
2021Z115	生态与环境学院	转录因子 Dof4.7 在拟南芥气孔发育中的功能研究	理工科	重点项目	余爽爽	硕士	和兆荣 陈小兰
2021Z116	生态与环境学院	金沙江河谷山枣空间分布格局及种子传播策略	理工科	重点项目	熊在秋	硕士	张志明
2021Z117	医学院	柯萨奇 A 组 10 型病毒在细胞焦亡中的作用及其分子机制	理工科	重点项目	李智强	硕士	刘文静
2021Z118	资源植物研究院	解析解淀粉芽孢杆菌 YN0904 的生物膜形成相关基因及对香蕉枯萎病生物防控的影响	理工科	重点项目	周光东	硕士	郑泗军

2021Z119	资源植物研究院	多组学解析黄毛草莓果实香味合成	理工科	重点项目	曹强	硕士	乔琴
2021Z120	资源植物研究院	一种提高渗透胁迫下大麻种子萌发的外源激素筛选及作用机制研究	理工科	重点项目	张涵雪	硕士	杜光辉
2021Z121	建筑与规划学院	基于偏椭球凸集模型的预应力混凝土桥梁正截面抗弯承载能力评估理论研究	理工科	重点项目	谢璐源	硕士	冉志红
2021Y001	新闻学院	网络意见表达：基于语义社会网络的超网络模型研究	人文社科	一般项目	程俊超	博士	廖圣清
2021Y002	法学院	元代判例制度与案例指导制度比较研究	人文社科	一般项目	董曦琰	博士	胡兴东
2021Y003	法学院	艺术品交易中“买者当心”原则的适用范围与责任机制研究	人文社科	一般项目	金荣婧	博士	梁慧星
2021Y004	工商管理与旅游管理学院	民族地区传统技艺从业人员可持续性评价及推进机制研究	人文社科	一般项目	李灿松	博士	王克岭

2021Y005	国际关系研究院	企业社会责任的国际传播：对湄公河五国中资企业的实证研究	人文社科	一般项目	任欣霖	博士	孔建勋
2021Y006	国际关系研究院	中国-东南亚区域非传统安全不对称合作与动态集体认同研究	人文社科	一般项目	陈伟琳	博士	卢光盛
2021Y007	国际关系研究院	“印太”战略下印度对外政策中的古杰拉尔主义研究	人文社科	一般项目	李铭恩	博士	叶海林
2021Y008	国际关系研究院	索马里邦特兰的部落政治与类国家构建	人文社科	一般项目	崔媛媛	博士	王涛
2021Y009	经济学院	数字化背景下“数字鸿沟”对区域不平衡发展的影响与机制研究	人文社科	一般项目	侯建翔	博士	李娅
2021Y010	经济学院	数字基础设施与中国全要素生产率研究	人文社科	一般项目	李文静	博士	张国胜
2021Y011	历史与档案学院	“南方丝绸之路”概念的内涵与外延	人文社科	一般项目	邵申申	博士	张轲风

2021Y012	历史与档案学院	面向文旅融合特色景点建设的云南民族档案文化创意服务研究	人文社科	一般项目	梁思思	博士	华林
2021Y013	历史与档案学院	以祈雨看明清岭南民族文化的交融	人文社科	一般项目	农颖	博士	刘正寅
2021Y014	马克思主义学院	中华民族共同体意识融入边疆民族地区高校思政课的路径研究	人文社科	一般项目	张瑜敏	博士	赵新国
2021Y015	马克思主义学院	恩格斯对泛斯拉夫主义批判研究	人文社科	一般项目	齐付清	博士	张健
2021Y016	民族学与社会学学院	西南少数民族地区幼儿教育研究：以云南省元阳县新街镇为例	人文社科	一般项目	张宝元	博士	马翀炜
2021Y017	民族学与社会学学院	云南勐海镇少数民族茶商的直播民族志研究	人文社科	一般项目	杨素	博士	郑宇
2021Y018	民族学与社会学学院	多民族共塑圣山——民族交往交流交融史视角下的鸡足山兴起	人文社科	一般项目	张晗	博士	朱映占

2021Y019	民族学与社会学学院	生态视角下边疆地区少数民族儿童异地适应问题研究	人文社科	一般项目	李晓娇	博士	高万红
2021Y020	文学院	稳定与新生：白族大本曲的曲目演述	人文社科	一般项目	杨识余	博士	董秀团
2021Y021	政府管理学院	新中国成立初期的人口国民化研究（1949-1956）	人文社科	一般项目	廖子夏	博士	周平
2021Y022	政府管理学院	实边——守边——通边：边民身份及其功能的演进逻辑	人文社科	一般项目	侯红霞	博士	刘永刚
2021Y023	信息学院	支持多维数据“感知+推理”分析的贝叶斯深度学习方法研究	理工科	一般项目	吴鑫然	博士	岳昆
2021Y024	信息学院	基于异质网络的异质社区搜索技术研究	理工科	一般项目	王家龙	博士	周丽华
2021Y025	信息学院	特征级融合的红外与可见光目标跟踪	理工科	一般项目	刘琰煜	博士	周冬明

2021Y026	生命科学学院	中国西南特有属——鹭鸶草属的系统与分类学研究	理工科	一般项目	杨凤	博士	王焕冲
2021Y027	生命科学学院	玉米对从枝菌根的自调节	理工科	一般项目	王博文	博士	赵之伟
2021Y028	生命科学学院	小鼠 Naïve 滋养层干细胞的构建及重构类胚胎的研究	理工科	一般项目	高亚可	博士	张建
2021Y029	生命科学学院	转录因子 TEAD 在结直肠癌中的表达调控研究	理工科	一般项目	段佳虹	博士	邹成钢
2021Y030	生命科学学院	宿主分泌蛋白 TAG-293 参与宿主免疫防御的调控机制研究	理工科	一般项目	李倩	博士	祁斌
2021Y031	生命科学学院	DNA 助力穿山甲非法贸易来源追溯	理工科	一般项目	谷桐桐	博士	于黎
2021Y032	数学与统计学院	一类可压缩 Navier-Stokes 方程经典解的整体存在性及其爆破准则	理工科	一般项目	王伟华	博士	杨汉春

2021Y033	物理与天文学院	具有高稳定性的柔性晶体管光探测器的制备与研究	理工科	一般项目	杨振新	博士	吕正红
2021Y034	物理与天文学院	伽马射线活动星系核光变的高斯过程模拟	理工科	一般项目	张海云	博士	张力
2021Y035	物理与天文学院	Ia 型超新星的光学观测研究	理工科	一般项目	李丽萍	博士	戴本忠
2021Y036	材料与能源学院	基于钙钛矿 Cs3Cu2I5 材料的低成本高性能光电探测器的开发与应用	理工科	一般项目	陈涛	博士	王莞
2021Y037	材料与能源学院	Au-Ag-Cu 三元合金电接触材料微纳结构与力学性能耦合关系的研究	理工科	一般项目	付丽	博士	毛勇
2021Y038	地球科学学院	大湄公河次区域秋季降水的年际变化及其成因	理工科	一般项目	田梦萍	博士	曹杰
2021Y039	国际河流与生态安全研究院	集成生态系统服务与生物多样性保护对象的系统保护规划研究	理工科	一般项目	周键	博士	武瑞东

2021Y040	国际河流与生态安全研究院	人类活动和自然过程对城市近地面 CO2、CH4 大气浓度及稳定碳同位素的影响	理工科	一般项目	吴彬	博士	田立德
2021Y041	化学科学与工程学院	钴催化的 3+2 环化得到高区域选择性吲哚衍生物的方法学研究	理工科	一般项目	田勋	博士	羊晓东
2021Y042	化学科学与工程学院	具有抗肿瘤活性的吡咯类衍生物的设计合成及活性研究	理工科	一般项目	李堃	博士	严胜骄
2021Y043	化学科学与工程学院	基于掺杂体系的光刺激响应超长室温磷光材料的制备及应用研究	理工科	一般项目	程艺	博士	曹秋娥
2021Y044	化学科学与工程学院	非环状β，β-二取代烯胺盐的亲核反应研究	理工科	一般项目	李正飞	博士	卢崇道
2021Y045	生态与环境学院	亚热带常绿阔叶林中梯度增温对凋落物的分解研究	理工科	一般项目	陈德赟	博士	吴建平
2021Y046	生态与环境学院	微囊藻毒素 MC-LR 探针的构建， 荧光检测及生物学研究	理工科	一般项目	张辉霞	博士	谢平 刘勇

2021Y047	生态与环境学院	酸性环境下Al^{3+}毒害对高山木本植物P吸收的作用机制	理工科	一般项目	王思齐	博士	申仕康
2021Y048	生态与环境学院	利用高山倭蛙近缘物种解析祖先适应和物种特异分化	理工科	一般项目	陈容梅	博士	孙艳波
2021Y049	生态与环境学院	玉龙雪山不同海拔梯度下典型植被类型土壤DOM特征与微生物互作机制	理工科	一般项目	鲍勇	博士	程晓莉
2021Y050	医学院	RRM2对妇科肿瘤的作用影响及其机制研究	理工科	一般项目	杨璐涵	博士	郑冰蓉
2021Y051	法学院	民族边疆地区的疫情防控与社会治理问题研究——以腾冲市为调研对象	人文社科	一般项目	杨继湘	硕士	李春
2021Y052	法学院	运输毒品罪罪刑规制实证化研究 ——以云南省720份判决书为样本	人文社科	一般项目	张鑫	硕士	赵春玉
2021Y053	法学院	云南省外籍人员法律服务的现状与对策研究	人文社科	一般项目	祝一方	硕士	刘红春

2021Y054	法学院	智慧侦查背景下犯罪嫌疑人的电子储存信息证据搜查制度构建研究	人文社科	一般项目	孙永润	硕士	牟军
2021Y055	法学院	老龄化社会背景下意定监护制度运行现状问题和对策研究	人文社科	一般项目	殷梨梨	硕士	李一娴
2021Y056	法学院	合作主义视阈下的法院调解方式进路研究	人文社科	一般项目	王璐璐	硕士	赵子尧
2021Y057	法学院	十年回望与前瞻：新时代云南指导性案例的适用困境与进路研究	人文社科	一般项目	韩成龙	硕士	徐清
2021Y058	法学院	市域社会治理法治化中网格员定位与作用实证研究——以曲靖市为例	人文社科	一般项目	张聪	硕士	徐清
2021Y059	法学院	土地经营权入股法律问题研究	人文社科	一般项目	聂娅楠	硕士	周昌发
2021Y060	法学院	紧急状态下的法治——阿甘本人权思想研究	人文社科	一般项目	付一帆	硕士	刘敏

2021Y061	法学院	民族立法中的“云南经验”研究——以云南省人大地方性法规为切入点	人文社科	一般项目	张智威	硕士	王鑫
2021Y062	法学院	COP15 背景下云南省生物多样性司法保护研究	人文社科	一般项目	孙瑞	硕士	张殿军
2021Y063	法学院	人工智能技术在定罪量刑中的运用和规制——以 Y 市法院为调查对象	人文社科	一般项目	马玉晖	硕士	赵春玉
2021Y064	法学院	人工智能时代无人驾驶车辆交通肇事的刑事责任研究	人文社科	一般项目	李亚倩	硕士	程龙
2021Y065	法学院	法官助理的现实角色和法律定位问题研究	人文社科	一般项目	王毅健	硕士	徐清
2021Y066	法学院	大数据背景下“校园贷”的刑事责任体系研究	人文社科	一般项目	宋欣恬	硕士	赵春玉
2021Y067	法学院	国家公园立法中“最严格保护”与“游憩”的平衡研究	人文社科	一般项目	覃泽伟	硕士	张殿军

2021Y068	法学院	大数据时代下侵犯公民个人信息罪适用问题研究	人文社科	一般项目	卢青	硕士	赵春玉
2021Y069	法学院	“法治乡村建设”中大学生村官的角色与功能实证研究	人文社科	一般项目	晏莉洁	硕士	杜健荣
2021Y070	法学院	公司高管提前套现理财产品获益的退还请求权依据研究——以恒大“暴雷”事件引入	人文社科	一般项目	陈静	硕士	赵忠龙
2021Y071	法学院	Y省L市基层法院刑事强制医疗程序的适用与完善	人文社科	一般项目	石晓婧	硕士	牟军
2021Y072	法学院	宅基地“三权分置”改革的实践探索	人文社科	一般项目	王博涵	硕士	刘国乾
2021Y073	法学院	疫情常态化下西南边境恐怖主义威胁评估及治理研究——以云南边境为例	人文社科	一般项目	王一帆	硕士	赵春玉
2021Y074	法学院	疫情常态化下跨境电信网络诈骗犯罪防控研究——以刑法应对为中心	人文社科	一般项目	李珞宁	硕士	李春

2021Y075	法学院	论“人肉搜索”行为的入罪界限—以侵犯公民个人信息罪视角展开	人文社科	一般项目	韦学鑫	硕士	赵春玉
2021Y076	工商管理与旅游管理学院	基于GIS的云南省非物质文化遗产项目空间分布特征及旅游响应研究	人文社科	一般项目	刀梦然	硕士	邓永进
2021Y077	工商管理与旅游管理学院	社区增权视角下鲁史古镇规模性返贫阻断机制研究	人文社科	一般项目	王娇	硕士	杨懿
2021Y078	工商管理与旅游管理学院	聂耳精神的红色基因传承与旅游化路径研究	人文社科	一般项目	王含含	硕士	杨懿
2021Y079	工商管理与旅游管理学院	基于PROMETHEE-GAIA云南民族地区旅游高质量发展空间差异与发展路径研究	人文社科	一般项目	袁梦	硕士	杜靖川
2021Y080	工商管理与旅游管理学院	中国长江流域水利遗产时空分布特征与旅游活化利用研究	人文社科	一般项目	王丹宁	硕士	李鹏
2021Y081	工商管理与旅游管理学院	滇西北区域旅游经济外部性效应及优化路径研究	人文社科	一般项目	程新杰	硕士	王克岭

2021Y082	工商管理与旅游管理学院	纳入征信系统后的大学生互联网消费信贷行为风险评估调查研究	人文社科	一般项目	李珊	硕士	赵璋
2021Y083	工商管理与旅游管理学院	代际视角下角色认同、内部人身份感知对建言行为的影响研究	人文社科	一般项目	刘静怡	硕士	张建民
2021Y084	工商管理与旅游管理学院	基于空间杜宾模型的云南省旅游经济韧性障碍因素诊断与破解机制研究	人文社科	一般项目	常春雨	硕士	李鹏
2021Y085	工商管理与旅游管理学院	基于 Flickr 元数据的云南省入境旅游流动态演变特征研究	人文社科	一般项目	裴艳霞	硕士	李鹏
2021Y086	工商管理与旅游管理学院	云南旅游气候舒适度评价及其康养效应研究	人文社科	一般项目	张颖	硕士	邓永进
2021Y087	工商管理与旅游管理学院	东道国制度环境、区位优势对中国 OFDI 效率的影响研究	人文社科	一般项目	余建华	硕士	赵德森
2021Y088	工商管理与旅游管理学院	零售行业全渠道供应链下各参与方因库存策略差异引起的“效益背反”问题研究	人文社科	一般项目	刘阳阳	硕士	宋乐

2021Y089	工商管理与旅游管理学院	研发人力资本效应下产业数字化对创新成果转化的影响研究	人文社科	一般项目	许濒文	硕士	李亚玲
2021Y090	建筑与规划学院	云南省基础设施投资对新型城镇化高质量发展影响的研究	人文社科	一般项目	邹红艳	硕士	李志英
2021Y091	建筑与规划学院	组织依恋对工程项目组织韧性的影响及作用机制研究	人文社科	一般项目	刘惠芬	硕士	卜炜玮
2021Y092	建筑与规划学院	城市蔓延对区域经济活力的影响研究—以长三角城市群为例	人文社科	一般项目	吴晓囡	硕士	陈涛
2021Y093	建筑与规划学院	政策工具对西部地区建筑业可持续发展的影响研究——基于生态位的视角	人文社科	一般项目	许馨丹	硕士	卜炜玮
2021Y094	昌新国际艺术学院	云南题材在当代中国花鸟画中的呈现与影响	人文社科	一般项目	于炀	硕士	陈孟昕
2021Y095	昌新国际艺术学院	汉字启蒙书法绘本——学龄前儿童书法教材研究与制作	人文社科	一般项目	郝苑婷	硕士	朱倩

2021Y096	昌新国际艺术学院	云南省非物质文化遗产东巴造纸的保护与创新应用研究	人文社科	一般项目	叶娅恒	硕士	王巍
2021Y097	昌新国际艺术学院	滇中状元-袁嘉谷书法风格及人文精神研究	人文社科	一般项目	刘伟明	硕士	唐楷之
2021Y098	昌新国际艺术学院	昆明“纸马”的艺术研究以及在绘画创作中的应用	人文社科	一般项目	郑美琪	硕士	袁玲玲
2021Y099	昌新国际艺术学院	乡村振兴视角下平地村“一颗印”式民居的现状研究与保护发展思考	人文社科	一般项目	冯帆	硕士	王路
2021Y100	昌新国际艺术学院	从《孟孝琚碑》探汉代儒学在昭通的延续	人文社科	一般项目	陈芳慧	硕士	邱正伦
2021Y101	高等教育研究院	云南大学硕士留学生教育质量保障研究	人文社科	一般项目	王诗润	硕士	张建新
2021Y102	高等教育研究院	云南大学专业型硕士人才培养的质量调查及对策研究	人文社科	一般项目	荆碧婷	硕士	刘康宁

2021Y103	国际关系研究院	萨赫勒五国组织的跨境安全合作研究	人文社科	一般项目	瞿诗怡	硕士	张春
2021Y104	国际关系研究院	撒哈拉以南非洲国家反恐政策有效性评估	人文社科	一般项目	王潇茹	硕士	卢凌宇
2021Y105	国际关系研究院	缅甸历史教科书中的中国形象	人文社科	一般项目	吴杨珍	硕士	李涛
2021Y106	国际关系研究院	东盟国家对“双循环新发展格局”的认知与对策研究	人文社科	一般项目	张新	硕士	罗圣荣
2021Y107	汉语国际教育学院	在线学习环境下汉语国际教育硕士数字素养与科研能力的关系研究	人文社科	一般项目	李维	硕士	李伟
2021Y108	汉语国际教育学院	提升南亚东南亚本科留学生学术适应能力的教学需求调查	人文社科	一般项目	龚何倩	硕士	王亚琼
2021Y109	汉语国际教育学院	中外儿童绘本创作中的中国文化表达方式研究	人文社科	一般项目	袁园	硕士	罗江文

2021Y110	汉语国际教育学院	昆明来华留学生非课堂环境言语适应调查研究	人文社科	一般项目	陈冰	硕士	杨晓雯
2021Y111	汉语国际教育学院	后疫情时期汉语国际教育硕士研究生专业认同的调查研究	人文社科	一般项目	李玉凤	硕士	李伟
2021Y112	汉语国际教育学院	汉语学术词表创建及其与《中文水平等级标准》词表的对比研究	人文社科	一般项目	吴亚	硕士	王亚琼
2021Y113	汉语国际教育学院	线上教学对提升华裔儿童自主学习能力的效能研究	人文社科	一般项目	户江祎	硕士	张艳萍
2021Y114	汉语国际教育学院	缅甸中资企业中文减贫效果调查研究	人文社科	一般项目	朱梦洁	硕士	余江英
2021Y115	汉语国际教育学院	汉语学术论文中框架语分级研究	人文社科	一般项目	赖攀	硕士	王亚琼
2021Y116	汉语国际教育学院	基于多模态教学模式的对外汉语成语教学研究	人文社科	一般项目	王娜	硕士	张艳萍

2021Y117	汉语国际教育学院	文化生态学视阈下“讲好中国故事”的有效叙事形态研究	人文社科	一般项目	刘双跃	硕士	杨晓雯
2021Y118	经济学院	规模经济还是范围经济？——中国企业数字化与生产方式变革	人文社科	一般项目	魏心贤	硕士	张国胜
2021Y119	经济学院	国内国际“双循环”下高端装备制造业高质量发展路径及应对策略研究	人文社科	一般项目	沈秋爽	硕士	郭树华
2021Y120	经济学院	政府政策与绿色金融效率关系的实证研究——以长江经济带为例	人文社科	一般项目	胡丁言	硕士	何树红
2021Y121	经济学院	后减贫时代少数民族农业转移人口社会融合机制研究—以昭通为例	人文社科	一般项目	段鹏	硕士	吕昭河
2021Y122	经济学院	全球价值链重构背景下中国与东盟双边贸易特征及潜力研究——基于本土市场规模的视角	人文社科	一般项目	何维鑫	硕士	杨先明
2021Y123	经济学院	数字经济、空间外溢与地区收入差距——兼论溢出带宽	人文社科	一般项目	钱美君	硕士	文传浩

2021Y124	经济学院	数字赋能是否提升了我国基本公共服务的效率——以社会保障为例	人文社科	一般项目	成晓栋	硕士	张国胜
2021Y125	经济学院	数字经济对地区间财力差异影响的实证研究	人文社科	一般项目	张洪雪	硕士	袁崇坚
2021Y126	经济学院	限购政策对上市房地产企业经营绩效的影响研究	人文社科	一般项目	刘雯	硕士	马子红
2021Y127	经济学院	金融错配、非金融企业影子银行化与系统性金融风险	人文社科	一般项目	许学茜	硕士	杨洋
2021Y128	经济学院	数字经济对劳动者权益的影响研究	人文社科	一般项目	周怡	硕士	马子红
2021Y129	经济学院	混合所有制改革对国有上市企业去杠杆的影响研究	人文社科	一般项目	高紫薇	硕士	马子红
2021Y130	经济学院	数字经济推动云南省制造业高质量发展研究	人文社科	一般项目	郭婧茹	硕士	秦开强

2021Y131	经济学院	绿色金融对我国企业绿色技术创新的影响	人文社科	一般项目	李浪涛	硕士	罗美娟
2021Y132	经济学院	数字金融对中小企业财务可持续的研究——基于融资约束视角	人文社科	一般项目	李润思	硕士	杨洋
2021Y133	经济学院	数字普惠金融对中小企业技术创新的影响研究	人文社科	一般项目	张玥	硕士	杨洋
2021Y134	经济学院	数字普惠金融与产业结构升级——基于金融资源错配角度的分析	人文社科	一般项目	甘星月	硕士	杨洋
2021Y135	经济学院	“一带一路”沿线国家中国企业社会责任履行与来源国劣势转变影响研究	人文社科	一般项目	王玮	硕士	陈瑛
2021Y136	经济学院	数字普惠金融推动乡村共同富裕的机制研究——以禄丰县为例	人文社科	一般项目	王雨桐	硕士	郭树华
2021Y137	经济学院	云南生态保护与修复专项基金绩效审计评价体系构建——以普达措为例	人文社科	一般项目	段琬琪	硕士	程士国

2021Y138	经济学院	数字基础设施对区域经济增长收敛的影响机制与实证研究	人文社科	一般项目	刘英标	硕士	张国胜
2021Y139	经济学院	城市旅游品牌的绿色全要素生产率效应——基于旅游景区评选的准自然实验	人文社科	一般项目	唐宝时	硕士	杨孟禹
2021Y140	经济学院	RCEP 构架下云南自由贸易试验区与东盟通道建设——基于贸易便利化的研究	人文社科	一般项目	陈洁筠	硕士	胡颖
2021Y141	经济学院	全球价值链视角下云南咖啡低端锁定破解路径研究	人文社科	一般项目	殷旭航	硕士	胡颖
2021Y142	经济学院	数字金融对区域技术创新的影响机制研究	人文社科	一般项目	黄光世	硕士	杨洋
2021Y143	经济学院	限购政策对房地产企业资本结构的影响：基于中国的经验证据	人文社科	一般项目	周璟泽	硕士	马子红
2021Y144	经济学院	数字经济与云南省文旅产业深度融合研究	人文社科	一般项目	蔡璐璐	硕士	李贤

2021Y145	经济学院	“双碳”目标下云南传统产业与新兴产业的不同转型升级模式	人文社科	一般项目	邢晋嘉	硕士	李贤
2021Y146	历史与档案学院	屯田与协饷：清中期新疆治理机制研究	人文社科	一般项目	范文恺	硕士	马琦
2021Y147	历史与档案学院	明清时期滇中地区基层区划与组织形态研究	人文社科	一般项目	徐超	硕士	刘灵坪
2021Y148	历史与档案学院	解放战争时期中国共产党对国统区的舆论宣传研究——以云南地区为例	人文社科	一般项目	曹锐锟	硕士	董雁伟
2021Y149	历史与档案学院	民国时期云南盐工管理及福利待遇	人文社科	一般项目	杜倩	硕士	赵小平
2021Y150	历史与档案学院	华夷一体：明清时期武陵土家族地区“大一统”思想实践研究	人文社科	一般项目	向斯瑶	硕士	段丽波
2021Y151	历史与档案学院	清代广西水旱灾害及其社会应对	人文社科	一般项目	张林凤	硕士	廖国强

2021Y152	历史与档案学院	从关索信仰看西南地区民族文化交融	人文社科	一般项目	张诗琦	硕士	刘正寅
2021Y153	历史与档案学院	陈宏谋社仓实践与雍乾时期国家治理深化研究	人文社科	一般项目	刘佳亮	硕士	祁志浩
2021Y154	历史与档案学院	1949 年中共在苏沪地区城市筹粮运动研究	人文社科	一般项目	王威杰	硕士	罗群
2021Y155	历史与档案学院	戴季陶与中国西北高等农林教育研究	人文社科	一般项目	徐安珏	硕士	潘先林
2021Y156	历史与档案学院	二战期间英帝国对印度的资源开发政策及影响	人文社科	一般项目	汤志杰	硕士	任有权
2021Y157	历史与档案学院	县级图书馆总分馆服务效能调查研究:以五华区为例	人文社科	一般项目	杜禹	硕士	王水乔
2021Y158	历史与档案学院	农村慢性病患者网络健康信息行为研究	人文社科	一般项目	郭莹凤	硕士	刘宇

2021Y159	历史与档案学院	跨境流动背景下中老边民信息适应研究	人文社科	一般项目	李程玲	硕士	朱明
2021Y160	历史与档案学院	面向民族文化传承的白族经典文献遴选研究	人文社科	一般项目	钟岚	硕士	黄体杨
2021Y161	历史与档案学院	云南少数民族“古方灸”文献整理及特色数据库开发研究	人文社科	一般项目	曹梦琪	硕士	陈海玉
2021Y162	历史与档案学院	聋人日常信息搜寻影响因素研究	人文社科	一般项目	王茗睿	硕士	王晋
2021Y163	历史与档案学院	云南省自然保护区文献资源的整合研究	人文社科	一般项目	王天佑	硕士	庄会富
2021Y164	历史与档案学院	以茶治边：北宋对西夏的策略选择	人文社科	一般项目	冯全镇	硕士	张锦鹏
2021Y165	历史与档案学院	汉唐时期的白马朱鬣	人文社科	一般项目	季晶	硕士	陈果

2021Y166	马克思主义学院	香格里拉金江镇乡村振兴的路径研究	人文社科	一般项目	涂敏	硕士	杨荣
2021Y167	马克思主义学院	红色主题影视在中华民族共同体意识建构中的功能与运用研究	人文社科	一般项目	普晓娟	硕士	杜帮云
2021Y168	马克思主义学院	新时代青年大学生中华优秀传统文化认同研究	人文社科	一般项目	陈兴惠	硕士	谢青松
2021Y169	马克思主义学院	边疆民族地区大学生中国民族共同体意识教育路径研究——以云南高校为例	人文社科	一般项目	刘蕊	硕士	杨志玲
2021Y170	马克思主义学院	新榨取资本主义的批判性研究	人文社科	一般项目	向燕妮	硕士	杜敏
2021Y171	民族学与社会学学院	云南少数民族题材影视作品的民族文化建构研究	人文社科	一般项目	早柔娟	硕士	马居里
2021Y172	民族学与社会学学院	线性文化遗产的历史记忆与文化认同研究	人文社科	一般项目	苏菊	硕士	李佳

2021Y173	民族学与社会学学院	民纪片摄制的设备技术条件和影音呈现方式	人文社科	一般项目	陈琳	硕士	陈学礼
2021Y174	民族学与社会学学院	传统藏医药创造性转化过程中的具身实践与族际互动——以尤阙疗法为例	人文社科	一般项目	杨雅鑫	硕士	桂榕
2021Y175	民族学与社会学学院	精准扶贫与乡村振兴有效衔接的思路和对策——以怒江州丙中洛扎那桶村为例	人文社科	一般项目	汪敬轩	硕士	何明
2021Y176	民族学与社会学学院	基于 IPA 分析的香巴拉民族团结主题公园的创新实践与满意度研究	人文社科	一般项目	李立夫	硕士	李志农
2021Y177	民族学与社会学学院	云南八蜡庙历史资料搜集整理与研究	人文社科	一般项目	齐荣	硕士	马居里
2021Y178	民族学与社会学学院	江苏佛学院青年女性出家动机及自我认同研究	人文社科	一般项目	王极	硕士	周娅
2021Y179	民族学与社会学学院	“稳在城市”：异地搬迁移民的社会融入研究——以香格里拉雅瑞安和社区为例	人文社科	一般项目	张佩佩	硕士	李志农

2021Y180	民族学与社会学学院	数字化时代大理白族手工艺从业女性的社会角色研究	人文社科	一般项目	钟岚	硕士	刘从水
2021Y181	民族学与社会学学院	楚雄大雪地村野生菌产业与彝族生计转型关系研究	人文社科	一般项目	韦丹妮	硕士	郑宇
2021Y182	民族学与社会学学院	传统村落风貌的保护及发展对策研究——基于腾冲水碓村的田野调查报告	人文社科	一般项目	高崎鑫	硕士	刘彦
2021Y183	民族学与社会学学院	柿华菁苗族入赘婚的生存理性选择研究	人文社科	一般项目	李忠努	硕士	郑宇
2021Y184	民族学与社会学学院	乡村振兴战略背景下非物质文化遗产的传承与发展——以云南省通海县高台为例	人文社科	一般项目	普玲	硕士	何明
2021Y185	民族学与社会学学院	东南亚中资企业的公共外交实践与中国形象建构研究	人文社科	一般项目	农惠婷	硕士	许庆红
2021Y186	民族学与社会学学院	易地扶贫搬迁背景下父母外出务工对初中生学习倦怠的影响研究	人文社科	一般项目	王德文	硕士	许庆红

2021Y187	民族学与社会学学院	云南省退休知青谋生生命体验口述史研究	人文社科	一般项目	梁艳	硕士	张美川
2021Y188	民族学与社会学学院	项目制劳工的跨国流动——以宁波 Z 公司建筑工人为例	人文社科	一般项目	张海静	硕士	刘建娥
2021Y189	体育学院	云南省体育中考改革对昆明市初中体育教学的影响研究	人文社科	一般项目	杨灿灿	硕士	朱永莉
2021Y190	体育学院	″双减”政策对云大附小课后体育服务的提升对策研究	人文社科	一般项目	杨会健	硕士	杨霞
2021Y191	体育学院	身体素养视域下促进大学生健康发展的路径研究——以云南大学为例	人文社科	一般项目	李硕	硕士	朱永莉
2021Y192	体育学院	大学生引体向上现状及提升对策研究——以云南大学为例	人文社科	一般项目	郭宇宸	硕士	杨霞
2021Y193	体育学院	昆明雁来湖浆板特色运动小镇发展规划研究	人文社科	一般项目	程一安	硕士	夏文

2021Y194	外国语学院	疫情背景下印度媒体涉华报道话语分析——以《今日印度》为例	人文社科	一般项目	王苗	硕士	刘军
2021Y195	外国语学院	云南高校英语专业教育与职业发展的调查研究	人文社科	一般项目	张宇	硕士	李佳
2021Y196	外国语学院	日本智库联盟的运行机制及特征研究	人文社科	一般项目	耿鑫	硕士	毕世鸿
2021Y197	外国语学院	功能翻译理论视角下的日本汉文训读效用研究	人文社科	一般项目	沐晶洁	硕士	郑雁天
2021Y198	外国语学院	印度神话传说在云南的本土化—以傣族、白族、纳西族为例	人文社科	一般项目	焦云鹤	硕士	邓兵
2021Y199	外国语学院	泰汉人称代词活用移情机制对比研究	人文社科	一般项目	林世英	硕士	李芳
2021Y200	外国语学院	多维视角下维克多•雨果《写给女儿的诗》中的时间书写	人文社科	一般项目	吴怡	硕士	孙芳

2021Y201	外国语学院	阐释学视角下《芙蓉女儿诔》的文学性英译研究	人文社科	一般项目	卫平君	硕士	刘志成
2021Y202	外国语学院	伊泽视话表音文字在汉语及日语教育中的应用	人文社科	一般项目	金征	硕士	卢骁
2021Y203	外国语学院	越南网络安全发展与治理探析	人文社科	一般项目	李灵晟	硕士	毕世鸿
2021Y204	外国语学院	莫迪政府对达赖流亡政府政策的演变及价值取向	人文社科	一般项目	李静	硕士	邓兵
2021Y205	文学院	艾米莉·狄金森在中国译介中的文化形象研究	人文社科	一般项目	王卓群	硕士	杨磊
2021Y206	文学院	同素同义单双音节动词语义研究	人文社科	一般项目	李文涛	硕士	赵倩
2021Y207	文学院	细小与南方——论朱文颖小说的城市想象	人文社科	一般项目	杨懿	硕士	降红燕

2021Y208	文学院	“搞音乐的苏格拉底”——尼采美学视野下的审美乌托邦如何可能	人文社科	一般项目	吴永鹏	硕士	王卫东
2021Y209	文学院	新世纪儿童诗歌创作研究	人文社科	一般项目	李霞	硕士	李森
2021Y210	文学院	松尾芭蕉俳句的空间叙事研究	人文社科	一般项目	张云尧	硕士	舒凌鸿
2021Y211	文学院	云南大姚“金马碧鸡”的神圣叙事研究	人文社科	一般项目	布晖	硕士	董秀团
2021Y212	文学院	湘南土话声调变异与变化实验研究	人文社科	一般项目	谢思雨	硕士	刘增慧
2021Y213	新闻学院	云南红色文化网络传播的创新与发展	人文社科	一般项目	张若涵	硕士	赵净秋
2021Y214	新闻学院	世界变局的中国叙事—《国民》杂志国际议题的历史考察	人文社科	一般项目	黄晓黎	硕士	杨星星

2021Y215	新闻学院	在线信息搜寻与大学生健康焦虑研究	人文社科	一般项目	温雨曦	硕士	黎藜
2021Y216	新闻学院	城市流动人口的媒介交往与关系建构研究	人文社科	一般项目	常芬	硕士	孙信茹
2021Y217	新闻学院	COP15 期间云南主流媒体生态文明建设报道内容探究	人文社科	一般项目	刘彦明	硕士	黎藜
2021Y218	新闻学院	抖音短视频对城市“地方感”的建构——以昆明为例	人文社科	一般项目	赵婧	硕士	林进桃
2021Y219	新闻学院	从“生态忧思”到“可爱中国”：云南亚洲象北迁案例舆论引导的启示	人文社科	一般项目	柴雨馨	硕士	左小麟
2021Y220	新闻学院	“逛老街”：昆明城市空间的媒介化与文化意涵研究	人文社科	一般项目	张喆一	硕士	杨星星
2021Y221	新闻学院	基于扎根理论的青年群体红色文化认同影响因素与提升路径研究	人文社科	一般项目	隆青泉	硕士	单晓红

2021Y222	艺术与设计学院	《五代至两宋道释画的继承与发展》	人文社科	一般项目	缪凯	硕士	于兰
2021Y223	艺术与设计学院	疫情境况下演出场景的经营现状与未来拓展研究	人文社科	一般项目	李臻	硕士	陈春轩
2021Y224	艺术与设计学院	罗斯金纯真之眼的绘画教育思想对我国高中绘画教学的启发	人文社科	一般项目	丁佳妮	硕士	龙晓滢
2021Y225	艺术与设计学院	古斯塔夫·多雷《乌鸦》插画的图像叙事学研究	人文社科	一般项目	冯天骄	硕士	龙晓滢
2021Y226	艺术与设计学院	傅抱石国画作品的爱国性与民族性对当下艺术创作的启示	人文社科	一般项目	黄霜雪	硕士	韩社林
2021Y227	艺术与设计学院	音乐叙事学中的叙事功能研究	人文社科	一般项目	廖望	硕士	陈春轩
2021Y228	艺术与设计学院	大理古陶釉与唐三彩的比较研究	人文社科	一般项目	潘良运	硕士	王新

2021Y229	艺术与设计学院	乡村振兴背景下侗族风雨桥建筑特征与营造技艺的发展与保护研究	人文社科	一般项目	陶鸿	硕士	杨晓翔
2021Y230	艺术与设计学院	滇剧年表简编	人文社科	一般项目	王静	硕士	牛东梅
2021Y231	艺术与设计学院	云南傣族竹编的现代传承与产品创新研究	人文社科	一般项目	魏微	硕士	于兰
2021Y232	艺术与设计学院	人口老龄化现状下新媒体艺术的实践研究	人文社科	一般项目	文瑾萱	硕士	管郁达
2021Y233	艺术与设计学院	文化认同视野下阿昌族和景颇族艺术研究	人文社科	一般项目	张丞寅	硕士	龙晓滢
2021Y234	艺术与设计学院	云南玉溪地区斑铜工艺传承及产销策略研究	人文社科	一般项目	张林杰	硕士	王新
2021Y235	政府管理学院	数字强边背景下云南省边疆传统战略资源数字化研究——基于数字孪生视角	人文社科	一般项目	杨迪	硕士	邓崧

2021Y236	政府管理学院	边疆民族地区基础教育在国家认同培育中的作用研究	人文社科	一般项目	李敏	硕士	方盛举
2021Y237	政府管理学院	“双减”政策网络教育舆情的话语分析——以人民网“领导留言板”为例	人文社科	一般项目	魏莉丽	硕士	侯江红
2021Y238	政府管理学院	乡村振兴视域下都匀市少数民族特色村寨建设的困境及路径研究	人文社科	一般项目	文齐花	硕士	刘新林
2021Y239	政府管理学院	《新时代云南省退役军人安置方式创新研究》	人文社科	一般项目	尹蕾	硕士	谢和均
2021Y240	政府管理学院	乡村振兴战略中白族照壁的家风传承研究	人文社科	一般项目	杨欢	硕士	蒋颖荣
2021Y241	政府管理学院	论《九章集》中的辩证法	人文社科	一般项目	任才怡	硕士	刘玉鹏
2021Y242	政府管理学院	周汝登伦理思想研究	人文社科	一般项目	张睿	硕士	谢青松

2021Y243	政府管理学院	民族互嵌式社区治理研究——以兰坪县易地扶贫安置Y社区为例	人文社科	一般项目	黎倩雯	硕士	李朝辉
2021Y244	政府管理学院	“乡政”与“村治”的工作衔接机制研究—以云南省N镇人居环境整治为例	人文社科	一般项目	秦秋玲	硕士	夏文贵
2021Y245	政府管理学院	情感型治理视域下多民族社区的互嵌研究：对云南省靖安新区移民搬迁定居点的分析	人文社科	一般项目	朱丽蓉	硕士	朱军
2021Y246	政府管理学院	乡村振兴战略下云南省蒲缥镇易地扶贫搬迁后续扶持政策研究	人文社科	一般项目	杨抒悦	硕士	卢瑾
2021Y247	政府管理学院	少数民族易地扶贫搬迁后的社会融入研究——以云南省南华县彝族为例	人文社科	一般项目	杨菊美	硕士	张会龙
2021Y248	职业与继续教育学院	创新创业教育与高职市场营销专业教学融合探究	人文社科	一般项目	邱萍	硕士	施芳
2021Y249	职业与继续教育学院	利益相关者视角下残疾人职业教育发展动力影响因素探析—以昆明市五华区残联为例	人文社科	一般项目	连会斌	硕士	聂琴

2021Y250	职业与继续教育学院	现代学徒制下中职旅游管理专业校企合作发展困境及对策研究	人文社科	一般项目	赵燕芹	硕士	周芳
2021Y251	职业与继续教育学院	中小学生“减负”政策背景下的教师专业能力提升	人文社科	一般项目	黄玉平	硕士	窦刚
2021Y252	职业与继续教育学院	分布式认知理论视角下 SNS 平台师生交互行为及交互效果探究	人文社科	一般项目	王益凡	硕士	刘俊玮
2021Y253	职业与继续教育学院	数字鸿沟视角下城乡数字化生活差异性特征研究	人文社科	一般项目	饶芳园	硕士	窦刚
2021Y254	信息学院	适用于 5G 毫米波的高增益、低剖面 ISGW 超表面透镜天线的研究和设计	理工科	一般项目	林思鸿	硕士	申东娅
2021Y255	信息学院	新型可吸收超声易显影补片固定锚钉的应用与制备关键技术研究	理工科	一般项目	王强	硕士	吴俊
2021Y256	信息学院	基于小波集成注意网络的糖尿病视网膜病变分级	理工科	一般项目	董晓芳	硕士	李海燕

2021Y257	信息学院	基于显著性细节约束的逐步恢复网络的红外与可见光图像融合	理工科	一般项目	李娅	硕士	官铮
2021Y258	信息学院	云南古壁画中缺陷自动标定关键技术研究	理工科	一般项目	邓小超	硕士	余映
2021Y259	信息学院	基于分类和实例分割的烟叶种植智能监测模型	理工科	一般项目	罗薇	硕士	梁虹
2021Y260	信息学院	基于归一化流的低质图像统一增强框架研究	理工科	一般项目	李修文	硕士	陶大鹏
2021Y261	信息学院	一种高效的支持随机访问的质量分数压缩器	理工科	一般项目	陈瀚森	硕士	陈建华
2021Y262	信息学院	基于腹壁疝疝修补手术的补片钉合固定器研究	理工科	一般项目	张兆民	硕士	吴俊
2021Y263	信息学院	基于循环神经网络的多模态医学图像融合	理工科	一般项目	张谷铖	硕士	聂仁灿

2021Y264	信息学院	基于物联网的区块链共识机制性能研究	理工科	一般项目	李路迟	硕士	何乐生
2021Y265	信息学院	α噪声下基于遗传算法和随机共振的频谱感知性能研究	理工科	一般项目	李如雪	硕士	鲁进
2021Y266	信息学院	基于 ISGW 的毫米波圆极化电磁超表面天线阵列的设计与实现	理工科	一般项目	李涛	硕士	申东娅
2021Y267	信息学院	基于图像相似度探究预训练模型进行新冠肺炎 CT 影像分类的合理性	理工科	一般项目	赵常	硕士	王顺芳
2021Y268	信息学院	异构云计算系统中多资源的公平-高效率分配的创新研究	理工科	一般项目	张凤月	硕士	张学杰
2021Y269	信息学院	基于 Transformer 的太阳射电爆发分类研究	理工科	一般项目	陈键	硕士	袁国武
2021Y270	信息学院	基于机器学习算法的卒中相关性肺炎预测模型研究	理工科	一般项目	马玉龙	硕士	孔兵

2021Y271	信息学院	实时视频目标检测模型及应用	理工科	一般项目	刘斌	硕士	段亮
2021Y272	信息学院	基于生成对抗网络的异源图像配准方法研究	理工科	一般项目	罗月英	硕士	贺康建
2021Y273	信息学院	多聚焦太阳斑点图高分辨率重建算法研究	理工科	一般项目	金亚辉	硕士	蒋慕容
2021Y274	信息学院	基于多任务学习的石油测井数据处理研究	理工科	一般项目	罗鸿鑫	硕士	丁海燕
2021Y275	信息学院	基于深度强化斯坦纳树搜索的 Mashup 发现研究	理工科	一般项目	陈海龙	硕士	武浩
2021Y276	信息学院	面向 CTR 预测的自门控因子机研究	理工科	一般项目	李忠雪	硕士	武浩
2021Y277	信息学院	基于物质点法的多相耦合气泡模拟及渲染研究	理工科	一般项目	熊罡宇	硕士	徐丹

2021Y278	信息学院	连接局部与全局关系的信息扩散预测	理工科	一般项目	徐劭凯	硕士	周丽华
2021Y279	信息学院	非冗余区域空间并置模式挖掘算法及应用研究	理工科	一般项目	蒋希文	硕士	王丽珍
2021Y280	信息学院	基于时空特征的甲型流感病毒抗原性预测	理工科	一般项目	李川	硕士	李维华
2021Y281	信息学院	基于动态矩估计的对抗攻击算法研究	理工科	一般项目	张结宝	硕士	钱文华
2021Y282	信息学院	基于元学习的预训练语言模型知识蒸馏方法研究	理工科	一般项目	马新革	硕士	王津
2021Y283	软件学院	可设计事件间隔时间的智能车辆事件触发横向动力学控制研究	理工科	一般项目	刘智	硕士	储星
2021Y284	建筑与规划学院	基于有机朗肯循环的平板-抛物面槽式太阳能集热系统性能研究	理工科	一般项目	姚东	硕士	葛众

2021Y285	建筑与规划学院	一种适用于箱型截面杆单层网壳新型装配式连接系统研究	理工科	一般项目	吴潮	硕士	翁振江
2021Y286	建筑与规划学院	环氧天然岩沥青复合改性钢桥面浇筑式混凝土性能评价研究	理工科	一般项目	谢汶秀	硕士	张林艳
2021Y287	建筑与规划学院	甘肃北山核废料处置库围岩气体突破性能研究	理工科	一般项目	尹航	硕士	张翔 雷真
2021Y288	建筑与规划学院	酸性干湿循环对石灰岩围观结构及物理力学性质的影响	理工科	一般项目	周文强	硕士	张翔
2021Y289	建筑与规划学院	多灾山地区域城乡韧性时空演变及动态模拟研究	理工科	一般项目	李知远	硕士	王培茗
2021Y290	生命科学学院	基于核基因片段的条蕨属分子系统学研究	理工科	一般项目	李预昕	硕士	和兆荣
2021Y291	生命科学学院	云南轿子山不同海拔梯度土壤微生物群落构建机制	理工科	一般项目	张蕊	硕士	申仕康

2021Y292	生命科学学院	云南瘤牛和普通牛梨形虫病抗性差异的分子调控机制初探	理工科	一般项目	陈钰明	硕士	陈善元
2021Y293	生命科学学院	热效应调控小鼠肠道菌群失调的分子机制	理工科	一般项目	邓艳玲	硕士	王兴红
2021Y294	生命科学学院	垫状植物可培养根际真菌和根内生真菌多样性及其适冷特性研究	理工科	一般项目	方琳琳	硕士	余泽芬
2021Y295	生命科学学院	重金属污染农田菌根际（Mycorrhizosphere）功能微生物的重塑及功能	理工科	一般项目	胡娜	硕士	李涛
2021Y296	生命科学学院	云南草莓主产区主要真菌病害病原菌的物种多样性研究	理工科	一般项目	严聪文	硕士	崔晓龙
2021Y297	生命科学学院	基于表观遗传学研究全反式维甲酸治疗急性早幼粒细胞白血病的机制	理工科	一般项目	韩文瑞	硕士	颜芳
2021Y298	生命科学学院	miR-941 调控人类细胞有丝分裂的研究及其靶基因 IQCG 的参与作用	理工科	一般项目	何霞	硕士	余海菁

2021Y299	生命科学学院	抗诺如病毒药物的筛选	理工科	一般项目	胡嘉明	硕士	路群
2021Y300	生命科学学院	GPX8 参与恶性胶质瘤细胞发展的生物学研究	理工科	一般项目	蒋佳依	硕士	林洁
2021Y301	生命科学学院	调控甲基化转移酶 1 的MicroRNA 在乳腺癌发生的机制研究	理工科	一般项目	马菱远	硕士	崔清华
2021Y302	生命科学学院	鸟嘌呤核苷酸交换因子 GEF-H1 在桥粒组装和皮肤屏障调节中的作用机制研究	理工科	一般项目	王琰	硕士	宁文秀
2021Y303	生命科学学院	C9orf72,SMCR8 影响多层膜细胞器的机制	理工科	一般项目	许世栋	硕士	杨玫
2021Y304	生命科学学院	过量食盐摄入对果蝇发育的调控机制研究	理工科	一般项目	杨会	硕士	陈严
2021Y305	生命科学学院	肝脏脂滴增大的蛋白、脂质组学研究	理工科	一般项目	陈明娇	硕士	梁斌

2021Y306	生命科学学院	微丝骨架调控纤毛膜蛋白 Ar113b 转运机制	理工科	一般项目	崔文娟	硕士	付文祥
2021Y307	生命科学学院	自噬基因 epg-5/epg5 在内吞运输途径中的作用	理工科	一般项目	董华川	硕士	路群
2021Y308	生命科学学院	翻译起始因子 eIF5A 的 hypusine 修饰协同 connexin43 调控肺癌发生的研究	理工科	一般项目	严祥	硕士	罗开珺
2021Y309	生命科学学院	RVS167 蛋白调控捕食线虫真菌少孢节丛孢的捕器形成、内吞作用及致病性	理工科	一般项目	崔培杰	硕士	张克勤
2021Y310	生命科学学院	寻找调控 C9ORF72-SMCR8 复合物蛋白稳定性的因子	理工科	一般项目	邓艳	硕士	杨玫
2021Y311	生命科学学院	RAPTOR 结合蛋白的调控机制研究	理工科	一般项目	姜润震	硕士	付文祥
2021Y312	生命科学学院	高温记忆效应蛋白 HTT5 调控植物免疫的分子机制	理工科	一般项目	李泰	硕士	刘军钟

2021Y313	生命科学学院	云南矿区废弃地旱柳-紫花苜蓿复垦过程中微生物的利他效应（facilitation effect）及机制	理工科	一般项目	秦志阳	硕士	李涛
2021Y314	生命科学学院	c-myc 调控代谢关键酶 PPAT 促进脑胶质瘤发生的分子机制研究	理工科	一般项目	陶颖	硕士	余敏
2021Y315	生命科学学院	丫蕊花叶片转录组及甾体皂苷生物合成途径 P450 酶基因分析及验证	理工科	一般项目	王婧伊	硕士	文孟良
2021Y316	生命科学学院	vibralactone 什么合成途径中 P450 单加氧酶 vibol 的体内功能研究	理工科	一般项目	谢娟	硕士	赵沛基
2021Y317	生命科学学院	基于 OSMAC 策略的一株印楝内生炭角菌的次级代谢产物研究	理工科	一般项目	杨晴榕	硕士	吴少华
2021Y318	数学与统计学院	手眼结合，智能科技入万家	理工科	一般项目	陈瑜阳	硕士	赵越
2021Y319	数学与统计学院	时滞模糊动力系统的解的复杂性研究	理工科	一般项目	郑欣	硕士	王超

2021Y320	数学与统计学院	抽象概周期动力方程的二分性研究	理工科	一般项目	李怡婷	硕士	王超
2021Y321	数学与统计学院	分数阶模糊离散系统的定性研究	理工科	一般项目	谢维玉	硕士	王超
2021Y322	数学与统计学院	模糊分数阶系统 Cauchy 问题的研究	理工科	一般项目	谭英	硕士	王超
2021Y323	数学与统计学院	一类变阶分数阶微分方程初值问题解的存在唯一性	理工科	一般项目	江昆	硕士	周见文
2021Y324	数学与统计学院	联邦学习中的安全性与隐私保护研究	理工科	一般项目	刘航麟	硕士	陆正福
2021Y325	数学与统计学院	带固定成本的两台同型机器上的最小总费用排序问题	理工科	一般项目	丁冉	硕士	李伟东
2021Y326	数学与统计学院	响应变量带有缺失数据的高维部分函数型线性回归模型的统计推断	理工科	一般项目	宋霄汉	硕士	唐年胜

