

申 报	系列：自然科学类
	专业：植物学
	职称：副教授

业绩成果材料

（申报人的业绩成果材料包括论文、科研项目、获奖以及其他成果等）

单 位（二级单位）生 命 科 学 学 院

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单位盖章：

核对时间：

华南农业大学制

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华南农业大学文件

华南农教〔2025〕7号

关于公布华南农业大学 2024 年度 课程思政示范项目立项名单的通知

各学院、部处、各单位：

根据《关于开展 2024 年度校级课程思政示范建设项目申报工作的通知》，学校组织开展了 2024 年度课程思政示范项目评选工作。

经项目负责人申请、所在单位遴选推荐、学校组织专家评审、校内公示等程序，决定立项建设华南农业大学 2024 年度课程思政示范项目 91 项，包括课程思政试点学院 4 个、课程思政示范团队 6 个、课程思政示范课程 14 门、课程思政示范课堂 25 个、课程思政典型案例 42 个（名单详见附件），现予以公布。

本次立项的课程思政示范项目建设期至 2026 年 12 月，建设期内，示范项目原则上不允许更换负责人或变更项目团队成员。

各项目负责人要严格按照 2024 年度申报通知要求，及时开展工作，加快推进课程思政改革，确保高质量完成建设目标和任务。

请各学院充分认识课程思政改革的重要意义，认真贯彻《华南农业大学课程思政实施方案》，加强对教师的相关培训、指导、引领和支持，带动教师全员积极参与课程思政教学改革，持续深入抓典型、树标杆、推经验，全面提升本科人才培养质量。

附件：华南农业大学 2024 年度课程思政示范项目立项名单

华南农业大学
2025 年 1 月 24 日

公开方式：主动公开

华南农业大学党政办公室 2025 年 1 月 24 日印发

附件

华南农业大学 2024 年度课程思政示范项目立项名单（部分）

序号	项目名称	所属单位	项目负责人	团队成员（不含负责人）
kcsz2024001	课程思政试点学院	动物科学学院	王文策	
kcsz2024002	课程思政试点学院	艺术学院	张艳河	
kcsz2024003	课程思政试点学院	数学与信息学院、软件学院	陈文艺	
kcsz2024004	课程思政试点学院	公共管理学院	蔡茂华	
kcsz2024005	《公共管理学》课程思政示范团队	公共管理学院	史传林	唐斌、姜国兵、方敏、曾小龙、吴彦
kcsz2024006	藏粮于“技”，“数”说农业：数字化转型课程思政教学团队	数学与信息学院、软件学院	熊俊涛	黄文玲、韩方珍、古万荣、邓金、张建桃、宋歌、韦婷婷、邓成剑、黄丽清
kcsz2024007	农业昆虫学课程群思政示范团队	植物保护学院	陆永跃	王磊、齐易香、岑伊静、黄振、程代凤、桑文、冼继东、吴建辉、潘慧鹏、何晓芳、汪荦荦、何娜芬
kcsz2024008	大数据与机器学习类课程思政示范团队	经济管理学院	陈有华	何勤英、伍敬文、文乐、李景荣

kcsz2024033	《空间分析原理与应用》第四章第4节(全局空间自相关分析)	资源环境学院	陈永康	钟晓兰、赵寒冰
kcsz2024034	《金工实习》--注射成型及零件检测专题	基础实验与实践训练中心	张殿武	温威、陈海波
kcsz2024035	《水球》--踩水与水球游戏专题	体育教学研究部	张俊龙	李梅、卢三妹
kcsz2024036	《大学英语III》unit 5 Gender Equality	外国语学院	陈国华	苏君、李志英
kcsz2024037	《国际政治学》第二章 第2节(世界格局)	公共管理学院	欧阳晓东	黄剑飞
kcsz2024038	《C 语言程序设计》第五章--循环结构程序设计之 while 语句专题	数学与信息学院、软件学院	郭艾侠	王栋、邢仲璟
kcsz2024039	《乒乓球》--正手攻球技术专题	体育教学研究部	黄燕玲	吕立、张晓萍、郭城
kcsz2024040	《移动应用开发》第九章--丰富你的程序，运用程序多媒体专题	数学与信息学院、软件学院	杨春	
kcsz2024041	《程序设计与算法语言》第三章第1部分--数据表示与数据类型专题	数学与信息学院、软件学院	廖彬	蔡贤资、张连宽
kcsz2024042	《社会保障基金管理》第四章(社会保障基金管理的对象专题)	公共管理学院	杨亚丽	
kcsz2024043	《流体力学》第七章第2节(黏性流体的两种流态)	水利与土木工程学院	黄俐	韦未
kcsz2024044	《种子的力量》第十章 第2节(植物生理学课堂)	生命科学学院	罗娜	刘亚林、刘慧丽