2021Y327	数学与统计学院	非随机缺失机制下基于部分线性模型的高维数据变量筛选	理工科	一般项目	张朝瑞	硕士	唐年胜
2021Y328	数学与统计学院	缺失数据下广义线性混合效应模型的稳健统计推断	理工科	一般项目	张治国	硕士	唐年胜
2021Y329	数学与统计学院	时间序列季节调整中的移动假日效应模型的改进方法研究	理工科	一般项目	华文杰	硕士	潘东东
2021Y330	物理与天文学院	脉冲星风云多波段辐射特性研究	理工科	一般项目	武珂瑶	硕士	方军
2021Y331	物理与天文学院	基于半导体可饱和吸收镜被动锁模皮秒超短脉冲激光器的研究	理工科	一般项目	魏飞飞	硕士	吕彦飞 刘会龙
2021Y332	物理与天文学院	周围环境分子对宽带隙有机半导体的退化过程的影响	理工科	一般项目	王有妹	硕士	赵勇彪
2021Y333	物理与天文学院	镝镨共掺氟化钡晶体光谱研究	理工科	一般项目	赵建斌	硕士	郑丽和

2021Y334	物理与天文学院	基于马尔科夫链蒙特卡洛算法的暗能量模型观测限制研究	理工科	一般项目	强龄萱	硕士	王俊
2021Y335	物理与天文学院	活动星系核光变特性研究	理工科	一般项目	张浩洋	硕士	戴本忠
2021Y336	材料与能源学院	基于苯系物添加剂材料空间效应的高效、稳定钙钛矿太阳能电池研究	理工科	一般项目	庄荣山	硕士	华雍
2021Y337	材料与能源学院	咖啡壳水热生物炭负载纳米铁氧化物催化过硫酸盐氧化抗生素的降解	理工科	一般项目	刘光蓉	硕士	黄强
2021Y338	材料与能源学院	铂铱合金热挤压工艺优化及微观组织演变研究	理工科	一般项目	张心培	硕士	何俊杰
2021Y339	材料与能源学院	钙插层水合氧化钒作为高负载量水系锌离子电池阴极的性能研究	理工科	一般项目	任杰	硕士	王毓德
2021Y340	材料与能源学院	Sn-MOF 基 SnO2 气敏材料的制备及其双金属改性研究	理工科	一般项目	邓宗明	硕士	张裕敏

2021Y341	材料与能源学院	基于碲磷酸盐体系镝离子发光特性研究及光谱调控	理工科	一般项目	宋睿童	硕士	朱静
2021Y342	材料与能源学院	生物模板砷自掺杂二氧化钛对 As(III)的协同去除研究	理工科	一般项目	王可心	硕士	王家强
2021Y343	材料与能源学院	交联凝胶电解质应用于锌-空气电池	理工科	一般项目	张金辉	硕士	张龙舟
2021Y344	材料与能源学院	基于多孔钴酸盐材料催化剂的直接葡萄糖燃料电池研究	理工科	一般项目	汪振龙	硕士	肖雪春
2021Y345	材料与能源学院	石墨烯基 MnGe 量子点的结构和性能研究	理工科	一般项目	叶书鸣	硕士	王莞
2021Y346	材料与能源学院	Co-MOFs 衍生钴镍基金属化合物柔性电极的制备及电化学性能研究	理工科	一般项目	李德全	硕士	柳清菊
2021Y347	材料与能源学院	Cu 单原子 TiO2 的制备及光催化分解水性能研究	理工科	一般项目	沈丛丛	硕士	柳清菊

2021Y348	材料与能源学院	功能性基团调控醚键类共价有机骨架在锂金属电池中的应用	理工科	一般项目	安琪	硕士	郭洪
2021Y349	地球科学学院	通过西北太平洋反气旋组合模态预测华南地区降水	理工科	一般项目	徐怡然	硕士	杨若文
2021Y350	地球科学学院	气候模型预报对澜沧江流域季节水文可预报性的影响研究	理工科	一般项目	张诗晓	硕士	朗杨
2021Y351	地球科学学院	基于 MCE－CA－Markov 模型的星云湖流域土地利用变化模拟和预测	理工科	一般项目	彭钰博	硕士	陈俊旭
2021Y352	地球科学学院	基于多因素耦合建模的城市洪涝韧性评价	理工科	一般项目	徐通	硕士	解智强
2021Y353	地球科学学院	碳中和背景下的中小城镇屋顶高精度光伏能源空间格局分析	理工科	一般项目	张素金	硕士	赵飞
2021Y354	地球科学学院	CMIP6 全球气候耦合模式对低纬高原地区平流层臭氧长期变化的评估	理工科	一般项目	祁宁	硕士	李祥 杨若文

2021Y355	地球科学学院	行星际激波附近的湍流观测研究	理工科	一般项目	王惠琳	硕士	王铁砚 刘瑾
2021Y356	地球科学学院	云南富源晚二叠世鳞木属解剖构造研究	理工科	一般项目	张荣豪	硕士	何小元
2021Y357	地球科学学院	中国西南晚二叠世 Germaropteris martinsii 古大气二氧化碳浓度指示意义	理工科	一般项目	盛紫慧	硕士	冯卓
2021Y358	地球科学学院	四川盆地五峰-龙马溪组黑色页岩有机质差异富集及主控因素	理工科	一般项目	焦梦妍	硕士	袁玉洁
2021Y359	地球科学学院	普朗斑岩型铜矿区矿化蚀变特征矿物填图及找矿潜力分析	理工科	一般项目	张新乐	硕士	赵志芳
2021Y360	国际河流与生态安全研究院	元江干热河谷侵蚀-产沙过程对土壤微塑料的迁移影响机制	理工科	一般项目	张云	硕士	钟荣华
2021Y361	国际河流与生态安全研究院	基于云南侵蚀土壤的微生物群落融合的效应与机理研究	理工科	一般项目	陈丹虹	硕士	车荣晓

2021Y362	国际河流与生态安全研究院	侵蚀对坡耕地土壤氮组分及氮转化过程的影响研究	理工科	一般项目	丁爽	硕士	段兴武
2021Y363	国际河流与生态安全研究院	基于延时摄影和多源遥感技术的明永冰川运动机理研究	理工科	一般项目	秦彩霞	硕士	刘时银
2021Y364	国际河流与生态安全研究院	多源土壤水分数据融合和降尺度方法研究	理工科	一般项目	明文婷	硕士	李运刚
2021Y365	国际河流与生态安全研究院	泥石流输砂影响下的大盈江河床演变研究	理工科	一般项目	杨成	硕士	黄江成
2021Y366	国际河流与生态安全研究院	雅鲁藏布江-布拉马普特拉河流域 陆地水储量时空变化特征研究	理工科	一般项目	蒋薇	硕士	季漩
2021Y367	国际河流与生态安全研究院	印度跨境水霸权的实施路径分析	理工科	一般项目	孙啸拓	硕士	冯彦
2021Y368	国际河流与生态安全研究院	云南省人象冲突区农村居民点空间布局优化研究	理工科	一般项目	许贝贝	硕士	樊辉

2021Y369	国际河流与生态安全研究院	基于空间优化选址算法的横断山南段保护分区的研究	理工科	一般项目	林婧文	硕士	杨飞龄
2021Y370	国际河流与生态安全研究院	中国濒危淡水鱼类分布特征及保护策略研究	理工科	一般项目	陈晋南	硕士	丁城志
2021Y371	国际河流与生态安全研究院	城市化对植物-植食性昆虫相互作用的影响	理工科	一般项目	王英杰	硕士	段兴武 张霜
2021Y372	国际河流与生态安全研究院	中国南北界限亚高山针叶林生长气候响应及其预估	理工科	一般项目	冉依林	硕士	陈峰
2021Y373	国际河流与生态安全研究院	十年来云南大规模桉树引种区林下植物多样性变化分析	理工科	一般项目	唐媛媛	硕士	赵筱青
2021Y374	国际河流与生态安全研究院	铁碳微电解人工湿地净化农田尾水及其作用机制研究	理工科	一般项目	江明	硕士	常军军
2021Y375	国际河流与生态安全研究院	水交换速度对沉水植物锚定力及分布下限影响研究	理工科	一般项目	解明丽	硕士	潘瑛

2021Y376	国际河流与生态安全研究院	纳米电催化材料制备及电化学还原水中的硝酸盐	理工科	一般项目	李雪	硕士	胡广志
2021Y377	国际河流与生态安全研究院	基于 Karsys 方法的白邑断陷盆地水文地质模拟	理工科	一般项目	刘晴	硕士	陈俊旭
2021Y378	化学科学与工程学院	银杏诱导其内生菌 *Paraphaeosphaeria sporulosa* 产生新颖倍半萜类化合物及其生物活性的研究	理工科	一般项目	苏爽	硕士	杨亚滨
2021Y379	化学科学与工程学院	西双版纳沟谷雨林来源的两株链霉菌活性化合物的分离	理工科	一般项目	庄璐	硕士	赵立兴
2021Y380	化学科学与工程学院	毛细管电泳筛选α-葡萄糖苷酶抑制剂的研究	理工科	一般项目	黎升倩	硕士	李菲
2021Y381	化学科学与工程学院	靶向炎症小体的天然丁烯内酯类化合物的发现及作用机制研究	理工科	一般项目	赵莹欣	硕士	张兴杰
2021Y382	化学科学与工程学院	亲氧双金属有机框架精确设计及不对称催化氧化	理工科	一般项目	尚嵘	硕士	方文浩

2021Y383	化学科学与工程学院	尿素调控阿氏芽孢杆菌合成高效稳定的生物铁镍电极用于析氧反应催化	理工科	一般项目	李光	硕士	屈庆
2021Y384	化学科学与工程学院	基于 Pt@Ti3C2 MXenes 纳米复合电极构建一种电化学传感器实现对 HIV 病毒的超灵敏检测	理工科	一般项目	马恩阳	硕士	屈庆
2021Y385	化学科学与工程学院	金纳米的光热效应对镓配合物抗癌机制研究	理工科	一般项目	周思翰	硕士	谢明进
2021Y386	化学科学与工程学院	几种功能性离子液体萃取 Pd 的性能研究	理工科	一般项目	邓慧	硕士	黄章杰
2021Y387	化学科学与工程学院	基于 COF 复合材料的光电化学传感器在卡那霉素检测中的应用	理工科	一般项目	张瑞盈	硕士	曹秋娥
2021Y388	化学科学与工程学院	氨基酸修饰的铜基金属有机框架材料的过氧化物模拟酶性质研究	理工科	一般项目	王保茹	硕士	郑立炎
2021Y389	化学科学与工程学院	基于取代咔唑和吡啶的液晶 TADF 材料	理工科	一般项目	杜旭洋	硕士	程晓红

2021Y390	化学科学与工程学院	改性磁性纳米微粒处理污水中重金属的研究	理工科	一般项目	时欣	硕士	汤立红
2021Y391	化学科学与工程学院	α-In_2Se_3负载双原子电催化还原二氧化碳的理论研究	理工科	一般项目	杨云	硕士	刘世熙
2021Y392	化学科学与工程学院	基于陷阱 Mn、Mo 双掺杂 KOH 活化生物炭对低浓度六价铬的吸附性能研究	理工科	一般项目	王显扬	硕士	袁申富
2021Y393	化学科学与工程学院	基于一株臭参内生真菌（Penicillium ochrochloron）的次生代谢产物及其生物活性研究	理工科	一般项目	岑荣焕	硕士	杨雪琼
2021Y394	化学科学与工程学院	氧-磷原位阻燃聚氨酯型离子塑晶全固态电解质的制备与表征	理工科	一般项目	谢佳颖	硕士	王继亮
2021Y395	化学科学与工程学院	Cu_2O/MoS_2模拟蛋白酶水解蛋白及抗癌活性研究	理工科	一般项目	梁晶晶	硕士	王家强
2021Y396	化学科学与工程学院	多取代 2-吡啶酮化合物的高效合成及其活性研究	理工科	一般项目	王思予	硕士	张从海

2021Y397	软件学院	基于迁移学习对离院帕金森病患的活动识别和病情监测评估的研究	理工科	一般项目	陶柳	硕士	杨珀
2021Y398	软件学院	云协作移动边缘计算中多异构服务器的计算卸载策略优化研究	理工科	一般项目	许娅男	硕士	何臻力
2021Y399	软件学院	以太坊生态系统研究	理工科	一般项目	黄河祥	硕士	张璇
2021Y400	软件学院	基于无人机的时敏多任务边缘计算卸载策略	理工科	一般项目	罗佳	硕士	赵明雄
2021Y401	软件学院	基于多方向索贝尔梯度算子的图像融合	理工科	一般项目	曹廷	硕士	王普明
2021Y402	软件学院	多纳什均衡博弈中社会公约的涌现和演化机制研究	理工科	一般项目	刘绪成	硕士	刘金卓
2021Y403	软件学院	工业遗产软件中海量行为过程片段自动化遗传深度融合	理工科	一般项目	何亚辉	硕士	朱锐

2021Y404	软件学院	边缘设备上的增量学习	理工科	一般项目	王庆	硕士	刘迪
2021Y405	软件学院	基于深度学习的红外图像彩色化方法研究	理工科	一般项目	廖航英	硕士	江倩
2021Y406	软件学院	基于自适应感受野 transformer 的遥感图像融合	理工科	一般项目	章平凡	硕士	金鑫
2021Y407	软件学院	基于蛋白质多模态数据融合的相互作用位点预测研究	理工科	一般项目	徐玉龙	硕士	康雁
2021Y408	软件学院	基于 ON-LSTM 的业务过程模型深度自动生成	理工科	一般项目	张存明	硕士	朱锐
2021Y409	生态与环境学院	乙醇脱氢酶调控印度梨形孢与兰科植物共生的机制研究	理工科	一般项目	朱薪蒙	硕士	韩佳嘉
2021Y410	生态与环境学院	亚洲禾叶蕨亚科的分子系统发育与生物地理学研究	理工科	一般项目	杨建军	硕士	周新茂

2021Y411	生态与环境学院	不同修复方式对云南昭通退化草地生态系统 CO2 通量的影响	理工科	一般项目	杨仕明	硕士	吴建平
2021Y412	生态与环境学院	滇中高原元江栲林下植物与大型真菌多样性关系的研究	理工科	一般项目	张秋雨	硕士	沈泽昊
2021Y413	生态与环境学院	滇西北地区凋落物输入调控对土壤呼吸的影响及其微生物学机制	理工科	一般项目	门修贤	硕士	程晓莉
2021Y414	生态与环境学院	基于三因子实验推测海林芋果蝇卵多型转换的环境预示信号	理工科	一般项目	肖伶	硕士	高建军
2021Y415	生态与环境学院	云南大学呈贡校区蝶类多样性及动态分析	理工科	一般项目	方盛权	硕士	胡劭骥
2021Y416	生态与环境学院	盗蜜对姜花属植物传粉行为和繁殖成功的影响	理工科	一般项目	文祥跃	硕士	李庆军 张志强
2021Y417	生态与环境学院	多组学联合解析象牙参属植物越冬的遗传基础	理工科	一般项目	林雅婷	硕士	赵建立

2021Y418	生态与环境学院	昭通草地退化对土壤微生物多样性和功能的影响	理工科	一般项目	刘开放	硕士	车荣晓
2021Y419	医学院	几种药用植物内生菌分离及其生物活性研究	理工科	一般项目	孙慧冰	硕士	尹敏
2021Y420	资源植物研究院	利用 NILs 群体对水稻苗期耐冷性的 QTL 分析	理工科	一般项目	袁圆	硕士	张石来
2021Y421	资源植物研究院	基于无人机影像的大豆地上生物量反演	理工科	一般项目	张清伟	硕士	王贤智
2021Y422	资源植物研究院	多年生稻 PR23 直链淀粉含量遗传定向改良	理工科	一般项目	李臻园	硕士	黄立钰
2021Y423	资源植物研究院	水稻苯达松敏感恢复系的制备	理工科	一般项目	石淑丹	硕士	张毅
2021Y424	资源植物研究院	利用合成生物学创制多年生黄金大米	理工科	一般项目	李贤	硕士	邵林

2021Y425	资源植物研究院	水稻雄性不育突变体 ms1（t）的遗传分析及其基因定位	理工科	一般项目	杨宽	硕士	张毅
2021Y426	资源植物研究院	云南地方籼稻抗稻瘟病鉴定及利用基因编辑技术改良水稻稻瘟病抗性	理工科	一般项目	薛珊	硕士	程在全
2021Y427	资源植物研究院	草地贪夜蛾适应冬季干热河谷变温的机制初探	理工科	一般项目	侯佳蓝	硕士	李永萍
2021Y428	资源植物研究院	草莓属 MYB 基因家族的进化及果色调控基因表达分析	理工科	一般项目	李佳敏	硕士	乔琴
2021Y429	资源植物研究院	咖啡脱胶菌株的筛选、鉴定及其功能特性分析	理工科	一般项目	张树竹	硕士	何飞飞 秦世雯
2021Y430	资源植物研究院	珠芽魔芋遗传转化体系的建立	理工科	一般项目	张利娜	硕士	吴学尉
2021Y431	资源植物研究院	AMF 共生条件下 ZmPHT1;11 基因对玉米磷吸收的影响	理工科	一般项目	卢昱帆	硕士	刘芳

2021Y432	资源植物研究院	野生蒜芥茄 VIGS 基因沉默体系的建立	理工科	一般项目	蔚亚楠	硕士	杜光辉
2021Y433	生命科学学院	GAs 调节拟南芥胚轴发育的分子机制研究	理工科	一般项目	刘月	硕士	陈小兰

研究生院

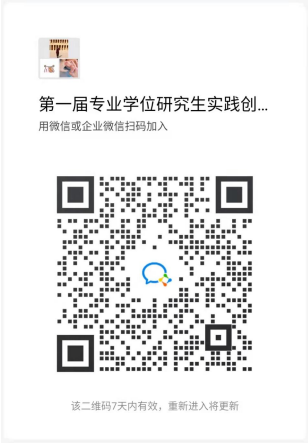
2021 年 11 月 10 日

云南大学第一届专业学位研究生实践创新项目拟立项名单公示

我校第一届专业学位研究生实践创新项目评审现已结束，共收到项目申报书 485 份，其中符合申报条件的有 469 项，重点项目、一般项目均为不限额申报，经各培养单位形式审查，校级评审委员会评议，拟对其中 320 项进行立项。

现对材料与能源学院《Pt-10Ir 超细键合丝连续退火中微组织取向-宏观性能关联性研究及数据库建设》等 320 项拟立项项目名单予以公示，若有异议，请于 2021 年 11 月 12 日 17:00 前以书面形式向研究生院反映，电话：65033585。若无异议，公示期满则自动生效。

公示期满获准立项的项目负责人请自动申请加入微信群：



项目编号	培养单位	项目名称	项目类别	专业学位类别代码及名称	项目负责人	指导教师
2021Z01	材料与能源学院	Pt-10Ir 超细键合丝连续退火中微组织取向-宏观性能关联性研究及数据库建设	重点	0856 材料与化工	朱丽仙	何俊杰
2021Z02	昌新国际艺术学院	云南社会文化塑造视阈下爨体字库的艺术性构建	重点	1351 艺术	聂子皓	吴迪
2021Z03	昌新国际艺术学院	云南民间传统纹饰探索——彝族纹饰在陶瓷高温颜色釉中装饰性实验研究	重点	1351 艺术	陈紫鹏	范敏祺
2021Z04	法学院	云南省彝族聚居区家庭暴力现状与对策研究	重点	0351 法律	侯敬峰	白纶
2021Z05	法学院	生态文明建设背景下洱海旅游区生态环境损害责任适用问题研究	重点	0351 法律	刘文林	李一娴
2021Z06	高等教育研究院	新课程标准下培养高中生语文核心素养的课堂教学实践研究	重点	0451 教育	孔令勇	范哗

2021Z07	高等教育研究院	1+X 制度下学生学习成果评价研究	重点	0451 教育	崔瑾	刘康宁
2021Z08	工商管理与旅游管理学院	乡村振兴背景下村集体企业发展困境和对策研究	重点	1251 工商管理	鲁怀义	赵德森
2021Z09	工商管理与旅游管理学院	基于人工智能+区块链创新技术的溯源产业数字化应用研究—以普洱茶为例	重点	1251 工商管理	涂亚运	陈伟
2021Z10	工商管理与旅游管理学院	碳排放权交易视角下环境规制对发电企业低碳创新影响机制实证研究 ——基于财务宽裕的中介效应	重点	1253 会计	乔智	蒋峻松
2021Z11	工商管理与旅游管理学院	数字普惠金融对易地扶贫搬迁农户的脱贫效应研究	重点	1253 会计	刘志远	武鹏
2021Z12	国际河流与生态安全研究院	多卫星遥感降水产品在澜沧江-湄公河流域下游水文模拟中的应用研究	重点	0857 资源与环境	王鹏翔	罗贤
2021Z13	国际河流与生态安全研究院	手机社交媒体对云南省农户管理外来入侵物种草地贪夜蛾的作用	重点	0857 资源与环境	陈东明	张立芸
2021Z14	国际河流与生态安全研究院	云南省滑坡易发性时空格局评价研究	重点	0857 资源与环境	贾涵姝	武瑞东

2021Z15	国际河流与生态安全研究院	基于 Cesium 的滇池周边湿地信息监测平台构建与应用研究	重点	0857 资源与环境	冯思雯	赵飞
2021Z16	汉语国际教育学院	基于 ARCS 动机模型的线上汉语综合课教学行动研究	重点	0453 汉语国际教育	朱志勇	张高翔
2021Z17	建筑与规划学院	微生物与植物脲酶诱导碳酸钙沉积对比加固云南泥炭质土研究	重点	0859 土木水利	郭再斌	谢建斌
2021Z18	建筑与规划学院	风荷载或其他扰动下截柱顶升过程的稳定性及其他要素研究	重点	0859 土木水利	王洪	秦云
2021Z19	建筑与规划学院	带肋钢管自密实混凝土加固 RC 柱偏压性能研究	重点	0859 土木水利	许兆杰	林帆
2021Z20	建筑与规划学院	突发公共卫生事件视角下的昆明市老旧社区的韧性建设研究	重点	0859 土木水利	马雯钰	徐坚
2021Z21	经济学院	科技创新企业创新发展效率与融资结构关系研究——基于科创板上市企业的实证分析	重点	0251 金融	高林海	待定
2021Z22	经济学院	云南咖啡价值链中的冰点与破解点——以德宏后谷咖啡为例	重点	0254 国际商务	熊显鑫	胡颖

2021Z23	经济学院	混合所有制改革对企业价值的影响研究——以云南白药为例	重点	0256 资产评估	刘千辉	刘志坚
2021Z24	经济学院	整合审计对审计质量的影响研究——基于 A 股主板上市公司的经验数据	重点	0257 审计	蔡权文	柴毅
2021Z25	经济学院	政府审计视角下 PPP 项目在跟踪审计中的应用研究	重点	0257 审计	何志强	待定
2021Z26	经济学院	审计收费价格管制会影响审计师的专业判断吗？	重点	0257 审计	柏惠子	杨孟禹
2021Z27	历史与档案学院	雕塑黄泥和石膏在青铜文物复制中无残留翻模的技术研究	重点	0651 文物与博物馆学	郭志成	陈果
2021Z28	历史与档案学院	聚丙烯酸酯用作古建筑防水材料的性能研究	重点	0651 文物与博物馆学	王楷文	刘强
2021Z29	历史与档案学院	《被遗忘的珍宝——古滇国的黄金、玉器和珠宝》原创展览研究与实践	重点	0651 文物与博物馆学	杨莲	戴宗品
2021Z30	历史与档案学院	胥河溧阳段新石器时代及商周古遗址古墓葬调查研究	重点	0651 文物与博物馆学	史骏	陈果

2021Z31	历史与档案学院	边疆生态治理视野下云南茶马古道生态档案创新性开发研究	重点	1255 图书情报	武思远	周铭
2021Z32	软件学院	基于物联网和深度学习户外智能露营安全帐篷系统设计和实现	重点	0854 电子信息	刘博	杨云
2021Z33	软件学院	面向社会经济调查大数据的不确定性知识图谱构建及应用	重点	0854 电子信息	袁子豪	张璇
2021Z34	软件学院	基于语义知识图谱和注意力机制的医学图像报告生成	重点	0854 电子信息	任安全	张德海
2021Z35	生命科学学院	寡孢节丛孢 milR309 通过细胞外囊泡跨界调控秀丽隐杆线虫的机制	重点	0860 生物与医药	周总	李娟
2021Z36	生命科学学院	OsTCP1 调控水稻分枝的分子机制研究	重点	0860 生物与医药	冯若宣	余迪求
2021Z37	生命科学学院	胰腺癌染色质结构变异及其转录调控机理研究	重点	0860 生物与医药	时德通	彭城
2021Z38	生命科学学院	少孢节丛孢中倍半萜环己烯类化合物三元环氧结构生物合成机制	重点	0860 生物与医药	王东楼	牛雪梅

2021Z39	数学与统计学院	基于投入产出技术的云南数字经济 高质量发展预警监测模型研究	重点	0252 应用统计	兀皓帆	潘东东
2021Z40	体育学院	冬奥会背景下冰雪项目进高校的策略研究——以昆明市高校为例	重点	0452 体育	孙鹏	刘春
2021Z41	体育学院	云南大学水域体育休闲实践创新基地构建研究	重点	0452 体育	曾建森	夏文
2021Z42	新闻学院	构筑“云端城市”——以本地媒体 cop15 报道为例	重点	0552 新闻与传播	王偲	孙信茹
2021Z43	新闻学院	“我和我的云大”影像计划——献礼云南大学百年华诞微纪录片	重点	0552 新闻与传播	郝康	杨星星
2021Z44	信息学院	面向智慧工建的安全检测模型	重点	0854 电子信息	杨明泽	岳昆
2021Z45	信息学院	基于无监督学习的多模态医学图像融合研究	重点	0854 电子信息	朱娅	聂仁灿
2021Z46	信息学院	基于超声与机器学习的智能肌体评估系统	重点	0854 电子信息	谭浩	张榆锋

2021Z47	信息学院	5G 毫米波超宽带、高增益基片间隙波导宽缝滤波天线阵列设计	重点	0854 电子信息	冯亚军	申东娅
2021Z48	信息学院	基于深度学习的烟支外观缺陷检测方法研究	重点	0854 电子信息	刘鸿瑜	袁国武
2021Z49	信息学院	可解释联合注意卷积神经网络的医学图像分割	重点	0854 电子信息	曾鹏	李海燕
2021Z50	信息学院	深度学习方法下的儿童骨龄自动评估系统	重点	0854 电子信息	张鑫	张俊华
2021Z51	艺术与设计学院	云南大理剑川木雕格子门图案的整理与研究	重点	1351 艺术	赵雨琛	寇元勋 高波
2021Z52	艺术与设计学院	武定彝族刺绣纹样在现代平面设计中的创新运用	重点	1351 艺术	普天琪	张星
2021Z53	艺术与设计学院	绘画在服饰中的二次创作实践	重点	1351 艺术	周钰	张志明
2021Z54	艺术与设计学院	基于百年云大的《途说云大 东陆》APP 交互设计研究	重点	1351 艺术	刘彦霞	王兢

2021Z55	艺术与设计学院	《基于五感体验的儿童活动空间设计》——以云大附小为例	重点	1351 艺术	杨嘉磊	余丽婴
2021Z56	艺术与设计学院	《国家非物质文化遗产傣族织绣纹样与书画装裱艺术结合的实践与推广》	重点	1351 艺术	冯嘉欣	屠潇
2021Z57	政府管理学院	根植性视角下云南省特色小镇发展模式研究——基于 5 个省级特色小镇的多案例分析	重点	1252 公共管理	彭煜玮	马桑
2021Z58	政府管理学院	西南边疆城市脆弱性评价、动态演变及其影响因素	重点	1252 公共管理	赵冠祥	谭立力
2021Z59	职业与继续教育学院	中小学综合实践活动教学模式研究与实践——“思迪创课”	重点	0451 教育	张意荣	何鸣皋
2021Z60	职业与继续教育学院	中职院校非遗文化传承与创新路径探究——以省级非遗项目“客家门匾习俗”为例	重点	0451 教育	林敏	周芳
2021Z61	职业与继续教育学院	“双减政策”下小学课后服务共育模式探究	重点	0451 教育	薛白钰	谢志昆
2021Z62	资源植物研究院	“丛枝菌根真菌-根际解钾细菌”新型复合微生物菌剂研发	重点	0951 农业	闫怡秀	刘芳

2021Z63	资源植物研究院	药用野生稻渗入系对稻瘟病优势菌系的抗性分析研究	重点	0951 农业	雷凌云	程在全 秦世雯
2021Y001	材料与能源学院	智慧能源管理平台的推广应用	一般	0856 材料与化工	崔秀秀	王毓德
2021Y002	材料与能源学院	多孔结构钴酸盐对高氯酸铵热分解的催化性能研究及应用	一般	0856 材料与化工	张国飞	肖雪春
2021Y003	材料与能源学院	界面工程及缺陷调控的 Nb 基光催化剂可控构筑及光催化还原 CO_2 研究及应用	一般	0856 材料与化工	蒋静雯	郭洪
2021Y004	材料与能源学院	液相烧结 $Na_3Zr_2Si_2PO_{12}$ 的离子电导率增强及其在固态电池中的应用	一般	0856 材料与化工	王晗	郭洪
2021Y005	昌新国际艺术学院	城市遗产保护视角下的云南大学东陆校区建筑人文精神研究与活化设计	一般	1351 艺术	魏环	吴迪
2021Y006	昌新国际艺术学院	非遗进校园在美术教育中的研究与实践	一般	1351 艺术	孙瑞阳	张东华
2021Y007	昌新国际艺术学院	基于乡村振兴背景下非遗文化传承与发展研究——以广东嵌瓷为例	一般	1351 艺术	黄晓芸	徐园

2021Y008	昌新国际艺术学院	新时代教育发展下云南地区书法实践	一般	1351 艺术	田佳洁	苏金成
2021Y009	昌新国际艺术学院	云南建水紫陶中的“残帖”装饰艺术在景德镇陶瓷上运用的可能性	一般	1351 艺术	王梦婷	范敏祺
2021Y010	昌新国际艺术学院	生物多样性视角下云南花鸟画的传承与创新	一般	1351 艺术	张艺凡	张东华
2021Y011	昌新国际艺术学院	富民县平地村传统建筑的测绘研究与保护发展	一般	1351 艺术	胡小娟	钱毅
2021Y012	法学院	多元纠纷下的诉调对接纠纷解决机制研究—以昆明市西山区人民法院为例	一般	0351 法律	刘英伟	赵忠龙
2021Y013	法学院	快递行业个人信息泄露的成因及对策研究——以昆明呈贡区为例	一般	0351 法律	木继莲	李一娴
2021Y014	法学院	“双减”政策下校外培训机构的困境与出路研究	一般	0351 法律	廖明慧	刘国乾
2021Y015	法学院	城镇房屋短期租赁法律问题研究——以在校大学生校外短期租赁为例	一般	0351 法律	刘明	戴琳

2021Y016	法学院	少数民族习惯与国家法律互动关系研究——基于云南 404 份裁判文书的分析	一般	0351 法律	杜晓艳	王鑫
2021Y017	高等教育研究院	双减政策背景下小学教师工作量实证研究	一般	0451 教育	李芬	董云川
2021Y018	高等教育研究院	民族地区小学校本课程开发的实践研究	一般	0451 教育	雷顺雯	张琪仁
2021Y019	工商管理与旅游管理学院	基于业财融合的医药制造企业风险管控研究	一般	1251 工商管理	陈琳	王健康
2021Y020	工商管理与旅游管理学院	锂电工厂的自动化物流系统设计与应用	一般	1251 工商管理	隋鑫	宋乐
2021Y021	工商管理与旅游管理学院	基于医疗设备物联网管理系统的大型医疗设备使用效益分析	一般	1251 工商管理	臧玉峰	高核
2021Y022	工商管理与旅游管理学院	顾客价值创新视域下昆明融创文旅城旅游体验提升策略研究	一般	1251 工商管理	高硕雁	陈伟
2021Y023	工商管理与旅游管理学院	云南西部大峡谷温泉康养旅游小镇开发模式与策略研究	一般	1251 工商管理	陶晓莹	杨懿

2021Y024	工商管理与旅游管理学院	新时代云南省红色旅游与生态旅游融合发展战略及路径研究--以昆明市为例	一般	1253 会计	王飞宇	光映炯
2021Y025	工商管理与旅游管理学院	边疆地区劳动教育实践基地建设路径及其效益研究	一般	1253 会计	杨健会	孔春丽
2021Y026	工商管理与旅游管理学院	普惠金融促进云南省乡村振兴发展中资源整合的机理与实践研究	一般	1253 会计	罗婧瑜	王健康
2021Y027	工商管理与旅游管理学院	基于游客满意度的云南民族文化主题公园服务质量提升策略研究	一般	1254 旅游管理	左健	待定
2021Y028	工商管理与旅游管理学院	长征国家文化公园建设背景下的滇西北红色旅游拓展研究	一般	1254 旅游管理	高白莲	邓永进
2021Y029	国际河流与生态安全研究院	桉树大规模引种区土壤持水能力对不同林地类型的响应	一般	0857 资源与环境	赵巧巧	赵筱青
2021Y030	国际河流与生态安全研究院	盈江地区基于资源一号 02D 高光谱遥感的土壤地球化学信息反演研究	一般	0857 资源与环境	李子洋	赵志芳
2021Y031	国际河流与生态安全研究院	夜光遥感大数据支持下的乡村振兴时空分异研究	一般	0857 资源与环境	朱思瑾	赵飞

2021Y032	国际河流与生态安全研究院	亚洲象肇事保险补偿标准的农户偏好及其对种植意愿的影响	一般	0857 资源与环境	张杰京	樊辉
2021Y033	国际河流与生态安全研究院	人工神经网络非破坏性测定表层土壤有机碳浓度	一般	0857 资源与环境	张舰	李世玉
2021Y034	国际河流与生态安全研究院	基于洪涝灾害感知的海绵城市用地布局优化时空模拟	一般	0857 资源与环境	杨寿泉	解智强
2021Y035	国际河流与生态安全研究院	云贵高原湖区湖泊富营养化评价模型适宜性选择	一般	0857 资源与环境	晏翠玲	陆颖
2021Y036	国际河流与生态安全研究院	哈尼梯田小流域土地利用变化的多主体模型建模	一般	0857 资源与环境	王晨星	李艳波
2021Y037	国际河流与生态安全研究院	磁性矿物转化对铁铝土区碳源汇的影响及其驱动因素研究	一般	0857 资源与环境	罗鹏飞	李世玉
2021Y038	国际河流与生态安全研究院	云南省道路网络修建对景观格局及土壤侵蚀的影响研究	一般	0857 资源与环境	刘娇	钟荣华
2021Y039	国际河流与生态安全研究院	干热河谷区柑橘种植的供需水差异研究	一般	0857 资源与环境	李青	段兴武

2021Y040	国际河流与生态安全研究院	美国国际开发署的澜沧江-湄公河流域涉水项目援助路径分析	一般	0857 资源与环境	耿彩彩	王文玲
2021Y041	国际河流与生态安全研究院	中缅边境山地道路系统外来植物薇甘菊（*Mikania micrantha*）可入侵性空间特征及时间变化	一般	0857 资源与环境	朱敏	张立芸
2021Y042	国际河流与生态安全研究院	三维实景的瓦片模型优化与高效渲染机制研究	一般	0857 资源与环境	沈克强	赵飞
2021Y043	汉语国际教育学院	疫情期间未返校孟加拉国留学生汉语学习焦虑调查研究	一般	0453 汉语国际教育	曹雅琼	杨晓雯
2021Y044	汉语国际教育学院	意大利散居华裔汉语继承语学习现状调查研究	一般	0453 汉语国际教育	刘莎	杨晓雯
2021Y045	汉语国际教育学院	转危为机：“双一流”背景下汉语国际教育学科高质量发展路径研究	一般	0453 汉语国际教育	梁雅慧	余江英
2021Y046	汉语国际教育学院	基于《国际中文教育中文水平等级标准》对新丝路华文系列教材成语选编研究	一般	0453 汉语国际教育	罗嫚	杨晓雯
2021Y047	汉语国际教育学院	云南大学汉语国际教育专业硕士就业状况调查与分析研究	一般	0453 汉语国际教育	宁梦雅	谭晓健

2021Y048	汉语国际教育学院	云南少数民族群体民族语言保护传承意识及其影响因素调查研究	一般	0453 汉语国际教育	陆艳芹	余江英
2021Y049	汉语国际教育学院	云南大学留学生《中国历史》文化课程适应研究	一般	0453 汉语国际教育	岳佳雨	谭晓健
2021Y050	化学科学与工程学院	固定床云南褐煤水蒸气加氢气化制天然气联产芳烃技术研究	一般	0856 材料与化工	李阔	袁申富
2021Y051	化学科学与工程学院	改性橄榄石负载铁镍催化剂应用于 CO_2 气化重整秸秆半焦的研究	一般	0856 材料与化工	陈鑫	袁申富
2021Y052	化学科学与工程学院	光致异构偶氮苯类化合物的合成及在药物化学方面的应用	一般	0860 生物与医药	张琳	程晓红
2021Y053	化学科学与工程学院	嵌入杂原子的马鞍型纳米石墨烯	一般	0860 生物与医药	马斌	安鹏
2021Y054	化学科学与工程学院	黔产乌头内生真菌次级代谢产物及其对乌头的改性研究	一般	0860 生物与医药	王诚曜	蔡乐
2021Y055	化学科学与工程学院	基于天然产物的新型 HSP90 C 端抑制剂改造	一般	0860 生物与医药	刘彪琦	张芮菡

2021Y056	化学科学与工程学院	新型苯并噻二唑/希夫碱类 CN-荧光探针的合成及性能研究	一般	0860 生物与医药	高云	高红飞
2021Y057	化学科学与工程学院	滇产桑黄的化学成分及其抗炎活性研究	一般	0860 生物与医药	符权	肖伟烈 李晓莉
2021Y058	化学科学与工程学院	二苯甲酮类化合物在抗阿尔茨海默病方面的筛选与研究	一般	0860 生物与医药	张睿朴	纪旭 曹光
2021Y059	建筑与规划学院	BRB 芯材端部与连接件接触点的优化研究	一般	0859 土木水利	李天赞	秦云
2021Y060	建筑与规划学院	硫酸盐环境下改性胶对 CFRP 加固混凝土的耐久性及力学性能研究	一般	0859 土木水利	林俊标	雷真 张翔
2021Y061	建筑与规划学院	温度载荷作用下花岗岩物理力学性质演化及其微观机制研究	一般	0859 土木水利	韦鳗珂	张翔 雷真
2021Y062	建筑与规划学院	新型颗粒阻尼惯容减振器的设计及试验研究	一般	0859 土木水利	郑飞云	王宪杰
2021Y063	建筑与规划学院	桥梁安全监测试验平台可行性研究与实践	一般	0859 土木水利	杨玉军	冉志红

2021Y064	建筑与规划学院	聚羧酸系高性能减水剂与云南复杂地材适应性研究与性能提升技术	一般	0859 土木水利	闫硕	任骏
2021Y065	建筑与规划学院	动态 SPC 技术在环氧沥青路面施工质量控制中的应用研究	一般	0859 土木水利	赵映琴	张林艳
2021Y066	建筑与规划学院	设备振动荷载下模块化钢结构栈桥动力响应分析及振动控制	一般	0859 土木水利	李玉洋	翁振江
2021Y067	建筑与规划学院	通讯基站信号塔倾斜摄影精细化建模的参数影响及优化研究	一般	0859 土木水利	李玲	刘杨
2021Y068	建筑与规划学院	建筑业领导底线思维对下属成员亲组织不道德行为的影响研究	一般	0859 土木水利	谢敏	王培茗
2021Y069	建筑与规划学院	南涧彝族自治县老村落住宅“拆改修”策略研究	一般	0859 土木水利	刘涛	张军
2021Y070	建筑与规划学院	高校建设 EPC 项目进度影响因素研究	一般	0859 土木水利	陈杰	赵平伟
2021Y071	建筑与规划学院	基于 AHP-shapley 值的交通 PPP 项目风险分担	一般	0859 土木水利	陈宥铭	李燕

2021Y072	建筑与规划学院	云南省山区农户耕地利用效率分析及影响机制研究	一般	0859 土木水利	昝俊宏	王丽娟
2021Y073	经济学院	绿色金融背景下云南省生物多样性保护自然融资渠道及机制研究	一般	0251 金融	陈琪琪	李娟
2021Y074	经济学院	数字经济背景下云南省制造业转型对经济高质量发展的影响研究	一般	0251 金融	王文俊	郭树华
2021Y075	经济学院	数字乡村建设与云南咖啡产业转型发展研究	一般	0251 金融	刘倩倩	刘志坚
2021Y076	经济学院	关于华夏银行昆明分行普惠金融信贷业务准入及运作的调查报告	一般	0251 金融	丁晗芮	付庆华
2021Y077	经济学院	云南省绿色金融业务发展存在的问题和对策研究	一般	0251 金融	陆思佳	郭树华
2021Y078	经济学院	“碳中和”对云南省产业结构优化升级的影响	一般	0251 金融	李凯	李贤
2021Y079	经济学院	“双循环”发展格局下云南省咖啡贸易发展的机遇、挑战与对策	一般	0254 国际商务	刘丽	待定

2021Y080	经济学院	后疫情时代云南文旅产业数字化创新发展路径研究	一般	0254 国际商务	何秋玥	姚书杰
2021Y081	经济学院	普洱市“乡村振兴”背景下茶旅产业融合研究	一般	0254 国际商务	张嘉悦	周观琪
2021Y082	经济学院	环滇沿线生态屏障与开发价值的评估分析	一般	0256 资产评估	许子贤	吕昭河
2021Y083	经济学院	基于物联网的智能温室应用与优化研究	一般	0256 资产评估	张宇	吴学尉
2021Y084	经济学院	云南中咖品牌价值评估——基于 DEA 和 AHP 改进下的 interbrand 模型研究	一般	0256 资产评估	毕福芳	刘志坚
2021Y085	经济学院	云南打造全国碳达峰、碳中和示范省的审计助推路径研究	一般	0257 审计	李霁桐	郭强华
2021Y086	经济学院	区块链+新能源行业内部审计平台运营与治理机制研究	一般	0257 审计	肖懿	潘小春
2021Y087	经济学院	保护生物多样性背景下的云南资源环境审计研究	一般	0257 审计	王雅茜	郭强华

2021Y088	经济学院	云南省 K 市自然资源资产离任审计路径优化研究	一般	0257 审计	高涵宇	梁双陆
2021Y089	经济学院	高校基建项目跟踪审计质量研究—以 Y 高校 XX 基建项目为例	一般	0257 审计	高明宇	待定
2021Y090	经济学院	大数据环境下云南省防疫专项资金审计研究	一般	0257 审计	尚思廷	罗美娟
2021Y091	经济学院	领导干部水资源资产离任审计评价体系构建及应用研究	一般	0257 审计	孙杰	董春
2021Y092	经济学院	美丽县城建设背景下云南镇雄县安居工程项目绩效审计研究	一般	0257 审计	徐万金	肖迎
2021Y093	经济学院	“后疫情”时代数字普惠金融支持昆明市中小微企业抒困实践研究	一般	0257 审计	杨茵茵	柴毅
2021Y094	经济学院	基于区块链技术创新云南省绿色金融体系建设研究	一般	0257 审计	殷姝	郭强华
2021Y095	经济学院	森林资源资产审计助力生态文明建设路径研究	一般	0257 审计	袁颖艳	龙小海

2021Y096	经济学院	“减税降费”对民营企业创新的影响研究	一般	0257 审计	邹芷欣	郭强华
2021Y097	经济学院	乡村振兴背景下专项资金审计及跟踪研究-以云南省江城哈尼族彝族为例	一般	0257 审计	邓咪	肖迎
2021Y098	经济学院	乡村振兴下农业产业专项资金审计研究—以云南曲靖蔬菜产业为例	一般	0257 审计	刘鹏翔	肖迎
2021Y099	经济学院	基于碳中和理论的农业碳审计评价体系构建与应用研究	一般	0257 审计	韩雨锦	程士国
2021Y100	经济学院	乡村振兴战略背景下云南大栗树茶企发展对乡镇共同富裕的作用机制研究	一般	0257 审计	余月	崔庆波
2021Y101	历史与档案学院	环球影城对中国博物馆的实践意义研究	一般	0651 文物与博物馆学	陈静滢	何强
2021Y102	历史与档案学院	留存在树叶上的记忆：文物保护视域下云南傣族贝叶经的传承发展研究	一般	0651 文物与博物馆学	何雨萌	李东红
2021Y103	历史与档案学院	怒江傈僳族自治州民族博物馆展览大纲	一般	0651 文物与博物馆学	王一岚	赵美

2021Y104	历史与档案学院	基于观众视角的博物馆佛教展览效果研究--以云南省博物馆为例	一般	0651 文物与博物馆学	吴姗	何强
2021Y105	历史与档案学院	传统拓印技术应用与文物保护的操作指导研究	一般	0651 文物与博物馆学	许汇文	刘强
2021Y106	历史与档案学院	以文创产品助推昆明市内博物馆的发展与调查研究	一般	0651 文物与博物馆学	张琪	陈果
2021Y107	历史与档案学院	以云南省博物馆为例的新社交媒体下博物馆文物活化研究	一般	0651 文物与博物馆学	张永坤	于兰
2021Y108	历史与档案学院	高校博物馆思想政治教育价值及途径研究——以云南大学历史博物馆为例	一般	0651 文物与博物馆学	朱明怡	李怀宇
2021Y109	历史与档案学院	浅析博物馆文化传播的新途径——文创	一般	0651 文物与博物馆学	于艾灵	陈果
2021Y110	历史与档案学院	关于大学生博物馆文创消费取向的研究--以昆明大学生为例	一般	0651 文物与博物馆学	韩梦	陈果
2021Y111	历史与档案学院	《生态博物馆与章朗村乡村振兴研究》	一般	0651 文物与博物馆学	郭晓璐	张海超

2021Y112	历史与档案学院	深度神经网络驱动的书画病害识别模型研究	一般	0651 文物与博物馆学	邓旭帅	张云春
2021Y113	历史与档案学院	云南考古 70 年——老中青三代考古学人访谈录	一般	0651 文物与博物馆学	苏航	李昆声
2021Y114	历史与档案学院	魏晋时期画像砖中的士人群体礼仪文化研究	一般	0651 文物与博物馆学	李文婷	陈果
2021Y115	历史与档案学院	面向戏曲文化传承的滇剧建档保护研究	一般	1255 图书情报	程淑芳	周铭
2021Y116	历史与档案学院	基于 FRBR 模型的裁判文书元数据模型设计与研究	一般	1255 图书情报	黄依涵	赵生辉
2021Y117	历史与档案学院	“互联网+医疗健康”背景下中老年慢病患者健康信息源的选择与利用研究	一般	1255 图书情报	罗林	王晋
2021Y118	历史与档案学院	昆明市城市公共阅读空间建设发展策略研究	一般	1255 图书情报	宋倩宁	朱明
2021Y119	历史与档案学院	中学生合作信息搜寻行为研究	一般	1255 图书情报	万素涵	朱明

2021Y120	历史与档案学院	高校校友档案的开发利用研究	一般	1255 图书情报	王雅婷	李怀宇
2021Y121	历史与档案学院	县级供销合作社历史档案资源建设研究——基于云南省陆良县调查	一般	1255 图书情报	孔懿璇	胡莹
2021Y122	历史与档案学院	全民阅读推广视域下高校读书会研究——以云南大学为例	一般	1255 图书情报	李珊	王水乔
2021Y123	历史与档案学院	文旅融合背景下公共图书馆服务空间重构研究	一般	1255 图书情报	吴紫伊	耿达
2021Y124	历史与档案学院	云南中医药名人档案数字化开发利用研究	一般	1255 图书情报	李芷萱	胡莹
2021Y125	民族学与社会学学院	云南省易地搬迁社区儿童家庭教养困境问题研究	一般	0352 社会工作	何颂一	王化起
2021Y126	民族学与社会学学院	社会工作视角下彝族村落空巢老人的居家养老服务的问题与对策研究	一般	0352 社会工作	王艳玲	高万红
2021Y127	软件学院	基于深度学习的稀贵金属材料结构建模与数据分析	一般	0854 电子信息	杨学昆	康雁

2021Y128	软件学院	基于图神经网络的口服生物利用度分类预测	一般	0854 电子信息	梁家树	张德海
2021Y129	软件学院	面向自动驾驶的交通场景理解技术研究	一般	0854 电子信息	马宇	张德海
2021Y130	软件学院	基于深度学习的高原地区儿童心电图分析与识别研究	一般	0854 电子信息	李小鹏	赵娜
2021Y131	软件学院	基于 PPG 信号面向心血管疾病的可穿戴设备研究	一般	0854 电子信息	余治国	蔡莉
2021Y132	生命科学学院	基于基因组浅层测序的蝎子草属物种界定	一般	0860 生物与医药	李志鹏	王跃华
2021Y133	生命科学学院	犬的 IPSCs 体系的建立	一般	0860 生物与医药	黄雪祯	张亚平
2021Y134	生命科学学院	基于分析少孢节丛孢交配型基因功能开发杀线虫生物防治剂	一般	0860 生物与医药	王达	张颖
2021Y135	生命科学学院	邻位标记技术在乙酰转移酶底物鉴定中的作用	一般	0860 生物与医药	谭玉晴	刘通

2021Y136	生命科学学院	名贵鸡枞菌菌丝体规模化纯培养体系的建立及其营养冻干粉的研制	一般	0860 生物与医药	陈鹏至	李宗菊
2021Y137	生命科学学院	指状德氏霉产生的孢子捕器的机制研究	一般	0860 生物与医药	张令	梁连铭
2021Y138	生命科学学院	防治南方根结线虫菌株的实践研究	一般	0860 生物与医药	吴彩蜜	刘亚君
2021Y139	生命科学学院	OsSAPK2-OsbZIP81 复合体调控水稻陆生适应性的功能研究	一般	0860 生物与医药	陈仕蝶	余迪求
2021Y140	生命科学学院	非编码 RNA 在云南瘤牛对梨形虫病抗性中的作用机理初探	一般	0860 生物与医药	刘榕	陈善元
2021Y141	数学与统计学院	交通道路信号灯智能控制系统优化	一般	0252 应用统计	黄治国	陈丹
2021Y142	数学与统计学院	半监督特征选择方法在医学图像识别领域中的应用	一般	0252 应用统计	马骏	李会琼
2021Y143	数学与统计学院	差分隐私 GAN 合成数据在医疗场景下的应用	一般	0252 应用统计	胡金梅	周建军

2021Y144	数学与统计学院	乡村文化重构与旅游振兴融合发展研究——以司莫拉佤族村为调研对象	一般	0252 应用统计	金娜	李维昌
2021Y145	数学与统计学院	基于半监督广义可加 Logistic 回归的企业信用异常检测方法	一般	0252 应用统计	杨柳清	李会琼
2021Y146	数学与统计学院	云南省数字经济发展指数研究	一般	0252 应用统计	唐弋超	张理
2021Y147	体育学院	在校大学生公共服务与社区公益需求结合的可行性分析——以呈贡碧潭社区为例	一般	0452 体育	王一铭	冼慧
2021Y148	体育学院	公共体育服务现状及优化路径研究	一般	0452 体育	朱苏芃	李年红
2021Y149	体育学院	云南大学定向运动创新发展对促进学生体质健康价值作用研究	一般	0452 体育	蔡恬甜	李国忠
2021Y150	体育学院	运动干预对大学生抑郁症干预效果的 meta 分析	一般	0452 体育	张富博	李年红
2021Y151	体育学院	运动干预对大学生体质健康弱势群体自我效能影响研究	一般	0452 体育	廖冰雪	李年红

2021Y152	体育学院	大学生体质健康弱势群体同伴支持模式研究	一般	0452 体育	张耘瑞	李年红
2021Y153	体育学院	打造云南大学“15 分钟健身圈”的策略研究	一般	0452 体育	高万岑	杨霞
2021Y154	外国语学院	云南旅游外宣文本语料的英译策略研究——基于丽江景点的考察与研究	一般	0551 翻译	胡添铁	刘树森
2021Y155	外国语学院	多模态话语分析视角下纪录片《云南》翻译实践与研究	一般	0551 翻译	李洁	崔凤娟
2021Y156	外国语学院	文化翻译观视域下的云南少数民族文化外宣翻译——以《云南特有民族文化知识》为例	一般	0551 翻译	施睿	谢萍
2021Y157	新闻学院	云南大学大学生就业服务及社会实践公众号的设计与推广	一般	0552 新闻与传播	陈旭东	蔡勇
2021Y158	新闻学院	斗南花卉网红营销路径研究——在“花匠赵大发”工作室的参与式观察	一般	0552 新闻与传播	赵玲	赵净秋
2021Y159	新闻学院	媒体实习经历对新闻学子择业意向的影响研究	一般	0552 新闻与传播	王舒	李凤萍

2021Y160	新闻学院	云南形象的国际传播效果研究——以云南象群北移故事的国际报道为例	一般	0552 新闻与传播	陈杨	李凤萍
2021Y161	新闻学院	短视频平台信息流广告脚本创作现状探究	一般	0552 新闻与传播	刘海霞	徐明卿
2021Y162	新闻学院	基于扎根理论的消费者对景区文创产品认知的影响因素分析	一般	0552 新闻与传播	章杭佳	韦平
2021Y163	新闻学院	民族地区乡村已婚女性健康传播研究——以禄劝彝族苗族自治县为例	一般	0552 新闻与传播	姚雪珂	王纪春
2021Y164	信息学院	ESAI——基于深度强化学习和点云的电子竞技战队战术模拟系统	一般	0854 电子信息	许潇	蒋慕蓉
2021Y165	信息学院	基于深度学习的蓝藻智能判别与监测	一般	0854 电子信息	徐瑞骋	梁虹
2021Y166	信息学院	基于超声的乳腺癌良恶性肿瘤早期快速精准分类的创新实践	一般	0854 电子信息	张月	张榆锋
2021Y167	信息学院	基于医学影像分析的 COVID-19 智能辅助诊断系统研究	一般	0854 电子信息	陆倩杰	柏正尧

2021Y168	信息学院	面向云南历史文物数字化呈现的三维重建和点云配准技术研究	一般	0854 电子信息	许祝	柏正尧
2021Y169	信息学院	用于 5G 毫米波的集成基片间隙波导电磁超材料宽带圆极化天线研究	一般	0854 电子信息	胡红	申东娅
2021Y170	信息学院	基于知识图谱的无线电监测数据管理系统	一般	0854 电子信息	陈舒平	杨晶晶
2021Y171	信息学院	基于实例信息和分布关系图神经网络的东巴画小样本分类	一般	0854 电子信息	徐启豪	钱文华
2021Y172	信息学院	甲型流感病毒抗原性预测开源项目	一般	0854 电子信息	王迎晖	李维华
2021Y173	信息学院	基于早停机制的机器阅读理解系统技术研究	一般	0854 电子信息	南福来	王津
2021Y174	信息学院	基于指代消解的民间文学文本数据关系抽取方法研究与应用	一般	0854 电子信息	魏静	岳昆
2021Y175	信息学院	基于空间高影响模式挖掘污染物与癌症病例的潜在关系	一般	0854 电子信息	束娟娟	王丽珍

2021Y176	信息学院	基于光照自适应图像融合的交通行人检测技术研究	一般	0854 电子信息	尹文霞	贺康建
2021Y177	信息学院	基于 GAN 网络的中国水墨画风格迁移研究	一般	0854 电子信息	刘昆晓	袁国武
2021Y178	信息学院	鼠疫宿主与媒介生物的智慧识别方法研究	一般	0854 电子信息	王利峰	岳昆
2021Y179	信息学院	基于 StarGAN 风格强度可控的图像风格迁移网络	一般	0854 电子信息	廖远鸿	钱文华
2021Y180	信息学院	基于 Yolov5 的补片可视化检测与三维重建	一般	0854 电子信息	夏芪芩	吴俊
2021Y181	信息学院	基于毫米波传感器的呼吸测量仪	一般	0854 电子信息	吴彭	常俊
2021Y182	信息学院	多深度特征与顶层信息引导的视网膜分割研究	一般	0854 电子信息	张树地	余鹏飞
2021Y183	信息学院	基于隐式语义增强的农作物病虫害检测与防治预警研究	一般	0854 电子信息	白雪松	吴建平

2021Y184	信息学院	基于等效电路分析的 THz 滤波器件设计	一般	0854 电子信息	曾一帆	陈哲
2021Y185	信息学院	基于生物电阻抗与压电传感器的脉搏信号检测系统	一般	0854 电子信息	李诗楠	梁竹关
2021Y186	信息学院	基于深度学习的图像恢复技术研究	一般	0854 电子信息	杨浩	周冬明
2021Y187	信息学院	基于强化学习的 MU-MIMO 带内全双工上下行链路的调度方案	一般	0854 电子信息	余胜千	官铮
2021Y188	信息学院	边缘计算场景下昆明电动自行车视频监控与分析研究	一般	0854 电子信息	李坤	李波
2021Y189	信息学院	基于空间分布式深度强化学习（DRL）协议	一般	0854 电子信息	周天玉	官铮
2021Y190	信息学院	基于毫米波雷达的手势识别研究	一般	0854 电子信息	许妍	常俊
2021Y191	信息学院	基于深度学习的重楼微性状鉴别方法研究	一般	0854 电子信息	罗晋	张俊华

2021Y192	信息学院	基于层次化时空特征结构的深度学习网络流量分类研究	一般	0854 电子信息	杨忠富	常俊
2021Y193	信息学院	基于 WiFi 指纹和图数据库的三维空间定位研究	一般	0854 电子信息	唐杰	黄铭
2021Y194	信息学院	边缘计算场景下春城高效交通拥堵调度方案的研究	一般	0854 电子信息	史宇豪	李波
2021Y195	艺术与设计学院	《傣族象脚鼓舞》云南傣族非遗艺术主题工笔组画创作	一般	1351 艺术	金小燕	王鹏程
2021Y196	艺术与设计学院	《丝路新语》云南布依族脱贫攻坚系列主题国画	一般	1351 艺术	卢国豪	寇元勋
2021Y197	艺术与设计学院	基于东巴画艺术语言的《纳西盛世歌》工笔重彩组画创作	一般	1351 艺术	彭晶	屠潇
2021Y198	艺术与设计学院	非遗传承下楚雄彝族酒歌的发展现状及传播形式探析	一般	1351 艺术	蔡晓羽	戴愿
2021Y199	艺术与设计学院	云南省大理白族地区石龙村白曲和大达村吹吹腔的传承与传播	一般	1351 艺术	钟孟智	赵兰芳

2021Y200	艺术与设计学院	基于丝网版画工艺的“苗岭新歌”主题海报设计与创作	一般	1351 艺术	赵洁	庞杰 唐军
2021Y201	艺术与设计学院	“百年云大”系列文创产品设计之《青铜古滇紫陶茶器》	一般	1351 艺术	张健	吴白雨
2021Y202	艺术与设计学院	设计管理理论下的建水紫陶品牌整合设计研究	一般	1351 艺术	胡倍榕	吴白雨
2021Y203	艺术与设计学院	基于数字化模型下云南大学东陆校区历史建筑的采集和测绘	一般	1351 艺术	罗江收	李沁
2021Y204	艺术与设计学院	“百年云大”基于声感体验的交互装置设计研究	一般	1351 艺术	何莉花	李沁
2021Y205	艺术与设计学院	“凤阳邑茶马古道”廊道遗产保护及旅游开发设计	一般	1351 艺术	黄清逸	李沁
2021Y206	艺术与设计学院	文化创新时代下的模玩产品个性化定制	一般	1351 艺术	陈林强	杨剑波
2021Y207	艺术与设计学院	校园画廊	一般	1351 艺术	丁希慧	张志明

2021Y208	艺术与设计学院	美术造型与色彩在城市立体绿化中的应用--以昆明COP15立体花坛作品为例	一般	1351 艺术	杨梅	陈玲洁
2021Y209	艺术与设计学院	曲靖会泽会馆古建筑艺术文创设计策略	一般	1351 艺术	卓楚源	屠潇
2021Y210	艺术与设计学院	云南非遗文化下白族酒歌的记谱及演唱	一般	1351 艺术	王力瑞	戴愿
2021Y211	艺术与设计学院	探究音乐游戏在音乐课堂中的应用	一般	1351 艺术	沈晓雨	赵兰芳
2021Y212	艺术与设计学院	叙事性傣族《孔雀》主题餐厅设计实践	一般	1351 艺术	李娜	陈春妮 黄欣友
2021Y213	艺术与设计学院	基于AR技术的《版纳花园》科普影像装置设计实践	一般	1351 艺术	徐洺敏	陈锋
2021Y214	艺术与设计学院	傣族剪纸艺术在现代交通设施站点设计中的应用	一般	1351 艺术	陈官亚	王兢
2021Y215	艺术与设计学院	村城共生下宏仁村人居环境空间再设计的实践与思考	一般	1351 艺术	袁子艳	杨晓翔

2021Y216	艺术与设计学院	服务人的无障碍地面公交站优化改造研究	一般	1351 艺术	易洋	余丽婴
2021Y217	艺术与设计学院	基于可持续设计理念的瓦猫主题儿童家具设计运用	一般	1351 艺术	黄晓敏	吴白雨
2021Y218	艺术与设计学院	云南民间玩具的当代设计转化实践——以陶制吹鸡为例	一般	1351 艺术	代红丽	吴白雨
2021Y219	艺术与设计学院	“百年云大”数字插画系列创作之《东陆植物志》	一般	1351 艺术	刘畅	吴白雨
2021Y220	艺术与设计学院	傣族特色建筑的中国画艺术表达	一般	1351 艺术	荆竺欣	高波
2021Y221	艺术与设计学院	云南音乐非遗文化下会泽海腔的再度创作及演绎	一般	1351 艺术	刘官迪	戴愿
2021Y222	艺术与设计学院	弘扬爱国主义——以艺术歌曲《故乡》、《勇士骨》演唱研究为例	一般	1351 艺术	仲永梅	杨沁文
2021Y223	艺术与设计学院	网络音乐课对改善优质教育资源分配不平衡问题的研究	一般	1351 艺术	钱多	赵兰芳

2021Y224	政府管理学院	基于零信任理论的政府网络系统高级持续威胁（APT）风险防范研究	一般	1252 公共管理	傅彬	罗红霞
2021Y225	政府管理学院	公共管理视域下乙肝病毒聚集传播风险防控策略研究	一般	1252 公共管理	刘立	马桑
2021Y226	政府管理学院	中越边境地区中方边民流动态势、机制与引导	一般	1252 公共管理	夏玉印	罗红霞
2021Y227	政府管理学院	强扭的瓜甜不甜：云南省“干部回乡规划乡村振兴行动”政策执行的多案例观察	一般	1252 公共管理	张宜玲	罗红霞
2021Y228	政府管理学院	第三次分配视野下社会组织推动乡村振兴的实现路径研究	一般	1252 公共管理	刘星明	罗红霞
2021Y229	政府管理学院	基于“模糊-冲突”模型的“拉闸限电”政策执行问题研究	一般	1252 公共管理	郭琳琳	马桑
2021Y230	政府管理学院	基于系统论的云南边境应急物资保障体系优化策略研究	一般	1252 公共管理	陈翔	陈然
2021Y231	政府管理学院	云南省经营性土地全生命周期管理的现实困境、体系构建及实现路径	一般	1252 公共管理	杨茜	陈然

2021Y232	政府管理学院	实名制管理下农民工薪酬权益保障问题研究——基于昆明市 A 建筑集团调研	一般	1252 公共管理	夏月蓉	马桑
2021Y233	职业与继续教育学院	双减政策下职业教育资历框架制度的研究	一般	0451 教育	刘晓娇	吴云
2021Y234	职业与继续教育学院	云南省中等职业学校校企教师双向流动研究	一般	0451 教育	陈圣杰	娄元元
2021Y235	职业与继续教育学院	“三方联动：全方位马背课堂”教学模式研究-以内蒙古兴安盟突泉县中等职业学校为例	一般	0451 教育	陈启月	杨永兵
2021Y236	职业与继续教育学院	“普职分流”背景下减少家长对职业教育偏见的实施建议	一般	0451 教育	饶静	陈云山
2021Y237	职业与继续教育学院	双高计划下双师型教师队伍的建设	一般	0451 教育	杨灵芝	周芳
2021Y238	职业与继续教育学院	关于课程长制度构想及可行性研究	一般	0451 教育	袁小蕊	陈云山
2021Y239	职业与继续教育学院	基于情景学习理论的中等职业学校旅游服务与管理专业教学模式研究	一般	0451 教育	孙紫竹	魏娜

2021Y240	职业与继续教育学院	双创背景下中职学校学分银行的实施建议	一般	0451 教育	罗岚	周芳
2021Y241	职业与继续教育学院	“大思政”背景下中职学校班主任心理健康教育实施研究	一般	0451 教育	蓝晓姣	王雯
2021Y242	职业与继续教育学院	“双减”政策下基于在线学习行为数据挖掘的学习绩效预测构建	一般	0451 教育	吴贝嘉	李甦
2021Y243	职业与继续教育学院	基于用户画像的在线教育个性化学习资源推荐技术的研究	一般	0451 教育	罗心雨	张熹
2021Y244	职业与继续教育学院	基于 RLO-RAO 模型的数字化课程资源与研学旅行实践活动的整合应用研究	一般	0451 教育	尹旭	刘俊玮
2021Y245	职业与继续教育学院	基于知识图谱的《Python 程序设计技术》课程答疑系统设计与应用研究	一般	0451 教育	李美姣	李继东
2021Y246	职业与继续教育学院	信息技术支持下的小学低年级语文单元学习的非纸笔评价方法	一般	0451 教育	郑湘莲	窦刚
2021Y247	职业与继续教育学院	面向创新思维培养的小学可视化编程校本课程的设计和开发	一般	0451 教育	余莉	何鸣皋

2021Y248	职业与继续教育学院	游戏型微课件在中小学信息技术课中的运用	一般	0451 教育	李新月	何鸣皋
2021Y249	资源植物研究院	水分胁迫下丛枝菌根真菌对玉米抗旱性的影响	一般	0951 农业	陈家斌	谭静
2021Y250	资源植物研究院	云南省马铃薯地方品种的鉴定评价与应用	一般	0951 农业	蔡婉华	黄斌全
2021Y251	资源植物研究院	生物胺降解菌的筛选及其在咖啡产业中的应用	一般	0951 农业	薛笑然	何飞飞 秦世雯
2021Y252	资源植物研究院	多年生稻氮高效利用的定向改良	一般	0951 农业	米胜南	邵林
2021Y253	资源植物研究院	珠芽魔芋的多叶连续生长现象探析	一般	0951 农业	李缘	吴学尉
2021Y254	资源植物研究院	基于五角栝楼活性物质分析的种质价值研究	一般	0951 农业	李怡洁	王杨
2021Y255	资源植物研究院	基于阿拉比卡咖啡果皮活性物质分析的综合利用探索	一般	0951 农业	张梁	王杨

2021Y256	资源植物研究院	外加直流电场对尖孢镰刀菌 Foc (F.oxysporum)侵染香蕉植株的防治效果研究	一般	0951 农业	李贝	高晶
2021Y257	资源植物研究院	小粒咖啡果皮营养成分及抗氧化活性的分析	一般	0951 农业	张佳欣	何飞飞

研究生院

2021 年 11 月 10 日

2021-2022学年常态化社会实践活动结项评审结果

编号	项目名称	是否同意结项
C202101	“党课进庭院”让红色精神薪火相传	同意结项
C202102	“讲好中国故事”：昆明老街红色文化实践育人研究	同意结项
C202103	云大学子助力“春城先锋——帮理想、帮学习、帮实践”志愿者服务项目	同意结项
C202104	爱在夕阳红-呈贡敬老院关爱调研活动	同意结项
C202105	斗南村花卉小镇建设与村民群体意见协调研究	同意结项
C202106	乡村振兴背景下昆明传统村落文化景观研究	同意结项
C202107	推动校园代步车管理，构建美丽和谐校园	同意结项
C202108	定格瞬间·书写生命：关爱社区孤寡老人志愿活动	同意结项
C202109	商品作为他者—昆明市外国店铺中的跨文化传播现象研究	同意结项
C202110	乡村振兴战略背景下昆明市寻甸县乡村特色旅游调研	同意结项
C202111	“我为大家代言”——云南大学硕士研究生校园服务意见调查行动	同意结项
C202112	昆明市禄劝县留守儿童心理支持现状及其系统构建	同意结项
C202113	乡村振兴战略视域下昆明传统村落的转型与发展路径调研	同意结项
C202114	全民阅读时代下视障群体数字阅读的价值实现路径分析	同意结项
C202115	‘我是你的眼’——文化助盲，我们在行动	同意结项

C202116	暖“馨”支教，爱在春城	同意结项
C202117	大墨雨村——乡村振兴下彝族古村到“昆明后花园”的蜕变	同意结项
C202118	云岭青年，爱心助弱	同意结项
C202119	昆明市民族团结进步示范社区的景观建设成效研究	同意结项
C202120	“快乐方舟”与爱同行	同意结项
C202121	乡村振兴背景下昆明市哈马者村的产业发展调研	同意结项
C202122	花都向阳——疫情常态化防控下斗南花卉市场调研实践	同意结项
C202123	诺克米自闭症儿童关爱活动	同意结项
C202124	云南大学及其周边生物科学类科研人员走访交流与科研现状实践调查	同意结项
C202125	下庄社区爱心支教	同意结项
C202126	“寻滇源·兴滇源”——商旅学子“三推一带一拓”助力滇源中村乡村振兴	同意结项
C202127	红色旅游资源开发助推乡村振兴模式探析—基于云南长征精神红色旅游路线的田野调查	同意结项
C202128	醉美梁王山——都市驱动型乡村振兴模式探析及劳动实践基地建设	同意结项
C202129	乡村振兴，教育先行——新媒体视角下昆明乡村智富新模式	同意结项
C202130	蒙多贝关爱自闭症儿童志愿服务活动	同意结项
C202131	一心人·一行人	同意结项
C202132	花从斗南来—基于花卉产业探索斗南村多方面发展潜力	同意结项
C202133	行者无疆	同意结项

C202134	走近腹壁疝科研基地——探究学习一种创新型超声易显影轻量补片及术后可视化评估系统	同意结项
C202135	“点亮星空，与爱同行”关爱自闭症儿童志愿公益活动	同意结项
C202136	岁稔时予农—云南高校助农工作室	同意结项
C202137	星光护航，让爱循环——特殊儿童爱心驿站志愿公益平台	同意结项
C202138	乡村振兴背景下关于创建昆明市呈贡区新型生态农家乐供需桥梁的实践调研	同意结项
C202139	“温暖阳光进社区”关心关爱行动	同意结项
C202140	定向越野对初中生“逆商”影响的研究	同意结项
C202141	云南省昆明市盲哑学校公益体育健身课堂	同意结项
C202142	乡村非遗文化振兴与产业振兴相促进的可行性机制研究——从昆明市呈贡区出发	同意结项
C202143	法润人心——普法志愿公益项目	同意结项
C202144	心手相牵五华志愿助残，与爱同行服务华山街道	同意结项
C202145	以花促农，以花兴业——昆明花卉产业发展研究	同意结项
C202146	“艺美万溪，文创助力”——文创产品助力万溪冲乡村振兴	同意结项
C202147	润物无声——博物馆与美术教育实践方案	同意结项
C202148	“实践育人”背景下从社会实际需求出发关爱孤寡老人——以昆明市五华区为例	同意结项
C202149	临床医生科研现状调研	同意结项
C202150	公益支教行动	同意结项
C202151	我与时间老人的独白	同意结项

C202152	梨园小镇的特色化振兴发展之路——万溪冲地缘优势和产业转型的个案研究	同意结项
C202153	基于云南省花卉技术创新中心建设及成果转化的深度实践调研	同意结项
C202154	云爱筑梦——官渡区金马街道曙光社区儿童课外课堂志愿公益行动	同意结项
C202155	“我是小小推普员”普通话志愿教学实践活动	同意结项
C202156	寓教于影——小学电影美育课堂	同意结项
C202157	走进光学实验室——设计一种基于光学成像测量液体粘滞系数的新方法	同意结项
C202158	上知天文支教活动	同意结项
C202159	研究疫情对昆明各大市场常见菜品菜价变动的影响	同意结项
C202160	万溪冲小学支教计划（萤光计划）	同意结项
C202161	“化”所学为所用——社区多方面志愿服务实践活动	同意结项
C202162	传承红色基因，讲好党的故事——从滇开始	同意结项
C202163	走进化学实验室：多聚吲哚光电子材料的研究进展	同意结项
C202164	2015-2020年昆明城市洪涝灾害韧性调查	同意结项
C202165	“地里”寻踪：云南地理知识志愿科普服务	同意结项
C202166	实践探真——万溪冲社区发展之路的研究与探索	同意结项
C202167	滇池护卫队	同意结项
C202168	云南南部和西南地区少数民族对野生稻药用认知情况调查	同意结项
C202169	关于脚步哨村发展林下经济可行性的调研与开展	同意结项

C202170	昆明都市驱动型乡村振兴美丽宜居示范村市级精品村调查研究	同意结项
C202171	云南省博物馆社会实践活动	同意结项
C202172	沐光兴农——生态科技筑梦乡村振兴的呈贡斗南实践	同意结项
C202173	彝族音乐发掘与保护	同意结项
C202174	“暮里流萤，熠耀桑榆”养老志愿服务项目——基于昆明市官渡区四个社区的试点实践	同意结项
C202175	乡村振兴背景下发展壮大村集体经济的现状对对策研究——基于对昆明市的调研分析	同意结项
C202176	从异质到共生：民族地区易地扶贫搬迁中“文化搬迁”实践研究——基于昆明市寻甸县钟灵社区的调查	同意结项
C202177	数字赋能乡村旅游的路径研究——基于昆明市都市驱动型乡村振兴创新实验区的调研	同意结项
C202178	昆明市城市驱动型乡村振兴创新实验区建设基本经验研究——以呈贡区万溪冲社区为例	同意结项
C202179	聚焦中华民族共同体仪式的基层党史宣讲——以呈贡万溪冲为例	同意结项
C202180	基于云南省水域生态状况调研的物联网水环境监测系统研发	同意结项
C202181	乡村古建筑文化振兴——以昆明市县街乡大石庄村清代古建筑群的测绘研究与保护发展为实践	同意结项
C202182	异地扶贫搬迁后的社区治理模式探索及其人民幸福感指数研究——以东川区对门山、起嘎安置区与卯家湾安置区为例	同意结项
C202183	益路童行——关爱福利院儿童计划	同意结项
C202184	唯有爱，让感动常在——爱之家	同意结项
C202185	体育筑梦活动	同意结项
C202186	“音”你而来校园广播公益活动	同意结项

C202187	基于社会视角的云南高校共青团社会实践高质量发展调研	申请延期
C202188	乡村振兴背景下村史馆与村落社会的互动研究	同意结项
C202189	立德树人背景下共青团开展大学生社会实践的现状与前景探析——基于昆明市6所高校的调查	申请延期
C202190	运动式治理附加议程的逻辑生成与风险管理：基于云南省昆明市“遛狗拴绳”的案例观察	同意结项
C202191	无逸长灯，为梦启明——云大青协文笔小学支教项目	同意结项
C202192	走进星星的世界	同意结项
C202193	善润童心，爱护理想——云大青协理想支教项目	同意结项
C202194	数字普惠金融消除农村家庭相对贫困问题的路径研究—基于昆明市农户调研数据	同意结项
C202195	呈贡区大新册村空巢老人存在原因情况分析	同意结项
C202196	“和你在一起”—对特教学校的长期志愿服务	同意结项
C202197	城市化进程中失地农民再就业现状探析——基于昆明市呈贡新区的实证调研	同意结项
万溪冲专项	万溪冲社区公共空间微更新改造	同意结项
万溪冲专项	万溪冲农村青年电商发展现况调查	同意结项
万溪冲专项	关于万溪冲村发展林下经济的路径研究	同意结项
万溪冲专项	“艺象万溪”常态化社会实践项目	同意结项
万溪冲专项	万溪冲小学支教分队	同意结项

2022年全国农科学子联合实践行动立项队伍信息公示

所属院校	队长姓名	指导教师姓名	实践地
东北农业大学	徐平	张大勇、刘丹	哈尔滨市尚志市元宝镇元宝村
东北农业大学	徐启华	石瑛、王春龙	黑龙江省齐齐哈尔市甘南县兴十四村
东北农业大学	夏畅鸿	宋爽、张华英	黑龙江省伊春市铁力市王杨乡建设村
东北农业大学	张浩然	郑洪亮、石云鹤	黑河市逊克县车陆乡卫东村
东北农业大学	郑梓豫	石云鹤	黑龙江省大庆市大同区八井子乡庆阳山村
东北农业大学	于瑞阳	王春龙	黑龙江省哈尔滨市南岗区黑龙江曲艺团
东北农业大学	赵浩妍	王敬国	黑龙江省哈尔滨市阿城区
东北农业大学	佟仕伦	张鑫琳	黑龙江省哈尔滨市双城区满族乡
福建农林大学	何秋娟	陈志伟、章清杞	福建省南平市浦城县莲塘镇山桥村、永兴镇庵后村，福建省莆田市仙游县石苍乡绿林农业创新农民专业合作社
福建农林大学	顾众	王衍坤	福建省龙岩市上杭县
福建农林大学	贾近	王松良	福建省宁德市屏南县
福建农林大学	何圣荐	叶德练	福建省南平市建瓯市东峰镇
福建农林大学	宋辉	杨志坚	福建省福州市闽侯县洋里乡茶苑村
福建农林大学	周伟杰	金呈蒙、李明杰	福建省泉州市德化县许厝村
福建农林大学	史夏蕾	林荔辉	福建省三明市泰宁县朱口镇里家源村
福建农林大学	包兴胜	吴仁烨	福建省泉州市惠安县惠川镇惠安家兴家庭农场
甘肃农业大学	田鑫	孟浩峰、王林林、谢军红、李玲玲、王凤	甘肃省定西市安定区李家堡镇、团结镇
甘肃农业大学	魏文龙	逄蕾、魏春楠	甘肃省白银市白银区桦皮川村
甘肃农业大学	马多吉	蒲媛媛　、毕真真　、许爱霞	甘肃省定西市通渭县北城铺镇北城铺学校
广西大学	方妍	李诺	广西壮族自治区柳州市
广西大学	郭桂颖	李诺	广西百色市乐业县
广西大学	兰弋	李诺	广西贺州市八步区仁义镇新联村
广西大学	张文选	李诺、宁云芬	广西壮族自治区柳州市融安县
广西大学	陈峻锐	李诺、贾永灵	广西百色市那坡县
广西大学	韦玉华	李诺	广西崇左市扶绥县新宁镇
广西大学	宋婧洁	许雄彪	广西壮族自治区百色市田东县江城镇大诺村
广西大学	刘洁	韦杰	广西壮族自治区柳州市融安县
广西大学	陈绪杨	韦杰	广西壮族自治区柳州市融安县长安镇融康社区
广西大学	王晓南	韦杰	广西壮族自治区柳州市融安县长安镇红卫村
广西大学	余冠学	韦杰	广西壮族自治区来宾市忻城县城关镇板河村
贵州大学	杨秀珍	肖宇	贵州省黔东南苗族侗族自治州黎平县坝寨乡高场村
贵州大学	晏丽娟	李经纬	贵州省贵阳市开阳县数谷贵阳市蔬菜良种繁殖基地
贵州大学	李超	肖宇	贵州省遵义市凤冈县琊川镇、贵州省黔南州惠水县好花红村、贵州省黔南州平塘县卡腊村、贵州省遵义市余庆县葛旁村等
贵州大学	郝振坤	潘学军	赫章县六曲河镇河边村、大田村及朱明镇全部村
贵州大学	代艺豪	任明见	贵州省毕节市黔西市杨家店村
贵州大学	张佳美	肖宇	贵州省六盘水市水城城区阿戛镇盐井村
贵州大学	殷艳菁	先桁	贵州省黔南州惠水县好花红镇好花红村

贵州大学	李首琴	肖宇	贵州省黔西南州望谟县石屯镇石屯村、贵州省黔东南州镇远县涌溪乡芽溪村、贵州省黄平县谷陇镇王坳村
贵州大学	罗明怡	冯跃华	贵州省遵义市绥阳县
贵州大学	杨婷	鲁敏	贵州省毕节市七星关区撒拉溪镇龙凤村
贵州大学	曾云诗	张彬	贵州省黔西南市贞丰县龙场镇坡柳村
贵州大学	蒙星聿	李刚	贵州省　六盘水市　水城县　红枫湖　民乐村　刘家寨
贵州大学	李小艳	肖宇	贵州省遵义市正安县、播州区
贵州大学	张文松	王勇老师	贵州省黔东南苗族侗族自治州从江县
贵州大学	黄欣然	罗雅雪	贵州省黔东南州施秉县甘溪乡甘溪村
河北科技师范学院	闫妍	赵鹏、吉志新、郭亚珂、温晓蕾、司增志	河北省秦皇岛市卢龙县印庄村河北中薯农业科技集团股份有限公司
河北科技师范学院	张瑞薇	李向岭、李哲、蔡瑞国、林小虎	秦皇岛市昌黎县荒佃庄镇信庄村
河北科技师范学院	齐芳宁	解海翠，韩玉翠	秦皇岛市海港区石门寨镇房庄村
河北科技师范学院	张笑瑜	苏文楠、张萌	河北省邯郸市永年区辛庄堡乡豆庄一村
河北科技师范学院	孟张林	王文颇、王健	河北省廊坊市文安县赵各庄乡
河北科技师范学院	陈思卓	韩金玲、杨晴	河北省邯郸市复兴区后郝村
河北科技师范学院	张鹏	张敬丹、范海荣、董永红	河北省张家口市尚义县
河北科技师范学院	王明静	郑金双、王艳丽	河北省承德市隆化县茅荆坝乡
河北科技师范学院	乔思蕊	杨敏，张敏	河北省衡水市饶阳县西九吉村
河北农业大学	刘坤	李琳	张家口市张北县小二台乡黑山堡村
河北农业大学	张世鑫	郭妍妍	邢台市威县梨园屯镇
河北农业大学	苗紫璇	武海娜	沧州市任丘市辛中驿镇
河南农业大学	范元奎	熊淑萍	河南省濮阳市范县吴桥村
河南农业大学	杨默含	熊淑萍	河南省洛阳市洛宁县中方村
河南农业大学	赵芷垚	麻兵继	河南省新乡市获嘉县铁官滩村
河南农业大学	李沛原	温鹏飞	河南省驻马店市泌阳县张铺村
河南农业大学	杨凡力	唐朝	全国青少年延安教育基地
黑龙江八一农垦大学	韩祥琪	曹亮	湖南省湘潭市韶山市清溪镇
黑龙江八一农垦大学	兰卓		吉林省公主岭市范家屯村
黑龙江八一农垦大学	毛鲁枭	曹亮	山东省菏泽市鄄城县陈王街道西曹村，引马镇范庄行政村
黑龙江八一农垦大学	刘艺莹	张明聪	黑龙江省大庆市春雷牧场
黑龙江八一农垦大学	钮湘煜	蒋雨洲	河南省新乡市延津县齐村
黑龙江八一农垦大学	李发广	邓杰	广西省桂平市大上村大充屯
黑龙江八一农垦大学	侯欣雨	尹志伟	辽宁省本溪市桓仁满族自治县雅河乡
黑龙江八一农垦大学	刘天生	张春秋	黑龙江省哈尔滨市巴彦县天增镇庆胜村
黑龙江八一农垦大学	郝冉	周园园	黑龙江省齐齐哈尔市富裕县关山村
黑龙江八一农垦大学	兰峥	尹志伟	湖南省邵阳市邵阳县五峰铺镇青云村、黑龙江省鹤岗市萝北县军川农场24队、新疆昌吉洲昌吉市北京南路地质村社区、黑龙江省齐齐哈尔市富拉尔基区前库勒村
黑龙江八一农垦大学	谷全伟	张明聪	黑龙江省大庆市春雷牧场
黑龙江八一农垦大学	宋佳亿	尹志伟	黑龙江省牡丹江市宁安市东京城镇
黑龙江八一农垦大学	刘思怡	刘明	吉林省四平市大孤山镇杏木村七组
黑龙江八一农垦大学	王启玮		河南省商丘市睢县城郊乡刘庄

黑龙江八一农垦大学	李博	曹亮	黑龙江省讷河市九井镇谦益村
黑龙江八一农垦大学	任紫星	曹亮	贵州省六盘水市盘州市城关镇九间楼社区，盘江镇盘江路
吉林农业大学	武高嫄	李丹	辽宁省阜新市海州区韩家店镇东瓦区、内蒙古自治区赤峰市翁牛特旗乌丹镇赛沁塔拉嘎查、内蒙古自治区包头市东河区河东镇王大汉村、吉林省松原市乾安县严字乡严字村
吉林农业大学	刘晴	唐少勋	吉林省长春市农安县烧锅镇中兴村
吉林农业大学	李想	李丹	吉林省松原市扶余市弓棚子镇双胜村
吉林农业大学	张晟博	李丹，周牲喆	吉林省长春市榆树市王家村孙户屯；吉林省吉林市永吉县北大壶镇松柏村；吉林省松原市前郭县查干花镇白音花村；安徽省安庆市岳西县白帽镇白帽村
吉林农业大学	杨嬰熙	李丹，周牲喆	吉林省长春市榆树市五棵树镇
宁夏大学	杨东梅	陈虎、马维宗	宁夏回族自治区吴忠市同心县丁塘镇吴家河湾村
宁夏大学	尹慧	李锦馨	宁夏省固原市泾源县米岗村
宁夏大学	马东升	马维宗	宁夏回族自治区固原市彭阳县新集乡沟口村
宁夏大学	马雨佳	靳磊	宁夏中卫市海原县李旺镇韩府七村、李家庄
宁夏大学	马民乐	马维宗	宁夏回族自治区吴忠市同心县丁塘镇团结村
宁夏大学	禹若晨	李惠霞	宁夏回族自治区吴忠市同心县石狮管委会惠安村、宁夏回族自治区固原市泾源县大湾乡杨岭村
青岛农学大学	王秋迪	石勇	山东省东营市垦利区
青岛农业大学	段新娇	刘源	临沂市沂水市金龙山农业专业合作社
青岛农业大学	王淑涵	刘源	淄博市临淄区伟强专业合作社
青岛农业大学	崔梦溪	刘源	山东省泰安市新泰市宫里镇，龙廷镇，羊流镇
青岛农业大学	杨欣瑜	刘源	山东省济南市天桥区
青岛农业大学	巩玉	刘源	山东省济南市市中区济南战役纪念馆
山东农业大学	刘凤欣	张大健	山东省东营市河口区义和镇
山东农业大学	尹承龙	代兴龙	山东省泰安市岱岳区道朗镇里裕村
山东农业大学	袁瑞	代兴龙	济宁市邹城市唐村镇
山东农业大学	朱百佳	宁堂原、田梦霞	山东省东营市垦利区黄河口生态保护区、山东省东营市垦利区黄河三角洲生态监测中心、山东省东营市广饶县三角洲现代农业高
山东农业大学	黄昊	宁堂原	山东省东营市广饶县农高区
山西农业大学	刘哲豪	刘黎明	山西省朔州市毛家皂镇
山西农业大学	刘哲豪	刘黎明	山西省忻州市繁峙县金山铺乡郝家湾村
山西农业大学	刘哲豪	刘黎明	山西省忻州市定襄县衡山村
山西农业大学	于建伟	刘黎明	山西省晋中市太谷区杨家庄
石河子大学	柏苏琴	冶建明	新疆生产建设兵团第三师53团4连
石河子大学	裴庆余	聂新辉、王龙	新疆生产建设兵团第三师图木舒克市44团、49团、51团
石河子大学	裴庆余	聂新辉、马亚东	新疆生产建设兵团第五师83团、84团、86团、87团、88团
石河子大学	杨秘	张泽	新疆生产建设兵团第三师51团
石河子大学	谢斯琦	冶建明、王登伟	新疆生产建设兵团第三师53团4连
石河子大学	丁乙明	冶建明	新疆维吾尔自治区和田地区和田县吾宗肖乡
石河子大学	张静	唐诚	新疆生产建设兵团五十一团十二连
塔里木大学	杨悦	罗新宁、赵书珍	新疆阿拉尔市十三团十七连
塔里木大学	杨楠	秦路兵	新疆省阿克苏地区阿克苏市依干其乡尤喀克科克巴什村

塔里木大学	陈宏深	肖海兵、黄英	新疆维吾尔自治区阿拉尔市12团、11团7连
塔里木大学	张永敬	胡强，翟云龙	新疆维吾尔自治区阿克苏地区五团沙河镇
塔里木大学	张嘉伟	何良荣	新疆阿克苏市拜城县
塔里木大学	姚春宇	梁继业	新疆省伊犁昭苏县别斯喀拉盖村
塔里木大学	周帆	刘义飞	新疆柯坪县阿恰勒其兰村
塔里木大学	于建华	曾勇	河南省开封市通许县练城乡翟岗村
塔里木大学	岳宗省	林敏娟	新疆建设生产兵团第十四市二二四团
塔里木大学	李爽	彭翠兰	新疆维吾尔族自治区和田地区和田县和谐新村
塔里木大学	贾艳贺	张宇阳	新疆维吾尔自治区昆玉市皮山农场
塔里木大学	黄旭	彭翠兰	新疆伊犁霍城县64团
塔里木大学	夏刘洋	梁继业	新疆阿拉尔市一师七团迎宾路社区和友谊路社区
塔里木大学	翟芮瑾	张川疆	新疆阿拉尔市一师四团永宁镇吊干杏基地
塔里木大学	合热阿尼	谭占明，黄芮	库木巴什乡
塔里木大学	周舒畅	张宇阳	新疆维吾尔自治区和田地区吉亚乡玉叶村
塔里木大学	王梦圆	张娟、阿依买木·沙吾提	新疆维吾尔自治区阿克苏地区新和县依其艾日克镇尤喀克英阿瓦
塔里木大学	姆纳瓦尔	徐崇志	新疆省昆玉市昆泉镇一连
塔里木大学	马梦琪	张宇阳	新疆维吾尔自治区和田市吉亚乡玉叶村
塔里木大学	彭泽熙	彭翠兰	阿拉尔市周边
塔里木大学	杜磊	金强	甘肃省白银市会宁县新庄镇杜岘村杨岘社
塔里木大学	杨玲丽	谭占明	新疆维吾尔自治区阿拉尔市兵团第一师12团
塔里木大学	王磊	吴翠云	新疆维吾尔族自治区和田市吉亚乡玉叶村
塔里木大学	高雅文	彭翠兰	巴音郭楞蒙古自治州尉型县兴平乡达西村
塔里木大学	吴忠祥	彭翠兰	克州乌恰县波斯坦铁列克乡凯勒敦村
塔里木大学	吕佩酌	阿布来提·依明林敏娟	新疆巴音郭楞自治州尉犁县兴平乡（达西村）
天津农学院	洪熙	帅磊	云南省昆明市晋宁区旧寨村四川省眉山市东坡区白马镇天津市武清区黄花店镇、泗村店镇浙江省金华市东阳市南马镇花园村四川省南充市高坪区搽耳镇四面山村天津市北辰区小淀镇温家房子村、东堤头村，滨海新区八堡村
天津农学院	王学彧	段苏虎	辽宁省抚顺市新抚区南阳街道青海省海西州蒙古族藏族自治州德令哈市火车站街道天津市武清区南蔡村镇畔水庭苑小区天津市静海区梁头镇东贾口村河南省濮阳市华龙区魏云楼甘肃省平凉市静宁县八里镇小山村一社67号
天津农学院	王茜	段苏虎	天津市蓟州区下仓镇北小胡村、宁夏回族自治区固原市原州区陈沟村、云南省曲靖市会泽县钟屏街道红石岩社区、甘肃省张掖市临泽县、天津市今日家园
西藏农牧学院	崔开通	次仁央金	西藏林芝市巴宜区嘎拉村、西藏林芝市巴宜区布久乡、西藏林芝市巴宜区米瑞乡米瑞村
新疆农业大学	房彦飞	唐江华	新疆昌吉州木垒哈萨克自治县东城镇孙家沟村
新疆农业大学	杨沆鑫	李超	新疆维吾尔自治区昌吉回族自治州阜康市城关镇
新疆农业大学	得里亚·依沙	牙森·沙力	新疆伊犁霍城县萨尔布拉克镇
扬州大学	肖颖	周立云	江苏省苏州市常熟市梅李镇瞿巷村、胜法村、塘桥村
扬州大学	居浩然	周立云	安徽六安金寨龚冲村、红湾村

扬州大学	张校瑜	周立云	江苏省宿迁市泗洪县上塘镇垫湖村、大楼街道面西村
云南大学	李怡洁	陈蕊	云南省玉溪市农科院、保山市农科所、云南省嵩明试验基地
云南农业大学	吴宇婧	安曈昕、杨友琼	云南省普洱市澜沧拉祜族自治县
云南农业大学	施怡彤	安曈昕、戴榕	云南省普洱市澜沧拉祜族自治县
黑龙江大学	张家赫	崔博涵、高健	黑龙江省牡丹江市海南乡
黑龙江大学	仲维婷	邹恩悦、孙阎	黑龙江省哈尔滨市阿城区
华中农业大学	闫雨萌	边银丙	湖北省十堰市房县土城镇和清河乡
华中农业大学	李津莹	梁伟军	河北省唐山市玉田县杨家套镇西高坦村
华中农业大学	张经纬	李萍	河南省安阳市水冶镇下河村
华中农业大学	赖妍伶	练人杰	贵州省铜仁市木树镇满地小学/川硐镇板栗园小学
华中农业大学	赵珈葳	张涛	海南省海口市瑶城古村
华中农业大学	杨雨婷	阮媛	湖南省吉首市土家族苗族自治州花垣县十八洞村
华中农业大学	朱冠臻	李林	湖北省襄阳是襄城区
华中农业大学	陈晓倩	刘立军	湖北苎麻产区
华中农业大学	陶灵湄	鄢文豪	湖北省襄阳市黄集镇湖北丰庆源面业有限公司
华中农业大学	谢裕乐	汪波	湖北省襄阳市农科院以及保康县高山蓝莓种植合作社、万花蜜土蜂蜜养殖专业合作社
华中农业大学	周志斐	李林	湖北省襄阳市襄州区双沟镇刘大湾村&襄阳正大种业
华中农业大学	梁沁怡	霍慧新	湖南省常德市津市市
华中农业大学	鞠慧柯	谢甲涛	湖北省宜昌市长阳县贺家坪镇
华中农业大学	王霄楠	汪金平	湖北省潜江市龙湾镇郑家湖村、潜江市积玉口积玉村、潜江市运粮湖新场办事处、潜江市熊口镇瞄场村、潜江市渔洋镇横堤村、潜江市总口管理区南东泓办事处、潜江市浩口镇许桥村、潜江市后湖管理区官苑办事处
西南科技大学	何琳	刘璐	四川省绵阳市平武县响岩镇青山村
西南科技大学	王硕	周蕾	四川省南充市阆中市天宫镇五龙村
西南科技大学	罗智健	袁小红、刘璐	四川省阿坝藏族羌族自治州九寨沟县黑河镇七里村、头道城村、二道城村、绕蜡村、达舍寨村、自玉村、碧历村、吊坝村、大叶子村、羌活沟村
西南科技大学	许紫欣	唐志康	四川省达州市达川区百节镇乌梅山村、肖家村，达川区景市镇茶园寺村
西南科技大学	刘熠铭	郑祥江、刘璐	四川省绵阳市游仙区小枧镇
中国农业大学	刘欣兰	张战营	安徽省合肥市肥东县白龙镇、河北省石家庄市栾城区柳林屯乡北十里铺村、云南省大理白族自治州大理市洱源县牛街乡福和村、广东省惠州市惠东县白花镇、河南省许昌市、广西百色市靖西市
中国农业大学	安明岳	陈源泉	林州市东姚镇西北泉村、林州市石板岩镇大垴村
中国农业大学	李杨	陈源泉	云南省昆明市石林县圭山镇糯黑村
中国农业大学	郑汀蕾	苏琳	湖北省孝感市孝昌县小悟乡、上海市崇明区城桥镇、江苏省常州市天宁区景福苑、陕西省西安市西咸新区泾河新城泾干镇
中国农业大学	张奚晨	陈源泉	云南省大理州洱源县
中国农业大学	姚若妍	袁承程	广西贵港市中里乡铁岭村
中国农业大学	杨皓然	陈倩	吉林省长春市公主岭市吉林省农科院
中国农业大学	唐睿航		山东省东营市、河北省保定市、广东省东莞市

中国农业大学	冀冉	廖波	山东省泰安市、济南市、烟台市、济宁市、德州市
中国农业大学	赵天宜	刘建一	北京市门头沟区妙峰山镇担礼村、上苇甸村、岭角村、涧沟村、樱桃沟村、谷山村景区
中国农业大学	颜玮纾	乔玉辉	浙江省衢州市开化县长虹乡
中国农业大学	张思涵	杨晓琳	云南省大理白族自治州洱源县三营镇永胜村菜园村组、云南省大理白族自治州巍山彝族回族自治县南诏镇、云南省大理白族自治州云龙县宝丰乡东山村
中国农业大学	曹景萱		吐鲁番地区吐鲁番市、葡萄沟、托克逊镇
南京农业大学	宋宇坤	孙小雯	江苏省泰州市兴化市、贵州省凯里市麻江县
南京农业大学	张子涵		山东省泰安市泰山区徂徕镇邓家庄，河北省邯郸市永年区小龙马乡何营村，江苏省南通市如东县掘港镇十里墩村，浙江省绍兴市嵊州市三界蒋镇翔联村飞翼农场，浙江省绍兴市嵊州市下相村，安徽省芜湖市繁昌县繁昌镇社区，湖南省株洲市炎陵县
南京农业大学	郭双奇	陈孙禄	河南省洛阳市洛龙区农科路一号洛阳农林科学院
南京农业大学	宋雅婧	陈长青	滁州市来安县施官镇
南京农业大学	陆仲骅		浙江省嘉兴市南湖区
南京农业大学	毕铭桓	孙小雯	辽宁省大连市金普新区华润社区、江苏省常州市武进区嘉泽镇晨山村委、江苏省扬州市邗江区邗上街道、山东省淄博市沂源县张家坡镇、山东省济南市商河县许商街道西常庄村、江苏省南京市江北新区大厂街道扬子第二社区
南京农业大学	杜阳	董淑凯	江苏省南京市革新镇王家边村
南京农业大学	洪金枝	孙小雯	江苏省泰州市兴化市安丰镇
南京农业大学	刘思辰	翁李胜	江苏省泰州市靖江市马桥镇徐周村

2022年春季学期云南大学暑期“三下乡”实践活动项目库
拟立项项目名单

序号	项目名称	项目类别	项目主题	备注
Z2022001	我国国家公园生态产品价值实现路径的观察与研究	社会调查类项目	发展成就观察团	重点项目、自筹经费
Z2022002	彩云之南描绘新画卷	社会调查类项目	发展成就观察团	重点项目
Z2022003	设计下乡——碗窑村人居环境改善提升服务社会实践活动	社会调查类、志愿服务类	乡村振兴	重点项目
Z2022004	曲靖马龙黄土坡红色文旅村低碳乡村振兴示范项目整体性策划与概念性规划设计	社会调查类项目	乡村振兴	重点项目
Z2022005	基于“廊道遗产”视角下的滇越铁路文化研学设计与改造——以云南省红河州腊哈村为例	社会调查类项目	乡村振兴促进团	重点项目
Z2022006	乡村振兴视域下脱贫民族村寨发展困境与治理研究——以勐弄乡龙门古寨为例	社会调查类项目	乡村振兴促进团	重点项目
Z2022007	边疆民族地区农村基层党组织建设在铸牢中华民族共同体意识中的作用研究	社会调查类项目	民族团结实践团	重点项目
Z2022008	大手牵小手“团~团”联动叙科普共话“多样性”促“双减”	志愿服务类项目	“团团陪伴·助力‘双减’”专项活动	重点项目
Z2022009	红色精神助力乡村绿色发展	社会调查类项目	发展成就观察团	重点项目
Z2022010	追光而行——云岭青年重走总书记寻访路线暑期三下乡研学夏令营	社会调查类项目	“牢记总书记教导奋进新征程”专项活动	重点项目
Z2022011	在“云岭之南”书写乡村振兴新篇章——基于红河金平县和昆明呈贡区的乡村振兴理论宣讲	理论宣讲类项目	“青春百团助力百村”服务乡村振兴专项活动	重点项目
Z2022012	“运动德宏”品牌打造下民族传统体育助推乡村振兴新征程 — 以芒市三台乡的民族 传统体育为例	社会调查类项目	乡村振兴促进团	重点项目
Z2022013	三下乡-共赴心生-先心病救助志愿服务	社会调查类项目	乡村振兴促进团	重点项目
Z2022014	红军长征过云南—红色资源开发利用调查（中线）	社会调查类项目	党史学习教育团	重点项目
Z2022015	走进乡土乡村 致力乡村振兴	社会调查类项目	乡村振兴促进团	重点项目
Z2022016	云南大学文学院“推普助力乡村振兴”小分队	理论宣讲类项目	通用语言文字普及实践团	重点项目
Y2022017	薪火相传，接力成长—西南民族地区《习近平与大学生朋友们》思想宣讲	理论宣讲类项目	理论普及宣讲团	
Y2022018	一村一站：国家通用语言助力少数民族村落文化振兴的构想与实践	志愿服务类项目	通用语言文字普及实践团	
Y2022019	共青团成立100周年下公众对“团史”的了解情况调查	社会调查类项目	“牢记总书记教导 奋进新征程”专项活动	
Y2022020	传承红色基因-实地走访调查东川区红色路线	社会调查类项目	党史学习教育团	
Y2022021	走进大墨雨村—用化学之笔为儿童世界添彩	志愿服务类项目	“青春百团助力百村”服务乡村振兴专项活动	

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Y2022022	寻“洲”迹，向“亮”行—发掘杨善洲精神中的青年大学生成才激励元素	社会调查类项目	“牢记总书记教导 奋进新征程”专项活动	
Y2022023	党的十八大后东北老工业基地所在城市的转型与发展——以黑龙江省哈尔滨市为例	志愿服务类项目	乡村振兴促进团	自筹经费
Y2022024	多视角、多元化发展禁毒宣传	志愿服务类项目	禁毒防艾实践宣讲团	
Y2022025	密语添颜——云南少数民族天然植物色素的开发与应用	社会调查类项目	乡村振兴促进团	自筹经费
Y2022026	沐浴祖国花朵——中学生素质培养	社会调查类项目	通用语言文字普及实践团	
Y2022027	农村职业教育助力乡村振兴调查研究——以红河州建水、开远两地为例	社会调查类	乡村振兴促进团	
Y2022028	科技赋能“乡村振兴”——云南省欠发达地区农业技术缺口与填补路径分析	社会调查类	乡村振兴促进团	
Y2022029	从易地扶贫到易地致富——基于多元帮扶的鲁甸定制化致富模式调查	社会调查类	乡村振兴促进团	
Y2022030	常态化疫情防控下旅游经济发展动能与模式创新研究----以大理白族自治州为例	社会调查类	乡村振兴促进团	
Y2022031	云南少数民族地区乡村振兴模式比较研究—以楚雄彝族自治州为例	社会调查类	乡村振兴促进团	
Y2022032	“果实奇遇”——基于朝鲜蓟种植及销售产业市场调查研究	社会调查类	乡村振兴促进团	
Y2022033	乡村振兴背景下广南县高原特色现代农业调查研究	社会调查类	乡村振兴促进团	
Y2022034	让幸福的地方更幸福——探究腾冲“司莫拉”村民族团结发展新路径	社会调查类	民族团结实践	
Y2022035	传承红色基因，喜迎党的二十大——探寻建校百年云南大学党员教师红色印迹	理论宣讲类	党史学习教育团	
Y2022036	云南青年发展型城市建设试点面临的难点与突破路径	社会调查类	发展成就观察团	
Y2022037	乡村振兴路径研究--以红塔区孙井社区为例	社会调查类	乡村振兴促进团	
Y2022038	一隅花溪 山屿万乐	社会调查类	乡村振兴促进团	自筹经费
Y2022039	针对云南省绿色食品牌建设及绿色溢价情况进行调查研究——以宾川县葡萄产业为例	社会调查类	发展成就观察团	自筹经费
Y2022040	你的房子“绿”了吗？—基于云南省各高校学生对绿色住宅支付意愿的调查	社会调查类	发展成就观察团	自筹经费
Y2022041	以会泽梨园扩红基地为中心的多方位党史学习调研	社会调查类	党史学习教育团	自筹经费
Y2022042	寻活非遗滇派内画	社会调查类	发展成就观察团	自筹经费