关于国家自然科学基金资助项目批准及有关事项的通知

华南农业大学 罗娜 先生/女士：

根据《国家自然科学基金条例》的规定和专家评审意见，国家自然科学基金委员会（以下简称自然科学基金委）决定批准资助您的申请项目。项目批准号：

31401906，项目名称：iTRAQ技术分析钝叶草抗旱的分子机理，资助金额：24.00万元，项目起止年月：2015年01月至2017年12月，有关项目的评审意见及修改意见附后。

请尽早登录科学基金网络信息系统（<https://isis.nsfc.gov.cn>），获取《国家自然科学基金资助项目计划书》（以下简称计划书）并按要求填写。对于有修改意见的项目，请按修改意见及时调整计划书相关内容；如对修改意见有异议，须在计划书电子版报送截止日期前提出。

计划书电子版通过科学基金网络信息系统（<https://isis.nsfc.gov.cn>）上传，由依托单位审核后提交至自然科学基金委进行审核。审核未通过者，返回修改后再行提交；审核通过者，打印（建议双面打印）为计划书纸质版（一式两份），由依托单位审核并加盖单位公章后报送至自然科学基金委项目材料接收工作组。计划书电子版和纸质版内容应当保证一致。

向自然科学基金委提交和报送计划书截止时间节点如下：

- 1、提交计划书电子版截止时间为**2014年9月11日16点**（视为计划书正式提交时间）；
- 2、提交计划书电子修改版截止时间为**2014年9月18日16点**；
- 3、报送计划书纸质版截止时间为**2014年9月26日16点**。

请按照以上规定及时提交计划书电子版，并报送计划书纸质版，未说明理由且逾期不报计划书者，视为自动放弃接受资助。

附件：项目评审意见及修改意见

国家自然科学基金委员会
生命科学部
2014年8月15日

附件：项目评审意见及修改意见表

项目批准号	31401906	项目负责人	罗娜	申请代码1	C150303
项目名称	iTRAQ技术分析钝叶草抗旱的分子机理				
资助类别	青年科学基金项目		亚类说明		
附注说明					
依托单位	华南农业大学				
资助金额	24.00 万元		起止年月	2015年01月 至 2017年12月	
通讯评审意见：					
<p><1> 干旱胁迫严重影响草坪草的应用和品质，从蛋白组学的层面对草坪草进行耐旱性研究具有新颖性和较好的理论研究意义和潜在应用价值。研究内容适中，重点比较突出，研究方案基本合理可行，项目组具有较好的研究能力和有一定的前期工作基础。但是，项目的申请仍存在问题：</p> <p>1. 研究结果难以有大的突破。利用相关技术研究植物的抗旱在模式植物拟南芥中已有大量研究报道，申请者难以得出新的研究结果。建议申请者先对已有的iTRAQ结果进行分析，如有突破，再做突变体的iTRAQ。</p> <p>2. 研究内容不够全面。钝叶草突变材料12-6的背景还不清楚，寻找其突变位点是一个很好的研究内容，可以进行基因定位和基因组测序。</p> <p>3. 研究方案有待完善。虽然项目组已经对普通钝叶草进行干旱处理做过iTRAQ分析，但只有生物信息量变化的统计结果，未见申请者进行后续验证。</p> <p>4. 项目申请书中书写存在笔误。如：图4的标注与内容叙述不一致；蛋白消化中所用的胰蛋白酶不可能是目的蛋白的30倍。</p> <p>鉴于以上问题，建议修改后可资助。</p> <p><2> 本项目选取钝叶草的抗旱突变植株和用作对照的敏感植株为材料，研究其在干旱胁迫下的差异表达蛋白的核心途径和蛋白互作网络图谱等，旨在从蛋白、基因和代谢三个方面探讨钝叶草抗旱分子机理。项目选题科学意义较好，文献分析透彻，前期积累深厚，关键科学问题明确，研究计划安排较为合理。具体意见如下：</p> <p>1、项目的科学意义和应用前景较好。草坪草需水量大，在水资源匮乏的今天，灌溉成本始终居高不下。因此草坪草的耐旱性研究具明显的科学意义，尤其是机理研究，有可能为增强草坪草抗旱性、培育耐旱新品种提供依据。</p> <p>2、前期积累深厚，实验条件较好。申请者在以往研究经历中，积累了较为深厚的文献阅读和科研经验，对重要实验技术能够熟练的掌握。申请者所在团队的实验条件较好，前期理论和实践成果较多，可以提供扎实的基础。经费预算合理。</p> <p>3、实验材料选择合理，研究内容较为丰富，部分研究成果能够很好地实现。本研究所采用的抗性突变体和敏感对比材料，也是前期科研成果的积累，是较为理想的实验材料，将二者对比研究，很有可能获得与抗旱性密切响应的蛋白。研究涉及蛋白、基因和代谢层面，研究内容较为丰富。</p> <p>4、具备一定的创新性。iTRAQ技术应用于草坪草抗旱性研究，且结合基因和代谢途径展开研究，在草坪草抗性研究领域具备一定的创新性。</p> <p>然而，本项目仍存在以下主要问题，部分内容需进一步调整。</p> <p>1) 研究目标过泛，不够聚焦，重点不突出。本项目以蛋白、基因和代谢三方面来研究钝叶草抗旱机理，固然比较全面，但三方面研究没有主次之分。从申请者所掌握的主要实验技能和行文篇幅来看，蛋白层面研究占据最大的比例，似乎是项目研究的重点，但又多次涉及钝叶草抗旱的“分子机理”，故基因水平似乎又是最终的研究技术层面和重点。</p> <p>如三方面同等对待，则研究目标过泛，不够集中深入，机理性的研究更多应该求深入而非广泛。建议研究者以最擅长的蛋白组学为主要研究内容，深入研究钝叶草抗旱中蛋白表达的研究。</p> <p>2) 调控差异表达蛋白的基因扩增和筛选的可行性不高。将已经获得的差异表达蛋白作为研究对象，扩增出其相关调控基因，此环节是非常重要的研究节点，在整个项目中承前启后，极为关键。然而，申请人却言之甚少，研究方案和实验设计均过于简单，使人对于申请人相关基因克隆和表达技术基础和研究技能产生质疑。</p> <p>此外，本项目要研究差异表达蛋白的相关“调控”基因，该研究目标恐较难实现。如果筛选克隆与该蛋白对应的基因，如何证实基因能够调控蛋白表达，这些均需要阐释清楚。</p>					