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Y2022043	后疫情时代下的西双版纳旅游业发展情况调研	社会调查类	发展成就观察团	自筹经费
Y2022044	疫情之下难以看到的角落：大理市社会边缘人群生存状态调查	社会调查类	发展成就观察团	自筹经费
Y2022045	致敬百年华章 勇担时代使命：党史宣讲活动	理论宣讲类	党史学习教育团	自筹经费
Y2022046	特色产业集聚与数字化破局：基于石屏县豆腐产业的调查研究	社会调查类	发展成就观察团	自筹经费
Y2022047	推动“民航强省”背景下云南地州机场的效益情况和存在问题研究—以西双版纳嘎洒机场和文山砚山机场为例	社会调查类	发展成就观察团	自筹经费
Y2022048	寻根问祖——乡村振兴背景下运城根祖文旅产业发展考察	社会调查类	乡村振兴促进团	自筹经费
Y2022049	乡村振兴战略背景下新农村环境和产业建设调查研究	社会调查类	乡村振兴促进团	自筹经费
Y2022050	乡村振兴背景下会泽县特色产业数字化融合发展研究	社会调查类	乡村振兴促进团	自筹经费
Y2022051	招云行动——护好就业路，奋进新征程	理论宣讲类	理论普及宣讲团	自筹经费
Y2022052	乡村振兴背景下东川农旅融合发展研究	社会调查类	乡村振兴促进团	自筹经费
Y2022053	打好世界一流“绿色食品牌”背景下保山咖啡绿色溢价研究	社会调查类	发展成就观察团	自筹经费
Y2022054	深挖掘“智慧”个性化的民族村寨景点—以石林县矣美堵为例	社会调查类	乡村振兴促进团	自筹经费
Y2022055	“万企进万村”，共绘振兴新图景—对“秦岭八村”乡村振兴示范村的调研和分析	社会调查类	乡村振兴促进团	自筹经费
Y2022056	乌蒙磅礴赫赫名，红军长征漫漫路——红军在滇足迹的开发、保护和利用	社会调查类	党史学习教育团	自筹经费
Y2022057	讲好党史故事，深种红色种子—“党史第一在井冈山”	社会调查类	党史学习教育团	自筹经费
Y2022058	勺窝之旅——穿青人村落发展现状调查与乡村振兴帮扶	社会调查类	乡村振兴促进团	自筹经费
Y2022059	乡村振兴背景下农业生猪保险发展研究——以云南省保山市为例	社会调查类	乡村振兴促进团	自筹经费
Y2022060	绘画艺术治疗——老年人心理健康干预项目以虹山东路社区为例	志愿服务类项目	乡村振兴促进团	
Y2022061	云南大学“蓝花楹”暑期实践队	志愿服务类项目	“团团陪伴·助力‘双减’”专项活动	
Y2022062	立夏社区党史学习教育理论宣讲	理论宣讲类项目	党史学习教育团	
Y2022063	民族团结视域下傣族传统舞蹈文化的传播与发展	社会调查类项目	民族团结实践团（以铸牢中华民族共同体意识为主题的其他实践活动）	
Y2022064	新源学校美育小课堂	志愿服务类项目	乡村振兴促进团	
Y2022065	红色理论普及及宣讲	理论宣讲类项目	理论普及宣讲团	
Y2022066	时代长征路，青春伴国行——寻甸红军长征柯渡纪念馆党史理论宣讲	理论宣讲类项目	党史学习教育团	

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Y2022067	红色“火种”燃我心--云南糯黑村和南昌八一起义纪念馆党史宣讲实践	志愿服务类项目	党史学习教育团	
Y2022068	云南省大理白族自治州宾川县“赶马调”调查研究	社会调查类项目	乡村振兴促进团	
Y2022069	云南大学助力临沧市临翔区乡村振兴的路径、方法、实效调查研究	社会调查类项目	乡村振兴促进团	
Y2022070	丽江市白沙古镇东巴文象形字传承与保护及发展现状调查研究	社会调查类项目	H.共青团云南省委专项活动	
Y2022071	多主体治理下的美丽乡村发展路径——以万溪冲为例	社会调查类一般项目	乡村振兴促进	
Y2022072	乡村振兴青创中心----嘎洒乡村振兴发展	志愿服务类一般项目	乡村振兴促进	
Y2022073	青春不毒行，防艾不容缓	理论宣讲类项目	禁毒防艾实践宣讲团	
Y2022074	防灾减灾——洪涝下文山州丘北县的烟草受灾情况调研	社会调查类	乡村振兴促进	
Y2022075	聚焦发展成就，感受祖国发展变化	社会调查类项目	发展成就观察团	自筹经费
Y2022076	传统风筝的调查与发展——以滇式风筝为例	社会调查类一般项目	发展成就观察团	
Y2022077	基于乡村振兴背景下抚仙湖的绿色发展之路研究	社会调查类项目	乡村振兴促进团	
Y2022078	禁毒防艾实践宣讲	社会调查类	禁毒防艾实践宣讲团	
Y2022079	基于云南省稀土资源现状的可持续发展对策调查研究	社会调查类一般项目	发展成就	
Y2022080	“追光者”—云南省稀土发光材料产业现状和发展路径调查研究	社会调查类一般项目	发展成就	
Y2022081	西双版纳民族红色文化艺术转化探索	社会调查类项目	民族团结实践团（以铸牢中华民族共同体意识为主题的其他实践活动）	
Y2022082	关于全国最大异地扶贫搬迁现状调查报告——基于鲁甸县卯家湾为例	社会调查类项目	乡村振兴促进团	
Y2022083	追溯红色记忆推进党史学习——鱼山新村社区宣传党的百年征程	社会调查类项目	党史学习教育团	
Y2022084	绿春县乡村振兴视野下的特色农产品开发实践调研	社会调查类项目	发展成就观察团	
Y2022085	屋脊上的守望者——瓦猫传统文化传承社会调研	社会调查类项目	发展成就观察团	
Y2022086	探究非遗背后的文化底蕴——非遗滇剧传承现状调查研究	社会调查类项目	发展成就观察团	
Y2022087	脱贫攻坚电商助农特色产业调研：以湖南省祁东县为例	社会调查类项目	发展成就观察团	
Y2022088	“艺”路有青年，助力乡村振兴	志愿服务类项目	乡村振兴促进团	
Y2022089	小树苗志愿服务团队	志愿服务类项目	发展成就观察团	
Y2022090	践行美育支教，助力乡村振兴	志愿服务类项目	乡村振兴促进团	
Y2022091	地理归乡：点燃地理兴趣，培养家国情怀	志愿服务类项目	“团团陪伴·助力‘双减’”专项活动	

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Y2022092	“览峥嵘革命历史，弘扬红色革命精神”党史学习之旅	社会调查类项目	党史学习教育团	
Y2022093	多元化禁毒防艾宣讲	理论宣讲类项目	禁毒防艾实践宣讲团	
Y2022094	乡村振兴背景下民族村寨文旅融合发展研究——以罗溪瑶族乡为例	社会调查类项目	乡村振兴促进团	
Y2022095	“蕴势腾飞”—油城大庆转型升级发展光明之路探寻	社会调查类项目	发展成就观察团	自筹经费
Y2022096	根植传统文化，助力乡村振兴——城市化背景下洱海流域传统文化的传承与发展调查研究	社会调查类项目	乡村振兴促进团	
Y2022097	助推乡村振兴和地震灾害防治——以昭通鲁甸县为调研对象	社会调查类项目	乡村振兴促进团	
Y2022098	昆明红色记忆——昆明党史理论宣讲学习教育	理论宣讲类项目	理论普及宣讲团	
Y2022099	无价之保·无人机植保技术推广现状调研——以云南省特色作物为例	社会调查类项目	乡村振兴促进团	
Y2022100	“黑土长歌”—纵观大庆市林甸县环境治理与生态旅游发展建设的不凡征程	社会调查类项目	发展成就观察团	自筹经费
Y2022101	“深入基层，饮水思源”党史学习教育团	理论宣讲类项目	党史学习教育团	
Y2022102	普法手段铸牢中华民族共同体意识实证调研——以大理白族自治州的庭审普法为例	社会调查类项目	民族团结实践团（以铸牢中华民族共同体意识为主题的其他实践活动）	
Y2022103	蒙以养正——云南大学暑期三下乡之晋宁区未成年人保护宣讲服务活动	理论宣讲类项目	理论普及宣讲团	
Y2022104	大理湾桥镇地区古迹古寺维护	志愿服务类项目	乡村振兴促进团	
Y2022105	乡村振兴背景下以非遗产品所形成的集体经济的发展研究——以呈贡区七 步场臭豆腐为例	社会调查类项目	乡村振兴促进团	
Y2022106	桃李金果，红星熠熠——以恩施桃李溪村为例，探索和研究乡村旅游产业	社会调查类项目	乡村振兴促进团	
Y2022107	链链有茶——乡村振兴背景下云南省临沧市云县茶产业高质量发展路径初探	社会调查类项目	乡村振兴促进团	
Y2022108	乡镇基层治理的法治化路径研究—以勐罕司法所为中心	社会调查类项目	发展成就观察团	
Y2022109	乡村振兴背景下禄丰市乡镇发展现状调查	社会调查类项目	乡村振兴促进团	
Y2022110	“送法下乡，助力乡村振兴”共享农庄调查研究	社会调查类项目	乡村振兴促进团	
Y2022111	国家治理下的司法治理经验——以昆明市基层法院治理财产刑“执行难”为例	社会调查类项目	发展成就观察团	
Y2022112	民族地区中学生共同体意识认知及培育的田野实验研究	社会调查类项目	民族团结实践团（以铸牢中华民族共同体意识为主题的其他实践活动）	自筹经费
Y2022113	乡村振兴战略中，乡村教师的作为	社会调查类项目	乡村振兴促进团	自筹经费
Y2022114	绽放科学之美，助力“双减”落实	志愿服务类项目	“团团陪伴·助力‘双减’”专项活动	
Y2022115	宣传教育对于农户管理外来入侵物种草地贪夜蛾的认知与管理作用	社会调查类项目	乡村振兴促进团	

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Y2022116	美丽乡村建设——就云南省宣威市宝山镇河长制、农村水污染及水环境监测现状调查研究	社会调查类项目	乡村振兴促进	
Y2022117	独龙江乡农户生计方式对家庭能源消费结构的影响	社会调查类项目	发展成就观察	
Y2022118	澄江市抚仙湖流域农户生计现状及生态保护情况的相关影响研究	社会调查类项目	乡村振兴促进团	
Y2022119	昆明市居民垃圾分类行为影响因素研究	社会调查类项目	发展成就观察团	
Y2022120	“保护母亲河 青春助力云南生态文明建设”主题宣讲——重走习近平总书记云南省考察线路	理论宣讲类项目	“牢记总书记教导 奋进新征程”专项活动	
Y2022121	大理州宾川县葡萄种植产业发展调查	社会调查类项目	发展成就观察团	
Y2022122	大理市巍山县巍宝山乡农村污水治理现状调查	社会调查类项目	乡村振兴促进团	
Y2022123	西双版纳三十年老龄茶园提取研究	社会调查类项目	乡村振兴促进团	
Y2022124	搬得出，稳得住 看易地扶贫搬迁的“云南奇迹”——昭通易地扶贫搬迁人员后续就业落实情况调研	社会调查类	发展成就观察	
Y2022125	开平碉楼下的党史学习和乡村振兴	社会调查类	党史学习教育	
Y2022126	乡村振兴背景下对东营黄河滩区房台村迁居的调查研究——以杨庙、龙口社区为例	社会调查类	乡村振兴	
Y2022127	聚焦农村 永远跟党走 党史教育进乡村	理论宣讲类	理论普及及宣讲 党史学习教育	
Y2022128	昆明市禄劝彝族苗族自治县乡村人居环境调研	社会调查类	乡村振兴	
Y2022129	银生古城乡村振兴实践团——基于银生古茶产业的全过程调研	社会调查类	乡村振兴	
Y2022130	“富源情·中国梦”	社会调查类	党史学习教育	
Y2022131	弘扬百年建党精神，学习云南红色人物	理论宣讲类	党史学习教育	
Y2022132	易地扶贫搬迁形成的多民族互嵌区治理研究——基于云南省新平县曼哈社区的个案分析	社会调查类项目	民族团结实践团（以铸牢中华民族共同体意识为主题的其他实践活动）	
Y2022133	基层社区治理体系和治理能力现代化的新模式——基于重庆市荣昌万灵社区“小院”模式的个案研究	理论宣讲类项目	乡村振兴促进团	
Y2022134	产业视角下乡村振兴与脱贫攻坚衔接的实现路径研究——以曲靖市大庄乡为例	社会调查类项目	乡村振兴促进团	
Y2022135	一心跟党走，“二德”育仪人	理论宣讲类项目	党史学习教育团	
Y2022136	甘肃乡村振兴重点帮扶县发展成果考察及红色理论宣讲	社会调查类项目	理论普及宣讲团	
Y2022137	农旅融合背景下普者黑村传统价值观的嬗变研究	理论宣讲类项目	发展成就观察团	
Y2022138	感西畴精神，助乡村振兴：后脱贫时代杉树坪发展状况调查研究	社会调查类项目	发展成就观察团	

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Y2022139	“土家史歌”—乡村振兴背景下土家族织锦数字化保护研究	社会调查类项目	乡村振兴促进团	
Y2022140	聚焦鼎新，文化赋能——地扪生态博物馆赋能乡村振兴	社会调查类项目	乡村振兴促进团	
Y2022141	民族交往交流交融视角下茶马古道历史文献整理研究——以临沧地区为例	社会调查类项目	民族团结实践团（以铸牢中华民族共同体意识为主题的其他实践活动）	
Y2022142	大理祥云红色文化助力中华民族共同体意识培育的实践路径研究	社会调查类项目	民族团结实践团（内地新疆、西藏籍大学生开展“民族团结我践行”社会实践活动	
Y2022143	讲好云南革命故事，争做红色传人——面向查尼皮红色文化的党史传播	理论宣讲类项目	理论普及宣讲团	
Y2022144	沿着总书记乡愁足迹：云南大理乡愁赋能乡村振兴的现状及路径研究	社会调查类项目	发展成就观察团	
Y2022145	云南省巧家县白鹤滩水电站大寨镇居民易地搬迁后续扶持工作实践研究	社会调查类项目	发展成就观察团	
Y2022146	乡村振兴战略视域下乡风传承的“云南经验”研究——基于云南省曲靖市三村实证调查	社会调查类项目	乡村振兴促进团	
Y2022147	乡村振兴视域下乡贤文化对民族地区的价值与实践路径研究——以思茅港镇少数民族村落为例	社会调查类项目	乡村振兴促进团	
Y2022148	花田喜事——横州市茉莉花产业发展调查研究	社会调查类项目	乡村振兴促进团	
Y2022149	洱源唢呐艺术的发展现状和传承保护研究	社会调查类项目	发展成就观察团	
Y2022150	云南民族村展演对促进民族“三交”与铸牢中华民族共同体意识的意义研究	社会调查类项目	民族团结实践团（以铸牢中华民族共同体意识为主题的其他实践活动）	
Y2022151	昆明市西山区富善社区草莓特色小镇的产业选择及发展路径研究	社会调查类项目	发展成就观察团	
Y2022152	“学党史，明实情”——民族地区医疗卫生事业发展现状汇报暨党史宣讲活	理论宣讲类项目	党史学习教育团	
Y2022153	文化传承与市场创新——鹤庆县银器的电子市场化历程	社会调查类项目	发展成就观察团	
Y2022154	寻访红色记忆，走入缅宁“红色小井冈”——平村红色革命历史调查与教育实践	社会调查类项目	党史学习教育团	
Y2022155	铸牢中华民族共同体意识的小凉山经验研究	社会调查类项目	民族团结实践团（以铸牢中华民族共同体意识为主题的其他实践活动）	
Y2022156	我生病了吗？血液病患者的疾病叙事	社会调查类项目	发展成就观察团	
Y2022157	共同富裕视域下云南现代化边境小康村的实证研究——以石笋村为例	社会调查类项目	发展成就观察团	
Y2022158	内生动力、社会资本与乡村发展：基于怒江丙中洛扎那桶村的调查研究	社会调查类项目	发展成就观察团	
Y2022159	乡村振兴视域下产业发展和生态实践融合推进的会泽模式探析	社会调查类项目	乡村振兴促进	
Y2022160	脱贫与赋能：易地扶贫搬迁“新移民”的二十年———基于永仁县查利么村 的调查	社会调查类项目	乡村振兴促进	

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Y2022161	乡村振兴背景下智慧农村数字建设发展路径研究——以云南省昆明市禄劝彝族苗族自治县转龙镇为例	社会调查类项目	乡村振兴促进团	
Y2022162	巩固脱贫成果，振兴赤水之都 ——关于昭通镇雄县以竹产业为依托的乡村振兴之路探索	社会调查类项目	乡村振兴促进团	
Y2022163	蒙自市乡村振兴与新时代文明站建设观察服务学习	志愿服务类项目	乡村振兴促进团	
Y2022164	脱贫攻坚与乡村振兴有效衔接路径调查——以原国家级贫困县屏边苗族自治县猕猴桃产业为例	志愿服务类项目	乡村振兴促进团	
Y2022165	新时代下生态公园建设与旅游资源可持续发展探究——以河南省为例	社会调查类项目	发展成就观察团	
Y2022166	重走红军长征路，缅怀先烈温初心	社会调查类项目	党史学习教育团	
Y2022167	乡村振兴战略背景下红色旅游的开发研究——以丽江雄古村为例	社会调查类项目	乡村振兴促进团	
Y2022168	文旅融合视角下少数民族民俗文化促进旅游可持续发展研究 ——以大理甸南镇狮河村为例	社会调查类项目	发展成就观察团	
Y2022169	香格里拉市旅游规划扶贫示范村脱贫效应研究	社会调查类项目	发展成就观察团	
Y2022170	基于数智化产地仓的高原水果产业供应链管理模式的优化 研究——以云南孟连牛油果为示范	社会调查类项目	乡村振兴	
Y2022171	“乡残励” ——少数民族地区重点帮扶县残疾人励志自主创业调研	社会调查类项目	乡村振兴促进团	
Y2022172	后疫情时代背景下的文旅再生推广助力乡村振兴 ——基于重庆“鬼城”丰都非物质文化遗产开发现状及推广办法调研	社会调查类项目	乡村振兴促进团	
Y2022173	乡村振兴视域下查尼皮村红色旅游资源开发的现状 、困境及对策研究	社会调查类项目	乡村振兴促进团	
Y2022174	“双减”背景下义务教育阶段家长教育焦虑的影响因素研究	社会调查类项目	“团团陪伴·助力‘双减’”专项活动	
Y2022175	乡村振兴背景下民族旅游村寨环境治理能力提升研究 —— 以基诺山寨为例	社会调查类	乡村振兴促进团	
Y2022176	农文旅融合助力乡村产业振兴 ——以宾川县新庄红军村为例	社会调查类项目	发展成就观察团	
Y2022177	山东省乡村振兴战略规 （2018-2022年）》实施成果及农民满意调-以山东省济南市归德镇为例	社会调查类项目	乡村振兴促进团	自筹经费
Y2022178	斗南地区高水平劳动力现状调研及分析	社会调查类项目	发展成就观察团	自筹经费
Y2022179	唐山红色学习小组	理论宣讲类	党史学习教育团	自筹经费
Y2022180	踏寻红色足迹，奋进新时代征程——勤丰镇红色教育基地实践学习	社会调查类	党史学习教育团	自筹经费
Y2022181	关于四川省雅安市余家村生态旅游业发展情况调研	社会调查类项目	乡村振兴促进团	自筹经费
Y2022182	以青春之力，探果产之业，助白乡发展	社会调查类项目	发展成就观察团	

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Y2022183	青春不"毒"行，防艾不容缓	理论宣讲类项目	禁毒防艾实践宣讲团	
Y2022184	紧跟新时代，青年与乡行——宣威六中夏日成长计划	志愿服务类项目	乡村振兴促进团	
Y2022185	跟着非遗的足迹寻求传承的方法	社会调查类	民族团结实践	
Y2022186	云上青年志愿者禁毒防艾宣传教育项目	志愿服务类项目	禁毒防艾实践宣讲团	
Y2022187	红丝带宣讲	理论宣讲类项目	禁毒防艾实践宣讲团	
Y2022188	品百年风雨砥砺 铸中华民族之魂——多民族自治县红色铸魂之旅	理论宣讲类项目	党史学习教育团	
Y2022189	探索高山乌龙茶产业发展“密钥”，寻求茶叶发展新路径—以云南腾冲市为例	社会调查类项目	乡村振兴促进团	
Y2022190	互嵌与耦合:高原湖泊治理的生态效益生成机制与转化路径调研——基于中国生态修复典型案例“抚仙湖流域治理”的成果观察	社会调查类项目	发展成就观察团	
Y2022191	乡村振兴背景下少数民族非遗创新成果的知识产权保护–以云南彝绣为例	社会调查类项目	乡村振兴促进团	
Y2022192	云守银发–人口老龄化形势下的养老服务中心新发展趋势研究	志愿服务类项目	发展成就观察团	
Y2022193	美丽家园促进乡村振兴—新农村卫生生活体系问题调研	社会调查类项目	乡村振兴促进团	
Y2022194	规划•治理•价值：生态视域下民族地区人与自然和谐共生现代化实践研究——以大理新洲村和才村为例	社会调查类项目	发展成就观察团	
Y2022195	咖农咖啡豆加工处理工艺调查及污染现状分析——以云南省普洱市为例	社会调查类项目	乡村振兴促进团	
Y2022196	乡村振兴背景下当地发展现状及生态产业赋能乡村振兴状况调研——以云南曲靖、西双版纳、红河州为例	社会调查类项目	发展成就观察团	
Y2022197	朴门永续理念下城郊乡村休闲农业规划与可行性探讨——以昆明市西山区大墨雨村为例	社会调查类项目	乡村振兴促进团	
Y2022198	玉溪市峨山县大龙潭中心小学“青春有你，绝不毒行”	理论宣讲类项目	禁毒防艾实践宣讲团	
Y2022199	“爱驻夕阳，从心开始”——云南玉溪、云南昆明、山西临汾、福建福州四地针对老年人群的爱心服务活动	志愿服务类项目	乡村振兴促进团	
Y2022200	天麻种植对镇雄县乡村人才回流的效用调查与研究——基于乌蒙山区云南贫困县空心化现状	社会调查类项目	乡村振兴促进团	
Y2022201	乡村振兴背景下对曲靖市富源县红色遗迹的调查研究——以黄泥河镇铁锁箐村为例	社会调查类项目	乡村振兴促进团	
Y2022202	“肖”遥游黔——以贵州省毕节市为中心开展的十二生肖街道文化调查与乡村振兴帮扶	社会调查类项目	乡村振兴促进团	
Y2022203	“同心同力学党史·同向同行践使命”，党史中的“云南印象”——曲靖三元宫	社会调查类项目	党史学习教育团	

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Y2022204	乡村振兴背景下农村宜居环境调研—以河北省三市为例	社会调查类项目	乡村振兴促进团	
Y2022205	“乡村振兴尤奇丽，苹果花开雀舌香”——助农扶贫视角下对栖霞县烟台苹果的种植现状及发展路径研究	社会调查类项目	乡村振兴促进团	
Y2022206	“走进红色基地，传递红色信念”——以云南省罗平县钟山革命老区为例	社会调查类项目	党史学习教育团	
Y2022207	“我家巍山美”—乡村振兴背景下巍山县美丽乡村建设调研	社会调查类项目	发展成就观察团	
Y2022208	产教融合赋能乡村振兴——以云南大学抚仙湖帆船基地为例	志愿服务类项目	乡村振兴促进团	
Y2022209	文体活动进寨下乡	志愿服务类项目	“青春百团助力百村”服务乡村振兴专项活动	
Y2022210	“健康中国”视域下体育三下乡增进民族村寨活力建设---以芒市三台山德昂族乡健身服务为例	志愿服务类项目	乡村振兴促进团	
Y2022211	《“喜迎二十大”辽中南地区群众体育文化活动调查研究》	社会调查类项目	发展成就观察团	
Y2022212	聚焦“三农”问题，情系乡村振兴，践行时代使命	理论宣讲类项目	乡村振兴促进团	
Y2022213	乡村振兴背景下民族地区农村产业发展现状及对策研究——以云南省楚雄市大过口乡为调查对象	社会调查类项目	乡村振兴促进团	
Y2022214	“为有担当多壮志，乡村振兴正当时“——疫情防控背景下协助村委会工作助力乡村振兴	志愿服务类项目	乡村振兴促进团	
Y2022215	“夕阳红” ——少数民族地区重点帮扶县老龄人口致贫原因和养老现状调研	社会调查类项目	发展成就观察团	自筹经费
Y2022216	探乡村振兴成果，听脱贫攻坚故事	社会调查类项目	发展成就观察团	自筹经费
Y2022217	艺术人才助力乡村振兴——以双廊《农民画社》为例	社会调查类项目	通用语言文字普及实践团	
Y2022218	枣乡枣香——基于柳林红枣产业成功经验助力襄汾官滩村乡村振兴研究	社会调查类项目	乡村振兴促进团	
Y2022219	普洱市咖农咖啡豆销售渠道调查及咖啡“豆贱伤农”现状分析——探寻普洱乡村振兴新模式	社会调查类项目	乡村振兴促进团	
Y2022220	新发展理念下乡镇花卉产业的转型模式研究——以“斗南花卉小镇”为例	社会调查类项目	发展成就观察团	
Y2022221	把云岭特色鱼资源优势转化为经济优势的可行性方法调研与实践探索——以软鳍新光唇鱼和金鳞鱼为例	社会调查类项目	乡村振兴促进团	
Y2022222	晋宁福安，以约共治：乡村振兴视野下基层治理新路径的探索	社会调查类项目	乡村振兴促进团	
Y2022223	“云上普洱”何以飘香——以西双版纳傣族自治州易武乡茶叶生产、销售现状为例	社会调查类项目	乡村振兴促进团	
Y2022224	中国十佳小康村——皇城村“乡村振兴背景下推进新农村建设范例调研	社会调查类项目	发展成就观察团	
Y2022225	大众媒体对文旅特色小镇的国际形象发展影响路径研究——以云南普者黑为例	社会调查类项目	发展成就观察团	
Y2022226	研宾川果 传白乡宝	社会调查类项目	发展成就观察团	

序号	项目名称	项目类别	项目主题	备注
Y2022227	减负担，句句入耳，规范字，行行入目。——“双减”政策下云南石林糯黑村的普通话宣传与规范字的使用推广服务	志愿服务类项目	通用语言文字普及实践团	
Y2022228	喜迎二十大：乡村振兴背景下弘扬党的精神和思想——以云南省曲靖市会泽县“乡村振兴，教育先行”为例	志愿服务类项目	乡村振兴促进团	
Y2022229	特色产业助力美丽新农村建设研究 —以雷洞村为例	社会调查类项目	发展成就观察团	
Y2022230	浙江新农村·乡村振兴模式社会情况调研	社会调查类项目	乡村振兴促进团	
Y2022231	阳光心情·延爱互动·希望田野	志愿服务类项目	乡村振兴促进	
Y2022232	乡村振兴在东北农业村落中的实践发展——以黑龙江省鹤岗市新华镇村落为例	社会调查类	乡村振兴促进	
Y2022233	地域文化助力居民文化自信的路径探究——基于四省四地文化典型的社会调查	社会调查类项目	发展成就观察团	
Y2022234	族际通婚与蒙汉双语习得的相关性调查—以内蒙古鄂尔多斯市为例	社会调查类项目	民族团结实践团（以铸牢中华民族共同体意识为主题的其他实践活动）	
Y2022235	入四省四地乡镇，颂红色青春精神——习近平同志《论党的青年工作》理论宣讲	理论宣讲类项目	理论普及宣讲团	
Y2022236	民族团结视角下的“访惠聚”	社会调查类项目	民族团结实践团（内地新疆、西藏籍大学生开展“民族团结我践行”社会实践活动	
Y2022237	阿拉彝族乡的经济发展成就观察	社会调查类项目	发展成就观察团	
Y2022238	铸牢中华民族共同体意识视域下边疆民族地区基础教育在国家认同培育中的作用机制	社会调查类项目	民族团结实践团（以铸牢中华民族共同体意识为主题的其他实践活动）	
Y2022239	乡村振兴下的昆明沙朗大村文化遗址保护与新发展调研	社会调查类项目	乡村振兴促进团	
Y2022240	一心许湘，传承“湘红”基因：云大青年暑期花垣县红色湘西党史教育调研活动	社会调查类项目	党史学习教育团	
Y2022241	“菌”子之交——易门县生态保护下的可持续发展乡村振兴产业调研	社会调查类项目	乡村振兴促进团	
Y2022242	昆明市呈贡区菱角编制非遗的传承保护与资源转化研究	社会调查类项目	乡村振兴促进团	
Y2022243	赋能乡村医生，服务乡村患者——数字医疗助推云南省边疆民族乡村地区医疗卫生健康事业发展	社会调查类项目	乡村振兴促进团	
Y2022244	“安'参'为乐”实地考察活动	社会调查类项目	乡村振兴促进团	
Y2022245	追寻习总书记云南之行足迹 见证云岭高质量发展新篇章	社会调查类项目	“牢记总书记教导 奋进新征程”专项活动	
Y2022246	携党史之光走进乡镇中学——运用新媒介技术在宣威市热水镇第三中学开展党史学习教育	志愿服务类项目	党史学习教育团	
Y2022247	昭阳区苹果产业科技助农成效调研	社会调查类项目	乡村振兴促进团	

序号	项目名称	项目类别	项目主题	备注
Y2022248	乡愁视域下传统村落文化景观的保护与发展研究——以昆明市乐居村为例	社会调查类项目	乡村振兴促进	
Y2022249	乡村振兴背景下云南农村健康传播活动现状与效果研究	社会调查类项目	乡村振兴促进团	
Y2022250	“学眼看云南，在昆解乡愁”在地情境下云上乡愁书院的文化实践与关系建构研究	社会调查类项目	发展成就观察团	
Y2022251	执“党建”之笔 绘就乡村“只此青绿”——云南五县十二地“厕所革命”成就考察记录	社会调查类项目	发展成就观察团	
Y2022252	新媒体时代下少数民族地区的政策传播情况调查——以勐腊县人民对中国-东盟援助的了解情况为例	社会调查类项目	乡村振兴促进团	
Y2022253	玉溪市峨山县大龙潭中心小学“禁毒有我，绝不毒行”主题理论宣讲	理论宣讲类项目	禁毒防艾实践宣讲团	
Y2022254	《情牵彝乡，共建美好家园》——“铸牢中华民族共同体意识”的青春行动方案	理论宣讲类项目	民族团结实践团（以铸牢中华民族共同体意识为主题的其他实践活动）	
Y2022255	禁毒防艾有你有我，刻不容缓	理论宣讲类项目	禁毒防艾实践宣讲团	
Y2022256	乡村振兴-关于农村垃圾处理现状调研	社会调查类项目	乡村振兴促进团	
Y2022257	乡村振兴背景下昆明市发展壮大村集体经济的对策方案	社会调查类项目	乡村振兴促进团	
Y2022258	基础设施辐射能力的实证研究——以镇雄县以勒高铁站为例	社会调查类项目	乡村振兴促进团	
Y2022259	本地区养老模式研究	社会调查类项目	乡村振兴促进团	
Y2022260	云南贫困民族地区返贫风险调查及“阻断”策略研究—以5个国家乡村振兴重点帮扶县为例	社会调查类项目	乡村振兴促进团	
Y2022261	爱心导医——助力人民共享发展成果	志愿服务类项目	发展成就观察团	
Y2022262	浙江大学、云南大学暑期联合调研项目——基于浙江省和云南省的地域经济优势互补	社会调查类项目	乡村振兴促进团	
Y2022263	铸牢中华民族共同体意识视域下边疆地区乡村治理与产业振兴实践调查研究	社会调查类项目	发展成就观察团	
Y2022264	乡村振兴视阈下民族地区文明新形态转型探析—基于重庆市少数民族地区调研与思考	社会调查类项目	乡村振兴促进团	
Y2022265	基层党建引领民族地区乡村治理的实践研究——以云南省禄劝县撒营盘镇为例	社会调查类项目	乡村振兴促进团	
Y2022266	“民族走廊”中的民族交往交流交融实践研究——以宁洱茶马古道为例	社会调查类项目	民族团结实践团（以铸牢中华民族共同体意识为主题的其他实践活动）	
Y2022267	“渝”说红色经典，“重”燃红色之火：红色旅游景点群的“根植性”及其效应研究——以白公馆和渣滓洞为例	社会调查类项目	党史学习教育团	自筹经费
Y2022268	“巧木生金”：大理白族特色工艺助力乡村产业振兴——以剑川县狮河村木雕产业发展为例	社会调查类项目	乡村振兴促进团	
Y2022269	滇东黑尔壮族村的民族产业振兴实践研究	社会调查类项目	乡村振兴促进团	

序号	项目名称	项目类别	项目主题	备注
Y2022270	滇藏茶马古道：铸牢中华民族共同体意识的民族交往交流交融历史调查	社会调查类项目	民族团结实践团（以铸牢中华民族共同体意识为主题的其他实践活动）	
Y2022271	稻梦人生—云南元江野生稻资源与民众保护意识调查	社会调查类项目	乡村振兴促进团	
Y2022272	基于乡村振兴战略的食用玫瑰产业研究	社会调查类项目	乡村振兴促进团	
Y2022273	乡村背景下云南保山潞江坝咖啡产业发展现状的调研分析——以赧亢村和丛岗村为例	社会调查类项目	乡村振兴促进团	
Y2022274	关于洱海流域古生片区农村生产生活情况调研	社会调查类项目	乡村振兴促进团	
Y2022275	“云上花卉”—以斗南花市为背景促进花卉增值	社会调查类项目	乡村振兴促进团	
Y2022276	滇西南地区对野生稻利用价值调查	社会调查类项目	乡村振兴促进团	
Y2022277	“心系三农，情暖花开”植物病害防控	志愿服务类项目	乡村振兴促进团	
Y2022278	关于户籍地青年学生对于人才回流看法--基于黑龙江省数字经济政策的访谈研究	社会调查类项目	发展成就观察团	
Y2022279	寻迹绿水青山——关于习近平总书记考察滇池、洱海生态建设的调查研究	社会调查类项目	发展成就观察团	
Y2022280	民族团结之花，春城沃土绽放——“石榴籽”宣讲团	理论宣讲类项目	民族团结实践团（以铸牢中华民族共同体意识为主题的其他实践活动）	
Y2022281	青春心向党，“乡”约河西村	社会调查类项目	乡村振兴促进团	
Y2022282	汉硕助力“双减”，金江逐梦童行	志愿服务类项目	“团团陪伴·助力‘双减’”专项活动	
Y2022283	云南涉藏地区铸牢中华民族共同体意识的形势分析——基于迪庆州香格里拉市的考察	社会调查类项目	民族团结实践团（以铸牢中华民族共同体意识为主题的其他实践活动）	
Y2022284	云南省猴桥口岸在中缅跨境经贸中的作用研究	社会调查类项目	发展成就观察团	
Y2022285	云端情丝牵万里，凉山晨星映满天——云大青协小凉山支教项目	志愿服务类项目	乡村振兴促进团	
Y2022286	云南部分高职院校对新型毒品和艾滋病认知度调查	社会调查类	禁毒防艾实践宣讲团	

“SKSUNNY幸福行动——2022年度大学生社会实践活动”资助实践团队名单

类别	序号	推荐学校名称	团队名称
产业振兴	1	北京科技大学	北京科技大学“星火”之桃蕴乡村振兴实践团
产业振兴	2	北京科技大学	北京科技大学“兴安”社会实践团
产业振兴	3	北京科技大学	北京科技大学晨阳实践团
产业振兴	4	北京科技大学	北京科技大学故渊乡村企业调研实践团
产业振兴	5	北京科技大学	北京科技大学鹿贝柯苹鹿桂昌乡村振兴实践团
产业振兴	6	北京科技大学	北京科技大学鹿贝柯踏鹿荨娲乡村调研实践团
产业振兴	7	北京科技大学	北京科技大学孟子居解兰振乡兴业实践团
产业振兴	8	北京科技大学	北京科技大学孟子居鲁韵儒风乡村振兴实践团
产业振兴	9	北京科技大学	北京科技大学孟子居群空冀北实践团
产业振兴	10	北京科技大学	北京科技大学孟子居霞暖乡情调研助农实践团
产业振兴	11	北京科技大学	北京科技大学孟子居新农科发展鼎薪调研实践团
产业振兴	12	北京科技大学	北京科技大学柒许实践团
产业振兴	13	北京科技大学	北京科技大学星火之粟泽乡村振兴实践团
产业振兴	14	北京科技大学	北京科技大学沂鹿同行电商助农实践团
产业振兴	15	北京理工大学	惠农慧农——经管书院赴通州西集镇社会实践
产业振兴	16	滨州医学院	“访聚产业乡镇，擎画振兴蓝图”
产业振兴	17	滨州医学院	无棣本草香，黄河中药行
产业振兴	18	河北工业大学	星星之火实践团
产业振兴	19	河北工业大学	共创幸福小队
产业振兴	20	河北工业大学	社会主义接班人队
产业振兴	21	河北工业大学	燕赵风骨巡红队
产业振兴	22	河北工业大学	青年担当行动队
产业振兴	23	河北工业大学	“乡约盛夏，艺路前行”实践队
产业振兴	24	河北工业大学	振兴乘风小分队

产业振兴	25	河北工业大学	樱满塘湖队
产业振兴	26	河北工业大学	“直”击人心
产业振兴	27	河北工业大学	塞罕坝红色小分队
产业振兴	28	河北工业大学	阜平红色实践小队
产业振兴	29	济南大学	黄河入海队
产业振兴	30	晋中信息学院	“鹏”抟汇商——淬炼·国际商学院青年益行发展成就观察
产业振兴	31	晋中信息学院	青春筑梦●创响五村——裕农兴业乡村振兴促进团
产业振兴	32	聊城大学	“E农行动“电商助农实践队
产业振兴	33	聊城大学	聊城大学新兴之光乡村振兴调研队
产业振兴	34	临沂大学	“访沂蒙，传初心 ”红色社会实践团
产业振兴	35	临沂大学	“政策送万家”实践队
产业振兴	36	齐鲁医药学院	“一起来摆摊呀”
产业振兴	37	青岛酒店管理职业技术学院	“益旅阳光”乡村振兴服务队
产业振兴	38	青岛理工大学	“乡海逐梦”——青岛理工大学乡村振兴实践团队
产业振兴	39	青岛理工大学	“青山风光•调起沂蒙”——沂蒙乡村实践团
产业振兴	40	青岛理工大学	“星星之火，归乡圆梦”暑期调研团
产业振兴	41	青岛理工大学	“枣”日致富团队
产业振兴	42	青岛理工大学	青岛理工大学赴山东省青岛市西海岸新区张家楼镇逄家桃园村乡村振兴促进团实践服务团
产业振兴	43	青岛理工大学	观“龙”图腾队
产业振兴	44	青岛理工大学	青春凝集，乡村振兴
产业振兴	45	青岛理工大学	薪火裕乡乡村振兴实践服务团
产业振兴	46	青岛理工大学	青岛理工大学兴沂实践队
产业振兴	47	青岛理工大学	义务编制村庄规划团队
产业振兴	48	青岛理工大学	青岛理工大学重走红色之路社会实践团
产业振兴	49	青岛理工大学	筑梦之光·滕州乡村振兴实践团

产业振兴	50	青岛农业大学	青岛农业大学巴瑟斯未来农业科技学院“探寻蒜都文化，打响振兴振兴‘蒜’盘”赴济宁金乡实践服务团
产业振兴	51	山东财经大学	“农旅融合”——疫情之下旅行社资源重组奏响乡村振兴新乐章
产业振兴	52	山东财经大学	山东财经大学计算机科学与技术学院“新农村里新生活 新时代里新面貌”——青岛和菏泽农村发展变化对比与参照烟台济宁优点深化调研团队
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教育振兴	208	河北工业大学	薪火永传实践队
教育振兴	209	河北工业大学	一路黔行大学生支教团
教育振兴	210	菏泽学院	菏泽学院教师教育学院“乡约一夏逐幸福”暑期社会实践团队
教育振兴	211	济南大学	济南大学益农乡村发展促进会B队
教育振兴	212	济南大学	济南大学益农乡村发展促进会c队
教育振兴	213	晋中信息学院	“传承红色基因”调研寻访团
教育振兴	214	聊城大学	聊城大学“你不孤单”爱心支教服务队
教育振兴	215	聊城大学	Music Heal The Country-音乐下乡队
教育振兴	216	临沂大学	“奥运情 健康行”乡村体育健康传播实践队
教育振兴	217	临沂大学	“智绘成长·与爱同行”
教育振兴	218	临沂大学	“星火传递”乡村阅读计划实践队
教育振兴	219	临沂大学	建设“沂”“莒”夺魁学习屋实践团
教育振兴	220	临沂大学	山水沂蒙情，和谐幸福行——抗大小学党史学习教育团
教育振兴	221	临沂大学	诵沂乡国学经典，助推普乡村振兴
教育振兴	222	鲁东大学	“心源计划”暑期实践团
教育振兴	223	鲁东大学	“幼师微伴”暑期社会实践团
教育振兴	224	鲁东大学	鲁东大学食品安全与营养宣讲团队

教育振兴	225	青岛黄海学院	湖滨镇柳童村党史学习教育团
教育振兴	226	青岛黄海学院	经管星火支教队
教育振兴	227	青岛黄海学院	青口镇下口村民族团结实践团
教育振兴	228	青岛理工大学	“希望之光”为乡村儿童而设计实践团
教育振兴	229	青岛理工大学	“阳光助苗”乡村振兴实践团
教育振兴	230	青岛理工大学	彩虹堂支教团——“星光闪耀点亮童年，彩虹逐梦共向未来”
教育振兴	231	青岛理工大学	青岛理工大学蒲公英支教团
教育振兴	232	青岛理工大学	青岛理工大学种子支教团
教育振兴	233	青岛职业技术学院	青岛职业技术学院振超先锋志愿服务队
教育振兴	234	清华大学	“藏书万里，清济同行”暑期调研支教支队
教育振兴	235	山东财经大学	山东财经大学“寻梦于乡，筑梦于心“三梦团队
教育振兴	236	山东财经大学	“乡村支教聚焦双减政策 互联网+助力素质教育”主题支教队
教育振兴	237	山东财经大学	山东财经大学陶行知协会扶摇直上支教队
教育振兴	238	山东财经大学	乡村振兴中的法治教育实施及效果调研团队
教育振兴	239	山东财经大学	山东财经大学 “文化下乡，筑梦未来”赴济南商河县农村幼儿园支教团队
教育振兴	240	山东财经大学	山东财经大学“小屋大爱”乡村困境儿童公益社会实践团队
教育振兴	241	山东第一医科大学	晨曦志愿服务队
教育振兴	242	山东第一医科大学	岐黄本草志愿者服务队
教育振兴	243	山东第一医科大学	泰山爱心支教团
教育振兴	244	山东第一医科大学	山东第一医科大学泰山爱心支教团 （东平分队）
教育振兴	245	山东第一医科大学	泰山爱心支教团（新疆分队）
教育振兴	246	山东理工大学	山东理工大学“赴陇援西”实践团
教育振兴	247	山东理工大学	向日葵暑期社会实践团
教育振兴	248	山东理工职业学院	机电工程学院彩虹人生志愿服务队
教育振兴	249	山东农业工程学院	“青春献礼二十大，逐梦畲乡促振兴”实践团

教育振兴	250	山东青年政治学院	“雏鹰起飞留守儿童关爱行动”团队
教育振兴	251	山东青年政治学院	山东青年政治学院薪火相传实践团队
教育振兴	252	山东师范大学	绿野堂——打造城乡教育共同体
教育振兴	253	山东师范大学	青翼乡村支教实践团
教育振兴	254	山东师范大学	师荟+——做未来乡村教育的助力者
教育振兴	255	山东师范大学	微微星光
教育振兴	256	山东师范大学	“禹你同行”志愿服务队
教育振兴	257	山东师范大学	“红色党史，薪火相传”——新闻与传媒学院暑期“三下乡”社会实践队
教育振兴	258	山东师范大学	“青春红心向党，青年服务国家”——山东师范大学商学院本硕博党员志愿服务先锋队
教育振兴	259	山东师范大学	山东师范大学教育学部“希望小屋”爱心服务团
教育振兴	260	山东师范大学	红桨队
教育振兴	261	山东职业学院	“易”起普法—“百千万”新职教法宣传暑期社会实践队
教育振兴	262	泰山护理职业学院	泰山护理职业学院青年志愿服务队
教育振兴	263	天津理工大学	“青春献礼二十大 师生奋进新征程 助力乡村再振兴”发展成就观察察团
教育振兴	264	天津理工大学	追随总书记的足迹“‘天理课堂进乡村 津陇情深向未来’”暑期社会实践服务团
教育振兴	265	同济大学	“藏书万里，清济同行”暑期调研支教支队
教育振兴	266	同济大学	“梦想家园”永安实践调研团
教育振兴	267	同济大学	同济大学乡村振兴甘肃小分队
教育振兴	268	同济大学	“生命之花”乡镇中小学女性健康知识科普实践团
教育振兴	269	同济大学	满天星济划——阜南双失儿童心理关怀暑期实践团
教育振兴	270	同济大学	“藏书万里，清济同行”暑期调研支教支队
教育振兴	271	潍坊学院	量子社会实践队
教育振兴	272	潍坊学院	潍坊学院体育学院爱心服务队
教育振兴	273	潍坊医学院	潍坊医学院康复医学院明天计划•百年风华，青春筑梦队
教育振兴	274	武汉大学	“趣学外语，度七彩假期”第一届武汉大学赴罗田县多语种支教夏令营

教育振兴	275	武汉大学	“玉露逐光珞施缘 萤火向阳侗水长”恩施支教志愿服务队
教育振兴	276	武汉理工大学	武汉理工大学退役军人大学生“向太阳”志愿服务分队
教育振兴	277	武汉理工大学	“茶园吐绿育新人，盖杯蕴叶助家‘香’”暑期社会实践队
教育振兴	278	武汉理工大学	“七彩社团，助童成长”暑期社会实践队
教育振兴	279	武汉理工大学	传播希望·“渝”你相遇 社会实践队
教育振兴	280	武汉理工大学	武汉理工大学材料科学与工程学院“启智铸材-STEAM科普教育”暑期社会实践队
教育振兴	281	武汉理工大学	武汉理工大学材料示范学院赴陕西省石泉县“STEAM科普教育”暑期社会实践队
教育振兴	282	武汉理工大学	武汉理工大学蛋蛋在行动志愿服务队
教育振兴	283	武汉理工大学	武汉理工大学资环学院“红心筑梦”农村留守儿童成长助航暑期实践队
教育振兴	284	烟台大学	“启明星”计划
教育振兴	285	烟台大学	护花使者实践队
教育振兴	286	烟台大学	西望喀什队
教育振兴	287	烟台大学	2022烟育菏梦支教队
教育振兴	288	云南大学	寓教于影
教育振兴	289	云南大学	振兴乡村——多年生稻科技小院在行动
教育振兴	290	云南大学	“童语同音·平安童行”实践服务团
教育振兴	291	云南大学	紧跟新时代，青年与乡行——宣威六中夏日成长计划
教育振兴	292	云南大学	健康你我他，行动传帮带
教育振兴	293	云南大学	爱华公益暑假支教团
教育振兴	294	云南大学	云南大学政府管理学院乡村振兴社会实践队
教育振兴	295	云南大学	花山镇农村小学美育教育关怀团
教育振兴	296	云南大学	云南省“日晞朝露”乡村教育帮扶计划-以禄劝彝族苗族自治县则黑乡为试点
教育振兴	297	中国海洋大学	中国海洋大学“鸿鹄”生涯志愿服务队
教育振兴	298	中国农业大学	“育苗计划”留守儿童乡村筑基行
教育振兴	299	中国农业大学	中国农业大学向日葵爱心社赴云拾光支教小队

教育振兴	300	中国石油大学（北京）	云果公益，同心筑梦——多元支教打造乡村学校云端“少年宫”
教育振兴	301	中国石油大学（北京）	云端筑梦，绽放青春
教育振兴	302	中国石油大学（北京）	心系乡间花朵，筑梦云端未来
教育振兴	303	中国石油大学（北京）	桃李灼华·异乡同行——重温红色初心
教育振兴	304	中国石油大学（北京）	石子筑梦云“滇”，京华心手相牵
教育振兴	305	中国石油大学（北京）	点亮火苗丨献礼二十大，青春支教行
教育振兴	306	中国石油大学（北京）	大地之光·圆梦丨助力乌龙乡水浒村，托举小孩子大梦想
教育振兴	307	中国石油大学（北京）	“薪火相传，童心向党”社会实践团队
教育振兴	308	中国石油大学（北京）	“连线濯水·信火相传”支教团
教育振兴	309	中国石油大学（北京）	“厚植山海情，筑梦闽宁行”社会实践团队
教育振兴	310	中国石油大学（北京）	“共育五美”新青年队
教育振兴	311	中南大学	中南大学赴湖南省吉首市保靖县毛沟镇素质教育展新貌公共卫生下乡村暑期社会实践团
教育振兴	312	中南大学	山那边文艺支教团
教育振兴	313	中南大学	中南大学赴新疆维吾尔自治区喀什地区伽师县 “新”火燎原支教暑期社会实践团
教育振兴	314	中南大学	中南大学赴湖南省湘西龙山县大安乡留守儿童心理健康调研团
教育振兴	315	中南大学	中南大学粉冶院赴湖南省永州市江华县萤火长照山明实践团
教育振兴	316	中南大学	中南大学湘雅社会实践团 “潇瑶游”健康教育支教队
教育振兴	317	淄博职业学院	薪火XH实践团
科技创新	318	北京科技大学	北京科技大学荷锄科技助农实践团
科技创新	319	北京科技大学	北京科技大学孟子居荷锄归科技助农实践团
科技创新	320	河北工业大学	用电安全守护队
科技创新	321	河北工业大学	“智检”小分队
科技创新	322	河北工业大学	老有所依队
科技创新	323	河北工业大学	智禾科技团队
科技创新	324	河北工业大学	译音技术团队

科技创新	325	河北工业大学	精检科技
科技创新	326	河北工业大学	圆梦助学·围场爱心行实践队
科技创新	327	聊城大学	“筑梦乡村”志愿服务队
科技创新	328	山东财经大学	山东财经大学工商管理学院村中有 “智”乡中见“数”：乡村振兴背景下农民数字素养现状与提升路径 ——山东省威海市乡中有 “数”实践调研团队
科技创新	329	山东财经大学	山东财经大学工商管理学院学院乡中有 “数”新疆喀什实践调研团队
科技创新	330	山东财经大学	基于疫情下的鲁地万能语音转换包研发与创新调研团队团队
科技创新	331	山东理工大学	创飞科技实践团
科技创新	332	同济大学	同济大学数字赋能乡村人居环境治理团队
科技创新	333	同济大学	新媒体与智能设计赋能乡村振兴实践团
科技创新	334	潍坊职业学院	潍坊职业学院无人机科技支农社会实践团队
科技创新	335	武汉理工大学	武汉理工大学“足迹印初心 青春路南延”暑期社会实践队
科技创新	336	云南大学	云南大学转龙镇调研队
科技创新	337	淄博职业学院	智造未来
科技振兴	338	河北工业大学	菇够科技小队
科技振兴	339	河北工业大学	医工融合：“AI+医生”赋能医疗乡村振兴的实地调研与推广
科技振兴	340	烟台大学	执手限塑 共创未来
人才振兴	341	北京科技大学	北京科技大学SPQ逐源求理科普实践团
人才振兴	342	北京科技大学	北京科技大学微梦想心行动之赤心筑梦乡村振兴实践团
人才振兴	343	东南大学	SEU晋行记
人才振兴	344	共青团固安县委员会	青聚固乡•筑梦方城实践队
人才振兴	345	济南大学	济南大学商学院“星火传递”——逐梦基层实践队
人才振兴	346	青岛理工大学	青岛理工大学久遇夕光助老服务实践团
人才振兴	347	青岛理工大学	青岛理工大学人外学院SKSUNNY幸福行动，助力成长实践团
人才振兴	348	山东财经大学	山东财经大学“生涯领航员”社会实践团队
人才振兴	349	山东财经大学	山东财经大学爱心公益协会暑期支教童心向阳团队

人才振兴	350	天津理工大学	天津理工大学“青春寻访闵都行，文化传承助振兴”暑假社会实践服务团
人才振兴	351	武汉大学	武汉大学动力与机械学院赴广西，陕西，湖北等地七省调研实践队
人才振兴	352	武汉大学	武汉大学马克思主义学院赴湖北省宜昌市“基层人才考核评价的‘秭归方案’——基于秭归县茅坪镇‘积分制’的运转观察”考察实践队
人才振兴	353	烟台大学	菁溯民工实践队
人才振兴	354	烟台大学	药我菁彩
人才振兴	355	云南大学	“乡残励” ——少数民族地区重点帮扶县残疾人励志自主创业调 研
人才振兴	356	中国石油大学（北京）	新时代新青年——六省九地喜迎“二十大”
人才振兴	357	中国石油大学（北京）	时代不减石油魂，石油精神辈辈存
人才振兴	358	中南大学	中南大学 “童心而来，医伴成长”乡村留守儿童身心健康教育支教社会实践团
人才振兴	359	中南大学	中南大学赴湖南省怀化市溆浦县同心筑梦服务乡村振兴实践团
生态振兴	360	北京科技大学	北京科技大学蜀乡农废调研实践团
生态振兴	361	北京科技大学	北京科技大学卧龙幸福实践团
生态振兴	362	北京中医药大学	“医”起振兴团队
生态振兴	363	滨州医学院	滴滴碧水缘，浓浓安康情——咕咚净水宣讲团
生态振兴	364	滨州医学院	投身乡村振兴，保护运河生态队
生态振兴	365	河北工业大学	河北乡建调研队
生态振兴	366	河北工业大学	低碳乡村小分队
生态振兴	367	河北工业大学	“青春行”垃圾分类实践队
生态振兴	368	河北工业大学	对对对对队
生态振兴	369	河北工业大学	“环环相扣，守护吾源”队
生态振兴	370	河北工业大学	家乡护卫队
生态振兴	371	河北工业大学	与时偕行实践队
生态振兴	372	晋中信息学院	青风环箕——食品与环境学院乡村振兴实践团
生态振兴	373	聊城大学	聊城大学“星火筑梦”大学生社会实践志愿服务队
生态振兴	374	齐鲁医药学院	“绿水青山，青春筑梦”服务队

生态振兴	375	齐鲁医药学院	点燃“星火”之光，助力乡村振兴社会实践队
生态振兴	376	齐鲁医药学院	医学影像学院萤火虫志愿服务队
生态振兴	377	青岛理工大学	“青鲤”护卫大沽河团队
生态振兴	378	青岛理工大学	辽宁小阳光队
生态振兴	379	青岛理工大学	绿水青山乡村水环境保护实践团
生态振兴	380	山东财经大学	山东财经大学乡村振兴背景下山东省农村环境污染现状与治理对 策实践调研团队
生态振兴	381	山东财经大学	经济学院吹响生态经济“集秸号”：双碳战略下针对秸秆回收各主体交易现状对秸秆原料供应链的调查研究 ——山东省聊城市实践调研团队
生态振兴	382	山东财经大学	山东财经大学乡村振兴背景下山东省泰安市农村环境污染现状与治理对策
生态振兴	383	山东第一医科大学	神采飞扬志愿者服务队
生态振兴	384	山东理工大学	砼护黄河之滨，描绘安澜新篇
生态振兴	385	山东理工大学	“齐稷社工”实践调研团
生态振兴	386	山东理工大学	“砼”心筑桥，情系黄河实践团
生态振兴	387	山东师范大学	“青绿公益”——山东省湿地生态文化调研团
生态振兴	388	山东师范大学	“师韵莲心”山东师范大学国际教育学院社会实践服务队
生态振兴	389	山东师范大学	山东师范大学法学院“垃圾分类，绿色先行”社会实践队
生态振兴	390	天津理工大学	“守护绿水青山，建设美丽乡村”——创“艺”花椒园乡村振兴实践团
生态振兴	391	天津理工大学	“美丽乡村，智净未来”暑期社会实践团
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生态振兴	394	潍坊学院	潍坊学院大学生阳光爱心协会服务队
生态振兴	395	潍坊学院	美术学院梦想团队
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生态振兴	397	潍坊学院	“小鲁班、大工匠”社会实践团队
生态振兴	398	潍坊学院	种子与设施农业工程学院志愿服务队
生态振兴	399	武汉大学	“一壶山水”山西壶关实践队

生态振兴	400	武汉理工大学	武汉理工大学航运学院“守一江春水，护两岸江滩”环保实践队
生态振兴	401	武汉理工大学	武汉理工大学航运学院助力木兰乡可持续发展暑期社会实践队
生态振兴	402	武汉理工大学	武汉理工大学天兴洲生态振兴社会实践队
生态振兴	403	烟台大学	烟台大学“零碳未来”社会实践队
生态振兴	404	烟台大学	“绿建未来”志愿队
生态振兴	405	烟台大学	“绿泽万物”社会实践队
生态振兴	406	云南大学	破解“垃圾桶”困境，助力乡村生态振兴
生态振兴	407	云南大学	昆明市乡村生态振兴帮扶小组
生态振兴	408	云南大学	守护稻源团队
生态振兴	409	云南大学	冀忆犹新
生态振兴	410	云南大学	寻迹青山绿水志愿队
生态振兴	411	云南大学	稻梦人生—云南元江野生稻资源与民众保护意识调查
生态振兴	412	云南大学	大理市巍山县巍宝山乡三角坪村农村污水治理现状调查团队
生态振兴	413	中国农业大学	屋顶“碳”秘队
生态振兴	414	中国农业大学	与梦咫尺实践队
生态振兴	415	中国农业大学	助力美丽乡村小队
生态振兴	416	中国农业大学	生态格桑花
生态振兴	417	中国农业大学	果力彩云实践小队
生态振兴	418	中国石油大学（北京）	“携手卫蓝天，同心护家园”——城市垃圾分类调研与科普团
生态振兴	419	淄博职业学院	赓续荣光志愿服务队
生态振兴	420	淄博职业学院	红话筒宣讲队
文化振兴	421	天津理工大学	“青春百年跟党走，服务乡村谱新篇”乡村振兴促进团
文化振兴	422	北京科技大学	北京科技大学长青支教团之青稚实践团
文化振兴	423	北京科技大学	北京科技大学京风燕情京郊村落民俗风情探访实践团
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文化振兴	426	北京科技大学	北京科技大学莎行乡村振兴实践团
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文化振兴	429	北京中医药大学	青稞飘香，格桑花开
文化振兴	430	北京中医药大学	“中医青年共奋进，筑梦健康新征程”青年实践团
文化振兴	431	滨州医学院	“文化振兴”小队
文化振兴	432	滨州医学院	“扎根齐鲁大地，邂逅红色足迹”星火社会实践队
文化振兴	433	滨州医学院	“重温路线 营迹于心 筑梦前行”社会实践团队
文化振兴	434	滨州医学院	地下的太阳守护者队
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文化振兴	438	共青团石楼县委员会	山西工商学院赴石楼县乡村振兴实践团
文化振兴	439	河北工业大学	红色文旅助力乡村振兴
文化振兴	440	河北工业大学	红星闪闪队
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文化振兴	442	河北工业大学	服基兴村先锋团
文化振兴	443	河北工业大学	津门红色考古队
文化振兴	444	河北工业大学	红篇巨制实践队
文化振兴	445	河北工业大学	“峪路青春行，艺心助振兴”唐山鲁家峪暑期社会实践队
文化振兴	446	河北工业大学	红色探索者
文化振兴	447	河北工业大学	“情系水泉”社会实践团
文化振兴	448	河北工业大学	“出类坝萃”——围场县地区设计赋能乡村振兴社会实践队
文化振兴	449	河北工业大学	超能振兴团

文化振兴	450	河北工业大学	重游红色老区感悟光荣传统助力乡村振兴
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文化振兴	452	河北工业大学	不忘初心小分队
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文化振兴	454	河北工业大学	赤心铸忠魂队
文化振兴	455	河北工业大学	党史勘探队
文化振兴	456	河北工业大学	红心铸魂学生理论宣讲团
文化振兴	457	河北工业大学	红色筑梦队
文化振兴	458	河北工业大学	筑梦青春队
文化振兴	459	河北工业大学	追红逐梦队
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文化振兴	463	河北工业大学	履践致远同行队
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文化振兴	465	河北工业大学	赤心向党小分队
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文化振兴	467	河北工业大学	一起努力福州行
文化振兴	468	菏泽学院	机电工程学院“拳拳向农心，献礼二十大”乡村振兴团队
文化振兴	469	菏泽学院	戏腔“幸”韵
文化振兴	470	济南大学	济南大学美术学院“传承红色文化，艺术带来希望”社会实践团
文化振兴	471	晋中信息学院	“红心向党映三晋”党史宣讲团
文化振兴	472	晋中信息学院	“信工学子百村行”乡村振兴实践团
文化振兴	473	晋中信息学院	“艺家人”家乡非遗传播官
文化振兴	474	晋中信息学院	百年同行·红韵石楼实践团

文化振兴	475	晋中信息学院	青春兴晋，筑梦有我——商务英语学院乡村振兴促进团
文化振兴	476	晋中信息学院	星火“乡”筑——经济与管理学院乡村振兴促进团
文化振兴	477	晋中信息学院	“传承红色基因，强化党史教育”——智能工程学院红色文化宣传实践团
文化振兴	478	聊城大学	“寻访耿店”乡村振兴青年观察服务队
文化振兴	479	聊城大学	聊城大学“四海同音”推普助力乡村振兴志愿服务队
文化振兴	480	聊城大学	“医”路有你青春行爱心医疗服务队
文化振兴	481	聊城职业技术学院	护理系“萤火虫”志愿服务队
文化振兴	482	聊城职业技术学院	建筑系“筑梦之光”志愿服务队
文化振兴	483	聊城职业技术学院	聊城职业技术学院旅游管理系 “小叶子”礼仪志愿服务队
文化振兴	484	临沂大学	“翰墨怀史·涵育初心·喜迎二十大”
文化振兴	485	临沂大学	沂蒙精神沉浸式艺术宣讲团
文化振兴	486	临沂大学	“字正腔圆话党史，青春献礼二十大”推普助力乡村振兴服务队
文化振兴	487	临沂大学	做时代新柳，创乡村新篇
文化振兴	488	临沂大学	“千家万户语同音”推普调研帮扶实践团
文化振兴	489	鲁东大学	暖心筑梦·健身气功推广实践团
文化振兴	490	齐鲁医药学院	口腔医学院微笑天使实践团
文化振兴	491	齐鲁医药学院	齐鲁医药学院公共卫生与检验学院筑基志愿者服务队
文化振兴	492	齐鲁医药学院	药学院蒲公英志愿服务队
文化振兴	493	青岛黄海学院	猴嘴镇黄沙坨村理论普及宣讲团
文化振兴	494	青岛黄海学院	青岛黄海学院
文化振兴	495	青岛黄海学院	谭坊镇高埠村乡村振兴促进团
文化振兴	496	青岛酒店管理职业技术学院	“百村计划”红色宣讲团
文化振兴	497	青岛酒店管理职业技术学院	味味道来乡村振兴服务队
文化振兴	498	青岛理工大学	“星火青春”大学生社会实践团
文化振兴	499	青岛理工大学	“筑梦寻光，红旅井冈”实践团

文化振兴	500	青岛理工大学	“筑梦延安”乡村振兴实践团
文化振兴	501	青岛理工大学	非遗所思——非物质文化遗产调研服务队
文化振兴	502	青岛理工大学	青岛理工大学皓首乡村振兴服务团
文化振兴	503	山东财经大学	从村落到社区—济宁钢山街道后八里沟村的韧性振兴策略研究
文化振兴	504	山东财经大学	助力乡村文化振兴，传承非遗文化经典——以青岛市平度市仁兆镇新时代文明实践基地为依托
文化振兴	505	山东财经大学	韧性治理视域下德州乡村社区新冠疫情防控策略研究
文化振兴	506	山东财经大学	韧性治理视域下青岛农村社区新冠疫情防控策略研究
文化振兴	507	山东财经大学	山东财经大学工商管理学院村中有“智”乡中见“数”：乡村振兴背景下农民数字素养现状与提升路径——新疆省和田市乡中有“数”实践调研团队
文化振兴	508	山东财经大学	韧性治理视域下青岛桑行社区新冠疫情防控策略研究
文化振兴	509	山东财经大学	韧性治理视域下淄博乡村新冠疫情防控策略研究团队
文化振兴	510	山东财经大学	“手拉手一‘语’你同行”推普助力乡村振兴团队
文化振兴	511	山东财经大学	“喜迎二十大，永远跟党走”百年风华正青春团队
文化振兴	512	山东财经大学	山东财经大学莫让乡音成乡愁方言保护团队
文化振兴	513	山东财经大学	韧性治理视域下潍坊乡村社区新冠疫情防控策略研究团队
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文化振兴	515	山东财经大学	山东省红色文化资源“赋能”县域高质量发展的现状调研团队
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文化振兴	517	山东第一医科大学	医保惠民大学生志愿服务队
文化振兴	518	山东理工大学	“兴”走龙泉调研实践团
文化振兴	519	山东理工大学	“情系三农，振兴乡村”实践团
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文化振兴	522	山东理工大学美术学院	“绘美逐梦”社会实践团队
文化振兴	523	山东理工职业学院	宏远队
文化振兴	524	山东农业工程学院	“乡约一夏，遇践青春”古村落复兴实践队

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文化振兴	527	山东师范大学	山东师范大学法学院“探家风•承国韵”社会实践团
文化振兴	528	山东艺术学院	山东艺术学院大学生暑期社会实践团
文化振兴	529	山东艺术学院	山东艺术学院赴无棣县推普助力乡村振兴服务队
文化振兴	530	山东艺术学院	艺梦琅琊社会实践团
文化振兴	531	山东职业学院	南山书院“指南车”暑期社会实践团
文化振兴	532	山西大同大学	诗和远方,赋能乡村振兴
文化振兴	533	山西大同大学	乡村振兴走基层，青春聚力正当时
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文化振兴	535	山西大学	山西大学共肯团助力乡村抵兴社会实跤队 （灵丘分队）
文化振兴	536	山西师范大学	晋美乡村•“艺”缕同心社会实践队
文化振兴	537	太原师范学院	太原师范学院赴灵丘县国情观察实践团
文化振兴	538	天津理工大学	“重走方舟路，青听实践行”
文化振兴	539	同济大学	特色产业赋能乡村振兴
文化振兴	540	同济大学	少数民族传统村落规划-湖南省张家界市牧笛溪村乡村整体风貌提质
文化振兴	541	同济大学	遗珠拾粹踏乡野，青春建功新时代
文化振兴	542	潍坊学院	“七色花”大学生志愿者服务队
文化振兴	543	潍坊医学院	“医风圣道，共定潍医”大学生暑期志愿服务队
文化振兴	544	潍坊医学院	“医行鸢都，同心逐梦”三下乡志愿服务队
文化振兴	545	武汉大学	“桑榆不晚，银发无忧”武汉大学法学院赴湖南省、河南省、安徽省、四川省等地农村失能老人长期照护状况调研实践队
文化振兴	546	武汉大学	“玉露逐光珞施缘 萤火向阳侗水长”芭蕉侗族乡文创推广实践队
文化振兴	547	武汉大学	武汉大学“珞珈职航之青樱计划”基层实习实践四川省队
文化振兴	548	武汉大学	青春献礼二十大，四海之村寻红章”——武汉大学赴多省村落探访红色 资源实践队
文化振兴	549	武汉大学	武汉大学赴湖北省恩施土家族苗族自治州 乡村文化阵地和少数民族文化建设实践考察队

文化振兴	550	武汉大学	揭榜挂帅|武汉大学信息管理学院赴多省市红色文化调研实践队
文化振兴	551	武汉理工大学	武汉理工大学艺术与设计学院“青春瞭望—美丽乡村建设”暑期社会实践队
文化振兴	552	武汉理工大学	武汉理工大学土木工程与建筑学院非物质文化遗产聂家庄泥塑 调研暑期社会实践队
文化振兴	553	烟台大学	“携手云游”暑期社会实践服务队
文化振兴	554	烟台大学	烟台大学微观鲁乡乡村振兴服务团
文化振兴	555	烟台大学	沂见倾心，蒙回红色
文化振兴	556	烟台大学	追光者实践队
文化振兴	557	云南大学	“池鱼思故渊”——以乡愁为关键的文化振兴促进团队
文化振兴	558	云南大学	民族团结的人民最幸福小队
文化振兴	559	云南大学	东阳镇空巢老人守护团队
文化振兴	560	云南大学	芳年华月
文化振兴	561	云南大学	甲乙丙丁队
文化振兴	562	云南大学	“艺”路有青年，助力乡村振兴
文化振兴	563	云南大学	洱海流域文化追觅——云南大学喜洲镇桃源村调研分队
文化振兴	564	云南大学	洱海流域文化追觅——云南大学湾桥镇调研分队
文化振兴	565	云南大学	探沙朗大村文化遗址实践队
文化振兴	566	云南大学	“红”篇“剧”制——实景高沉浸式剧本杀助力红色旅游发展
文化振兴	567	云南大学	践知践行队
文化振兴	568	云南大学	保山潞江镇的乡村振兴之路：云南咖啡庄园的品牌建设
文化振兴	569	云南大学	微光闪闪服务队——血液病患者的疾病叙事
文化振兴	570	云南大学	饭桶之家
文化振兴	571	云南大学	文体活动进寨下乡
文化振兴	572	云南大学	益直爱
文化振兴	573	云南大学	扬善小队
文化振兴	574	云南大学	城市逃离计划

文化振兴	575	云南大学	云南腾冲红色文化志愿宣传服务小组
文化振兴	576	云南大学	在“云岭之南”书写乡村振兴新篇章——基于红河金平县和昆明呈贡区的乡村振兴理论宣讲队
文化振兴	577	云南大学	青衿远志队
文化振兴	578	云南大学	阿细记忆--彝族阿细人非物质文化遗产数据库调研团队
文化振兴	579	云南大学	筝心实意队
文化振兴	580	中国海洋大学	中国海洋大学“文化进万家”青州实践团
文化振兴	581	中国海洋大学	“苍洱大地上的乡村艺术”实践调研团
文化振兴	582	中国海洋大学	海大学子画家村特色村庄可持续发展调研队
文化振兴	583	中国海洋大学	牛头镇文化振兴小队
文化振兴	584	中国农业大学	微湖红游小队
文化振兴	585	中国农业大学	稼穑耀辉实践小队
文化振兴	586	中国农业大学	艺心艺意，情系乡土——农大学子服务“乡村振兴”实践探索
文化振兴	587	中国农业大学	走进鲁西——鲁西乡村文化调查
文化振兴	588	中国农业大学	文化聚力促振兴小队
文化振兴	589	中国农业大学	承蓝--风花雪月，乡愁大理
文化振兴	590	中国农业大学	“观浙里繁荣，延良渚遗风”小队
文化振兴	591	中国石油大学（北京）	倾听真实声音，弘扬劳动精神团队
文化振兴	592	中国石油大学（北京）	百年荣光使命重，今朝担当吾辈承
文化振兴	593	中国石油大学（北京）	“追光者”——触摸团的记忆
文化振兴	594	中国石油大学（北京）	“油你油我”志愿服务队
文化振兴	595	中国石油大学（北京）	“红旅英情”团队——追溯红色足迹 倾听历史回声
文化振兴	596	中国石油大学（北京）	“百年荣光、红色传承”暑假社会实践小组
文化振兴	597	中南大学	中南大学赴湖南省汨罗市禁毒宣传与调研暑期社会实践团
文化振兴	598	中南大学	中南大学赴湖南省衡阳市衡东县基于虚拟现实技术的 创新红色文化传承与发展暑期社会实践团
文化振兴	599	淄博职业学院	“凝聚青春力量，传承红色基因”文化振兴团队

文化振兴	600	淄博职业学院	“齐心筑梦”社会实践团队

教 育 部 办 公 厅

教研厅函〔2022〕7号

教育部办公厅　农业农村部办公厅
中国科协办公厅关于支持建设
一批科技小院的通知

各省、自治区、直辖市教育厅(教委),农业农村(农牧、畜牧兽医、渔业)厅(局、委)、科协,新疆生产建设兵团教育局、农业农村局、科协,有关研究生培养单位:

为深入贯彻习近平总书记对研究生教育的重要指示精神,落实《全民科学素质行动规划纲要(2021—2035年)》和中共中央办公厅、国务院办公厅《关于加快推进乡村人才振兴的意见》以及科技部、农业农村部、教育部等七部门《关于加强农业科技社会化服务体系建设的若干意见》等文件精神,根据《教育部办公厅 农业农村部办公厅 中国科协办公厅关于推广科技小院研究生培养模式助力乡村人才振兴的通知》(教研厅函〔2022〕2号)要求,经研究生培养单位自愿申请,省级教育、农业农村行政部门和科协联合推荐,专家咨询,确定对68个单位的780个科技小院予以支持建设(名单见附件)。现将有关事宜通知如下。

一、请各研究生培养单位深入贯彻落实习近平总书记对研究生教育工作重要指示精神，把农业农村领域高层次应用型人才培养摆在学校工作的重要位置，巩固政府、社会组织、企业、大学、科研机构协同合作的政产学研一体化人才培养模式，加强组织协调和条件建设，确保科技小院人才培养方案高质量实施，推动研究生教育与生产实践紧密结合、与社会需求紧密结合、与农业农村发展紧密结合，引导广大研究生在乡村振兴中建功立业，绽放青春风采，为全面推进乡村振兴、加快农业农村现代化提供坚实的人才支撑。

二、有关省级教育行政部门要引导相关研究生培养单位，通过增量倾斜和存量调整，优先满足科技小院农业专业学位人才培养的招生计划需求。有关科技小院所在区县农业农村部门要将科技小院纳入当地农业技术服务和农民培训体系。各级科协要鼓励发挥基层农技协的作用，支持基层农技协参与科技小院建设。

三、教育部、农业农村部、中国科协将持续加大对科技小院建设的支持力度，充分发挥全国农业专业学位研究生教育指导委员会、中国农村专业技术协会等专家组织作用，适时组织专家就科技小院建设情况进行跟踪指导，对科技小院人才培养成效突出的研究生培养单位，在学科建设和研究生教育教学改革方面给予相应支持。科技小院人才培养质量将作为农业专业学位授权点及涉农学位授权点学科建设质量评价的重要指标。

四、请各研究生培养单位根据全国农业专业学位研究生教育

指导委员会反馈的专家咨询意见(另发),完善《科技小院人才培养实施方案》,不断改进人才培养工作。

联系方式:教育部研究生司培养处,陆敏,010-66096528, 010-66096882,xwbpyc@ moe.edu.cn。

农业农村部科教司教育处,王馨,010-59193017, kjsjyc@ agri.gov.cn。

中国科协科普部基层工作处,吴沛,010-68578249, wupei@ cast.org.cn。

附件:1. 支持建设科技小院名单(按培养单位排序)

2. 支持建设科技小院名单(按所在省市排序)

教育部办公厅　　农业农村部办公厅　　中国科协办公厅

2022年7月29日

附件1

支持建设科技小院名单
（按培养单位排序）

序号	单位所在地区	单位名称	小院名称
1	北京市	中国农业大学	安徽黟县水稻科技小院
2			北京昌平果蔬科技小院
3			北京昌平草莓科技小院
4			北京昌平奶牛科技小院
5			北京大兴蔬菜科技小院
6			北京门头沟苹果科技小院
7			北京密云果蔬科技小院
8			北京平谷大华山大桃科技小院
9			北京平谷峪口大桃科技小院
10			北京平谷甜玉米科技小院
11			北京顺义设施蔬菜科技小院
12			北京顺义果蔬科技小院
13			北京通州蔬菜科技小院
14			甘肃临泽玉米科技小院
15			广东佛山番茄科技小院
16			广西隆安火龙果科技小院
17			贵州七星关刺梨科技小院（共建）
18			贵州遵义辣椒科技小院
19			河北丰宁生猪科技小院
20			河北沽源蔬菜科技小院
21			河北广宗葡萄科技小院
22			河北白塞种养结合科技小院
23			河北曲周苹果科技小院
24			河北曲周葡萄科技小院
25			河北曲周小麦玉米科技小院
26			河北塞北草牧业科技小院
27			河北阳原驴科技小院
28			河北张北燕麦科技小院
29			河南邓州小麦科技小院
30			黑龙江建三江水稻科技小院
31			吉林梨树草莓科技小院
32			吉林梨树玉米科技小院
33			辽宁大洼水稻科技小院
34			辽宁东港水稻科技小院
35			内蒙古杭锦后旗种养结合科技小院
36			内蒙古杭锦后旗奶牛科技小院
37			内蒙古杭锦后旗玉米科技小院
38			内蒙古呼伦贝尔草业科技小院
39			内蒙古科右前旗番茄科技小院
40			内蒙古临河羊科技小院
41			内蒙古锡林浩特草业科技小院
42			内蒙古锡林浩特羊科技小院
43			内蒙古和林奶山羊科技小院
44			内蒙古五原胡羊科技小院
45			内蒙古武川马铃薯科技小院
46			宁夏永宁肉牛科技小院（共建）
47			山东滨州洼地绵羊科技小院
48			山东济阳小麦玉米科技小院
49			山东寿光番茄科技小院
50			山东寿光蔬菜科技小院

序号	单位所在地区	单位名称	小院名称
51	北京市	中国农业大学	上海崇明水稻科技小院
52			云南勐海水稻科技小院（共建）
53			云南大理稻油轮作科技小院
54			云南晋宁花卉科技小院
55			云南蒙自石榴科技小院
56			云南沧澳洲坚果科技小院
57			云南新平褚橙科技小院
58			重庆涪陵种养结合科技小院
59		北京农学院	北京平谷北寨红杏科技小院
60			北京房山蒲洼镇蜂业科技小院
61			北京昌平蔬菜小院
62			北京昌平苹果科技小院
63			北京延庆黄土梁香草科技小院
64			北京密云蔬菜科技小院
65			北京平谷食用菌科技小院
66			北京延庆蚯蚓科技小院
67			北京延庆旧县智慧果园科技小院
68			北京延庆花卉科技小院
69			北京延庆浆果科技小院
70		中国农业科学院	北京昌平牛羊科技小院
71			福建漳浦食用菌科技小院
72			甘肃镇原肉羊科技小院
73			甘肃舟曲食用菌科技小院
74			广东高明生猪科技小院
75			广东顺德马铃薯科技小院
76			广西巴马香猪科技小院
77			河北沧县枣科技小院
78			河北察北马铃薯科技小院
79			河北黄骅旱碱麦科技小院
80			河北廊坊水产饲料科技小院
81			河北廊坊生猪科技小院
82			河北平泉食用菌科技小院
83			河北泊头桑椹科技小院
84			河北沽源玉米科技小院
85			河北任丘小麦科技小院
86			河北赵县小麦科技小院
87			河北献县肉鸭科技小院
88			河北涿州蛋鸡科技小院
89			河南新乡大豆科技小院
90			河南新乡小麦科技小院
91			黑龙江宝清水稻科技小院
92			黑龙江哈尔滨民主水稻科技小院
93			黑龙江黑河大豆科技小院
94			湖北安陆蛋禽科技小院
95			湖北东西湖区食用菌科技小院
96			湖北五峰茶叶小院
97			湖南龙山百合科技小院
98			湖南雨湖水稻科技小院
99			吉林公主岭玉米杂粮科技小院
100			江苏东台西瓜科技小院
101			江西萍乡早稻科技小院
102			内蒙古察哈尔右翼前旗玉米科技小院
103			内蒙古五原肉羊科技小院
104			山东陵城小麦科技小院
105			山东寿光洛城设施蔬菜科技小院
106			山东寿光润宏设施蔬菜科技小院

序号	单位所在地区	单位名称	小院名称
107	北京市	中国农业科学院	山东汶上大豆科技小院
108			山东禹城大豆科技小院
109			山东滨州种养结合科技小院
110			山西寿阳有机旱作科技小院
111			上海金山奶牛科技小院
112			四川西昌烟草科技小院
113			西藏那曲草业科技小院
114			云南会泽燕麦科技小院
115			浙江平湖水稻科技小院
116			浙江西湖茶叶科技小院
117			重庆璧山柑橘科技小院
118	天津市	天津农学院	天津滨海对虾科技小院
119			天津大港贝类科技小院
120			天津西青蛋鸡科技小院
121	河北省	河北工程大学	河北肥乡设施蔬菜科技小院
122			河北复兴魔芋科技小院
123			河北馆陶黄瓜科技小院
124			河北鸡泽辣椒科技小院
125			河北涉县红花科技小院
126			河北魏县梨科技小院
127			河北魏县魏城梨科技小院
128		河北农业大学	河北阜平梨科技小院
129			河北高阳棉花科技小院
130			河北石家庄奶牛科技小院
131			河北辛集玉米科技小院
132			河北藁城大豆科技小院
133			河北沽源莴苣科技小院
134			河北宁晋玉米科技小院
135			河北饶阳肉羊科技小院
136			河北顺平樱桃科技小院
137			河北辛集小麦科技小院
138			河北张北藜麦科技小院
139			河北涿州赤松茸科技小院
140		河北北方学院	河北尚义西瓜科技小院
141			河北省宣化甘蓝科技小院
142			河北蔚县玉米科技小院
143			河北尚义燕麦科技小院
144			河北宣化谷子科技小院
145			河北张北马铃薯科技小院
146		河北科技师范学院	河北昌黎黄瓜科技小院
147			河北昌黎葡萄科技小院
148			河北崇礼长尾鸡科技小院
149			河北怀来葡萄科技小院
150			河北卢龙甘薯科技小院
151			河北青龙板栗科技小院
152	山西省	山西农业大学	山西定襄谷子科技小院
153			山西繁峙谷子科技小院
154			山西汾西肉鸡科技小院
155			山西高平黄梨科技小院
156			山西和顺牛科技小院
157			山西洪洞小麦科技小院
158			山西怀仁杂粮科技小院
159			山西交口食用菌科技小院
160			山西晋祠水稻科技小院
161			山西岢岚绒山羊科技小院
162			山西岚县马铃薯科技小院

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163	山西省	山西农业大学	山西临县食用菌科技小院
164			山西柳林红枣科技小院
165			山西祁县果蔬科技小院
166			山西沁县蛋鸡科技小院
167			山西石楼红枣科技小院
168			山西寿阳玉米科技小院
169			山西太谷生猪科技小院
170			山西太原奶牛科技小院
171			山西屯留辣椒科技小院
172			山西万荣苹果科技小院
173			山西闻喜小麦科技小院
174			山西五寨中药材科技小院
175			山西阳高渔植种养科技小院
176			山西应县玉米科技小院
177			山西右玉肉羊科技小院
178			山西云州黄花菜科技小院
179			山西中阳木耳科技小院
180	内蒙古自治区	内蒙古农业大学	内蒙古达拉特旗苜蓿科技小院
181			内蒙古杭锦旗向日葵科技小院
182			内蒙古阿拉善右旗骆驼科技小院
183			内蒙古达拉特旗沙葱科技小院
184			内蒙古杭锦后旗小麦科技小院
185			内蒙古凉城藜麦科技小院
186			内蒙古通辽玉米科技小院
187			内蒙古土默特右旗甘露子科技小院
188			内蒙古乌拉特前旗蒙中药材科技小院
189			内蒙古武川燕麦科技小院
190			内蒙古西乌马科技小院
191			内蒙古锡林郭勒草业科技小院
192			内蒙古牙克石马铃薯科技小院
193			内蒙古伊金霍洛旗玫瑰小院
194			内蒙古乌兰察布马铃薯科技小院
195			内蒙古五原葵花科技小院
196			内蒙古五原玉米科技小院
197	辽宁省	沈阳农业大学	辽宁朝阳羊肚菌科技小院
198			辽宁鲅鱼圈葡萄科技小院
199			辽宁凤城玉米科技小院
200			辽宁喀左山杏科技小院
201			辽宁盘锦河蟹科技小院
202		大连海洋大学	辽宁大洼稻渔科技小院
203			辽宁东港蟾蜍科技小院
204			辽宁东港孤山杂色蛤科技小院
205			辽宁普兰店海参科技小院
206			辽宁长海虾夷扇贝科技小院
207			辽宁庄河扇贝科技小院
208		渤海大学	辽宁北镇水稻科技小院
209			辽宁簸鱼圈海蜇科技小院
210			辽宁大洼对虾科技小院
211			辽宁东港长山杂色蛤科技小院
212			辽宁法库大豆科技小院
213			辽宁桓仁水稻科技小院
214			辽宁喀左山猪科技小院
215			辽宁凌海蛤蜊科技小院
216			辽宁凌海海参科技小院
217			辽宁沈北肉鸡科技小院
218			辽宁台安肉鸡科技小院

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219	辽宁省	渤海大学	辽宁兴城多宝鱼科技小院
220			辽宁庄河大骨鸡科技小院
221	吉林省	吉林农业大学	湖北东西湖食用菌科技小院
222			吉林安图西洋参科技小院
223			吉林大安农业智能装备科技小院
224			吉林德惠甜百合科技小院
225			吉林抚松参科技小院
226			吉林公主岭稻渔科技小院
227			吉林公主岭黑猪科技小院
228			吉林和龙桑黄科技小院
229			吉林桦甸桦牛科技小院
230			吉林珲春孟岭苹果科技小院
231			吉林集安山葡萄科技小院
232			吉林靖宇蓝莓科技小院
233			吉林九台稻米科技小院
234			吉林梨树付家街玉米科技小院
235			吉林龙山蛋鸡科技小院
236			吉林宁江果菜科技小院
237			吉林乾安肉羊科技小院
238			吉林舒兰水稻科技小院
239			吉林双辽花生科技小院
240			吉林双阳梅花鹿科技小院
241			吉林通榆谷物科技小院
242			吉林延边牛科技小院
243			吉林永春食用菌科技小院
244			吉林农安黑猪科技小院
245			江苏淮安双孢蘑菇科技小院
246			浙江武义灵芝科技小院
247	黑龙江省	黑龙江大学	黑龙江兰西油豆角科技小院
248			黑龙江七台河油豆角科技小院
249			黑龙江双城玉米科技小院
250			黑龙江依安甜菜科技小院
251			内蒙古呼伦贝尔甜菜科技小院
252		黑龙江八一农垦大学	黑龙江大同杂粮科技小院
253			黑龙江大同甜瓜科技小院
254			黑龙江大同中药材科技小院
255			黑龙江抚远蔓越莓科技小院
256			黑龙江富锦水稻科技小院
257			黑龙江嘉荫刺五加科技小院
258			黑龙江牡丹江杂粮科技小院
259			黑龙江萨尔图盐碱稻科技小院
260			黑龙江嫩江大豆科技小院
261			黑龙江肇东蔬菜科技小院
262			黑龙江肇源果树科技小院
263			黑龙江依安狮白鹅科技小院
264			黑龙江肇州糯玉米科技小院
265		东北农业大学	黑龙江甘南大豆科技小院
266			黑龙江甘南小浆果科技小院
267			黑龙江红卫农场水稻科技小院
268			黑龙江克山马铃薯科技小院
269			黑龙江尚志红小豆科技小院
270			黑龙江尚志小浆果科技小院
271			黑龙江铁力中药材科技小院
272			黑龙江五常水稻科技小院
273			黑龙江逊克大豆科技小院
274			黑龙江亚布力生猪科技小院

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275	黑龙江省	东北林业大学	黑龙江大兴安岭森林食品科技小院
276			黑龙江尚志食用菌科技小院
277			黑龙江鸡西刺五加科技小院
278			黑龙江亚布力黑木耳科技小院
279			黑龙江伊春浆果科技小院
280	上海市	上海交通大学	内蒙巴盟红驼科技小院
281			上海崇明蔬菜科技小院
282			上海嘉定葡萄科技小院
283			上海嘉定种养科技小院
284			上海金山茶叶科技小院
285			上海金山果蔬科技小院
286		上海海洋大学	安徽池州鳜鱼科技小院
287			福建闽威花鲈科技小院
288			上海崇明河蟹科技小院
289			上海金山虾类科技小院
290			浙江杭州鲑鱼鳙鱼科技小院
291			浙江武义淡水珍珠科技小院
292			浙江舟山鲅鱼科技小院
293	江苏省	苏州大学	江苏丹阳圣桑科技小院
294			江苏东台蚕科技小院
295			江苏溧阳蚕科技小院
296			江苏如皋桑蚕科技小院
297			江苏吴江蚕桑科技小院
298			江苏相城大闸蟹科技小院
299		扬州大学	江苏淮阴黄瓜科技小院
300			江苏常州芋头科技小院
301			江苏大丰奶牛科技小院
302			江苏海安稻麦科技小院
303			江苏江都农机科技小院
304			江苏金湖种鸡科技小院
305			江苏泗洪奶牛科技小院
306			江苏兴化梨科技小院
307			江苏高邮奶牛科技小院
308	江苏省	江苏科技大学	安徽岳西蚕桑科技小院
309			广西德保蚕桑科技小院
310			江苏海安桑蚕科技小院
311		南京农业大学	安徽埇桥大豆科技小院
312			江苏大丰棉花科技小院
313			江苏湖熟梨科技小院
314			江苏江宁水稻科技小院
315			江苏昆山水稻科技小院
316			江苏溧阳白茶科技小院
317			江苏如皋黑塌菜科技小院
318			江苏如皋水稻科技小院
319			江苏泰兴萝卜科技小院
320			云南开远切花菊科技小院
321	浙江省	浙江大学	广西苍梧茶科技小院
322			河南荥阳玉米科技小院
323			山东临沂枸杞番茄科技小院
324			山东临沂设施蔬菜科技小院
325			浙江常山胡柚科技小院
326			浙江海盐猪科技小院
327			浙江黄岩蜜橘科技小院
328			浙江建德草莓科技小院
329			浙江建德蛋鸡科技小院
330			浙江临安奶牛科技小院

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331	浙江省	浙江大学	浙江龙湾南美白对虾科技小院
332			浙江宁波蔬菜育种科技小院
333			浙江宁海白枇杷科技小院
334			浙江嵊州蚕桑科技小院
335			浙江松阳茶科技小院
336			浙江桐乡杭白菊科技小院
337			浙江温岭水稻科技小院
338			浙江文成山药科技小院
339			浙江余杭稻米科技小院
340			浙江余杭蜜梨科技小院
341			浙江长兴羊科技小院
342			浙江长兴紫笋茶科技小院
343		浙江工业大学	浙江德清岛鳖科技小院
344			浙江德清早园笋科技小院
345		浙江海洋大学	浙江平阳大黄鱼科技小院
346			浙江嵊泗贻贝科技小院
347	安徽省	安徽农业大学	安徽和县蔬菜科技小院
348			安徽怀宁蓝莓科技小院
349			安徽黄山稻渔科技小院
350			安徽金安鹅科技小院
351			安徽金寨灵芝科技小院
352			安徽砀山酥梨科技小院
353			安徽临泉芥菜科技小院
354			安徽庐江稻麦科技小院
355			安徽庐江灵台水稻科技小院
356			安徽庐江水稻科技小院
357			安徽明光甜叶菊科技小院
358			安徽埇桥麦玉科技小院
359			安徽潜山瓜蒌科技小院
360			安徽铜陵稻虾科技小院
361			安徽长丰草莓科技小院
362			安徽长丰甜瓜科技小院
363		安徽师范大学	安徽南陵稻渔科技小院
364			安徽南陵紫云英科技小院
365		安徽科技学院	安徽利辛生猪科技小院
366			安徽临泉牛羊科技小院
367			安徽歙县贡菊科技小院
368			安徽当涂水稻科技小院
369	福建省	福建农林大学	福建安溪铁观音茶科技小院
370			福建福安红茶科技小院
371			福建建瓯乌龙茶科技小院
372			福建连江海带科技小院
373			福建闽侯青梗菜科技小院
374			福建平和蜜柚科技小院
375			福建三明兰花科技小院
376			福建上杭萝卜科技小院
377			福建松溪甘蔗科技小院
378			福建泰宁红藜科技小院
379			福建永安蔬菜科技小院
380			福建永春芦柑科技小院
381			福建尤溪红茶科技小院
382		集美大学	福建德化棘胸蛙科技小院
383			福建福清上迳鳗鲡科技小院
384	江西省	江西农业大学	江西安远蜜蜂科技小院
385			江西大余麻鸭科技小院
386			江西东乡泥鳅科技小院

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387	江西省	江西农业大学	江西奉新猕猴桃科技小院
388			江西赣粮再生稻科技小院
389			江西赣县湖羊科技小院
390			江西章贡食用菌科技小院
391			江西广昌白莲科技小院
392			江西贵溪紫山药科技小院
393			江西泰和生猪科技小院
394			江西井冈蜜柚科技小院
395			江西南丰蜜桔科技小院
396			江西彭泽虾蟹科技小院
397			江西铅山红芽芋科技小院
398			江西瑞昌黑芝麻科技小院
399			江西上高水稻科技小院
400			江西上栗兔产业科技小院
401			江西泰和乌鸡科技小院
402			江西万载百合科技小院
403			江西新余蜜桔科技小院
404			江西修水杭猪科技小院
405			江西修水宁红茶科技小院
406			江西宜春毛竹科技小院
407			江西宜丰茶叶科技小院
408			江西弋阳龟科技小院
409			江西永丰蔬菜科技小院
410			江西袁州油茶科技小院
411	山东省	中国海洋大学	广东湛江牡蛎科技小院
412			河北滦南海水鱼科技小院
413			江苏赣榆贝虾科技小院
414			山东莱州牡蛎科技小院
415			山东利津海铲科技小院
416			山东日照虾蟹科技小院
417			山东荣成鳗草科技小院
418			山东诸城鱼糜科技小院
419			浙江衢州鲟鱼科技小院
420		山东理工大学	山东滨州棉花科技小院
421			山东河东水稻科技小院
422			山东临淄设施蔬菜科技小院
423			山东无棣棉花科技小院
424			山东淄博禾丰小麦科技小院
425		青岛农业大学	广东珠海生猪科技小院
426			山东高密五龙鹅科技小院
427			山东胶州大白菜科技小院
428			山东胶州黑猪科技小院
429			山东莱西胡萝卜科技小院
430			山东莱西玉米科技小院
431			山东莱州甘薯科技小院
432			山东兰陵梅花鹿科技小院
433			山东乐陵金丝小枣科技小院
434			山东乐陵小麦-玉米科技小院
435			山东临淄小麦-玉米科技小院
436			山东平度花生科技小院
437			山东平度蔬菜科技小院
438			山东平度小麦科技小院
439			山东栖霞黑绒山羊科技小院
440			山东青岛生猪科技小院
441			山东青岛猪饲料科技小院
442			山东青岛琅琊鸡科技小院

序号	单位所在地区	单位名称	小院名称
443	山东省	青岛农业大学	山东威海苹果科技小院
444			山东文登奶山羊科技小院
445			山东无棣苜蓿科技小院
446			山东禹城驴科技小院
447			山东诸城肉鸡科技小院
448			山东诸城水貂科技小院
449		临沂大学	山东兰陵肉羊科技小院
450			山东兰山沂蒙鸡科技小院
451			山东沂南种兔科技小院
452		烟台大学	山东烟台大樱桃科技小院
453			山东烟台番茄科技小院
454			山东烟台苹果科技小院
455			山东烟台小麦科技小院
456	河南省	河南农业大学	河南浚县生猪科技小院
457			河南开封肉鸡科技小院
458			河南兰考饲草生产科技小院
459			河南兰考小麦科技小院
460			河南临颍辣椒科技小院
461			河南内乡生猪科技小院
462			河南平舆芝麻科技小院
463			河南杞县大蒜科技小院
464			河南汝南番茄科技小院
465			河南汝阳香菇科技小院
466			河南固始鹅科技小院
467			河南郑州草莓科技小院
468			河南睢阳西瓜科技小院
469			河南唐庄桃科技小院
470			河南温县铁棍山药科技小院
471			河南淅川石榴科技小院
472			河南新乡花生科技小院
473			河南新野甘蓝科技小院
474			河南荥阳河阴软籽石榴科技小院
475			河南新郑生猪科技小院
476			河南禹州小麦科技小院
477			河南长垣草莓科技小院
478			河南柘城辣椒科技小院
479			河南正阳花生科技小院
480	湖北省	长江大学	湖北江陵“稻-油”科技小院
481			湖北仙桃稻虾科技小院
482		华中农业大学	湖北鄂州武昌鱼科技小院
483			湖北汉南番茄科技小院
484			湖北黄陂鳜鱼科技小院
485			湖北监利稻虾科技小院
486			湖北潜江水稻-小龙虾科技小院
487			湖北沙洋油菜科技小院
488			湖北武汉花卉科技小院
489			湖北随县食用菌科技小院
490			湖北武穴水稻科技小院
491			湖北襄阳麦作科技小院
492			湖北襄阳小麦科技小院
493			湖北襄州稻麦科技小院
494			湖北枣阳烟草科技小院
495		湖北大学	广东英德麻竹笋科技小院
496			湖北鄂州虾蟹科技小院
497			湖北嘉鱼水稻科技小院
498			湖北江陵水稻科技小院

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499	湖北省	湖北大学	湖北武湖水稻科技小院
500	湖南省	湖南农业大学	湖南凤凰猕猴桃科技小院
501			湖南邵阳瓜菜科技小院
502			湖南桃源稻米科技小院
503			湖南永兴冰糖橙科技小院
504			湖南长沙生猪科技小院
505			湖南长沙马铃薯科技小院
506		中南林业科技大学	湖南靖州山核桃科技小院
507			湖南浏阳油茶科技小院
508			湖南龙山锥栗科技小院
509			湖南祁东枣科技小院
510			湖南通道中草药科技小院
511			湖南武冈油桐科技小院
512			湖南新化黄精科技小院
513			湖南炎陵蒙李科技小院
514			湖南长沙油茶科技小院
515	广东省	华南农业大学	广东从化蛋鸡科技小院
516			广东从化荔枝科技小院
517			广东从化香米科技小院
518			广东电白沉香科技小院
519			广东和平山羊科技小院
520			广东河源柠檬科技小院
521			广东惠东叶菜科技小院
522			广东乐昌香芋科技小院
523			广东清远稻渔种养科技小院
524			广东清远麻鸡科技小院
525			广东汕尾猪科技小院
526			广东郁南黄皮科技小院
527			广东中山花木科技小院
528			广东中山生鱼科技小院
529			广东珠海白蕉海鲈鱼科技小院
530		广东海洋大学	广东惠来鲍鱼科技小院
531			广东雷州海水珍珠科技小院
532		仲恺农业工程学院	广东普宁青梅科技小院
533			广东三水胡萝卜科技小院
534			广东开平马冈鹅科技小院
535			广东饶平狮头鹅科技小院
536			广东兴宁肉鸽科技小院
537			广东中山罗非鱼科技小院
538			广东白云罗汉松科技小院
539		佛山科学技术学院	广东梅县金柚科技小院
540			广西贵港生猪科技小院
541	广西壮族自治区	广西大学	广西八步番茄科技小院
542			广西宾阳水稻科技小院
543			广西东兴石斑鱼科技小院
544			广西扶绥甘庶科技小院
545			广西扶绥坚果科技小院
546			广西合浦香苹科技小院
547			广西环江油茶科技小院
548			广西灵山香鸡科技小院
549			广西柳南螺蜂科技小院
550			广西容县三黄鸡科技小院
551			广西容县沙田柚科技小院
552			广西融安脆蜜金桔科技小院
553			广西万秀食用菌科技小院
554			广西武鸣沃柑科技小院

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555	广西壮族自治区	广西大学	广西兴宾小龙虾科技小院
556			广西兴宁富凤鸡科技小院
557			广西阳朔金桔科技小院
558			广西邕宁桑蚕科技小院
559			广西右江芒果科技小院
560		北部湾大学	广西钦南对虾科技小院
561			广西钦南青蟹科技小院
562			广西银海施氏獭蛤科技小院
563	海南省	海南大学	海南白沙百香果科技小院
564			海南昌江黑山羊科技小院
565			海南昌江蜜瓜科技小院
566			海南澄迈和牛科技小院
567			海南东方水稻科技小院
568			海南东方燕窝果科技小院
569			海南儋州食用菌科技小院
570			海南乐东火龙果科技小院
571			海南临高波萝科技小院
572			海南琼海手指柠檬科技小院
573			海南万宁诺丽科技小院
574	重庆市	西南大学	广西宜州蚕桑科技小院
575			广西邕宁蚕桑科技小院
576			贵州湄潭茶叶科技小院
577			四川南部柑橘科技小院
578			四川丹棱桔橙科技小院
579			四川嘉陵蚕桑科技小院
580			四川宁南蚕桑科技小院
581			四川武胜蚕桑科技小院
582			四川仪陇蚕桑科技小院
583			重庆大足油菜科技小院
584			重庆丰都肉牛科技小院
585			重庆涪陵榨菜科技小院
586			重庆江津高粱科技小院
587			重庆梁平水稻科技小院
588			重庆南川鸡科技小院
589			重庆彭水烟草科技小院
590			重庆彭水紫苏科技小院
591			重庆荣昌奶牛科技小院
592			重庆荣昌肉兔科技小院
593			重庆石柱黄连科技小院
594			重庆铜梁稻鱼科技小院
595			重庆铜梁桑蚕科技小院
596			重庆铜梁蔬菜科技小院
597			重庆万州榨菜科技小院
598			重庆永川豆豉科技小院
599			重庆忠县柑橘科技小院
600			重庆北碚蚕桑科技小院
601			重庆北碚蜡梅科技小院
602			重庆北碚蜜桃科技小院
603			重庆城口茶叶科技小院
604			重庆大足花卉科技小院
605			重庆奉节脐橙科技小院
606			重庆合川蚕桑科技小院
607			重庆开州生猪科技小院
608			重庆黔江蚕桑科技小院
609			重庆铜梁肉兔科技小院
610			重庆潼南柠檬科技小院

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611	重庆市	西南大学	重庆巫溪脆李科技小院
612			重庆武隆蚕桑科技小院
613			重庆渝北柑橘科技小院
614			重庆云阳蜂业科技小院
615	四川省	四川农业大学	四川安岳柠檬科技小院
616			四川布拖马铃薯科技小院
617			四川大英乌骨鸡科技小院
618			四川东坡鹌鹑科技小院
619			四川都江堰弥猴桃科技小院
620			四川会理烟草科技小院
621			四川米易枇杷科技小院
622			四川蒲江果业科技小院
623			四川邛崃蜜蜂科技小院
624		西南民族大学	四川红原牧草科技小院
625			四川简州大耳羊科技小院
626			四川乐至黑山羊科技小院
627			四川小金牦牛科技小院
628	贵州省	贵州大学	贵州赤水竹乡鸡科技小院
629			贵州从江百香果科技小院
630			贵州德江黄牛科技小院
631			贵州独山蜂糖李科技小院
632			贵州关岭肉牛科技小院
633			贵州赫章柯乐猪科技小院
634			贵州惠水蜂糖李科技小院
635			贵州锦屏铁皮石斛科技小院
636			贵州开阳蛋鸡科技小院
637			贵州黎平天麻科技小院
638			贵州六枝跑山猪科技小院
639			贵州盘州刺梨科技小院
640			贵州普定茶叶科技小院
641			贵州七星关刺梨科技小院（共建）
642			贵州黔西高粱科技小院
643			贵州清镇茶叶科技小院
644			贵州榕江西瓜科技小院
645			贵州榕江小香鸡科技小院
646			贵州三都马桑菌科技小院
647			贵州纳雍滚山鸡科技小院
648			贵州水城猕猴桃科技小院
649			贵州威宁蔬菜科技小院
650			贵州息烽鲈鱼科技小院
651			贵州玉屏水稻科技小院
652			贵州长顺蛋鸡科技小院
653			贵州遵义播州辣椒科技小院
654	云南省	云南大学	云南呈贡花卉科技小院
655			云南临翔苹果科技小院
656			云南隆阳咖啡科技小院
657			云南勐海多年生稻科技小院
658		云南农业大学	云南宾川葡萄科技小院
659			云南广南高峰牛科技小院
660			云南江川山猪科技小院
661			云南澜沧马铃薯科技小院
662			云南龙陵石斛科技小院
663			云南陇川黄精科技小院
664			云南罗平蜂业科技小院
665			云南勐海水稻科技小院（共建）
666			云南麒麟蓝莓科技小院

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667	云南省	云南农业大学	云南石林人参果科技小院
668			云南寻甸草莓科技小院
669			云南姚安花卉科技小院
670			云南宜良土著鱼科技小院
671			云南永胜软籽石榴科技小院
672			云南元谋果菜科技小院
673		昆明学院	云南安宁玫瑰科技小院
674			云南马龙水蛭科技小院
675			云南弥勒珠芽魔芋科技小院
676			云南嵩明菊花科技小院
677			云南西山草莓科技小院
678			云南香格里拉奇异莓科技小院
679			云南寻甸胡蜂科技小院
680	陕西省	陕西科技大学	陕西眉县猕猴桃科技小院
681			陕西三原苹果科技小院
682			陕西柞水木耳科技小院
683		西北农林科技大学	河南临颍小麦科技小院
684			内蒙古准格尔杂粮科技小院
685			宁夏红寺堡滩羊科技小院（共建）
686			宁夏灵武奶牛科技小院
687			陕西泾阳蔬菜科技小院
688			陕西陇县小麦科技小院
689			陕西略阳草莓科技小院
690			陕西千阳苜蓿科技小院
691			陕西汉滨蚕桑科技小院
692			陕西佳县红枣科技小院
693			陕西太白冷凉蔬菜科技小院
694			陕西延安蔬菜科技小院
695			陕西永寿小麦科技小院
696			陕西周至猕猴桃科技小院
697			新疆图木舒克棉花科技小院
698		榆林学院	陕西榆林绿豆科技小院
699			陕西佳县绒山羊科技小院
700			陕西绥德黄芪科技小院
701			陕西榆阳湖羊科技小院
702			陕西榆阳桑树科技小院
703			陕西榆阳沙棘科技小院
704			陕西榆阳羊饲料科技小院
705	甘肃省	兰州大学	甘肃环县滩羊科技小院
706			甘肃临泽草业科技小院
707			甘肃玛曲牦牛科技小院
708			甘肃永昌清河苜蓿科技小院
709		甘肃农业大学	甘肃安定团结马铃薯科技小院
710			甘肃安定凤翔马铃薯科技小院
711			甘肃安定玉米科技小院
712			甘肃白银玉米科技小院
713			甘肃迭部蕨麻猪科技小院
714			甘肃甘谷小麦科技小院
715			甘肃甘州玉米科技小院
716			甘肃景泰枸杞科技小院
717			甘肃皋兰月季科技小院
718			甘肃凉州绿肥科技小院
719			甘肃凉州玉米科技小院
720			甘肃临洮马铃薯科技小院
721			甘肃灵台矮砧苹果科技小院
722			甘肃庆阳冬油菜科技小院

序号	单位所在地区	单位名称	小院名称
723	甘肃省	甘肃农业大学	甘肃山丹马铃薯科技小院
724			甘肃天祝细毛羊科技小院
725			甘肃夏河牦牛科技小院
726			甘肃永昌苜蓿科技小院
727			甘肃永昌奶绵羊科技小院
728			甘肃榆中高原夏菜科技小院
729			甘肃镇原玉米科技小院
730	青海省	青海大学	青海都兰枸杞科技小院
731			青海共和藏羊科技小院
732			青海河南草业科技小院
733			青海互助青稞科技小院
734			青海尖扎果蔬科技小院
735	宁夏回族自治区	宁夏大学	宁夏固原肉牛科技小院
736			宁夏海原马铃薯科技小院
737			宁夏贺兰瓜菜科技小院
738			宁夏红寺堡滩羊科技小院（共建）
739			宁夏平吉堡玉米科技小院
740			宁夏平罗水稻科技小院
741			宁夏同心枸杞科技小院
742			宁夏盐池滩羊科技小院
743			宁夏永宁肉牛科技小院（共建）
744			宁夏中宁肉羊科技小院
745	新疆维吾尔自治区	塔里木大学	新疆皮山无花果科技小院
746			新疆阿拉尔棉花科技小院
747			新疆和田大棚瓜菜科技小院
748			新疆喀什湖羊科技小院
749			新疆库车县驴科技小院
750			新疆昆玉红枣科技小院
751			新疆阿克苏绿肥科技小院
752			新疆皮山肉兔科技小院
753			新疆且末肉牛科技小院
754			新疆若羌猪科技小院
755			新疆铁门关香梨科技小院
756			新疆图木舒克小麦科技小院
757			新疆温宿核桃科技小院
758		新疆农业大学	新疆阿瓦提棉花科技小院
759			新疆呼图壁苜蓿科技小院
760			新疆玛纳斯虫子鸡科技小院
761			新疆墨玉肉羊科技小院
762			新疆沙雅棉花科技小院
763			新疆乌鲁木齐南瓜科技小院
764			新疆乌鲁木齐草业科技小院
765			新疆昭苏马科技小院
766		石河子大学	新疆图木舒克鸽鹌科技小院
767			新疆石河子葡萄科技小院
768			新疆图木舒克枣科技小院
769			新疆图木舒克西梅科技小院
770			新疆昌吉棉花科技小院
771			新疆昆玉皮墨垦区红枣科技小院
772			新疆昌吉生猪科技小院
773			新疆库尔勒棉花科技小院
774			新疆奇台小麦作物科技小院
775			新疆石河子棉花科技小院
776			新疆图木舒克肉牛科技小院
777			新疆图木舒克苹果科技小院
778			新疆图木舒克石榴科技小院

序号	单位所在地区	单位名称	小院名称
779	新疆维吾尔自治区	石河子大学	新疆托里饲草科技小院
780			新疆乌兰乌苏棉花科技小院

附件2

支持建设科技小院名单
（按所在省市排序）

序号	小院所在地区	单位名称	小院名称
1	北京市	北京农学院	北京平谷北寨红杏科技小院
2			北京房山蒲洼镇蜂业科技小院
3			北京昌平蔬菜小院
4			北京昌平苹果科技小院
5			北京延庆黄土梁香草科技小院
6			北京密云蔬菜科技小院
7			北京平谷食用菌科技小院
8			北京延庆蚯蚓科技小院
9			北京延庆旧县智慧果园科技小院
10			北京延庆花卉科技小院
11			北京延庆浆果科技小院
12		中国农业大学	北京昌平果蔬科技小院
13			北京昌平草莓科技小院
14			北京昌平奶牛科技小院
15			北京大兴蔬菜科技小院
16			北京门头沟苹果科技小院
17			北京密云果蔬科技小院
18			北京平谷大华山大桃科技小院
19			北京平谷峪口大桃科技小院
20			北京平谷甜玉米科技小院
21			北京顺义设施蔬菜科技小院
22			北京顺义果蔬科技小院
23			北京通州蔬菜科技小院
24		中国农业科学院	北京昌平牛羊科技小院
25	天津市	天津农学院	天津滨海对虾科技小院
26			天津大港贝类科技小院
27			天津西青蛋鸡科技小院
28	河北省	河北北方学院	河北尚义西瓜科技小院
29			河北省宣化甘蓝科技小院
30			河北蔚县玉米科技小院
31			河北尚义燕麦科技小院
32			河北宣化谷子科技小院
33			河北张北马铃薯科技小院
34		河北工程大学	河北肥乡设施蔬菜科技小院
35			河北复兴魔芋科技小院
36			河北馆陶黄瓜科技小院
37			河北鸡泽辣椒科技小院
38			河北涉县红花科技小院
39			河北魏县梨科技小院
40			河北魏县魏城梨科技小院
41		河北科技师范学院	河北昌黎黄瓜科技小院
42			河北昌黎葡萄科技小院
43			河北崇礼长尾鸡科技小院
44			河北怀来葡萄科技小院
45			河北卢龙甘薯科技小院
46			河北青龙板栗科技小院
47		河北农业大学	河北阜平梨科技小院
48			河北高阳棉花科技小院
49			河北石家庄奶牛科技小院
50			河北辛集玉米科技小院

序号	小院所在地区	单位名称	小院名称
51	河北省	河北农业大学	河北藁城大豆科技小院
52			河北沽源莴苣科技小院
53			河北宁晋玉米科技小院
54			河北饶阳肉羊科技小院
55			河北顺平樱桃科技小院
56			河北辛集小麦科技小院
57			河北张北藜麦科技小院
58			河北涿州赤松茸科技小院
59		中国海洋大学	河北滦南海水鱼科技小院
60		中国农业大学	河北丰宁生猪科技小院
61			河北沽源蔬菜科技小院
62			河北广宗葡萄科技小院
63			河北白寨种养结合科技小院
64			河北曲周苹果科技小院
65			河北曲周葡萄科技小院
66			河北曲周小麦玉米科技小院
67			河北塞北草牧业科技小院
68			河北阳原驴科技小院
69			河北张北燕麦科技小院
70		中国农业科学院	河北沧县枣科技小院
71			河北察北马铃薯科技小院
72			河北黄骅旱碱麦科技小院
73			河北廊坊水产饲料科技小院
74			河北廊坊生猪科技小院
75			河北平泉食用菌科技小院
76			河北泊头桑椹科技小院
77			河北沽源玉米科技小院
78			河北任丘小麦科技小院
79			河北赵县小麦科技小院
80			河北献县肉鸭科技小院
81			河北涿州蛋鸡科技小院
82	山西省	山西农业大学	山西定襄谷子科技小院
83			山西繁峙谷子科技小院
84			山西汾西肉鸡科技小院
85			山西高平黄梨科技小院
86			山西和顺牛科技小院
87			山西洪洞小麦科技小院
88			山西怀仁杂粮科技小院
89			山西交口食用菌科技小院
90			山西晋祠水稻科技小院
91			山西岢岚绒山羊科技小院
92			山西岚县马铃薯科技小院
93			山西临县食用菌科技小院
94			山西柳林红枣科技小院
95			山西祁县果蔬科技小院
96			山西沁县蛋鸡科技小院
97			山西石楼红枣科技小院
98			山西寿阳玉米科技小院
99			山西太谷生猪科技小院
100			山西太原奶牛科技小院
101			山西屯留辣椒科技小院
102			山西万荣苹果科技小院
103			山西闻喜小麦科技小院
104			山西五寨中药材科技小院
105			山西阳高渔植种养科技小院
106			山西应县玉米科技小院

序号	小院所在地区	单位名称	小院名称
107	山西省	山西农业大学	山西右玉肉羊科技小院
108			山西云州黄花菜科技小院
109			山西中阳木耳科技小院
110		中国农业科学院	山西寿阳有机旱作科技小院
111	内蒙古自治区	黑龙江大学	内蒙古呼伦贝尔甜菜科技小院
112		内蒙古农业大学	内蒙古达拉特旗苜蓿科技小院
113			内蒙古杭锦旗向日葵科技小院
114			内蒙古阿拉善右旗骆驼科技小院
115			内蒙古达拉特旗沙葱科技小院
116			内蒙古杭锦后旗小麦科技小院
117			内蒙古凉城藜麦科技小院
118			内蒙古通辽玉米科技小院
119			内蒙古土默特右旗甘露子科技小院
120			内蒙古乌拉特前旗蒙中药材科技小院
121			内蒙古武川燕麦科技小院
122			内蒙古西乌马科技小院
123			内蒙古锡林郭勒草业科技小院
124			内蒙古牙克石马铃薯科技小院
125			内蒙古伊金霍洛旗玫瑰小院
126			内蒙古乌兰察布马铃薯科技小院
127			内蒙古五原葵花科技小院
128			内蒙古五原玉米科技小院
129		上海交通大学	内蒙巴盟红驼科技小院
130		西北农林科技大学	内蒙古准格尔杂粮科技小院
131		中国农业大学	内蒙古杭锦后旗种养结合科技小院
132			内蒙古杭锦后旗奶牛科技小院
133			内蒙古杭锦后旗玉米科技小院
134			内蒙古呼伦贝尔草业科技小院
135			内蒙古科右前旗番茄科技小院
136			内蒙古临河羊科技小院
137			内蒙古锡林浩特草业科技小院
138			内蒙古锡林浩特羊科技小院
139			内蒙古和林奶山羊科技小院
140			内蒙古五原胡羊科技小院
141			内蒙古武川马铃薯科技小院
142		中国农业科学院	内蒙古察哈尔右翼前旗玉米科技小院
143			内蒙古五原肉羊科技小院
144	辽宁省	渤海大学	辽宁北镇水稻科技小院
145			辽宁簸鱼圈海蜇科技小院
146			辽宁大洼对虾科技小院
147			辽宁东港长山杂色蛤科技小院
148			辽宁法库大豆科技小院
149			辽宁桓仁水稻科技小院
150			辽宁喀左山猪科技小院
151			辽宁凌海蛤蜊科技小院
152			辽宁凌海海参科技小院
153			辽宁沈北肉鸡科技小院
154			辽宁台安肉鸡科技小院
155			辽宁兴城多宝鱼科技小院
156			辽宁庄河大骨鸡科技小院
157		大连海洋大学	辽宁大洼稻渔科技小院
158			辽宁东港蟾蜍科技小院
159			辽宁东港孤山杂色蛤科技小院
160			辽宁普兰店海参科技小院
161			辽宁长海虾夷扇贝科技小院
162			辽宁庄河扇贝科技小院

序号	小院所在地区	单位名称	小院名称
163	辽宁省	沈阳农业大学	辽宁朝阳羊肚菌科技小院
164			辽宁鲅鱼圈葡萄科技小院
165			辽宁凤城玉米科技小院
166			辽宁喀左山杏科技小院
167			辽宁盘锦河蟹科技小院
168		中国农业大学	辽宁大洼水稻科技小院
169			辽宁东港水稻科技小院
170	吉林省	吉林农业大学	吉林安图西洋参科技小院
171			吉林大安农业智能装备科技小院
172			吉林德惠甜百合科技小院
173			吉林抚松参科技小院
174			吉林公主岭稻渔科技小院
175			吉林公主岭黑猪科技小院
176			吉林和龙桑黄科技小院
177			吉林桦甸桦牛科技小院
178			吉林珲春孟岭苹果科技小院
179			吉林集安山葡萄科技小院
180			吉林靖宇蓝莓科技小院
181			吉林九台稻米科技小院
182			吉林梨树付家街玉米科技小院
183			吉林龙山蛋鸡科技小院
184			吉林宁江果菜科技小院
185			吉林乾安肉羊科技小院
186			吉林舒兰水稻科技小院
187			吉林双辽花生科技小院
188			吉林双阳梅花鹿科技小院
189			吉林通榆谷物科技小院
190			吉林延边牛科技小院
191			吉林永春食用菌科技小院
192			吉林农安黑猪科技小院
193		中国农业大学	吉林梨树草莓科技小院
194			吉林梨树玉米科技小院
195		中国农业科学院	吉林公主岭玉米杂粮科技小院
196	黑龙江省	东北林业大学	黑龙江大兴安岭森林食品科技小院
197			黑龙江尚志食用菌科技小院
198			黑龙江鸡西刺五加科技小院
199			黑龙江亚布力黑木耳科技小院
200			黑龙江伊春浆果科技小院
201			黑龙江甘南大豆科技小院
202			黑龙江甘南小浆果科技小院
203			黑龙江红卫农场水稻科技小院
204			黑龙江克山马铃薯科技小院
205			黑龙江尚志红小豆科技小院
206			黑龙江尚志小浆果科技小院
207			黑龙江铁力中药材科技小院
208			黑龙江五常水稻科技小院
209			黑龙江逊克大豆科技小院
210			黑龙江亚布力生猪科技小院
211		黑龙江八一农垦大学	黑龙江大同杂粮科技小院
212			黑龙江大同甜瓜科技小院
213			黑龙江大同中药材科技小院
214			黑龙江抚远蔓越莓科技小院
215			黑龙江富锦水稻科技小院
216			黑龙江嘉荫刺五加科技小院
217			黑龙江牡丹江杂粮科技小院
218			黑龙江萨尔图盐碱稻科技小院

序号	小院所在地区	单位名称	小院名称
219	黑龙江省	黑龙江八一农垦大学	黑龙江嫩江大豆科技小院
220			黑龙江肇东蔬菜科技小院
221			黑龙江肇源果树科技小院
222			黑龙江依安狮白鹅科技小院
223			黑龙江肇州糯玉米科技小院
224		黑龙江大学	黑龙江兰西油豆角科技小院
225			黑龙江七台河油豆角科技小院
226			黑龙江双城玉米科技小院
227			黑龙江依安甜菜科技小院
228		中国农业大学	黑龙江建三江水稻科技小院
229		中国农业科学院	黑龙江宝清水稻科技小院
230			黑龙江哈尔滨民主水稻科技小院
231			黑龙江黑河大豆科技小院
232	上海市	上海海洋大学	上海崇明河蟹科技小院
233			上海金山虾类科技小院
234		上海交通大学	上海崇明蔬菜科技小院
235			上海嘉定葡萄科技小院
236			上海嘉定种养科技小院
237			上海金山茶叶科技小院
238			上海金山果蔬科技小院
239		中国农业大学	上海崇明水稻科技小院
240		中国农业科学院	上海金山奶牛科技小院
241	江苏省	吉林农业大学	江苏淮安双孢蘑菇科技小院
242		江苏科技大学	江苏海安桑蚕科技小院
243		南京农业大学	江苏大丰棉花科技小院
244			江苏湖熟梨科技小院
245			江苏江宁水稻科技小院
246			江苏昆山水稻科技小院
247			江苏溧阳白茶科技小院
248			江苏如皋黑塌菜科技小院
249			江苏如皋水稻科技小院
250			江苏泰兴萝卜科技小院
251		苏州大学	江苏丹阳圣桑科技小院
252			江苏东台蚕科技小院
253			江苏溧阳蚕科技小院
254			江苏如皋桑蚕科技小院
255			江苏吴江蚕桑科技小院
256			江苏相城大闸蟹科技小院
257		扬州大学	江苏淮阴黄瓜科技小院
258			江苏常州芋头科技小院
259			江苏大丰奶牛科技小院
260			江苏海安稻麦科技小院
261			江苏江都农机科技小院
262			江苏金湖种鸡科技小院
263			江苏泗洪奶牛科技小院
264			江苏兴化梨科技小院
265			江苏高邮奶牛科技小院
266		中国海洋大学	江苏赣榆贝虾科技小院
267		中国农业科学院	江苏东台西瓜科技小院
268	浙江省	吉林农业大学	浙江武义灵芝科技小院
269		上海海洋大学	浙江杭州鲢鱼鳙鱼科技小院
270			浙江武义淡水珍珠科技小院
271			浙江舟山鱿鱼科技小院
272		浙江大学	浙江常山胡柚科技小院
273			浙江海盐猪科技小院
274			浙江黄岩蜜橘科技小院

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275	浙江省	浙江大学	浙江建德草莓科技小院
276			浙江建德蛋鸡科技小院
277			浙江临安奶牛科技小院
278			浙江龙湾南美白对虾科技小院
279			浙江宁波蔬菜育种科技小院
280			浙江宁海白枇杷科技小院
281			浙江嵊州蚕桑科技小院
282			浙江松阳茶科技小院
283			浙江桐乡杭白菊科技小院
284			浙江温岭水稻科技小院
285			浙江文成山药科技小院
286			浙江余杭稻米科技小院
287			浙江余杭蜜梨科技小院
288			浙江长兴羊科技小院
289			浙江长兴紫笋茶科技小院
290		浙江工业大学	浙江德清岛鳖科技小院
291			浙江德清早园笋科技小院
292		浙江海洋大学	浙江平阳大黄鱼科技小院
293			浙江嵊泗贻贝科技小院
294		中国海洋大学	浙江衢州鲟鱼科技小院
295		中国农业科学院	浙江平湖水稻科技小院
296			浙江西湖茶叶科技小院
297	安徽省	安徽科技学院	安徽利辛生猪科技小院
298			安徽临泉牛羊科技小院
299			安徽歙县贡菊科技小院
300			安徽当涂水稻科技小院
301		安徽农业大学	安徽和县蔬菜科技小院
302			安徽怀宁蓝莓科技小院
303			安徽黄山稻渔科技小院
304			安徽金安鹅科技小院
305			安徽金寨灵芝科技小院
306			安徽砀山酥梨科技小院
307			安徽临泉芥菜科技小院
308			安徽庐江稻麦科技小院
309			安徽庐江灵台水稻科技小院
310			安徽庐江水稻科技小院
311			安徽明光甜叶菊科技小院
312			安徽埇桥麦玉科技小院
313			安徽潜山瓜蒌科技小院
314			安徽铜陵稻虾科技小院
315			安徽长丰草莓科技小院
316			安徽长丰甜瓜科技小院
317		安徽师范大学	安徽南陵稻渔科技小院
318			安徽南陵紫云英科技小院
319		江苏科技大学	安徽岳西蚕桑科技小院
320		南京农业大学	安徽埇桥大豆科技小院
321		上海海洋大学	安徽池州鳜鱼科技小院
322		中国农业大学	安徽黟县水稻科技小院
323	福建省	福建农林大学	福建安溪铁观音茶科技小院
324			福建福安红茶科技小院
325			福建建瓯乌龙茶科技小院
326			福建连江海带科技小院
327			福建闽侯青梗菜科技小院
328			福建平和蜜柚科技小院
329			福建三明兰花科技小院
330			福建上杭萝卜科技小院

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331	福建省	福建农林大学	福建松溪甘蔗科技小院
332			福建泰宁红藜科技小院
333			福建永安蔬菜科技小院
334			福建永春芦柑科技小院
335			福建尤溪红茶科技小院
336		集美大学	福建德化棘胸蛙科技小院
337			福建福清上迳鳗鲡科技小院
338		上海海洋大学	福建闽威花鲈科技小院
339		中国农业科学院	福建漳浦食用菌科技小院
340	江西省	江西农业大学	江西安远蜜蜂科技小院
341			江西大余麻鸭科技小院
342			江西东乡泥鳅科技小院
343			江西奉新猕猴桃科技小院
344			江西赣粮再生稻科技小院
345			江西赣县湖羊科技小院
346			江西章贡食用菌科技小院
347			江西广昌白莲科技小院
348			江西贵溪紫山药科技小院
349			江西泰和生猪科技小院
350			江西井冈蜜柚科技小院
351			江西南丰蜜桔科技小院
352			江西彭泽虾蟹科技小院
353			江西铅山红芽芋科技小院
354			江西瑞昌黑芝麻科技小院
355			江西上高水稻科技小院
356			江西上栗兔产业科技小院
357			江西泰和乌鸡科技小院
358			江西万载百合科技小院
359			江西新余蜜桔科技小院
360			江西修水杭猪科技小院
361			江西修水宁红茶科技小院
362			江西宜春毛竹科技小院
363			江西宜丰茶叶科技小院
364			江西弋阳龟科技小院
365			江西永丰蔬菜科技小院
366			江西袁州油茶科技小院
367		中国农业科学院	江西萍乡早稻科技小院
368	山东省	临沂大学	山东兰陵肉羊科技小院
369			山东兰山沂蒙鸡科技小院
370			山东沂南种兔科技小院
371		青岛农业大学	山东高密五龙鹅科技小院
372			山东胶州大白菜科技小院
373			山东胶州黑猪科技小院
374			山东莱西胡萝卜科技小院
375			山东莱西玉米科技小院
376			山东莱州甘薯科技小院
377			山东兰陵梅花鹿科技小院
378			山东乐陵金丝小枣科技小院
379			山东乐陵小麦-玉米科技小院
380			山东临淄小麦-玉米科技小院
381			山东平度花生科技小院
382			山东平度蔬菜科技小院
383			山东平度小麦科技小院
384			山东栖霞黑绒山羊科技小院
385			山东青岛生猪科技小院
386			山东青岛猪饲料科技小院

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387	山东省	青岛农业大学	山东青岛琅琊鸡科技小院
388			山东威海苹果科技小院
389			山东文登奶山羊科技小院
390			山东无棣苜蓿科技小院
391			山东禹城驴科技小院
392			山东诸城肉鸡科技小院
393			山东诸城水貂科技小院
394		山东理工大学	山东滨州棉花科技小院
395			山东河东水稻科技小院
396			山东临淄设施蔬菜科技小院
397			山东无棣棉花科技小院
398			山东淄博禾丰小麦科技小院
399		烟台大学	山东烟台大樱桃科技小院
400			山东烟台番茄科技小院
401			山东烟台苹果科技小院
402			山东烟台小麦科技小院
403		浙江大学	山东临沂枸杞番茄科技小院
404			山东临沂设施蔬菜科技小院
405		中国海洋大学	山东莱州牡蛎科技小院
406			山东利津海铲科技小院
407			山东日照虾蟹科技小院
408			山东荣成鳗草科技小院
409			山东诸城鱼糜科技小院
410		中国农业大学	山东滨州洼地绵羊科技小院
411			山东济阳小麦玉米科技小院
412			山东寿光番茄科技小院
413			山东寿光蔬菜科技小院
414		中国农业科学院	山东陵城小麦科技小院
415			山东寿光洛城设施蔬菜科技小院
416			山东寿光润宏设施蔬菜科技小院
417			山东汶上大豆科技小院
418			山东禹城大豆科技小院
419			山东滨州种养结合科技小院
420	河南省	河南农业大学	河南浚县生猪科技小院
421			河南开封肉鸡科技小院
422			河南兰考饲草生产科技小院
423			河南兰考小麦科技小院
424			河南临颍辣椒科技小院
425			河南内乡生猪科技小院
426			河南平舆芝麻科技小院
427			河南杞县大蒜科技小院
428			河南汝南番茄科技小院
429			河南汝阳香菇科技小院
430			河南固始鹅科技小院
431			河南郑州草莓科技小院
432			河南睢阳西瓜科技小院
433			河南唐庄桃科技小院
434			河南温县铁棍山药科技小院
435			河南淅川石榴科技小院
436			河南新乡花生科技小院
437			河南新野甘蓝科技小院
438			河南荥阳河阴软籽石榴科技小院
439			河南新郑生猪科技小院
440			河南禹州小麦科技小院
441			河南长垣草莓科技小院
442			河南柘城辣椒科技小院

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443	河南省	河南农业大学	河南正阳花生科技小院
444		西北农林科技大学	河南临颍小麦科技小院
445		浙江大学	河南荥阳玉米科技小院
446		中国农业大学	河南邓州小麦科技小院
447		中国农业科学院	河南新乡大豆科技小院
448			河南新乡小麦科技小院
449	湖北省	湖北大学	湖北鄂州虾蟹科技小院
450			湖北嘉鱼水稻科技小院
451			湖北江陵水稻科技小院
452			湖北武湖水稻科技小院
453		华中农业大学	湖北鄂州武昌鱼科技小院
454			湖北汉南番茄科技小院
455			湖北黄陂鳜鱼科技小院
456			湖北监利稻虾科技小院
457			湖北潜江水稻-小龙虾科技小院
458			湖北沙洋油菜科技小院
459			湖北武汉花卉科技小院
460			湖北随县食用菌科技小院
461			湖北武穴水稻科技小院
462			湖北襄阳麦作科技小院
463			湖北襄阳小麦科技小院
464			湖北襄州稻麦科技小院
465			湖北枣阳烟草科技小院
466		吉林农业大学	湖北东西湖食用菌科技小院
467		长江大学	湖北江陵“稻-油”科技小院
468			湖北仙桃稻虾科技小院
469		中国农业科学院	湖北安陆蛋禽科技小院
470			湖北东西湖区食用菌科技小院
471			湖北五峰茶叶小院
472	湖南省	湖南农业大学	湖南凤凰猕猴桃科技小院
473			湖南邵阳瓜菜科技小院
474			湖南桃源稻米科技小院
475			湖南永兴冰糖橙科技小院
476			湖南长沙生猪科技小院
477			湖南长沙马铃薯科技小院
478		中国农业科学院	湖南龙山百合科技小院
479			湖南雨湖水稻科技小院
480		中南林业科技大学	湖南靖州山核桃科技小院
481			湖南浏阳油茶科技小院
482			湖南龙山锥栗科技小院
483			湖南祁东枣科技小院
484			湖南通道中草药科技小院
485			湖南武冈油桐科技小院
486			湖南新化黄精科技小院
487			湖南炎陵紫李科技小院
488			湖南长沙油茶科技小院
489	广东省	佛山科学技术学院	广东梅县金柚科技小院
490		广东海洋大学	广东惠来鲍鱼科技小院
491			广东雷州海水珍珠科技小院
492		湖北大学	广东英德麻竹笋科技小院
493		华南农业大学	广东从化蛋鸡科技小院
494			广东从化荔枝科技小院
495			广东从化香米科技小院
496			广东电白沉香科技小院
497			广东和平山羊科技小院
498			广东河源柠檬科技小院

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499	广东省	华南农业大学	广东惠东叶菜科技小院
500			广东乐昌香芋科技小院
501			广东清远稻渔种养科技小院
502			广东清远麻鸡科技小院
503			广东汕尾猪科技小院
504			广东郁南黄皮科技小院
505			广东中山花木科技小院
506			广东中山生鱼科技小院
507			广东珠海白蕉海鲈鱼科技小院
508		青岛农业大学	广东珠海生猪科技小院
509		中国海洋大学	广东湛江牡蛎科技小院
510		中国农业大学	广东佛山番茄科技小院
511		中国农业科学院	广东高明生猪科技小院
512			广东顺德马铃薯科技小院
513		仲恺农业工程学院	广东普宁青梅科技小院
514			广东三水胡萝卜科技小院
515			广东开平马冈鹅科技小院
516			广东饶平狮头鹅科技小院
517			广东兴宁肉鸽科技小院
518			广东中山罗非鱼科技小院
519			广东白云罗汉松科技小院
520	广西壮族自治区	北部湾大学	广西钦南对虾科技小院
521			广西钦南青蟹科技小院
522			广西银海施氏獭蛤科技小院
523		佛山科学技术学院	广西贵港生猪科技小院
524		广西大学	广西八步番茄科技小院
525			广西宾阳水稻科技小院
526			广西东兴石斑鱼科技小院
527			广西扶绥甘庶科技小院
528			广西扶绥坚果科技小院
529			广西合浦香苹科技小院
530			广西环江油茶科技小院
531			广西灵山香鸡科技小院
532			广西柳南螺蜂科技小院
533			广西容县三黄鸡科技小院
534			广西容县沙田柚科技小院
535			广西融安脆蜜金桔科技小院
536			广西万秀食用菌科技小院
537			广西武鸣沃柑科技小院
538			广西兴宾小龙虾科技小院
539			广西兴宁富凤鸡科技小院
540			广西阳朔金桔科技小院
541			广西邕宁桑蚕科技小院
542			广西右江芒果科技小院
543		江苏科技大学	广西德保蚕桑科技小院
544		西南大学	广西宜州蚕桑科技小院
545		西南大学	广西邕宁蚕桑科技小院
546		浙江大学	广西苍梧茶科技小院
547		中国农业大学	广西隆安火龙果科技小院
548		中国农业科学院	广西巴马香猪科技小院
549	海南省	海南大学	海南白沙百香果科技小院
550			海南昌江黑山羊科技小院
551			海南昌江蜜瓜科技小院
552			海南澄迈和牛科技小院
553			海南东方水稻科技小院
554			海南东方燕窝果科技小院

序号	小院所在地区	单位名称	小院名称
555	海南省	海南大学	海南儋州食用菌科技小院
556			海南乐东火龙果科技小院
557			海南临高波萝科技小院
558			海南琼海手指柠檬科技小院
559			海南万宁诺丽科技小院
560	重庆市	西南大学	重庆大足油菜科技小院
561			重庆丰都肉牛科技小院
562			重庆涪陵榨菜科技小院
563			重庆江津高粱科技小院
564			重庆梁平水稻科技小院
565			重庆南川鸡科技小院
566			重庆彭水烟草科技小院
567			重庆彭水紫苏科技小院
568			重庆荣昌奶牛科技小院
569			重庆荣昌肉兔科技小院
570			重庆石柱黄连科技小院
571			重庆铜梁稻鱼科技小院
572			重庆铜梁桑蚕科技小院
573			重庆铜梁蔬菜科技小院
574			重庆万州榨菜科技小院
575			重庆永川豆豉科技小院
576			重庆忠县柑橘科技小院
577			重庆北碚蚕桑科技小院
578			重庆北碚蜡梅科技小院
579			重庆北碚蜜桃科技小院
580			重庆城口茶叶科技小院
581			重庆大足花卉科技小院
582			重庆奉节脐橙科技小院
583			重庆合川蚕桑科技小院
584			重庆开州生猪科技小院
585			重庆黔江蚕桑科技小院
586			重庆铜梁肉兔科技小院
587			重庆潼南柠檬科技小院
588			重庆巫溪脆李科技小院
589			重庆武隆蚕桑科技小院
590			重庆渝北柑橘科技小院
591			重庆云阳蜂业科技小院
592		中国农业大学	重庆涪陵种养结合科技小院
593		中国农业科学院	重庆璧山柑橘科技小院
594	四川省	四川农业大学	四川安岳柠檬科技小院
595			四川布拖马铃薯科技小院
596			四川大英乌骨鸡科技小院
597			四川东坡鹌鹑科技小院
598			四川都江堰弥猴桃科技小院
599			四川会理烟草科技小院
600			四川米易枇杷科技小院
601			四川蒲江果业科技小院
602			四川邛崃蜜蜂科技小院
603		西南大学	四川南部柑橘科技小院
604			四川丹棱桔橙科技小院
605			四川嘉陵蚕桑科技小院
606			四川宁南蚕桑科技小院
607			四川武胜蚕桑科技小院
608			四川仪陇蚕桑科技小院
609		西南民族大学	四川红原牧草科技小院
610			四川简州大耳羊科技小院

序号	小院所在地区	单位名称	小院名称
611	四川省	西南民族大学	四川乐至黑山羊科技小院
612			四川小金牦牛科技小院
613		中国农业科学院	四川西昌烟草科技小院
614	贵州省	贵州大学	贵州赤水竹乡鸡科技小院
615			贵州从江百香果科技小院
616			贵州德江黄牛科技小院
617			贵州独山蜂糖李科技小院
618			贵州关岭肉牛科技小院
619			贵州赫章柯乐猪科技小院
620			贵州惠水蜂糖李科技小院
621			贵州锦屏铁皮石斛科技小院
622			贵州开阳蛋鸡科技小院
623			贵州黎平天麻科技小院
624			贵州六枝跑山猪科技小院
625			贵州盘州刺梨科技小院
626			贵州普定茶叶科技小院
627			贵州七星关刺梨科技小院（共建）
628			贵州黔西高粱科技小院
629			贵州清镇茶叶科技小院
630			贵州榕江西瓜科技小院
631			贵州榕江小香鸡科技小院
632			贵州三都马桑菌科技小院
633			贵州纳雍滚山鸡科技小院
634			贵州水城猕猴桃科技小院
635			贵州威宁蔬菜科技小院
636			贵州息烽鲈鱼科技小院
637			贵州玉屏水稻科技小院
638			贵州长顺蛋鸡科技小院
639			贵州遵义播州辣椒科技小院
640		西南大学	贵州湄潭茶叶科技小院
641		中国农业大学	贵州七星关刺梨科技小院（共建）
642			贵州遵义辣椒科技小院
643	云南省	昆明学院	云南安宁玫瑰科技小院
644			云南马龙水蛭科技小院
645			云南弥勒珠芽魔芋科技小院
646			云南嵩明菊花科技小院
647			云南西山草莓科技小院
648			云南香格里拉奇异莓科技小院
649			云南寻甸胡蜂科技小院
650		南京农业大学	云南开远切花菊科技小院
651		云南大学	云南呈贡花卉科技小院
652			云南临翔苹果科技小院
653			云南隆阳咖啡科技小院
654			云南勐海多年生稻科技小院
655		云南农业大学	云南宾川葡萄科技小院
656			云南广南高峰牛科技小院
657			云南江川山猪科技小院
658			云南澜沧马铃薯科技小院
659			云南龙陵石斛科技小院
660			云南陇川黄精科技小院
661			云南罗平蜂业科技小院
662			云南勐海水稻科技小院（共建）
663			云南麒麟蓝莓科技小院
664			云南石林人参果科技小院
665			云南寻甸草莓科技小院
666			云南姚安花卉科技小院

序号	小院所在地区	单位名称	小院名称
667	云南省	云南农业大学	云南宜良土著鱼科技小院
668			云南永胜软籽石榴科技小院
669			云南元谋果菜科技小院
670		中国农业大学	云南勐海水稻科技小院（共建）
671			云南大理稻油轮作科技小院
672			云南晋宁花卉科技小院
673			云南蒙自石榴科技小院
674			云南沧澳洲坚果科技小院
675			云南新平褚橙科技小院
676		中国农业科学院	云南会泽燕麦科技小院
677	西藏自治区	中国农业科学院	西藏那曲草业科技小院
678	陕西省	陕西科技大学	陕西眉县猕猴桃科技小院
679			陕西三原苹果科技小院
680			陕西柞水木耳科技小院
681		西北农林科技大学	陕西泾阳蔬菜科技小院
682			陕西陇县小麦科技小院
683			陕西略阳草莓科技小院
684			陕西千阳苜蓿科技小院
685			陕西汉滨蚕桑科技小院
686			陕西佳县红枣科技小院
687			陕西太白冷凉蔬菜科技小院
688			陕西延安蔬菜科技小院
689			陕西永寿小麦科技小院
690			陕西周至猕猴桃科技小院
691		榆林学院	陕西榆林绿豆科技小院
692			陕西佳县绒山羊科技小院
693			陕西绥德黄芪科技小院
694			陕西榆阳湖羊科技小院
695			陕西榆阳桑树科技小院
696			陕西榆阳沙棘科技小院
697			陕西榆阳羊饲料科技小院
698	甘肃省	甘肃农业大学	甘肃安定团结马铃薯科技小院
699			甘肃安定凤翔马铃薯科技小院
700			甘肃安定玉米科技小院
701			甘肃白银玉米科技小院
702			甘肃迭部蕨麻猪科技小院
703			甘肃甘谷小麦科技小院
704			甘肃甘州玉米科技小院
705			甘肃景泰枸杞科技小院
706			甘肃皋兰月季科技小院
707			甘肃凉州绿肥科技小院
708			甘肃凉州玉米科技小院
709			甘肃临洮马铃薯科技小院
710			甘肃灵台矮砧苹果科技小院
711			甘肃庆阳冬油菜科技小院
712			甘肃山丹马铃薯科技小院
713			甘肃天祝细毛羊科技小院
714			甘肃夏河牦牛科技小院
715			甘肃永昌苜蓿科技小院
716			甘肃永昌奶绵羊科技小院
717			甘肃榆中高原夏菜科技小院
718			甘肃镇原玉米科技小院
719		兰州大学	甘肃环县滩羊科技小院
720			甘肃临泽草业科技小院
721			甘肃玛曲牦牛科技小院
722			甘肃永昌清河苜蓿科技小院

序号	小院所在地区	单位名称	小院名称
723	甘肃省	中国农业大学	甘肃临泽玉米科技小院
724		中国农业科学院	甘肃镇原肉羊科技小院
725			甘肃舟曲食用菌科技小院
726	青海省	青海大学	青海都兰枸杞科技小院
727			青海共和藏羊科技小院
728			青海河南草业科技小院
729			青海互助青稞科技小院
730			青海尖扎果蔬科技小院
731	宁夏回族自治区	宁夏大学	宁夏固原肉牛科技小院
732			宁夏海原马铃薯科技小院
733			宁夏贺兰瓜菜科技小院
734			宁夏红寺堡滩羊科技小院（共建）
735			宁夏平吉堡玉米科技小院
736			宁夏平罗水稻科技小院
737			宁夏同心枸杞科技小院
738			宁夏盐池滩羊科技小院
739			宁夏永宁肉牛科技小院（共建）
740			宁夏中宁肉羊科技小院
741		西北农林科技大学	宁夏红寺堡滩羊科技小院（共建）
742			宁夏灵武奶牛科技小院
743		中国农业大学	宁夏永宁肉牛科技小院（共建）
744	新疆维吾尔自治区	石河子大学	新疆图木舒克鸽鹑科技小院
745			新疆石河子葡萄科技小院
746			新疆图木舒克枣科技小院
747			新疆图木舒克西梅科技小院
748			新疆昌吉棉花科技小院
749			新疆昆玉皮墨垦区红枣科技小院
750			新疆昌吉生猪科技小院
751			新疆库尔勒棉花科技小院
752			新疆奇台小麦作物科技小院
753			新疆石河子棉花科技小院
754			新疆图木舒克肉牛科技小院
755			新疆图木舒克苹果科技小院
756			新疆图木舒克石榴科技小院
757			新疆托里饲草科技小院
758			新疆乌兰乌苏棉花科技小院
759		塔里木大学	新疆皮山无花果科技小院
760			新疆阿拉尔棉花科技小院
761			新疆和田大棚瓜菜科技小院
762			新疆喀什湖羊科技小院
763			新疆库车县驴科技小院
764			新疆昆玉红枣科技小院
765			新疆阿克苏绿肥科技小院
766			新疆皮山肉兔科技小院
767			新疆且末肉牛科技小院
768			新疆若羌猪科技小院
769			新疆铁门关香梨科技小院
770			新疆图木舒克小麦科技小院
771			新疆温宿核桃科技小院
772		西北农林科技大学	新疆图木舒克棉花科技小院
773		新疆农业大学	新疆阿瓦提棉花科技小院
774			新疆呼图壁首蓿科技小院
775			新疆玛纳斯虫子鸡科技小院
776			新疆墨玉肉羊科技小院
777			新疆沙雅棉花科技小院
778			新疆乌鲁木齐南瓜科技小院

序号	小院所在地区	单位名称	小院名称
779	新疆维吾尔自治区	新疆农业大学	新疆乌鲁木齐草业科技小院
780			新疆昭苏马科技小院

全国农业专业学位研究生教育指导委员会
2019年研究生教育管理课题·立项通知书

胡凤益：

“云南高原特色农业科技小院开放共享运行实践与机制研究”获准全国农业专业学位研究生教育指导委员会2019年重点课题立项（编号：2019-NYZD-25），由云南农业大学谢世清主持，共设置9个子课题，参加单位云南农业大学、云南大学、西南林业大学、云南师范大学、昆明学院。经重点课题主持人推荐，全国农业专业学位研究生教育指导委员会秘书处审核，你负责子课题有关事项通知如下：

1、主持子课题5：勐海曼拉多年生稻科技小院（编号：2019-NYZD-25-5）；子课题承担单位：云南大学农学院；执行年限：2019年11月-2021年11月。

2、接此通知后尽快和重点课题签署任务书，制定实施方案并按照《全国农业专业学位研究生教育指导委员会研究课题管理办法》（试行）组织实施。

3、承诺若岗位职务变更，保证继续完成好课题。做好课题下拨经费和单位配套经费管理，按照重点课题要求做好中期检查和结题验收等工作。

全国农业专业学位研究生教育指导委员会秘书处

2019年12月13日

全国农业专业学位研究生教育指导委员会
2019年研究生教育管理课题·立项通知书

吴学尉：

“云南高原特色农业科技小院开放共享运行实践与机制研究”获准全国农业专业学位研究生教育指导委员会2019年重点课题立项（编号：2019-NYZD-25），由云南农业大学谢世清主持，共设置9个子课题，参加单位云南农业大学、云南大学、西南林业大学、云南师范大学、昆明学院。经重点课题主持人推荐，全国农业专业学位研究生教育指导委员会秘书处审核，你负责子课题有关事项通知如下：

1、主持子课题6：花卉产业科技小院建设探索（编号：2019-NYZD-25-6）；子课题承担单位：云南大学农学院；执行年限：2019年11月-2021年11月。

2、接此通知后尽快和重点课题签署任务书，制定实施方案并按照《全国农业专业学位研究生教育指导委员会研究课题管理办法》（试行）组织实施。

3、承诺若岗位职务变更，保证继续完成好课题。做好课题下拨经费和单位配套经费管理，按照重点课题要求做好中期检查和结题验收等工作。

全国农业专业学位研究生教育指导委员会秘书处

2019年12月13日

全国农业专业学位研究生教育指导委员会

农业教指委〔2021〕3号

关于推选认定全国农业专业学位研究生实践教育特色基地及培育基地的通知

各培养单位:

为深入贯彻全国研究生教育工作会议精神，积极落实教育部 国家发展改革委 财政部《关于加快新时代研究生教育改革发展的意见》（教研〔2020〕9号）、国务院学位委员会 教育部《关于印发<专业学位研究生教育发展方案>（2020-2025）的通知》（学位〔2020〕20号）等文件精神，全国农业专业学位研究生教育指导委员会（以下简称“教指委”）组织开展了第三批农业专业学位研究生实践教育特色基地遴选工作。

根据评选办法，经部分教指委委员和领域分委员会专家回避式评审、教指委委员表决及公示等程序，最终推选出全国农业专业学位研究生实践教育特色基地8个、培育基地12个（详见附件）。

特色基地和培育基地证书、基地建设管理办法等，将于近期邮寄有关单位。

附件：第三批全国农业专业学位研究生实践教育特色基地及培育基地名单

全国农业专业学位研究生教育指导委员会

二零二一年十二月二十一日

附件：

第三批全国农业专业学位研究生实践教育特色基地及培育基地名单

序号	实践基地名称	依托培养单位	实践基地负责人	评审结果	基地编号
1	黑土地保护与利用“梨树模式”实践教育基地	中国农业大学	李保国	特色基地	2021-NYTSJD-01
2	宁夏滩羊专业学位研究生实践教育特色基地	西北农林科技大学	王小龙	特色基地	2021-NYTSJD-02
3	华中农业大学-广西扬翔股份有限公司研究生实践教育基地	华中农业大学	彭贵青	特色基地	2021-NYTSJD-03
4	南京农业大学宿迁研究院实践教育特色基地	南京农业大学	束　胜	特色基地	2021-NYTSJD-04
5	扬州大学-立华牧业研究生教育创新基地	扬州大学	常国斌	特色基地	2021-NYTSJD-05
6	东北农业大学与哈尔滨市农业科学院新农科建设协同育人创新基地	东北农业大学	彭显龙	特色基地	2021-NYTSJD-06
7	华南农业大学-诺普信研究生实践教育特色基地	华南农业大学	钟国华	特色基地	2021-NYTSJD-07
8	山东农业大学畜牧学研究生联合培养基地	山东农业大学	林　海	特色基地	2021-NYTSJD-08
9	浙江大学—嘉兴市农业科学研究院农业硕士专业学位研究生实践教育特色基地	浙江大学	张国平	培育基地	2021-NYPYJD-01
10	沈阳农业大学-丹玉种业专业学位硕士研究生实践基地	沈阳农业大学	于海秋	培育基地	2021-NYPYJD-02

序号	实践基地名称	依托培养单位	实践基地负责人	评审结果	基地编号
11	现代化大农业研究生培养创新实践示范基地	黑龙江八一农垦大学	杨克军	培育基地	2021-NYPYJD-03
12	江西上高水稻科技小院	江西农业大学	曾勇军	培育基地	2021-NYPYJD-04
13	江淮分水岭现代农业研究生实践教育基地	安徽农业大学	张子军	培育基地	2021-NYPYJD-05
14	青岛农业大学-青岛瑞滋集团有限公司专业学位研究生实践教育基地	青岛农业大学	任贻超	培育基地	2021-NYPYJD-06
15	多年生稻科技小院实践教育基地	云南大学	胡凤益	培育基地	2021-NYPYJD-07
16	长春国信现代农业科技发展股份有限公司	吉林大学	杨柏明	培育基地	2021-NYPYJD-08
17	北京首农畜牧发展有限公司奶牛中心	北京农学院	方洛云	培育基地	2021-NYPYJD-09
18	上海海洋大学农业硕士虾蟹育种与生态养殖实践基地	上海海洋大学	成永旭	培育基地	2021-NYPYJD-10
19	天津科技大学农业硕士食品高新技术研究生实践基地	天津科技大学	刘继锋	培育基地	2021-NYPYJD-11
20	广东天农食品集团股份有限公司	佛山科学技术学院	李　华	培育基地	2021-NYPYJD-12

云南大学　　云南省农业科学院
联合培养硕士研究生合作协议

甲方：云南大学

乙方：云南省农业科学院

为深入贯彻落实党的十九大精神，全面实施科教兴国、科教兴农、科教兴滇战略，适应产业升级转型需要，推进教育与科研紧密结合，培养高层次人才和技能型、实用型人才，为实施乡村振兴、打造云南“三张牌”和八大产业发展提供人才支撑。经云南大学（甲方）、云南省农业科学院（乙方）友好协商，本着“优势互补、资源共享、合作共赢、共同发展”的原则，就联合培养硕士研究生达成以下合作协议。

一、合作内容

甲乙双方联合建设研究生培养基地，联合培养硕士研究生。为充分挖掘和发挥甲乙双方在农业高级人才培养上的优势，培养更多的农业高级科教人才，促进双方科教队伍建设。依托甲方研究生教育体系、乙方高级专业技术人员和科研条件，扩大研究生招生规模，联合培养作物学和农业硕士（农艺与种业）研究生。研究生由乙方具有硕士研究生导师资格的导师指导，在甲方完成基础课程学习后，进入乙方相关科研团队和课题组，在导师指导下完成科研任务和毕业论文。

联合培养硕士研究生期间，乙方指导联合培养硕士研究生以第一作者在中文核心期刊及以上刊物发表论文至少1篇，署名的第一单位为甲方，乙方导师为通讯作者。

二、双方职责

（一）甲方的职责任务

1.甲方根据教育部有关规定和本校硕士研究生招生计划、管理办法，负责联合培养硕士研究生培养方案制定、招生录取、教学组织、学位授予、导师管理考核等工作。

2.甲方根据本校硕士生导师的遴选资格条件和程序，遴选聘用乙方在相应专业学术水平较高、实践经验丰富的高级职称人员为甲方导师，由甲方发给“云南大学硕士研究生指导教师”聘书，统一进行管理和考核。受聘导师聘任期间违反法律法规、学校规章制度，或明显不适于担任导师工作、不符合导师要求的，甲方有权暂停或取消其导师资格。

3.甲方每年选派不少于20名联合培养硕士研究生给乙方受聘导师指导，进入联合培养基地进行农业实践和课题研究。

4.根据乙方需要，甲方配合完成联合培养研究生农业实践或论文相关课题的研究任务。按甲方相关规定，甲方给予乙方受聘导师相应的导师费。

（二）乙方的职责任务

1.乙方须协助甲方做好联合培养硕士研究生招生、培养、

管理以及导师遴选、技能培训与课题研究、论文指导等工作。

2. 乙方负责为联合培养的硕士研究生提供必要的实验室、试验基地等科研平台和研究条件，提供生活食宿便利，并给予研究生相应补助。

3. 乙方根据甲方硕士研究生导师的遴选条件和程序，推荐政治素质硬、科研道德好、学术造诣高的高级职称人员作为联合培养硕士研究生导师，并按照甲方的相关规定和要求，做好联合培养研究生培养期间受聘导师的管理和考评工作。

4. 乙方负责对进入培养基地的硕士研究生进行思想教育、纪律教育、安全教育和日常管理。

三、组织领导

为确保联合培养硕士研究生工作顺利进行，双方成立“云南大学 云南省农业科学院联合培养硕士研究生领导小组”及相应的办事机构，由双方分管领导和相关人员组成。

领导小组负责联合培养工作的领导、统筹和协调工作。联合培养的日常组织、管理和实施工作，甲方由研究生院、农学院负责；乙方由组织人事处、科研管理处负责，办公室设在组织人事处，安排专人管理。

四、 其他事项

（一）甲乙双方建立联合培养合作关系，不改变各自现行的行政领导管理体制。双方参与各项工作的人员，人事、组织等各种关系不变。

（二）本协议作为甲乙双方科教合作的基本框架协议。合作项目的实施，由双方根据本协议确定的基本内容和原则，商定合作项目的具体协议和实施方案。

（三）甲乙双方如有一方欲终止本协议，需提前一个月向另一方提出书面说明材料，对方在收到终止协议的书面说明材料后一个月内未作表示的，视为默认，本协议即告终止。

（四）本协议一式四份，甲、乙双方各执二份。

（五）本协议自甲乙双方代表签字盖章之日起生效。未尽事宜，由双方协商解决。

甲方：云南大学（签章）

代表（签字）：

2018年10月24日

乙方：云南省农业科学院（签章）

代表（签字）：

2018年10月24日

云南大学 云南云天化股份有限公司
联合培养农业硕士专业学位研究生合作协议

甲方：云南大学

乙方：云南云天化股份有限公司

为了进一步提高人才培养质量，为云南省经济发展提供高级农业科技人才支撑，本着科研资源互相补充、人才共享、共同发展的原则，经甲乙双方友好协商，就甲乙双方开展联合培养农业硕士专业学位研究生相关事宜达成如下协议：

一、合作原则和合作内容

为充分挖掘和发挥甲乙双方在农业高级人才培养上的优势，培养更多农业高级人才，依托甲方研究生教育体系、乙方产业化条件，扩大研究生招生规模，联合培养专业型研究生，努力实现“校企合作、产学共赢”。

甲乙双方联合建设研究生培养基地，联合培养专业学位研究生。甲方研究生以联合培养研究生身份到乙方开展培养和毕业论文研究。研究生由乙方具有硕士研究生导师资格的导师作为第一导师、甲方导师作为第二导师协助进行指导，在甲方学生完成基础课程学习后，进入乙方相关科研团队和课题组，在甲、乙双方导师指导下完成培养任务和毕业论文。

二、双方的权利和义务

（一）甲方的职责任务

1. 甲方根据教育部有关规定和本校研究生招生计划、管理办法，负责联合培养研究生培养方案制定、招生录取、教学组织、学位授予、导师管理考核等工作。

2. 甲方根据本校研究生导师的遴选资格条件和程序，遴选聘用乙方相关人员为甲方导师，统一进行管理和考核。受聘导师聘任期间违反法律法规、学校规章制度，或明显不适于导师工作，不符合导师要求的，甲方有权暂停或取消导师资格。

3. 甲方每年招收不少于15名联合培养研究生进入联合培养基地进行农业实践和课题研究，如有变化双方在招生前进行协商。

4. 根据乙方需求，甲方配合完成联合培养研究生农业实践和论文相关课题的研究任务。

5. 联培学生不转户口和人事档案，医保报费及国家有关研究生待遇由甲方负责。甲方学生在乙方培养期间，应保障学生合法权利，不安排与专业实践和学位论文无关的工作。乙方负责为甲方学生购买人身意外保险，甲方学生应严格遵守乙方的各项规章制度，服从乙方的日常管理。

6. 甲方学生在乙方工作期间的一切成果，包括但不限于发表的文章、专利、开发的产品等，归乙方所有，权利人为乙方。但学生获得学位需要取得的论文和科研成果，署名第一作者为学生本人，署名第一单位为甲方，署名第二单位为乙方。

7. 甲方及相关学生承诺对参与乙方的研究工作内容和成果承担保密责任，未经乙方许可，不得向任何第三方披露。如因甲方或相关学生过错或者过失造成相关科研项目信息外露，甲方或相关学生对乙方承担赔偿责

任。

（二）乙方的职责任务

1. 乙方须协助甲方做好联合培养研究生招生、培养、管理以及导师遴选等工作。经过双方同意通过遴选的乙方导师，作为联合培养研究生的第一导师，负有研究生培养的第一导师责任，对研究生培养全过程负主要责任，甲方第二导师负共同指导责任。

2. 乙方应为联合培养研究生的技能培训与课题研究、论文指导负责。乙方需指导联合培养研究生以第一作者（或完成人）完成甲方规定的取得学位的相关成果，署名第一单位为甲方，第二单位为乙方。

3. 乙方负责为联合培养研究生提供必要的实验室、试验基地等科研平台和研究条件，提供生活食宿便利，并给予研究生相应补助（具体按乙方标准执行）。

4. 乙方根据甲方研究生导师的遴选条件和程序，推荐乙方相关人员作为联合培养研究生导师，并按照甲方的相关规定和要求，做好联合培养研究生受聘导师的管理和考评工作。

5. 乙方负责对进入培养基地的研究生进行思想教育、纪律教育、安全教育和日常管理，甲方配合乙方完成相关教育与管理工作。非工作时间发生的人生伤害等安全事故按国家相关规定办理。

6. 乙方根据岗位需求，优先聘用联合培养的毕业生到乙方工作。

三、组织领导

为确保联合培养研究生顺利进行，双方成立“云南大学一云南云天化股份有限公司联合培养研究生领导小组”及相应的办事机构，由双方分管

领导和相关人员组成。

领导小组负责联合培养工作的领导、统筹和协调工作。联合培养的日常组织、管理和实施工作，甲方由研究生院、资源植物研究院负责，办公室设在资源植物研究院；乙方由云南云天化股份有限公司研发中心、人力资源部负责，办公室设在研发中心，安排专人管理。

四、其他事项

（一）甲乙双方建立联合培养合作关系，不改变各自现有的行政领导管理体制。双方参与各项工作的人员，人事、组织等各种关系不变。

（二）甲乙双方如有一方欲终止本协议，需提前三个月向另一方提出书面说明材料，对方在收到终止协议的书面说明材料后一个月内未作表示的，视为默认，本协议即告终止。但已经形成事实招收的联合培养研究生，必须按照招收年级时的培养方案培养，直至该届研究生毕业。

（三）本协议一式六份，甲乙双方各执三份。本协议自双方签字盖章之日起生效，有效期至2025年12月31日。协议履行过程中发生任何纠纷，协议各方应协商解决，协商无效或不愿协商的，任何一方均可向有关部门提起诉讼。

甲方：云南大学

（盖章）

甲方代表（签字）：

2021年9月15日

乙方：云南云天化股份有限公司

（盖章）

乙方代表（签字）：

2021年9月15日

2022年度云南大学科教融合研究生联合培养项目

序号	负责单位	一级学科代码/专业学位类别代码	一级学科名称/专业学位类别名称	项目名称	项目负责人	建设周期	立项经费
1	经济学院	0201	理论经济学	云南大学-中央财经大学理论经济学科教融合研究生联合培养项目	陈瑛	2年	5万
2	经济学院	0202	应用经济学	云南大学-复旦大学经济学科教融合研究生联合培养项目	赵鑫铖	2年	5万
3	民族学与社会学学院	0303	社会学	云南大学社会学科教融合研究生联合培养项目	关凯	2年	5万
4	体育学院	0452	体育	云南大学-北京体育大学科教融合研究生联合培养项目	杨霞	2年	8万
5	文学院	0501	中国语言文学	云南大学-孟加拉达卡大学科教融合研究生联合培养项目	王卫东	2年	5万
6	外国语学院	0502	外国语言文学	云南大学-北京大学非通语种泰缅越科教融合研究生联合培养项目	李芳	2年	5万
7	外国语学院	0502	外国语言文学	云南大学-北京大学日语语言文学科教融合研究生联合培养项目	罗椿咏	2年	5万
8	历史与档案学院	0602	中国史	云南大学-南京大学中国史科教融合研究生联合培养项目	刘正寅	2年	5万

9	化学科学与工程学院	0703	化学	云南大学-湖南大学 科教融合研究生联合培养项目	方文浩	2 年	8 万
10	国际河流与生态安全研究院	0705	地理学	云南大学-中国科学院生态环境研究中心科教融合研究生联合培养项目	李艳波	2 年	8 万
11	地球科学学院	0706	大气科学	云南大学-中国科学院大气物理研究所 科教融合研究生联合培养项目	苏秦	2 年	8 万
12	生命科学学院	0710	生物学	云南大学-中国科学院微生物研究所 科教融合研究生联合培养项目	赖凡	2 年	8 万
13	生命科学学院	0710	生物学	云南大学-中国科学院遗传与发育研究所科教融合研究生联合培养项目	孙建伟	2 年	8 万
14	生物医药研究院	0710	生物学	云南大学-广州生物岛 科教融合研究生联合培养项目	陈大华	2 年	8 万
15	数学与统计学院	0714	统计学	云南大学-普利茅斯大学统计学 科教融合研究生联合培养项目	唐年胜	2 年	5 万
16	信息学院	0812	计算机科学与技术	云南大学-东南大学计算机科学与技术 科教融合研究生联合培养项目	钱文华	2 年	8 万
17	软件学院	0835	软件工程	云南大学-北京大学软件工程 科教融合研究生联合培养项目	张璇	2 年	8 万
18	软件学院	0839	网络空间安全	云南大学-北京航空航天大学网络空间安全科教融合研究生联合培养项目	姚绍文	2 年	8 万

2022年度云南大学产教融合研究生联合培养基地项目

序号	负责单位	一级学科代码/专业学位类别代码	一级学科名称/专业学位类别名称	项目名称	项目负责人	建设周期	立项经费
1	经济学院	0251	金融	云南大学-大童保险产教融合研究生联合培养基地项目	姚书杰	2年	5万
2	数学与统计学院	0252	应用统计	云南大学-广东泰迪智能科技产教融合研究生联合培养基地项目	李会琼	2年	5万
3	经济学院	0254	国际商务	云南大学-云南自由贸易试验区产教融合研究生联合培养基地项目	李隽	2年	8万
4	经济学院	0256	资产评估	云南大学-中和资产评估产教融合研究生联合培养基地项目	刘志坚	2年	8万
5	经济学院	0257	审计	云南大学-云南省审计厅产教融合研究生联合培养基地项目	施谦	2年	10万
6	法学院	0351	法律	云南大学-建纬（昆明）律师事务所产教融合研究生联合培养基地项目	刘国乾	2年	5万
7	民族学与社会学学院	0352	社会工作	云南大学“高校、医院、社会组织”共建医务社会工作人才产教融合研究生联合培养基地项目	高万红	2年	8万
8	职业与继续教育学院	0451	教育	云南大学职业技术教育产教融合研究生联合培养基地项目	罗昆	2年	10万

9	体育学院	0452	体育	云南大学-云南省体育科学研究所 产教融合研究生联合培养基地项目	李年红	2 年	5 万
10	体育学院	0452	体育	云南大学-云南云湖水上运动 产教融合研究生联合培养基地项目	杨雪芹	2 年	10 万
11	软件学院	0854	电子信息	云南大学-云南网际科技软件工程 产教融合研究生联合培养基地项目	张德海	2 年	8 万
12	软件学院	0854	电子信息	云南大学-奇安信网络空间安全 产教融合研究生联合培养基地项目	姚绍文	2 年	8 万
13	软件学院	0854	电子信息	云南大学-云南南天电子信息产业 产教融合研究生联合培养基地项目	张璇	2 年	5 万
14	信息学院	0854	电子信息	云南大学-百度计算机技术 产教融合研究生联合培养基地项目	钱文华	2 年	8 万
15	信息学院	0854	电子信息	云南大学-云南贝泰妮生物电子科技 产教融合研究生联合培养基地项目	张榆锋	2 年	5 万
16	地球科学学院	0857	资源与环境	云南大学-云南风蓝项目投资咨询 产教融合研究生联合培养基地项目	赵筱青	2 年	5 万
17	地球科学学院	0857	资源与环境	云南大学-云南省国土资源规划设计研究院 产教融合研究生联合培养基地项目	赵筱青	2 年	5 万
18	国际河流与生态安全研究院	0857	资源与环境	云南大学-昆明市滇池高原湖泊研究院 产教融合研究生联合培养基地项目	丁城志	2 年	10 万
19	国际河流与生态安全研究院 地球科学学院	0857	资源与环境	云南大学-中国电建集团昆明勘测设计研究院有限公司产教融合研究生联合培养基地项目	陆颖 解智强	2 年	10 万

20	国际河流与生态安全研究院	0857	资源与环境	云南大学-云南褚氏农业产教融合研究生联合培养基地项目	钟荣华	2 年	5 万
21	建筑与规划学院	0859	土木水利	云南大学-云南省建筑科学研究院产教融合研究生联合培养基地项目	冉志红	2 年	5 万
22	建筑与规划学院	0859	土木水利	云南大学-北京中外建建筑设计产教融合研究生联合培养基地项目	徐坚	2 年	5 万
23	建筑与规划学院	0859	土木水利	云南大学边疆民族地区规划与治理产教融合研究生联合培养基地项目	杨庆	2 年	5 万
24	化学科学与工程学院	0860	生物与医药	云南大学-云南昊邦制药产教融合研究生联合培养基地项目	韦琨	2 年	5 万
25	生命科学学院	0860	生物与医药	云南大学-北京协和医学院医学生物学研究所产教融合研究生联合培养基地项目	梁斌	2 年	8 万
26	资源植物研究院	0951	农业	云南大学多年生稻科技小院产教融合研究生联合培养基地项目	胡凤益	2 年	8 万
27	资源植物研究院	0951	农业	云南大学-保山比顿咖啡产教融合研究生联合培养基地项目	何飞飞	2 年	5 万
28	资源植物研究院	0951	农业	云南大学-汉晟丰工业大麻种植产教融合研究生联合培养基地项目	杜光辉	2 年	5 万
29	资源植物研究院	0951	农业	云南大学-云南省农业科学院产教融合研究生联合培养基地项目	胡凤益 吴学尉	2 年	10 万
30	资源植物研究院	0951	农业	云南大学-云天化产教融合研究生联合培养基地项目	何飞飞	2 年	5 万
31	工商管理与旅游管理学院	1251	工商管理	云南大学-云南省能投物流产教融合研究生联合培养基地项目	赵德森	2 年	5 万

32	政府管理学院	1252	公共管理	云南大学-云南省机关事务管理局 产教融合研究生联合培养基地项目	木永跃	2 年	8 万
33	工商管理与旅游管理学院	1253	会计	云南大学-中审众环会计 产教融合研究生联合培养基地项目	王健康	2 年	5 万
34	工商管理与旅游管理学院	1254	旅游管理	云南大学-怡美实业旅游管理 产教融合研究生联合培养基地项目	赵书虹	2 年	5 万
35	工商管理与旅游管理学院	1256	工程管理	云南大学-云南阳光道桥 产教融合研究生联合培养基地项目	雷晓凌	2 年	5 万

云南大学农学院与云南省农业科学院花卉研究所联合培养硕士研究生合作协议书

甲方：云南大学农学院（简称甲方）

乙方：云南省农业科学院花卉研究所（简称乙方）

为加强联合培养研究生的管理，提高研究生培养质量，理顺研究生联合培养过程中的各种关系，使培养工作能顺利开展，云南大学农学院与云南省农业科学院花卉研究所经过协商，就有关事项达成如下协议。

一、甲方的权利与义务

1、甲方在与乙方建立研究生联合培养基地，并共同成立研究生联合培养基地领导小组，协调研究生联合培养基地的建设、研究生的培养等工作。

2、研究生新生入学后，第一学年在学校学习学位课程，第二学年开始进入乙方开展学位论文研究工作。甲方根据具体情况将一定数量的研究生名额经双向选择分配到乙方。按导师遴选程序遴选导师，并对联合培养基地的导师进行培训和考核。

3、负责研究生招生、组织研究生的课程学习，并按学校规定发放全日制硕士研究生奖助学金。

4、负责研究生论文开题答辩，论文中期检查和论文答辩工作及硕士研究生培养相关工作。

二、乙方的权利与义务

1、负责研究生联合培养基地的条件建设，积极筹措和投入经费保证基地的运行，为研究生培养提供便利条件。

2、负责确定和承担研究生培养期间的研究生待遇。

3、加强对研究生的管理，在研究生培养时要实行导师负责制。

4、乙方导师要负责研究生的思想政治、安全管理和其它日常管理工作，应将研究生的思想、学习和工作情况及时与甲方沟通。并为研究生提供完备的住宿条件和科研条件。

5、乙方导师应遵守甲方关于研究生指导教师和导师组管理等有关规定，履行指导培养研究生的义务，严格按照甲方制定的研究生培养方案、培养计划和培养进度进行，包括进行开题报告、中期考核、论文答辩等相关的工作，按期完成研究生培养计划。

6、研究成果原则上归甲、乙双方共享。乙方导师指导培养的研究生所发表的学术论文，甲方应作为作者单位之一，至少有一篇论文署名作者第一单位为云南大学，其乙方导师为通讯作者。

7、在与研究生培养的有关信息收集及工作总结等方面，乙方应协助甲方的工作。

三、其他条款

1、本协议未尽事宜，双方协商解决。

2、本协议一式贰份，双方各持壹份，协议经双方签字盖章后生效，有效期为 2025 年 12 月 31 日。

3、若双方未提出终止协议，则本协议长期有效。若一方提出终止协议，须提前三个月通知对方，经双方认可后本协议终止。

甲方（盖章）：

甲方代表（签字）：

2015 年 11 月 3 日

乙方（盖章）：

乙方代表（签字）：

2015 年 11 月 3 日

云南大学农学院与云南省农业科学院园艺作物研究所联合培养硕士研究生合作协议书

甲方：云南大学农学院（简称甲方）

乙方：云南省农业科学院园艺作物研究所（简称乙方）

为加强联合培养研究生的管理，提高研究生培养质量，理顺研究生联合培养过程中的各种关系，使培养工作能顺利开展，云南大学农学院与云南省农业科学院花卉研究所经过协商，就有关事项达成如下协议。

一、甲方的权利与义务

1、甲方在与乙方建立研究生联合培养基地，并共同成立研究生联合培养基地领导小组，协调研究生联合培养基地的建设、研究生的培养等工作。

2、研究生新生入学后，第一学年在学校学习学位课程，第二学年开始进入乙方开展学位论文研究工作。甲方根据具体情况将一定数量的研究生名额经双向选择分配到乙方。按导师遴选程序遴选导师，并对联合培养基地的导师进行培训和考核。

3、负责研究生招生、组织研究生的课程学习，并按学校规定发放全日制硕士研究生奖助学金。

4、负责研究生论文开题答辩，论文中期检查和论文答辩工作及硕士研究生培养相关工作。

二、乙方的权利与义务

1、负责研究生联合培养基地的条件建设，积极筹措和投入经费保证基地的运行，为研究生培养提供便利条件。

2、负责确定和承担研究生培养期间的研究生待遇。

3、加强对研究生的管理，在研究生培养时要实行导师负责制。

4、乙方导师要负责研究生的思想政治、安全管理和其它日常管理工作，应将研究生的思想、学习和工作情况及时与甲方沟通。并为研究生提供完备的住宿条件和科研条件。

5、乙方导师应遵守甲方关于研究生指导教师和导师组管理等有关规定，履行指导培养研究生的义务，严格按照甲方制定的研究生培养方案、培养计划和培养进度进行，包括进行开题报告、中期考核、论文答辩等相关的工作，按期完成研究生培养计划。

6、研究成果原则上归甲、乙双方共享。乙方导师指导培养的研究生所发表的学术论文，甲方应作为作者单位之一，至少有一篇论文署名作者第一单位为云南大学，其乙方导师为通讯作者。

7、在与研究生培养的有关信息收集及工作总结等方面，乙方应协助甲方的工作。

三、其他条款

1、本协议未尽事宜，双方协商解决。

2、本协议一式贰份，双方各持壹份，协议经双方签字盖章后生效，有效期为 2025 年 12 月 31 日。

3、若双方未提出终止协议，则本协议长期有效。若一方提出终止协议，须提前三个月通知对方，经双方认可后本协议终止。

甲方（盖章）：

甲方代表（签字）：

2025年11月3日

乙方（盖章）：

乙方代表（签字）：

2025年11月3日

云南大学农学院与云南省农业科学院经济作物研究所联合培养硕士研究生合作协议书

甲方：云南大学农学院（以下简称甲方）

乙方：云南省农业科学院经济作物研究所（以下简称乙方）

为了加强联合培养研究生的管理，提高研究生培养质量，理顺研究生联合培养过程中的各种关系，使培养工作能顺利开展，云南大学农学院与云南省农业科学院经济作物研究所经过协商，就有关事项达成以下协议。

一、甲方的权利和义务

1、甲方在乙方建立研究生联合培养基地，并共同成立研究生联合培养基地领导小组，协调研究生联合培养基地的建设、研究生的培养等工作。

2、研究生新生入学后，第一学年在学校学习学位课程，第二学年开始进入乙方开展学位论文研究工作。甲方根据具体情况将一定数量的研究生名额经双向选择分配到乙方。按导师遴选程序遴选导师，并对联合培养基地的导师进行培训和考核。

3．负责研究生招生，组织研究生的课程教学，并按学校规定发放全日制硕士研究生奖助学金。

4、负责研究生论文的开题答辩，论文中期检查和论文答辩工作及硕士研究生培养相关工作。

二、乙方的权利和义务

1、负责研究生联合培养基地的条件建设，积极筹措和投入经费保证基地的运行，为研究生培养提供便利条件。

2、负责确定和承担研究生培养期间的研究生待遇。

3、加强对研究生的管理，在研究生培养时要实行导师负责制。

4、乙方导师要负责研究生的思想政治、安全管理和其它日常管理工作，应将研究生的思想、学习和工作情况及时与甲方沟通。并为研究生提供完备的住宿条件和科研条件。

5、乙方导师应遵守甲方关于研究生指导教师和导师组管理等有关规定，履行指导培养研究生的义务，严格按照甲方制定的研究生培养方案、培养计划和培养进度进行，包括进行开题报告、中期考核、论文答辩等相关的工作，按期完成研究生培养计划。

6、研究成果原则上归甲、乙双方共享。乙方导师指导培养的研究生所发表的学术论文，甲方应为作者单位之一，至少有一篇论文署名作者第一单位为云南大学，其乙方导师为通讯作者。

7、在与研究生培养的有关信息收集及工作总结等方面，乙方应协助甲方的工作。

三、其他条款。

1、本协议未尽事宜，双方协商解决。

2、本协议一式贰份，双方各持壹份，协议经双方签字盖章后生效。

3、若双方未提出终止协议，则本协议长期有效。若一方提出终止协议，须提前三个月通知对方，经双方认可后本协议终止。

甲方代表（签字）：

甲方单位（签章）：

2016年10月16日

乙方代表（签字）：

乙方单位（签章）：

2016年10月16日

云南大学农学院与云南禾韵科技有限公司联合培养硕士研究生合作协议书

甲方：云南大学农学院（以下简称甲方）

乙方：云南禾韵科技有限公司（以下简称乙方）

为了适应中国产业升级转型需要，更好地培养出既有良好专业知识又有实际操作技能的实用型人才，甲、乙双方就联合培养硕士研究生事宜达成如下协议：

一、合作内容

甲乙双方联合建设研究生培养基地，为硕士研究生的联合培养提供支撑。乙方根据生产和科研的需要，以若干生产环节或部门纳入培养基地范畴，在培养基地设置有关农业项目或研究课题任务。甲方定期组织选派硕士研究生进入培养基地开展相关实践和课题研究。

二、甲方权利与义务

1、积极与乙方沟通信息，共同做好培养基地建设的各项组织工作。

2、根据乙方需求，选派优秀学生进入培养基地进行农业实践和课题研究，并安排指导教师负责学生的思想教育和业务指导工作，协助乙方对进入培养基地的研究生进行纪律、安全和相关制度方面的岗前培训。

3、负责或协助完成乙方为研究生农业实践或论文工作提供的相关课题的研究任务。

4、优先聘请乙方具有丰富实践经验、较高学术水平或技术专长的专家作为甲方的兼职研究生指导教师，共同联合培养硕士研究生。

5、有计划地接受乙方有关人员的进修、培训或旁听，针对学习情况颁发结业证书。对乙方的教育培训、高层次人才需求等提供优先支持。利用学校优势有条件地开展科技合作或技术攻关，有偿为乙方解决生产实践中的技术难题。

6、甲方参与课题研究或农业实践的教师和研究生须遵守乙方的保密制度、安全制度和其他有关的规章制度。

7、甲方教师和研究生参与乙方的课题研究或农业项目取得的研究成果和知

识产权由双方共享。

三、乙方权利与义务

1、为培养基地提供必要的场地和研究条件，协助安排甲方教师和学生在培养基地工作期间的生活条件。

2、为研究生农业实践和论文研究提供相应的实践岗位和课题支持。

3、推荐乙方的有关专家和农业技术人员担任研究生副指导教师，并协助做好研究生在培养基地实践期间的管理工作。

4、做好研究生在培养基地实践期间的考评工作，并将考评意见反馈甲方。

5、在政策许可的前提下，基于本人自愿，乙方可优先选聘甲方的毕业生。

6、甲方教师和学生在培养基地学习期间患病所发生的费用，与乙方无关。

7、甲方人员在培养基地学习期间，若违反国家法律，乙方有权取消其在培养基地的学习资格，甲方人员自行承担法律责任。因甲方造成乙方财产损失的，按乙方的规章制度处理。如乙方发现甲方学生不符合联合培养要求时，可以向甲方提出终止联合培养。

8、有计划地向甲方推荐有关人员进入基地进修、培训或旁听。

四、附则

1、本协议未尽事宜，双方协商解决。

2、本协议一式贰份，双方各持壹份，协议经双方签字盖章后生效。

3、若双方未提出终止协议，则本协议长期有效。若一方提出终止协议，须提前三个月通知对方，经双方认可后本协议终止。

甲方代表（签字）：

甲方单位（签章）：

2016 年 4 月 6 日

乙方代表（签字）：

乙方单位（签章）：

2016 年 4 月 6 日

雲南大學
University Town, Chenggong, Kunming, Yunnan, China
Tel: +86-871-65155045 Fax: +86-871-65147713
Http: //www.ynu.edu.cn Zip code: 650500

校外实习基地协议书

甲方：**云南大学农学院**

乙方：**云南省热带作物科学研究所**

根据云南大学“双一流”培养目标及要求，为加强实践性教学环节，提高学生的认知能力，本着“资源共享，优势互补，相互协作，互惠互利，共同发展”的原则，甲、乙双方共同建设“云南大学农学院教学实习基地”，兹拟定如下协议：

第一条　甲方职责

1、发挥学校人才优势，提供技术支持和服务，讲授相关专业理论知识，向乙方推荐优秀毕业生。

2、学生实习期间，甲方带队老师和实习指导教师负责学生实习指导，学生思想政治、组织纪律、道德诚信、安全教育，配合乙方搞好专业教育和日常管理以及其它相关工作。

3、在实习前甲方向乙方提交实习计划（如学生人数、专业、实习时间、实习项目等）。

4、甲方制作并在乙方适当位置挂“云南大学农学院教学实习基地”牌匾。

第二条　乙方职责

1、在不影响乙方正常工作的情况下，乙方接收甲方学生实习，并提供实习必要的条件和便利，协助甲方完成实习教学任务。

2、根据甲方实习计划和要求，乙方做好实习方案与安排，经双方协商确定后执行。

雲南大學
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Http: //www.ynu.edu.cn Zip code: 650500

3、在实习期间，乙方派出责任心强、有实践经验的专业技术人员和管理人员担任实习指导教师，配合甲方对实习学生进行安全管理和纪律监督。

4、实习结束后，乙方如实提供学生实习期间表现的说明材料，协助甲方对实习学生做出实习鉴定。

第三条　其他

1、其它未尽事宜，经双方协商解决。

2、本协议一式两份，经双方签字盖章后生效，甲、乙双方各执一份。

3、本协议书有效期限：从 2018 年 7 月 23 日至 2022 年 7 月 23 日。

甲方（盖章）：
云南大学农学院

甲方代表（签字）：
2018年7月23日

乙方（盖章）：
云南省热带作物科学研究所

乙方代表（签字）：
2018年 7月23日

雲南大學
University Town, Chenggong, Kunming, Yunnan, China
Tel: +86-871-65155045 Fax: +86-871-65147713
Http: //www.ynu.edu.cn Zip code: 650500

校外实习基地协议书

甲方：**云南大学农学院**

乙方：**云南天泉生物科技股份有限公司**

根据云南大学“双一流”培养目标及要求，为加强实践性教学环节，提高学生的认知能力，本着“资源共享，优势互补，相互协作，互惠互利，共同发展”的原则，甲、乙双方共同建设“云南大学农学院教学实习基地”，兹拟定如下协议：

第一条　甲方职责

1、发挥学校人才优势，提供技术支持和服务，讲授相关专业理论知识，向乙方推荐优秀毕业生。

2、学生实习期间，甲方带队老师和实习指导教师负责学生实习指导，学生思想政治、组织纪律、道德诚信、安全教育，配合乙方搞好专业教育和日常管理以及其它相关工作。

3、在实习前甲方向乙方提交实习计划（如学生人数、专业、实习时间、实习项目等）。

4、甲方制作并在乙方适当位置挂“云南大学农学院教学实习基地”牌匾。

第二条　乙方职责

1、在不影响乙方正常工作的情况下，乙方接收甲方学生实习，并提供实习必要的条件和便利，协助甲方完成实习教学任务。

2、根据甲方实习计划和要求，乙方做好实习方案与安排，经双方协商确定后执行。

3、在实习期间，乙方派出责任心强、有实践经验的专业技术人员和管理人员担任实习指导教师，配合甲方对实习学生进行安全管理和纪律监督。

4、实习结束后，乙方如实提供学生实习期间表现的说明材料，协助甲方对实习学生做出实习鉴定。

第三条　其他

1、其它未尽事宜，经双方协商解决。

2、本协议一式两份，经双方签字盖章后生效，甲、乙双方各执一份。

3、本协议书有效期限：从 2019 年 月 日至 2023 年 月 日。

甲方（盖章）：
云南大学农学院

甲方代表（签字）：何飞飞
2019年9月4日

乙方（盖章）：
云南天泉生物科技股份有限公司

乙方代表（签字）：张伟
2019 年 9 月 4 日

云南大学农学院与玉溪市农业科学院联合培养硕士研究生合作协议书

甲方：云南大学农学院（以下简称甲方）

乙方：玉溪市农业科学院（以下简称乙方）

为了适应中国产业升级转型需要，更好地培养出既有良好专业知识又有实际操作技能的实用型人才，甲、乙双方就联合培养硕士研究生事宜达成如下协议：

一、合作内容

甲乙双方联合建设研究生培养基地，为硕士研究生的联合培养提供支撑。乙方根据生产和科研的需要，以若干生产环节或部门纳入培养基地范畴，在培养基地设置有关农业项目或研究课题任务。甲方定期组织选派硕士研究生进入培养基地开展相关实践和课题研究。

二、甲方权利与义务

1、积极与乙方沟通信息，共同做好培养基地建设的各项组织工作。

2、根据乙方需求，选派优秀学生进入培养基地进行农业实践和课题研究，并安排指导教师负责学生的思想教育和业务指导工作，协助乙方对进入培养基地的研究生进行纪律、安全和相关制度方面的岗前培训。

3、负责或协助完成乙方为研究生农业实践或论文工作提供的相关课题的研究任务。

4、优先聘请乙方具有丰富实践经验、较高学术水平或技术专长的专家作为甲方的兼职研究生指导教师，共同联合培养硕士研究生。

5、有计划地接受乙方有关人员的进修、培训或旁听，针对学习情况颁发结业证书。对乙方的教育培训、高层次人才需求等提供优先支持。利用学校优势有条件地开展科技合作或技术攻关，有偿为乙方解决生产实践中的技术难题。

6、甲方参与课题研究或农业实践的教师和研究生须遵守乙方的保密制度、安全制度和其他有关的规章制度。

7、甲方教师和研究生参与乙方的课题研究或农业项目取得的研究成果和知

识产权由双方共享。

三、乙方权利与义务

1、为培养基地提供必要的场地和研究条件，协助安排甲方教师和学生在培养基地工作期间的生活条件。

2、为研究生农业实践和论文研究提供相应的实践岗位和课题支持。

3、推荐乙方的有关专家和农业技术人员担任研究生副指导教师，并协助做好研究生在培养基地实践期间的管理工作。

4、做好研究生在培养基地实践期间的考评工作，并将考评意见反馈甲方。

5、在政策许可的前提下，基于本人自愿，乙方可优先选聘甲方的毕业生。

6、甲方教师和学生在培养基地学习期间患病所发生的费用，与乙方无关。

7、甲方人员在培养基地学习期间，若违反国家法律，乙方有权取消其在培养基地的学习资格，甲方人员自行承担法律责任。因甲方造成乙方财产损失的，按乙方的规章制度处理。如乙方发现甲方学生不符合联合培养要求时，可以向甲方提出终止联合培养。

8、有计划地向甲方推荐有关人员进入基地进修、培训或旁听。

四、附则

1、本协议未尽事宜，双方协商解决。

2、本协议一式四份，双方各持两份，协议经双方签字盖章后生效。

3、若双方未提出终止协议，则本协议长期有效。若一方提出终止协议，须提前三个月通知对方，经双方认可后本协议终止。

甲方代表（签字）：

甲方单位（签章）：

2017年12月3日

乙方代表（签字）：

乙方单位（签章）：

2017年12月3日

雲南大學
University Town, Chenggong, Kunming, Yunnan, China
Tel: +86-871-65155045 Fax: +86-871-65147713
Http: //www.ynu.edu.cn Zip code: 650500

校外教学实习基地协议书

甲方：云南大学农学院

乙方：曲靖市沾益区汉晟丰工业大麻种植有限公司

根据云南大学“双一流”培养目标及要求，为加强实践性教学环节，提高学生的认知能力，本着“资源共享，优势互补，相互协作，互惠互利，共同发展”的原则，甲、乙双方共同建设“云南大学农学院教学实习基地”，兹拟定如下协议：

第一条 甲方职责

1、发挥学校人才优势，提供技术支持和服务，讲授相关专业理论知识，向乙方推荐优秀毕业生。

2、学生实习期间，甲方带队老师和实习指导教师负责学生实习指导，学生思想政治、组织纪律、道德诚信、安全教育，配合乙方搞好专业教育和日常管理以及其它相关工作。

3、在实习前甲方向乙方提交实习计划（如学生人数、专业、实习时间、实习项目等）。

4、甲方制作并在乙方适当位置挂“云南大学农学院教学实习基地”牌匾。

第二条 乙方职责

1、在不影响乙方正常工作的情况下，乙方接收甲方学生实习，并提供实习必要的条件和便利，协助甲方完成实习教学任务。

2、根据甲方实习计划和要求，乙方做好实习方案与安排，经双方协商确定后执行。

雲南大學
University Town, Chenggong, Kunming, Yunnan, China
Tel: +86-871-65155045 Fax: +86-871-65147713
Http: //www.ynu.edu.cn Zip code: 650500

3、在实习期间，乙方派出责任心强、有实践经验的专业技术人员和管理人员担任实习指导教师，配合甲方对实习学生进行安全管理和纪律监督。

4、根据甲方要求，为甲方开展田间试验提供合适的试验用地。

5、实习结束后，乙方如实提供学生实习期间表现的说明材料，协助甲方对实习学生做出实习鉴定。

第三条 其他

1、其它未尽事宜，经双方协商解决。

2、本协议一式两份，经双方签字盖章后生效，甲、乙双方各执一份。

3、本协议书有效期限：从 2019 年 3 月 日至 2022 年 7 月 日。

甲方（盖章）：
云南农学院

甲方代表（签字）：

2019年3月14日

乙方（盖章）：
曲靖市沾益区汉晟丰工业大麻种植有限公司

乙方代表（签字）：

2019年3月14日

雲南大學
University Town, Chenggong, Kunming, Yunnan, China
Tel: +86-871-65155045 Fax: +86-871-65147713
Http: //www.ynu.edu.cn Zip code: 650500

校外实习基地协议书

甲方：云南大学农学院

乙方：普洱茶研究院

根据云南大学“双一流”培养目标及要求，为加强实践性教学环节，提高学生的认知能力，本着资源共享，优势互补，相互协作，互惠互利，共同发展的原则，甲、乙双方共同建设“云南大学农学院教学实习基地”，兹拟定如下协议：

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4、甲方制作并在乙方适当位置挂“云南大学农学院教学实习基地”牌匾。

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1、在不影响乙方正常工作的情况下，乙方接收甲方学生实习，并提供实习必要的条件和便利，协助甲方完成实习教学任务。

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3、在实习期间，乙方派出责任心强、有实践经验的专业技术人员和管理人员担任实习指导教师，配合甲方对实习学生进行安全管理和纪律监督。

4、实习结束后，乙方如实提供学生实习期间表现的说明材料，协助甲方对实习学生做出实习鉴定。

第三条　其他

1、其它未尽事宜，经双方协商解决。

2、本协议一式两份，经双方签字盖章后生效，甲、乙双方各执一份。

3、本协议书有效期限：从 2018 年 7 月 日至 2022 年 7 月 日。

甲方（盖章）：
云南大学农学院
甲方代表（签字）：
2018年 7月21日

乙方（盖章）：
普洱茶研究院
乙方代表（签字）：
2018年 7月21日

云南大学农学院与景洪市农业技术推广中心联合培养硕士研究生合作协议书

甲方：云南大学农学院（以下简称甲方）

乙方：景洪市农业技术推广中心（以下简称乙方）

为了适应中国产业升级转型需要，更好地培养出既有良好专业知识又有实际操作技能的实用型人才，甲、乙双方就联合培养硕士研究生事宜达成如下协议：

一、合作内容

甲乙双方联合建设研究生培养基地，为硕士研究生的联合培养提供支撑。乙方根据生产和科研的需要，以若干生产环节或部门纳入培养基地范畴，在培养基地设置有关农业项目或研究课题任务。甲方定期组织选派硕士研究生进入培养基地开展相关实践和课题研究。

二、甲方权利与义务

1、积极与乙方沟通信息，共同做好培养基地建设的各项组织工作。

2、根据乙方需求，选派优秀学生进入培养基地进行农业实践和课题研究，并安排指导教师负责学生的思想教育和业务指导工作，协助乙方对进入培养基地的研究生进行纪律、安全和相关制度方面的岗前培训。

3、负责或协助完成乙方为研究生农业实践或论文工作提供的相关课题的研究任务。

4、优先聘请乙方具有丰富实践经验、较高学术水平或技术专长的专家作为甲方的兼职研究生指导教师，共同联合培养硕士研究生。

5、有计划地接受乙方有关人员的进修、培训或旁听，针对学习情况颁发结业证书。对乙方的教育培训、高层次人才需求等提供优先支持。利用学校优势有条件地开展科技合作或技术攻关，有偿为乙方解决生产实践中的技术难题。

6、甲方参与课题研究或农业实践的教师和研究生须遵守乙方的保密制度、安全制度和其他有关的规章制度。

7、甲方教师和研究生参与乙方的课题研究或农业项目取得的研究成果和知

识产权由双方共享。

三、乙方权利与义务

1、为培养基地提供必要的场地和研究条件，协助安排甲方教师和学生在培养基地工作期间的生活条件。

2、为研究生农业实践和论文研究提供相应的实践岗位和课题支持。

3、推荐乙方的有关专家和农业技术人员担任研究生副指导教师，并协助做好研究生在培养基地实践期间的管理工作。

4、做好研究生在培养基地实践期间的考评工作，并将考评意见反馈甲方。

5、在政策许可的前提下，基于本人自愿，乙方可优先选聘甲方的毕业生。

6、甲方教师和学生在培养基地学习期间患病所发生的费用，与乙方无关。

7、甲方人员在培养基地学习期间，若违反国家法律，乙方有权取消其在培养基地的学习资格，甲方人员自行承担法律责任。因甲方造成乙方财产损失的，按乙方的规章制度处理。如乙方发现甲方学生不符合联合培养要求时，可以向甲方提出终止联合培养。

8、有计划地向甲方推荐有关人员进入基地进修、培训或旁听。

四、附则

1、本协议未尽事宜，双方协商解决。

2、本协议一式四份，双方各持两份，协议经双方签字盖章后生效。

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甲方代表（签字）：

甲方单位（签章）：

2017 年 9 月 20 日

乙方代表（签字）：

乙方单位（签章）：

2017 年 9 月 20 日

云南大学农学院与西双版纳傣族自治州农业科学研究所联合培养硕士研究生合作协议书

甲方：云南大学农学院（以下简称甲方）

乙方：西双版纳傣族自治州农业科学研究所（以下简称乙方）

为了适应中国产业升级转型需要，更好地培养出既有良好专业知识又有实际操作技能的实用型人才，甲、乙双方就联合培养硕士研究生事宜达成如下协议：

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甲乙双方联合建设研究生培养基地，为硕士研究生的联合培养提供支撑。乙方根据生产和科研的需要，以若干生产环节或部门纳入培养基地范畴，在培养基地设置有关农业项目或研究课题任务。甲方定期组织选派硕士研究生进入培养基地开展相关实践和课题研究。

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2、根据乙方需求，选派优秀学生进入培养基地进行农业实践和课题研究，并安排指导教师负责学生的思想教育和业务指导工作，协助乙方对进入培养基地的研究生进行纪律、安全和相关制度方面的岗前培训。

3、负责或协助完成乙方为研究生农业实践或论文工作提供的相关课题的研究任务。

4、优先聘请乙方具有丰富实践经验、较高学术水平或技术专长的专家作为甲方的兼职研究生指导教师，共同联合培养硕士研究生。

5、有计划地接受乙方有关人员的进修、培训或旁听，针对学习情况颁发结业证书。对乙方的教育培训、高层次人才需求等提供优先支持。利用学校优势有条件地开展科技合作或技术攻关，有偿为乙方解决生产实践中的技术难题。

6、甲方参与课题研究或农业实践的教师和研究生须遵守乙方的保密制度、安全制度和其他有关的规章制度。

7、甲方教师和研究生参与乙方的课题研究或农业项目取得的研究成果和知

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2、为研究生农业实践和论文研究提供相应的实践岗位和课题支持。

3、推荐乙方的有关专家和农业技术人员担任研究生副指导教师，并协助做好研究生在培养基地实践期间的管理工作。

4、做好研究生在培养基地实践期间的考评工作，并将考评意见反馈甲方。

5、在政策许可的前提下，基于本人自愿，乙方可优先选聘甲方的毕业生。

6、甲方教师和学生在培养基地学习期间患病所发生的费用，与乙方无关。

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甲方代表（签字）：

甲方单位（签章）：

2016 年 10 月 11 日

乙方代表（签字）：

乙方单位（签章）：

2016 年 10 月 11 日

雲南大學
University Town, Chenggong, Kunming, Yunnan, China
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Http: //www.ynu.edu.cn Zip code: 650500

校外实习基地协议书

甲方：**云南大学农学院**

乙方：**云南景兰热作科技有限责任公司**

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第三条 其他

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2、本协议一式两份，经双方签字盖章后生效，甲、乙双方各执一份。

3、本协议书有效期限：从 2019 年 11 月 15 日至 2023 年 11 月 15 日。

甲方（盖章）：
云南大学农学院

甲方代表（签字）：何飞

2019年11月15日

乙方（盖章）：
云南景兰热作科技有限责任公司

乙方代表（签字）：

2019 年 11 月 15 日

云南省学位委员会
云南省教育厅文件

云学位〔2021〕17号

云南省学位委员会 云南省教育厅关于公布2021年度云南省研究生导师团队建设项目名单的通知

各研究生培养单位：

根据《云南省学位委员会办公室关于开展2021年度研究生导师团队建设项目申报工作的通知》（云学位办〔2021〕11号）要求，经各研究生培养单位推荐、省学位委员会办公室审核和公示等程序，决定立项建设116个省级研究生导师团队（具体名单见附件）。

项目建设期限为2年，每个导师团队在建设期限内由申报单位投入经费不少于5万元进行建设。各培养单位要严格按照《云南省研究生导师团队建设管理实施方案》要求，确保建设经费投入，加强经费管理，督促项目负责人切实加强导师团队建设质量，全面提高全省研究生导师队伍整体水平。

附件：2021年度云南省研究生导师团队建设项目名单

云南省学位委员会　　云南省教育厅

2021年11月26日

（此件公开发布）

附件

2021年度云南省研究生导师团队建设项目名单

序号	团队名称	学校名称	所在学科（专业学位类别）	团队带头人
1	人口资源与环境经济学博士生导师团队	云南大学	理论经济学	张国胜
2	中国近现代史与党史基本问题研究博士生导师团队	云南大学	马克思主义理论	张巨成
3	中国现当代文学研究博士生导师团队	云南大学	中国语言文学	张志平
4	运筹学博士生导师团队	云南大学	数学	李建平
5	手性合成化学博士生导师团队	云南大学	化学	邵志会
6	动物学科博士生导师团队	云南大学	生物学	罗静
7	水资源与水灾害博士生导师团队	云南大学	地理学	刘时银
8	机器人与智能系统博士生导师团队	云南大学	通信与信息工程	陶大鹏
9	数据科学与人工智能博士生导师团队	云南大学	计算机科学与技术	岳昆
10	旅游管理博士生导师团队	云南大学	工商管理	田卫民
11	经济民族学硕士生导师团队	云南大学	民族学	郑宇
12	区域水土资源-环境演变与国土空间优化管控硕士生导师团队	云南大学	地理学	赵筱青
13	横断山区灾害性天气气候发生机理及预测硕士生导师团队	云南大学	大气科学	杨若文
14	岩浆岩与内生金属矿床硕士生导师团队	云南大学	地质学	谈树成
15	多年生稻种质资源创制与应用硕士生导师团队	云南大学	作物学	胡凤益
16	视觉媒体与文化计算硕士生导师团队	云南大学	计算机科学与技术	徐丹
17	网络空间安全硕士生导师团队	云南大学	网络空间安全	姚绍文
18	高原遥感博士生导师团队	昆明理工大学	测绘科学与技术	唐伯惠
19	矿山岩土灾害与固废生态资源化博士生导师团队	昆明理工大学	矿业工程	王光进

序号	团队名称	学校名称	所在学科（专业学位类别）	团队带头人
20	成矿动力学与隐伏矿预测博士生导师团队	昆明理工大学	地质资源与地质工程	韩润生
21	二氧化碳减排及利用博士生导师团队	昆明理工大学	动力工程及工程热物理	祝星
22	新能源电池材料博士生导师团队	昆明理工大学	冶金工程	张英杰
23	复杂机电系统智能控制博士生导师团队	昆明理工大学	机械工程	那靖
24	车辆智能控制与运行安全博士生导师团队	昆明理工大学	交通运输工程	陈峥
25	水环境新型污染物防治博士生导师团队	昆明理工大学	环境科学与工程	潘学军
26	磷化工博士生导师团队	昆明理工大学	化学工程与技术	梅毅
27	细胞分子生物学博士生导师团队	昆明理工大学	生物学	白洁
28	工程风险防控理论与应用博士生导师团队	昆明理工大学	土木工程	宋志刚
29	复杂系统建模与调控博士生导师团队	昆明理工大学	系统科学	刘文奇
30	西南三江成矿规律与找矿硕士生导师团队	昆明理工大学	地质资源与地质工程	邓明国
31	战略金属矿产资源绿色分离与富集硕士生导师团队	昆明理工大学	矿业工程	刘殿文
32	离子液体冶金硕士生导师团队	昆明理工大学	冶金工程	徐存英
33	电力系统安全稳定运行与控制硕士生导师团队	昆明理工大学	电气工程	刘志坚
34	地域性建筑设计及理论研究硕士生导师团队	昆明理工大学	建筑学	王冬
35	光电信息技术博士生导师团队	云南师范大学	光学工程	冯小波
36	政治地理与边疆发展博士生导师团队	云南师范大学	地理学	熊理然
37	新能源材料与器件博士生导师团队	云南师范大学	农业工程	杨培志
38	资源与环境遥感应用硕士生导师团队	云南师范大学	地理学	王金亮
39	模式识别与人工智能硕士生导师团队	云南师范大学	计算机科学与技术	杨扬

序号	团队名称	学校名称	所在学科（专业学位类别）	团队带头人
40	古代文学与文献学硕士生导师团队	云南师范大学	中国语言文学	傅宇斌
41	功能材料化学硕士生导师团队	云南师范大学	化学	谢生明
42	“澜湄国家”国际中文教育与传播硕士生导师团队	云南师范大学	汉语国际教育	王秀成
43	金融创新与风险管理硕士生导师团队	云南师范大学	金融	李富昌
44	新闻与传播硕士专业学位硕士生导师团队	云南师范大学	新闻与传播	杜忠锋
45	人力资源管理硕士生导师团队	云南师范大学	工商管理	李正彪
46	翻译（英语）硕士生导师团队	云南师范大学	翻译	黄　瑛
47	美术领域中国画方向硕士生导师团队	云南师范大学	艺术	戴　杰
48	法律硕士专业学位硕士生导师团队	云南师范大学	法律	周梁云
49	学科教学（数学领域）硕士生导师团队	云南师范大学	教育（学科教学数学)	王永生
50	常见病基础医学博士生导师团队	昆明医科大学	基础医学	杨照青
51	消化病学博士生导师团队	昆明医科大学	临床医学	唐映梅
52	普通外科学博士生导师团队	昆明医科大学	临床医学	曾仲
53	盆腔肿瘤学博士生导师团队	昆明医科大学	临床医学	董坚
54	药理学博士生导师团队	昆明医科大学	药学	陈鹏
55	生物学硕士生导师团队	昆明医科大学	生物学	朱月春
56	医学技术硕士生导师团队	昆明医科大学	医学技术	杨毅
57	绿色食品加工与安全控制博士生导师团队	云南农业大学	食品科学与工程	龚加顺
58	动物遗传育种与繁殖博士生导师团队	云南农业大学	畜牧学	邓卫东
59	马克思主义基本原理硕士生导师团队	云南农业大学	马克思主义理论	杨玲
60	智慧农业水土工程硕士生导师团队	云南农业大学	农业工程	李靖
61	食药同源功能性食品硕士生导师团队	云南农业大学	食品科学与工程	田洋
62	茶学硕士生导师团队	云南农业大学	园艺学	王白娟

序号	团队名称	学校名称	所在学科（专业学位类别）	团队带头人
63	农药开发与利用硕士生导师团队	云南农业大学	植物保护	吴国星
64	土壤学硕士生导师团队	云南农业大学	农业资源与环境	李永梅
65	农业经济管理硕士生导师团队	云南农业大学	农林经济管理	起建凌
66	体育教学硕士生导师团队	云南农业大学	体育	王钧
67	电力信息物理系统硕士生导师团队	云南民族大学	能源动力	徐天奇
68	有机功能分子及材料硕士生导师团队	云南民族大学	化学	杨丽娟
69	少数民族文学硕士生导师团队	云南民族大学	中国语言文学	龙珊
70	复杂系统理论与应用硕士生导师团队	云南民族大学	数学	熊良林
71	中国古代文学硕士生导师团队	云南民族大学	中国语言文学	周雪根
72	政治学硕士生导师团队	云南民族大学	政治学	陆海发
73	社会体育指导硕士生导师团队	云南民族大学	体育	李莹
74	宏观经济（周期与增长理论创新研究）博士生导师团队	云南财经大学	应用经济学	陈昆亭
75	应用统计博士生导师团队	云南财经大学	统计学	费宇
76	财政学学术训练与创新人才培养硕士生导师团队	云南财经大学	应用经济学	缪小林
77	知识管理与高质量创新硕士生导师团队	云南财经大学	管理科学与工程	段云龙
78	新兴信息技术与数字经济硕士生导师团队	云南财经大学	计算机科学与技术	姜茸
79	运筹与优化硕士生导师团队	云南财经大学	数学	陈龙伟
80	组织行为与边疆治理硕士生导师团队	云南财经大学	公共管理	马国芳
81	预测理论硕士生导师团队	云南财经大学	统计学	喻达磊
82	数理统计及应用硕士生导师团队	云南财经大学	统计学	陈飞
83	南亚东南亚区域经济合作硕士生导师团队	云南财经大学	国际商务	王荣党
84	边疆社会治理法治化硕士生导师团队	云南财经大学	法学	佴澎

序号	团队名称	学校名称	所在学科（专业学位类别）	团队带头人
85	工作能量与工作健康研究硕士生导师团队	云南财经大学	工商管理	江新会
86	中医痹病博士生导师团队	云南中医药大学	中医学	李兆福
87	中医生殖博士生导师团队	云南中医药大学	中医学	赵荣
88	云南省道地濒危中药材繁育与栽培技术创新硕士生导师团队	云南中医药大学	中药学	徐福荣
89	中药防治代谢综合征硕士生导师团队	云南中医药大学	中药学	俞捷
90	慢性肺病硕士生导师团队	云南中医药大学	中西医结合	付义
91	中药（民族药）标准应用硕士生导师团队	云南中医药大学	中药学	周志宏
92	扶阳理论治疗中医脾胃病硕士生导师团队	云南中医药大学	中医学	姜莉云
93	水土保持博士生导师团队	西南林业大学	林学	陈奇伯
94	木材胶黏剂研发博士生导师团队	西南林业大学	林业工程	雷洪
95	园林植物遗传改良与高效繁育博士生导师团队	西南林业大学	风景园林学	黄海泉
96	数字林业及森林资源管理硕士生导师团队	西南林业大学	林学	欧光龙
97	系统科学硕士生导师团队	西南林业大学	系统科学	代飞
98	生态旅游与乡村振兴硕士生导师团队	西南林业大学	地理学	唐雪琼
99	林下中药材生态评估硕士生导师团队	西南林业大学	农业	何霞红
100	植物多样性与资源利用硕士生导师团队	西南林业大学	林业	张大才
101	生物质树脂及材料硕士生导师团队	西南林业大学	材料与化工	周晓剑

序号	团队名称	学校名称	所在学科（专业学位类别）	团队带头人
102	医学细胞生物学硕士生导师团队	大理大学	基础医学	周玥
103	美洲大蠊系列药物深度研发硕士生导师团队	大理大学	药学	彭芳
104	中药及民族药资源开发利用硕士生导师团队	大理大学	药学	夏从龙
105	药理学硕士生导师团队	大理大学	药学	赖泳
106	天然活性成分发现与结构优化硕士生导师团队	大理大学	药学	王福生
107	马克思主义基本原理硕士生导师团队	大理大学	马克思主义理论	赵金元
108	小学教育硕士生导师团队	大理大学	教育	田夏彪
109	中西方音乐史研究硕士生导师团队	云南艺术学院	音乐与舞蹈学	师向宁
110	中国民族民间舞蹈教育方向硕士生导师团队	云南艺术学院	艺术	徐梅
111	戏剧人类学的理论与实践硕士生导师团队	云南艺术学院	戏剧与影视学	曾静
112	版画语言研究硕士生导师团队	云南艺术学院	艺术	陈光勇
113	云南民间工艺文化研究与设计硕士生导师团队	云南艺术学院	设计学	万凡
114	人工微结构材料硕士生导师团队	昆明学院	物理学	杨海
115	资源利用硕士生导师团队	昆明学院	农业	徐胜光
116	智慧农业与分子遗传学硕士生导师团队	昆明学院	农业	李晶

云南省教育厅办公室　　2021年11月30日印发

云南省学位委员会
云南省教育厅文件

云学位〔2021〕16号

云南省学位委员会 云南省教育厅关于公布2021年度云南省研究生优质课程建设项目名单的通知

各研究生培养单位：

为贯彻落实《教育部关于改进和加强研究生课程建设的意见》（教研〔2014〕5号），根据《云南省学位委员会办公室关于做好2021年度云南省研究生优质课程项目申报工作的通知》（云学位〔2020〕8号）要求，经各研究生培养单位评审推荐、省学位委员会办公室审核和公示等程序，决定立项建设云南大学等12

个研究生培养单位的98门研究生优质课程（以下简称优质课程，名单见附件）。

项目建设期限为2年，每门优质课程在建设期限内由申报单位投入经费不少于5万元进行建设。各培养单位要严格按照《云南省研究生优质课程建设实施方案》要求，确保建设经费投入，加强经费管理，督促项目负责人切实提高研究生优质课程建设质量，推动全省研究生课程教学内容、教学模式和教学方法创新。

附件：2021年度云南省研究生优质课程建设项目名单

云南省学位委员会　　云南省教育厅

2021年11月26日

（此件公开发布）

附件

2021年度云南省研究生优质课程建设项目名单

序号	课程名称	培养单位	负责人姓名	课程类型(博士/硕士)	课程类别（公共学位课/专业学位课）	所属学科（专业学位类别）
1	植物分类与系统学	云南大学	周新茂	硕士	专业学位课	生态学
2	旅游产业经济学	云南大学	吕宛青	硕士	专业学位课	工商管理/旅游管理（专硕）
3	市场营销	云南大学	赵书虹	硕士	专业学位课	工商管理（MBA）
4	运营管理	云南大学	吴奇志	硕士	专业学位课	工商管理（MBA）
5	企业战略管理	云南大学	高核	硕士	专业学位课	工商管理（MBA）
6	金融法原理	云南大学	周昌发	硕士	专业学位课	法学
7	国外马克思主义专题研究	云南大学	袁群	博士	专业学位课	马克思主义理论
8	高等岩石学	云南大学	孙涛	硕士	专业学位课	地质学
9	陆气相互作用研究进展	云南大学	杨启东	硕士	专业学位课	大气科学
10	土地覆被与土地评价	云南大学	陈俊旭	硕士	专业学位课	地理学
11	多元统计分析	云南大学	李会琼	硕士	专业学位课	统计学
12	算法图论	云南大学	李建平	硕士	专业学位课	数学
13	现代农业创新与乡村振兴战略	云南大学	胡凤益	硕士	专业学位课	农业（农艺与种业）
14	材料结构与性能	云南大学	郭洪	博士	专业学位课	材料科学与工程
15	中级微观经济学	云南大学	姚书杰	硕士	专业学位课	理论经济学/金融
16	国际贸易政策与实务	云南大学	胡颖	硕士	专业学位课	国际商务

序号	课程名称	培养单位	负责人姓名	课程类型(博士/硕士)	课程类别（公共学位课/专业学位课）	所属学科（专业学位类别）
17	智能数据工程	云南大学	岳昆	硕士	专业学位课	电子信息
18	经济人类学	云南大学	郑宇	硕士	专业学位课	民族学
19	民事诉讼法专题	昆明理工大学	王嘎利	硕士	专业学位课	法学
20	选择性磨矿	昆明理工大学	肖庆飞	硕士	专业学位课	矿业工程
21	建筑与城市设计2	昆明理工大学	吴志宏	硕士	专业学位课	建筑学
22	数据仓库与数据挖掘	昆明理工大学	丁家满	硕士	专业学位课	计算机科学与技术
23	硅冶金与硅材料	昆明理工大学	魏奎先	硕士	专业学位课	冶金工程
24	地质灾害防治	昆明理工大学	杨志全	硕士	专业学位课	安全科学与工程
25	反应工程	昆明理工大学	马丽萍	博士/硕士	公共学位课	环境科学与工程
26	管理会计理论与实务	昆明理工大学	胡元林	硕士	专业学位课	工商管理
27	交通运输数据分析理论与实践	昆明理工大学	帅春燕	博士/硕士	专业学位课	交通运输工程
28	对外汉语课堂教学案例	昆明理工大学	潘玉华	硕士	专业学位课	汉语国际教育
29	岩土加固技术	昆明理工大学	吴顺川	硕士	专业学位课	矿业工程
30	乡村聚落研究专题	昆明理工大学	车震宇	硕士	专业学位课	城市规划
31	专业英语（双语）	昆明理工大学	易俊洁	硕士	专业学位课	食品科学与工程
32	功能陶瓷物理基础	昆明理工大学	陈清明	硕士	专业学位课	材料科学与工程
33	高等金属学	昆明理工大学	曹建春	硕士	专业学位课	材料科学与工程
34	地理学思想方法	云南师范大学	潘玉君	博士	专业学位课	地理学
35	高等经济地理	云南师范大学	熊理然	博士	专业学位课	地理学

序号	课程名称	培养单位	负责人姓名	课程类型(博士/硕士)	课程类别（公共学位课/专业学位课）	所属学科（专业学位类别）
36	质性研究方法	云南师范大学	尹可丽	博士	专业学位课	教育
37	教育基本理论专题	云南师范大学	陈瑶	博士	专业学位课	教育学
38	论文写作指导	云南师范大学	刘利平	博士	专业学位课	历史学
39	高等光子学	云南师范大学	刘刚	博士	专业学位课	光学工程
40	光伏科学理论与工程设计	云南师范大学	涂洁磊	博士	专业学位课	农业工程
41	细胞与分子生物学	云南师范大学	龚明	博士	专业学位课	生物学
42	中国马克思主义与当代	云南师范大学	刘化军	博士	公共学位课	
43	英文科技论文写作	云南师范大学	尚轶	博士	专业学位课	生物学
44	现场流行病学	昆明医科大学	陈莹	硕士	专业学位课	公共卫生与预防医学
45	高级健康评估	昆明医科大学	冯蕾	硕士	专业学位课	护理学
46	实用 DNA 分析技术	昆明医科大学	聂胜洁	博士	专业学位课	基础医学
47	人体断层与影像解剖学	昆明医科大学	邹智荣	博士	专业学位课	基础医学
48	神经病学	昆明医科大学	殷梅	硕士	专业学位课	临床医学
49	现代动物遗传学	云南农业大学	苗永旺	硕士	专业学位课	畜牧学
50	数值分析	云南农业大学	黄峻伟	硕士	专业学位课	农业工程
51	农业工程专论	云南农业大学	张刘东	硕士	专业学位课	土木水利
52	生物化学与分子生物学(双语)	云南农业大学	赵昶灵	硕士	专业学位课	生物学
53	现代兽医病理学	云南农业大学	高洪	硕士	专业学位课	兽医学
54	环境生态与生态恢复	云南农业大学	祖艳群	硕士	专业学位课	资源与环境

序号	课程名称	培养单位	负责人姓名	课程类型(博士/硕士)	课程类别（公共学位课/专业学位课）	所属学科（专业学位类别）
55	社会体育学	云南农业大学	岁金	硕士	专业学位课	体育
56	社会工作理论	云南农业大学	王晓艳	硕士	专业学位课	社会工作
57	社会理论	云南民族大学	朴光星	博士	专业学位课	社会学
58	中国政治思想史	云南民族大学	吕朝辉	硕士	专业学位课	政治学
59	电分析化学	云南民族大学	张艳丽	硕士	专业学位课	化学
60	高级管理会计理论与实务	云南民族大学	赵馨燕	硕士	专业学位课	工商管理
61	高级生物化学	云南民族大学	袁燕	硕士	专业学位课	化学
62	运动技能学习原理	云南民族大学	李莹	硕士	专业学位课	体育
63	文化语言学	云南民族大学	李强	硕士	专业学位课	外国语言文学
64	中国现当代作家作品研究	云南民族大学	刘玉霞	硕士	专业学位课	中国语言文学
65	高级宏观经济学	云南财经大学	龚刚	博士	专业学位课	理论经济学
66	货币金融理论	云南财经大学	唐柳洁	硕士	专业学位课	应用经济学
67	营销管理	云南财经大学	鲁平俊	博士/硕士	专业学位课	工商管理
68	国际商务	云南财经大学	尹豪	硕士	专业学位课	国际商务
69	人力资源管理	云南财经大学	黄波	硕士	专业学位课	工商管理
70	经济法学	云南财经大学	马慧娟	硕士	专业学位课	法学
71	人工智能	云南财经大学	陈韬伟	硕士	专业学位课	计算机科学与技术
72	社会研究方法	云南财经大学	韩全芳	硕士	专业学位课	公共管理

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73	国际税收管理	云南财经大学	陈庆萍	硕士	专业学位课	应用经济学
74	中医临床基础辨证方法概论	云南中医药大学	李兆福	硕士	专业学位课	中医学
75	高级护理实践能力培养	云南中医药大学	郭趣	硕士	专业学位课	护理
76	云南吴氏扶阳学术思想中的脾胃病理论与实践	云南中医药大学	吴文笛	硕士	专业学位课	中医
77	分子药理学	云南中医药大学	王睿睿	硕士	专业学位课	药学
78	中药药理学与临床应用	云南中医药大学	李秀芳	硕士	专业学位课	中药学
79	现代药物分析技术	云南中医药大学	杨兴鑫	硕士	专业学位课	药学
80	农村发展规划	西南林业大学	付伟	硕士	专业学位课	农业
81	胶黏剂与人造板工艺学	西南林业大学	雷洪	硕士	专业学位课	林业工程
82	新时代中国特色社会主义理论与实践	西南林业大学	王俊勇	硕士	公共学位课	马克思主义理论
83	现代植物生产理论与技术	西南林业大学	李宗艳	硕士	专业学位课	农业
84	系统科学专业英语	西南林业大学	吕丹桔	硕士	专业学位课	系统科学
85	中级应用统计学	西南林业大学	李春波	硕士	专业学位课	工商管理
86	循证护理	大理大学	付艳芬	硕士	专业学位课	护理学
87	体内药物分析	大理大学	王燕	硕士	专业学位课	药学
88	数学教育测量与评价	大理大学	王彭德	硕士	专业学位课	教育学
89	临床流行病学	大理大学	张态	硕士	专业学位课	公共卫生与预防医学

序号	课程名称	培养单位	负责人姓名	课程类型(博士/硕士)	课程类别（公共学位课/专业学位课）	所属学科（专业学位类别）
90	高级护理实践	大理大学	汤琚	硕士	专业学位课	护理学
91	艺术理论	云南艺术学院	吴婉婷	硕士	专业学位课	艺术学理论
92	舞蹈教育学	云南艺术学院	额瑜婷	硕士	专业学位课	音乐与舞蹈学
93	表演训练-1	云南艺术学院	张天欣	硕士	专业学位课	戏剧与影视学
94	雕塑创作与表现	云南艺术学院	张仲夏	硕士	专业学位课	美术学
95	民居建筑装饰艺术专题研究	云南艺术学院	王锐	硕士	专业学位课	设计学
96	新时代中国特色社会主义理论与实践	昆明学院	张春梅	硕士	公共学位课	
97	现代农业创新与乡村振兴战略	昆明学院	李育川	硕士	基础学位课	农业
98	中国语言文学研究方法论	昆明学院	邓瑶	硕士	基础学位课	中国语言文学

云南省教育厅办公室　　2021年11月30日印发

云南省学位委员会
云南省教育厅文件

云学位〔2021〕18号

云南省学位委员会　云南省教育厅关于公布2021年度云南省专业学位研究生教学案例库建设项目名单的通知

各研究生培养单位：

根据《云南省学位委员会关于做好2021年度云南省专业学位研究生教学案例库项目申报工作的通知》（云学位〔2021〕6号）要求，经各研究生培养单位评审推荐和省学位委员会办公室审核，决定立项建设234个省级专业学位研究生教学案例库项目（名单见附件）。

项目建设期限为2年，每个案例库建设项目在建设期限内由

申报单位投入经费不少于3万元进行建设。各培养单位要严格按照《云南省专业学位研究生教学案例库建设实施方案》要求，确保建设经费投入，加强经费管理，督促项目负责人切实提高专业学位研究生教学案例库建设质量，提高全省专业学位研究生教育教学质量。

附件：2021年度云南省专业学位研究生教学案例库建设项目名单

云南省学位委员会　　云南省教育厅

2021年11月30日

（此件公开发布）

附件

2021年度云南省专业学位研究生案例库建设项目名单

序号	项目名称	学校	培养层次	专业学位类别	负责人
1	应用统计建模教学案例数据库	云南大学	硕士	应用统计	李会琼
2	现代农业发展与实践案例库	云南大学	硕士	农业	吴学尉
3	植物生理学研究进展教学案例库	云南大学	硕士	生物与医药	陈小兰
4	人工智能导论教学案例库	云南大学	硕士	电子信息	刘松岩
5	控制工程综合实践课程案例库	云南大学	硕士	电子信息	余鹏飞
6	软件工程专业硕士培养教学案例库	云南大学	硕士	电子信息	张德海
7	水土保持技术案例库	云南大学	硕士	资源与环境	钟荣华
8	跨境水资源管理教学案例库	云南大学	硕士	资源与环境	王文玲
9	面向新文科建设的知识产权教学案例库	云南大学	硕士	法律	马碧玉
10	金融法研究生课程教学案例库	云南大学	硕士	法律	周昌发
11	行政法学理论与实务教学案例库	云南大学	硕士	法律	刘国乾
12	工程项目风险管理课程案例库	云南大学	硕士	工程管理	尤荻
13	数学模型与决策课程案例库	云南大学	硕士	工商管理	罗裕梅
14	课程思政融入的《企业战略管理》课程案例库	云南大学	硕士	工商管理	陈伟
15	数字治理赋能国家治理体系案例库	云南大学	硕士	公共管理	邓崧
16	边疆治理创新实践教学案例库	云南大学	硕士	公共管理	谭立力
17	聚焦汉语学习者的汉语国际教育硕士教学案例库	云南大学	硕士	汉语国际教育	王亚琼
18	面向两亚的汉语国际教育硕士课程教学案例库	云南大学	硕士	汉语国际教育	赵玮
19	乡村教育振兴教学案例库	云南大学	硕士	教育	张琪仁
20	教育政策与法律教学案例库	云南大学	硕士	教育	邓凡
21	信息技术课程和教材研究教学案例库	云南大学	硕士	教育	杨胜林
22	职业教育测量与评价案例库	云南大学	硕士	教育	魏娜

序号	项目名称	学校	培养层次	专业学位类别	负责人
23	教学设计与案例分析案例库	云南大学	硕士	教育	章光洁
24	金融市场与投资案例库	云南大学	硕士	金融	姚书杰
25	中级计量经济学教学案例库	云南大学	硕士	金融	娄锋
26	注册会计师审计教学案例库	云南大学	硕士	审计	施谦
27	“一带一路”贸易与投资教学案例库	云南大学	硕士	国际商务	胡颖
28	体育心理学理论与方法教学案例库	云南大学	硕士	体育	李年红
29	交互式知识产权研究生教学案例库	昆明理工大学	硕士	法律	杨静
30	岩土工程稳定性分析研究生教学案例库	昆明理工大学	博士	资源与环境	王光进
31	爆破新技术研究生教学案例库	昆明理工大学	硕士	资源与环境	李祥龙
32	现代设计方法研究生教学案例库	昆明理工大学	硕士	机械	刘孝保
33	风景旅游区规划研究生教学案例库	昆明理工大学	硕士	风景园林、城市规划	车震宇
34	云南乡村人居环境建设实践研究生教学案例库	昆明理工大学	硕士	建筑学、城市规划、风景园林	李莉萍
35	传统聚落空间环境特色保护研究生教学案例库	昆明理工大学	硕士	建筑学	姚青石
36	典籍英译研究生教学案例库	昆明理工大学	硕士	翻译	吴涛
37	高等燃烧学研究生教学案例库	昆明理工大学	博士/硕士	能源动力	李法社
38	湿法冶金研究生教学案例库	昆明理工大学	硕士	材料与化工	李存兄
39	冶金动力学研究生教学案例库	昆明理工大学	博士/硕士	材料与化工	徐存英
40	创新设计服务社会研究生教学案例库	昆明理工大学	硕士	艺术、机械	许佳
41	地质灾害防治研究生教学案例库	昆明理工大学	硕士	资源与环境	杨志全
42	公私法交叉案件研究生教学案例库	昆明理工大学	硕士	法律	曾娜
43	高梯度磁选研究生教学案例库	昆明理工大学	博士/硕士	资源与环境	陈禄政
44	恢复生态学研究生教学案例库	昆明理工大学	硕士	资源与环境	王海娟

序号	项目名称	学校	培养层次	专业学位类别	负责人
45	现代给水与废水处理研究生教学案例库	昆明理工大学	硕士	资源与环境	王向宇
46	生产运作管理研究生教学案例库	昆明理工大学	硕士	机械	李莹
47	工程管理哲学、模型与综合研究生教学案例库	昆明理工大学	硕士	工程管理	李国良
48	交通系统安全研究生教学案例库	昆明理工大学	硕士	交通运输	万华森
49	设计符号语意研究生教学案例库	昆明理工大学	硕士	艺术	王坤茜
50	男性生殖及泌尿系统疾病诊治研究生教学案例库	昆明理工大学	硕士	临床医学	赵良运
51	外科手术术前3D手术规划及评价研究生教学案例库	昆明理工大学	硕士	临床医学	王峻峰
52	现代测绘理论与技术研究生教学案例库	昆明理工大学	硕士	资源与环境	黄亮
53	选择性磨矿研究生教学案例库	昆明理工大学	硕士	资源与环境	肖庆飞
54	土木工程建造与管理学科前沿讲座研究生教学案例库	昆明理工大学	硕士	土木水利	陈永鸿
55	特色城市设计研究生教学案例库	昆明理工大学	硕士	建筑学	吴志宏
56	车辆动力节能与环保技术研究生教学案例库	昆明理工大学	硕士	交通运输	黄粉莲
57	交通系统仿真技术及应用研究生教学案例库	昆明理工大学	硕士	交通运输	郭凤香
58	功能食品研究进展研究生教学案例库	昆明理工大学	硕士	生物与医药	程桂广
59	桥梁加固设计研究生教学案例库	昆明理工大学	硕士	土木水利	李睿
60	少数民族民间工艺研究生教学案例库	昆明理工大学	硕士	艺术	张瑞
61	基于土动力学的大盈江地震小区划研究生教学案例库	昆明理工大学	硕士	土木水利	刘海明
62	商法研究生教学案例库	昆明理工大学	硕士	法律	罗芳
63	数字音视频专题研究生教学案例库	昆明理工大学	硕士	出版	张名章
64	坡面土壤侵蚀与生态修复典型案例解析研究生教学案例库	昆明理工大学	硕士	资源与环境	孔志岗
65	汉语国际教育课程与教学专题教学案例库	云南师范大学	博士	教育	郭中丽
66	高级数据库技术教学案例库	云南师范大学	硕士	电子信息	甘健侯
67	非文学翻译教学案例库	云南师范大学	硕士	翻译	李彦

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68	人力资源管理教学案例库	云南师范大学	硕士	工商管理	王俊程
69	东南亚南亚商业环境研究教学案例库	云南师范大学	硕士	工商管理	韩越
70	组织行为学教学案例库	云南师范大学	硕士	工商管理	李正彪
71	管理沟通教学案例库	云南师范大学	硕士	工商管理	李艳
72	财务会计理论与实务教学案例库	云南师范大学	硕士	会计	赵明元
73	会计教学设计与案例分析教学案例库	云南师范大学	硕士	教育	师佳英
74	中学历史教学设计与实施教学案例库	云南师范大学	硕士	教育	李庆华
75	美术教育测量与评价教学案例库	云南师范大学	硕士	教育	刘连杰
76	心理健康教育课程与教材研究教学案例库	云南师范大学	硕士	教育	周泓
77	幼儿园课程理论与实践教学案例库	云南师范大学	硕士	教育	樊倞
78	农村公共管理教学案例库	云南师范大学	硕士	农业	李富昌
79	当代植物种业发展概论教学案例库	云南师范大学	硕士	农业	李灿辉
80	高级社会工作实务教学案例库	云南师范大学	硕士	社会工作	曹丽
81	社会工作理论教学案例库	云南师范大学	硕士	社会工作	刘婷
82	体育教材教法教学案例库	云南师范大学	硕士	体育	赵泽顺
83	体能训练理论与方法教学案例库	云南师范大学	硕士	体育	杨勇
84	舆情危机处理和应对教学案例库	云南师范大学	硕士	新闻与传播	杜忠锋
85	专业创演课教学案例库	云南师范大学	硕士	艺术	何纾
86	环境空间形态基础研究教学案例库	云南师范大学	硕士	艺术	何浩
87	景观空间设计教学案例库	云南师范大学	硕士	艺术	余玲
88	视觉传达设计研究与实践教学案例库	云南师范大学	硕士	艺术	向云波
89	高级心理测量教学案例库	云南师范大学	硕士	应用心理	李鹏
90	超声医学科研究生教学案例库	昆明医科大学	硕士	临床医学	姜 岚
91	核医学科研究生教学案例库	昆明医科大学	硕士	临床医学	朱高红
92	医学遗传科研究生教学案例库	昆明医科大学	硕士	临床医学	刘 洋
93	骨科研究生教学案例库	昆明医科大学	硕士	临床医学	张 俊
94	放射肿瘤科研究生教学案例库	昆明医科大学	硕士	临床医学	吴星娆

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95	儿外科研究生教学案例库	昆明医科大学	硕士	临床医学	康晓鹏
96	感染科研究生教学案例库	昆明医科大学	硕士	临床医学	周 菊
97	心血管内科研究生教学案例库	昆明医科大学	硕士	临床医学	蒲里津
98	血液内科研究生教学案例库	昆明医科大学	硕士	临床医学	黎承平
99	风湿免疫科研究生教学案例库	昆明医科大学	硕士	临床医学	傅 萍
100	神经外科研究生教学案例库	昆明医科大学	硕士	临床医学	李经辉
101	肿瘤内科研究生教学案例库	昆明医科大学	硕士	临床医学	杨 梅
102	胸外科研究生教学案例库	昆明医科大学	硕士	临床医学	李高峰
103	心脏外科研究生教学案例库	昆明医科大学	硕士	临床医学	李 鹏
104	泌尿外科研究生教学案例库	昆明医科大学	硕士	临床医学	王剑松
105	肿瘤外科研究生教学案例库	昆明医科大学	硕士	临床医学	肖砚斌
106	整形外科研究生教学案例库	昆明医科大学	硕士	临床医学	代晓明
107	高级环境工程学课程教学案例库	云南农业大学	硕士	资源与环境	陈建军
108	农业机器人课程教学案例库	云南农业大学	硕士	农业	张艳诚
109	农艺与种业领域核心课程教学案例库	云南农业大学	硕士	农业	林春
110	民族社会工作课程教学案例库	云南农业大学	硕士	社会工作	王晓艳
111	茶文化学教学案例库	云南农业大学	硕士	农业	李亚莉
112	生态学专题课程案例库	云南农业大学	硕士	风景园林	张敬丽
113	数学物理方法课程教学案例库	云南农业大学	硕士	土木水利	徐兴倩
114	草地植物栽培管理课程教学案例库	云南农业大学	硕士	农业	段新慧
115	动物遗传原理与育种方法课程教学案例库	云南农业大学	硕士	农业	严达伟
116	兽医寄生虫学专题课程教学案例库	云南农业大学	硕士	兽医	杨建发
117	农业科技阅读与翻译课程教学案例库	云南农业大学	硕士	翻译	姜梅
118	英汉比较与翻译课程教学案例库	云南农业大学	硕士	翻译	吴广平
119	应用翻译课程案例库	云南农业大学	硕士	翻译	刘丹丹
120	现代管理学教学案例库	云南农业大学	硕士	农业	郭颖梅
121	农业政策学课程教学案例库	云南农业大学	硕士	农业	普雁翔

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122	农业发展理论与实践课程教学案例库	云南农业大学	硕士	农业	路遥
123	体育管理学课程教学案例库	云南农业大学	硕士	体育	王钧
124	社会体育学课程教学案例库	云南农业大学	硕士	体育	岁金
125	运动训练理论与方法课程案例库	云南农业大学	硕士	体育	李少华
126	基于优秀传统文化的园本活动案例库	云南民族大学	硕士	教育	罗利群
127	运动技能学习原理教学案例库	云南民族大学	硕士	体育	李莹
128	中华文化传播与跨文化交际教学案例库	云南民族大学	硕士	汉语国际教育	沈峥
129	国际汉语课堂教学案例库	云南民族大学	硕士	汉语国际教育	康泳
130	“民族文化传播”理论与实践教学案例库	云南民族大学	硕士	新闻与传播	苏涛
131	MBA管理沟通案例库	云南民族大学	硕士	工商管理	李兴宽
132	高级审计理论与实务教学案例库	云南民族大学	硕士	会计	吴可夫
133	经济责任审计教学案例库	云南民族大学	硕士	会计	张筱
134	高级社会工作实务教学案例库	云南民族大学	硕士	社会工作	曾黎
135	译文审校课程案例库	云南民族大学	硕士	翻译	赵俊海
136	电力系统运行与控制虚拟仿真教学案例库	云南民族大学	硕士	能源动力	徐武
137	民族舞蹈教育教学案例库	云南民族大学	硕士	艺术	赵云艳
138	公共经济学教学案例库	云南财经大学	硕士	公共管理	朱俊立
139	公司治理教务案例库	云南财经大学	硕士	会计	余怒涛
140	地方特色产品期货设计与风险管理案例库	云南财经大学	硕士	金融	周伟
141	我国税法适用与优化案例库	云南财经大学	硕士	税务	李蕾
142	保险经济学理论与实践案例库	云南财经大学	硕士	保险	孙正成
143	基于云南本土企业的人力资源管理教学案例库	云南财经大学	硕士	工商管理	黄波
144	精编经济法教学案例库	云南财经大学	硕士	法律	欧阳杉
145	中国驰名商标“猫哆哩”营销管理案例库	云南财经大学	硕士	工商管理	聂元昆
146	时间序列分析案例库	云南财经大学	硕士	应用统计	谢佳春
147	“社会政策”课程思政教学案例库	云南财经大学	硕士	社会工作	木薇

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148	多元统计分析的案例分析	云南财经大学	硕士	应用统计	鲁筠
149	公共服务课程案例库	云南财经大学	硕士	公共管理	李鹏飞
150	房地产评估研究生教学案例库	云南财经大学	硕士	资产评估	金杰
151	泰国高铁工程国际采购决策困境	云南财经大学	硕士	国际商务	蒋其发
152	审计全案例教学库	云南财经大学	硕士	审计	余晓燕
153	多元化还是归核化：云南龙润集团的战略抉择	云南财经大学	硕士	工商管理	杨增雄
154	财务管理理论与实务教学案例库	云南财经大学	硕士	会计	徐慧
155	法理学“个案全过程教学”实例	云南财经大学	硕士	法律	刘新星
156	农村电商仅是帮农户卖农产品吗	云南财经大学	硕士	工商管理	钱瑛
157	公共管理教学案例库	云南财经大学	硕士	公共管理	李永康
158	税务会计与纳税筹划教学案例库	云南财经大学	硕士	会计、审计	王红云
159	公共危机与应急管理典型案例库	云南财经大学	硕士	公共管理	肖兴安
160	文化旅游类研究生课程教学案例库	云南财经大学	硕士	旅游管理	晏雄
161	散落的共同体：空心化古村落振兴之困 --以茶马古道古村落“一字格”村为个案	云南财经大学	硕士	公共管理	郗春媛
162	内部控制与风险管理教学案例库	云南财经大学	硕士	审计	李正
163	不走寻常路？—葵花户外的商业模式迭代之惑	云南财经大学	硕士	工商管理	唐泳
164	资产评估实务与案例	云南财经大学	硕士	资产评估	王晓丽
165	茶香也怕巷子深：龙润茶业的新媒体营销之路	云南财经大学	硕士	工商管理	张立明
166	职业生涯规划教学案例库	云南财经大学	硕士	会计、审计	刘丽芳
167	“古滇颐泉”天然气泡水市场赛道的探索之路	云南财经大学	硕士	工商管理	陈昆玉
168	中医专硕住培思政教学案例库	云南中医药大学	硕士	中医	张琨
169	“妇科常见血证”诊治策略案例库	云南中医药大学	硕士	中医	杨丽娟
170	中医生殖医学教学案例库	云南中医药大学	硕士	中医	袁卓珺
171	中医痔病教学案例库	云南中医药大学	硕士	中医	张志云
172	基于扶阳学术特色的中医脾胃病教学案例库	云南中医药大学	硕士	中医	吴文笛
173	针灸推拿经典选读教学案例库	云南中医药大学	硕士	中医	郭太品

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174	中西结合特色推拿临床教学案例库	云南中医药大学	硕士	中医	李应志
175	中药分析学教学案例库	云南中医药大学	硕士	中药学	俞捷
176	现代生物学技术在中药研发中的应用教学案例库	云南中医药大学	硕士	中药学	庄馨瑛
177	药物剂型设计与应用教学案例库	云南中医药大学	硕士	药学	马云淑
178	《现代药物设计》教学案例库	云南中医药大学	硕士	药学	毛泽伟
179	中医临床护理实践教学案例库	云南中医药大学	硕士	护理	钱凤娥
180	高级健康评估教学案例库	云南中医药大学	硕士	护理	李潇
181	护理理论与实践案例库	云南中医药大学	硕士	护理	张朔玮
182	地震反应谱 MATLAB 程序案例库	西南林业大学	硕士	土木水利	徐国林
183	基于现代力学概念的竹木结构计算方法案例库	西南林业大学	硕士	土木水利	张庆文
184	家具设计与制造案例库	西南林业大学	硕士	材料与化工	强明礼
185	综合交通运输规划案例库	西南林业大学	硕士	交通运输	杨圣文
186	农业推广理论与实践案例库	西南林业大学	硕士	农业	杨洁
187	面向农业专硕《生态经济与绿色发展》教学案例库	西南林业大学	硕士	农业	付伟
188	农村公共管理课程教学案例库	西南林业大学	硕士	农业	苏建兰
189	食品检测新技术研究生教学案例库	西南林业大学	硕士	农业	刘云
190	面向农业专硕的植物与病原物互作教学案例库	西南林业大学	硕士	农业	韩长志
191	面向资产评估的统计案例库	西南林业大学	硕士	资产评估	李春波
192	新发展阶段风景园林研究进展案例库	西南林业大学	硕士	风景园林	刘娟娟
193	民族园林（传统村落保护规划）教学案例库	西南林业大学	硕士	风景园林	张云
194	面向新工科的电子与通信工程专业硕士研究生教学案例库	大理大学	硕士	电子信息	赵恩铭
195	公共卫生类突发事件研究生教学案例库	大理大学	硕士	公共卫生	张　态
196	高级健康评估教学案例库	大理大学	硕士	护理	赵　媛
197	探究案例教学法在提高神经病学专业硕士研究生的临床思维能力中的应用	大理大学	硕士	临床医学	杨　林

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198	体内药物分析教学案例库	大理大学	硕士	药学	王　燕
199	消化系统疾病学教学病案库	大理大学	硕士	临床医学	施荣杰
200	社区护理理论与实践教学案例库	大理大学	硕士	护理	张筱岚
201	护理管理理论与实践案例库	大理大学	硕士	护理	柴世学
202	临床护理新进展教学案例库	大理大学	硕士	护理	贺启莲
203	临床药学专硕典型案例收集与分析	大理大学	硕士	药学	杨志勇
204	教育硕士《学前课程改革》双语课程教学案例库	大理大学	硕士	教育	房慧
205	《学前儿童观察与分析》课程教学案例库	大理大学	硕士	教育	李秀芳
206	耳鼻咽喉科学教学案例库	大理大学	硕士	临床医学	陈银忠
207	妇产科学专业学位研究生教学案例库	大理大学	硕士	临床医学	史纪芳
208	儿科专业教学案例库	大理大学	硕士	临床医学	丁传刚
209	舞蹈思政艺术作品研习教学案例库	云南艺术学院	硕士	艺术	刘丽
210	戏剧应用与服务社会实践教学案例库	云南艺术学院	硕士	艺术	杨俊霞
211	雕塑创作与表现教学案例库	云南艺术学院	硕士	艺术	张仲夏
212	山水画专业写生教学案例库	云南艺术学院	硕士	艺术	温泉沁
213	互联网介入艺术实践教学案例库	云南艺术学院	硕士	艺术	李艳丽
214	《视听心理学》课程教学案例库	云南艺术学院	硕士	艺术	洪志明
215	民族器乐重奏教学案例库	云南艺术学院	硕士	艺术	王静
216	云南民间服饰研究教学案例库	云南艺术学院	硕士	艺术	孙琦
217	中西方音乐史专题教学案例库	云南艺术学院	硕士	艺术	师向宁
218	室内乐教学案例库	云南艺术学院	硕士	艺术	范琴雯
219	油画风景写生与创作教学案例库	云南艺术学院	硕士	艺术	边小强
220	云南少数民族器乐教学案例库	云南艺术学院	硕士	艺术	任俊宇
221	民族声乐演唱教学案例库	云南艺术学院	硕士	艺术	陈红玲
222	美术专题研究教学案例库	云南艺术学院	硕士	艺术	马宁
223	植物保护技术与应用案例库	昆明学院	硕士	农业	刘佳妮

序号	项目名称	学校	培养层次	专业学位类别	负责人
224	植物有害生物综合防控案例库	昆明学院	硕士	农业	黄 敏
225	农化产品的高效利用与管理案例库	昆明学院	硕士	农业	宋知春
226	农业环境保护与生态工程案例库	昆明学院	硕士	农业	侯秀丽
227	天然产物开发与研究案例库	昆明学院	硕士	材料与化工	袁 琳
228	跨境物流与供应链管理案例库	昆明学院	硕士	国际商务	张必清
229	行业研究案例库	昆明学院	硕士	国际商务	林 涛
230	材料与化工现代研究方法案例库	昆明学院	硕士	材料与化工	杨婉秋
231	社会科学量化研究方法案例库	昆明学院	硕士	国际商务	夏 异
232	植物病害诊断技术案例库	昆明学院	硕士	农业	裴徐梨
233	农业科技论文写作案例库	昆明学院	硕士	农业	钟 宇
234	高等分离工程案例库	昆明学院	硕士	材料与化工	葛丹丹

云南省教育厅办公室　　2021年11月30日印发

云南大学2022年度云南省研究生优质课程、专业学位研究生教学案例库建设项目、研究生导师团队建设项目评审结果公示

根据2022年度云南省研究生优质课程项目、专业学位研究生教学案例库项目、研究生导师团队建设项目申报工作要求，我校评审工作现已结束。本次申报经申报人申请、学院推荐，共收到研究生优质课程项目47项，专业学位研究生教学案例库项目26项，研究生导师团队建设项目53项。经校内外专家评审，拟推荐18门课程参评2022年度云南省研究生优质课程项目，23个案例库参评云南省专业学位研究生教学案例库建设项目，22个团队参评云南省研究生导师团队建设项目。

现对项目评审结果进行公示，公示时间：2022年7月7日至2022年7月11日。若有异议，请于2022年7月11日17:00前以书面形式向研究生院反映，若无异议则自动生效。

联系人：刘钰65031879；成希65033585。

云南大学研究生院

2022年7月7日

云南大学2022年度云南省研究生优质课程项目评审推荐结果

序号	课程名称	培养单位	课程负责人	课程类型(博士/硕士)	课程类别（公共学位课/专业学位课）	所属学科	学科类别	评审结果
1	高等药物合成	化学科学与工程学院	严胜骄	硕士	专业学位课	化学	学术学位、专业学位	推荐参评省级优质课程
2	现代波谱分析	化学科学与工程学院	汤峨	硕士	专业学位课	药学	学术学位、专业学位	推荐参评省级优质课程
3	高级分子生物学与生物化学	生命科学学院	孙建伟	硕士	专业学位课	生物学	学术学位	推荐参评省级优质课程
4	高等大气物理学	地球科学学院	陈兵	硕士	专业学位课	大气科学	学术学位	推荐参评省级优质课程
5	进化生态学原理与方法	生态与环境学院	王崇云	硕士	专业学位课	生态学	学术学位	推荐参评省级优质课程
6	高等激光物理学	物理与天文学院	张远宪	博士、硕士	专业学位课	物理学	学术学位	推荐参评省级优质课程
7	计量经济学	数学与统计学院	陈黎	硕士	专业学位课	统计学	学术学位、专业学位	推荐参评省级优质课程
8	现代通信原理	信息学院	李波	硕士	专业学位课	信息与通信工程	学术学位	推荐参评省级优质课程
9	铸牢中华民族共同体意识研究	民族学与社会学学院	关凯	博士、硕士	专业学位课	民族学	学术学位	推荐参评省级优质课程

10	区域国别学研究	国际关系研究院	祝湘辉	博士、硕士	专业学位课	区域国别学	学术学位	推荐参评 省级优质课程
11	国际问题研究方法	国际关系研究院	卢凌宇	博士、硕士	专业学位课	政治学	学术学位	推荐参评 省级优质课程
12	中国马克思主义 与当代	马克思主义学院	王新红	博士	公共学位课	马克思主义 理论	学术学位	推荐参评 省级优质课程
13	比较文学专题	文学院	李娟	硕士	专业学位课	中国语言文学	学术学位	推荐参评 省级优质课程
14	组织行为学	工商管理与旅游管理学院	成竹	硕士	专业学位课	管理学	专业学位	推荐参评 省级优质课程
15	战略管理研究	工商管理与旅游管理学院	邓伟升	硕士	专业学位课	工商管理	学术学位	推荐参评 省级优质课程
16	管理学	工商管理与旅游管理学院	赵德森	硕士	专业学位课	工商管理	专业学位	推荐参评 省级优质课程
17	教育史	职业与继续教育学院	杨永兵	硕士	学科基础课	教育学	学术学位	推荐参评 省级优质课程
18	体适能评定与方法	体育学院	杨霞	硕士	专业学位课	体育学	专业学位	推荐参评 省级优质课程
19	高等催化化学	化学科学与工程学院	方文浩	教授	专业学位课	化学	专业学位	校级优质课程
20	学术规范与 学位论文写作指导	材料与能源学院	王毓德	教授	专业学位课	材料科学 与工程	学术学位、 专业学位	校级优质课程
21	环境监测与 生态安全评价	地球科学学院	邓福英	副教授	专业选修课	地理学	学术学位	校级优质课程

22	高级计算机网络与分布式计算	信息学院	王津	副教授	专业学位课	计算机科学与技术	学术学位	校级优质课程
23	社会学研究方法	民族学与社会学学院	许庆红	副研究员	专业学位课	社会学	学术学位	校级优质课程
24	南亚地区研究	国际关系研究院	胡潇文	副研究员	专业学位课	政治学	学术学位	校级优质课程
25	国际政治理论研究	国际关系研究院	贺鉴	教授	专业学位课	政治学	学术学位	校级优质课程
26	新时代中国特色社会主义理论与实践	马克思主义学院	李雪章	副教授	公共学位课	思想政治教育	学术学位、专业学位	校级优质课程
27	历史自然地理	历史与档案学院	潘威	副教授	专业学位课	历史学	学术学位	校级优质课程
28	日本文化史	外国语学院	赵毅达	副教授	专业学位课	外国语言文学	学术学位	校级优质课程
29	数据、模型与决策	工商管理与旅游管理学院	罗裕梅	副教授	专业学位课	工商管理	专业学位	校级优质课程
30	工程安全与风险管理	工商管理与旅游管理学院	尤荻	副研究员	专业学位课	管理学	专业学位	校级优质课程
31	管理经济学	经济学院	崔庆波	副教授	专业学位课	审计专硕	专业学位	校级优质课程
32	社会科学研究方法（定量）	政府管理学院	谭立力	教授	专业学位课	公共管理学	学术学位	校级优质课程
33	信息技术课程和教材研究	职业与继续教育学院	杨胜林	副研究员	专业学位课	教育学	专业学位	校级优质课程

云南大学2022年度云南省专业学位研究生教学案例库建设项目评审推荐结果

序号	项目名称	申请人	所在学院	专业学位类别（领域）	评审结果
1	信息存储载体破坏损毁与保护现状教学案例库	侯明昌	历史与档案学院	图书情报硕士	推荐参评省级案例库
2	政府运行保障管理	徐应萍	政府管理学院	公共管理硕士	推荐参评省级案例库
3	“公共管理案例研发实务”课程案例库	罗红霞	政府管理学院	公共管理硕士	推荐参评省级案例库
4	世界政治经济视野下的地方公共管理	夏维勇	政府管理学院	公共管理硕士	推荐参评省级案例库
5	公共经济学教学案例库建设	马桑	政府管理学院	公共管理硕士	推荐参评省级案例库
6	知识产权法课程案例库	卿越	法学院	法律硕士	推荐参评省级案例库
7	国际法课程案例库	陈志平	法学院	法律硕士	推荐参评省级案例库
8	金融市场与金融机构教学案例库	付庆华	经济学院	金融专业硕士	推荐参评省级案例库
9	数字资产价值评估案例库	刘志坚	经济学院	资产评估专业硕士	推荐参评省级案例库
10	中级宏观经济学课程思政教学案例库建设	王旭	经济学院	经济类专业硕士	推荐参评省级案例库

11	企业跨国经营面临的金融风险及风险规避	陈锐	经济学院	金融专业硕士	推荐参评省级案例库
12	东南亚南亚经济与贸易教学案例库	李隽	经济学院	国际商务硕士	推荐参评省级案例库
13	管理信息系统教学案例	张俊梅	工商管理与旅游管理学院	工商管理硕士	推荐参评省级案例库
14	《管理学》课程教学案例库	孔莉	工商管理与旅游管理学院	工商管理硕士	推荐参评省级案例库
15	基于产学协同的《供应链管理》课程案例库建设	宋乐	工商管理与旅游管理学院	工商管理硕士	推荐参评省级案例库
16	乡村振兴管理案例库	邓伟升	工商管理与旅游管理学院	工商管理硕士	推荐参评省级案例库
17	《国学与现代管理》课程教学案例	晏钢	工商管理与旅游管理学院	工商管理硕士	推荐参评省级案例库
18	软件工程研究生数学类课程教学案例库	郁湧	软件学院	工程硕士（电子信息）	推荐参评省级案例库
19	土木工程项目合同管理教学案例库	卜炜玮	建筑与规划学院	工程硕士（土木水利）	推荐参评省级案例库
20	工业药剂学教学案例库的建设	韦琨	化学科学与工程学院	工程硕士（生物与医药）	推荐参评省级案例库
21	现代能源化工技术课程案例库建设	袁申富	化学科学与工程学院	工程硕士（材料与化工）	推荐参评省级案例库
22	地理信息技术应用案例库	季漩	国际河流与生态安全研究院	工程硕士（资源与环境）	推荐参评省级案例库

23	农业资源高效利用技术案例库建设	何飞飞	资源植物研究院	农业硕士	推荐参评 省级案例库
24	金融计量经济学教学案例库	曹骥	经济学院	金融专业硕士	校级案例库
25	《工程数学》教学案例库建设	黄亚群	信息学院	工程硕士（电子信息）	校级案例库
26	数据驱动决策教学案例数据库	彭程	数学与统计学院	应用统计硕士	校级案例库

云南大学2022年度研究生导师团队建设项目评审结果

序号	团队名称	团队类别	所在学科 （专业学位类别）	团队 带头人	评审结果
1	“经济发展与结构转型研究”导师团队	博士生导师团队	理论经济学（学博）	杨先明	推荐参评 省级导师团队
2	南亚东南亚区域国别研究导师团队	博士生导师团队	政治学（学博）	卢光盛	推荐参评 省级导师团队
3	云南民族工作研究导师团队	博士生导师团队	民族学（学博）	李志农	推荐参评 省级导师团队
4	中国边疆学构筑导师团队	博士生导师团队	中国史（学博）	罗群	推荐参评 省级导师团队
5	云南大学分析化学研究生导师团队	博士生导师团队	化学（学博）	郑立炎	推荐参评 省级导师团队

6	星系宇宙学导师团队	博士生导师团队	天文学（学博）	刘晓为	推荐参评省级导师团队
7	细胞代谢与疾病	博士生导师团队	生物学（学博）	杨崇林	推荐参评省级导师团队
8	食线虫真菌功能基因组学研究导师团队	博士生导师团队	生物学（学博）	杨金奎	推荐参评省级导师团队
9	生物多样性保护导师团队	博士生导师团队	生态学（学博）	申仕康	推荐参评省级导师团队
10	新能源材料与器件导师团队	博士生导师团队	材料科学与工程（学博）	郭洪	推荐参评省级导师团队
11	高性能计算与智能计算团队	博士生导师团队	计算机科学与技术(学博）	张学杰	推荐参评省级导师团队
12	工商管理一级学科博士点导师团队	博士生导师团队	工商管理（学博）	王克岭	推荐参评省级导师团队
13	地图学与地理信息系统导师团队	硕士生导师团队	地理学（学硕）	夏既胜	推荐参评省级导师团队
14	药理学导师团队	硕士生导师团队	药学（学硕）	李艳	推荐参评省级导师团队
15	生物资源利用化学研究导师团队	博士生导师团队	资源与环境（专博）	罗晓东	推荐参评省级导师团队
16	高原山地土壤侵蚀与水土保持	博士生导师团队	资源与环境（专博）	段兴武	推荐参评省级导师团队
17	云南大学应用统计专业硕士生导师团队	硕士生导师团队	应用统计专业硕士(专硕）	李会琼	推荐参评省级导师团队

18	体育教育与运动健康促进导师团队	硕士生导师团队	体育（专硕）	李年红	推荐参评省级导师团队
19	天然产物生物转化导师团队导师团队	硕士生导师团队	生物与医药（专硕）	蔡乐	推荐参评省级导师团队
20	“电子信息工程与应用”专业硕士生导师团队	硕士生导师团队	电子信息（专硕）	钱文华	推荐参评省级导师团队
21	公共管理导师团队	硕士生导师团队	公共管理（专硕）	马桑	推荐参评省级导师团队
22	产业需求导向下的高级旅游管理人才培养导师团队	硕士生导师团队	旅游管理（专硕）	赵书虹	推荐参评省级导师团队
23	世界经济博士研究生导师团队	博士生导师团队	理论经济学（学博）	黄宁	校级导师团队
24	“乡村振兴与基层治理”导师团队	博士生导师团队	政治学（学博）	罗强强	校级导师团队
25	民族政治学导师团队	博士生导师团队	政治学（学博）	黄清吉	校级导师团队
26	理论物理创新团队	博士生导师团队	物理学（学博）	郑波	校级导师团队
27	新型光电器件研究创新团队	博士生导师团队	物理学（学博）	史衍丽	校级导师团队
28	云南大学天体物理和宇宙线研究博士生导师团队	博士生导师团队	天文学（学博）	张力	校级导师团队
29	生物医药创新研究导师团队	博士生导师团队	生物学（学博）	陈大华	校级导师团队

30	脂质代谢与疾病	博士生导师团队	生物学（学博）	梁斌	校级导师团队
31	中国近现代史导师团队	硕士生导师团队	中国史（学硕）	齐春风	校级导师团队
32	世界史导师团队	硕士生导师团队	世界史（学硕）	钱金飞	校级导师团队
33	关键金属成矿与找矿预测导师团队	硕士生导师团队	地质学（学硕）	周家喜	校级导师团队
34	云南高原山地人居环境保护与建设团队	硕士生导师团队	城乡规划学（学硕）	徐坚	校级导师团队
35	管理科学与工程硕导团队	硕士生导师团队	管理科学与工程（学硕）	张正华	校级导师团队
36	西南少数民族古籍保护研究导师团队	硕士生导师团队	图书情报与档案管理（学硕）	王水乔	校级导师团队
37	新闻与传播专业硕士导师团队	硕士生导师团队	新闻与传播（专硕）	单晓红	校级导师团队
38	现代农业导师团队	硕士生导师团队	农业（专硕）	吴学尉	校级导师团队

立志做有理想、有道德、有文化、有纪律、有本领、有担当的社会主义建设者和接班人，为建设中国特色社会主义事业而奋斗！

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中共云南省委教育工委　云南省教育厅

二〇二二年六月