3) 生理机制研究的目的不明, 与其他研究脱节。在2.3.1部分测定4个生理指标, 但该部分内容的研究目的不够明确, 且与其余研究环节之间的关系不明。本项目在“研究目的和意义”、“研究目标”、“拟解决的关键科学问题”等多处内容都提到要结合蛋白、基因和代谢三个水平来探讨钝叶草的抗旱机理, 却没有相关生理研究内容支撑。

建议在获得差异表达蛋白之后, 研究该蛋白具体的调控通路, 究竟在哪个方面对钝叶草抗旱带来影响, 例如是干旱胁迫信号转导或渗透调节方面, 还是抗氧化系统或水孔通道蛋白方面。明确了差异表达蛋白的具体通路, 再进行生理实验予以验证。

4) 表述不清。拟解决的关键科学问题、技术手段和特色、创新之处相互混淆, 区分模糊。“拟解决的关键科学问题”表述更接近实验技术手段。如“研究钝叶草响应干旱的蛋白”是拟解决的问题, 但加上“利用iTRAQ技术”之后, 只是对研究技术手段的一种阐述; “分析钝叶草抗旱性的分子机理”也是拟解决的问题, 但“综合基因水平、蛋白水平和代谢水平来分析”则是阐述拟采用的技术手段和分析方法。而且, 关键问题与特色、创新之处完全重复, 说明申请人理解较为混乱。

5) 预期研究结果略显单薄。“发表论文和培养研究生”只能是预期成果形式, 而实质性的研究成果仅有分子机理的阐述, 略显空洞和单薄。应补充说明, 如是否有抗旱差异蛋白的筛选、调控蛋白基因文库的制备、相关代谢图或蛋白互作网络图的构建, 等等。

此外, 如综述中提到“在基因组和蛋白组方面草坪草抗逆研究的很少”, 但随后又举出了很多和基因组、蛋白组相关的研究报道, 如黑麦草、剪股颖、高羊茅、百慕大等; 仅详细论述了实验方法和研究方案, “可行性分析”部分未见详述。

鉴于以上意见, 建议可进行资助。

<3> 利用钝叶草抗旱突变体和对干旱敏感材料, 通过iTRAQ技术, 研究钝叶草的分子机理, 构建蛋白调控及互作的网络图谱, 对我国草坪草的研究具有很重要的科学意义和应用前景。申请人及其团队长期开展草坪草的研究, 有雄厚的工作积累, 发表了一系列相关的论文。申请书中所选的关键问题较准确, 整体研究方案和技术路线切实可行, 研究团队实力雄厚。建议优先资助。

对研究方案的修改意见:

生命科学部

2014年8月15日



项目批准号	31401906
申请代码	C150303
归口管理部门	
依托单位代码	51064208A0499-0932



3 1401906 1006923

国家自然科学基金委员会 资助项目计划书

资助类别：青年科学基金项目

亚类说明：

附注说明：

项目名称：iTRAQ技术分析钝叶草抗旱的分子机理

资助经费：24万元 执行年限：2015.01-2017.12

负责人：罗娜

通讯地址：广州市天河区五山路华南农业大学生命科学学院

邮政编码：510642 电 话：02085282180

电子邮件：naluo@scau.edu.cn

依托单位：华南农业大学

联系人：苏弟华 电 话：020-85280070

填表日期：2014年08月25日

国家自然科学基金委员会制



国家自然科学基金委员会资助项目计划书填报说明

- 一、项目负责人收到《关于国家自然科学基金资助项目批准及有关事项的通知》（以下简称《批准通知》）后，请认真阅读本填报说明和自然科学基金相关项目及财务管理办法（查阅<http://www.nsfc.gov.cn/>），按《批准通知》的要求认真填写《国家自然科学基金委员会资助项目计划书》（以下简称《计划书》）。
- 二、填写《计划书》时要求科学严谨、实事求是、表述清晰、准确。《计划书》经主管科学部审核批准后，将作为项目研究计划执行和检查、验收的依据。
- 三、《计划书》简表部分自动生成，其他部分按以下要求填写：
 - （一）各类获资助项目都必须填写中、英文摘要及主题词，按批准经费填报经费预算表。
 - （二）正文撰写：
 1. 对于面上项目、青年科学基金项目、地区科学基金项目，如果《批准通知》中没有修改要求的，只需选择“研究内容和研究目标按照申请书执行”即可；如果《批准通知》中明确要求调整研究内容的，须选择“根据研究方案修改意见更改”并填报相关修改内容。
 2. 对于重点项目、重大项目、科学仪器基础研究专款项目及国家重大科研仪器设备研制专项（自由申请）项目，须选择“根据研究方案修改意见更改”，根据《批准通知》的要求填报研究内容，不得自行降低、更改研究目标（或仪器研制指标）或缩减关键的研究内容。此外，还要突出以下几点：
 - 1) 研究的难点和在实施过程中可能碰到的问题（或仪器研制风险），拟采用的研究方案和技术路线；
 - 2) 项目主要参与者分工，并请说明课题及合作单位之间的关系与分工。
 3. 对于国家杰出青年科学基金、优秀青年科学基金和海外及港澳学者合作研究基金项目，须选择“根据研究方案修改意见更改”，按下列提纲撰写：
 - 1) 研究方向；
 - 2) 结合国内外研究现状，说明研究工作的学术思想和科学意义（限两个页面）；
 - 3) 研究内容、研究方案及预期目标（限两个页面）；
 - 4) 分年度进度安排；
 - 5) 研究队伍的组成情况。
 4. 对于其他类型项目，参照面上项目填报。



简表

申请者信息	姓 名	罗娜	性 别	女	出生年月	1982年04月	民 族	汉族
	学 位	博士			职称	助理研究员		
	电 话	02085282180		电子邮件	naluo@scau.edu.cn			
	传 真			个人网页				
	工 作 单 位	华南农业大学						
	所 在 院 系 所	生命科学学院						
依托单位信息	名 称	华南农业大学					代码	51064208A0499
	联 系 人	苏弟华		电子邮件	kycjkh@scau.edu.cn			
	电 话	020-85280070		网站地址	http://web.scau.edu.cn/kjc/			
合作单位信息	单 位 名 称							代 码
项目基本信息	项 目 名 称	iTRAQ技术分析钝叶草抗旱的分子机理						
	资 助 类 别	青年科学基金项目			亚 类 说 明			
	附 注 说 明							
	申 请 代 码	C150303:观赏作物分子生物学						
	基 地 类 别							
	执 行 年 限	2015.01-2017.12						
	资 助 经 费	24.0000万元						



项目摘要

中文摘要(500字以内):

干旱胁迫严重影响了草坪草的应用和品质,研究草坪草的抗旱机理尤为重要。蛋白组学iTRAQ技术的应用可以从蛋白角度揭示植物抗逆的分子机理。本研究将以申请者实验室已鉴定的抗性材料突变体12-6钝叶草和敏感材料普通钝叶草(CK)为材料,利用iTRAQ技术分析两种抗性不同的材料响应干旱胁迫的蛋白及蛋白表达的差异,同时利用Western-blot验证iTRAQ实验结果;对调控差异表达蛋白的基因进行表达分析,分析差异表达蛋白与差异表达基因的关系;明确差异表达蛋白调控的核心代谢途径,构建核心代谢途径中的蛋白互作的网络图谱,应用双分子荧光互补技术验证蛋白互作的关系,并且分析核心代谢途径中的产物响应干旱的情况。本研究是从蛋白水平、基因水平和代谢水平三个方面揭示钝叶草抗旱的分子机理,并且三个方面互相验证,保证研究结果的准确性与真实性。本研究将对钝叶草种质资源的改良与分子遗传育种具有重大意义。

关键词: 钝叶草; 抗旱性; 相对和绝对定量的同位素标记法; 基因; 代谢途径

Abstract(limited to 500 words):

Drought stress is a major factor affects the using and quality of turf grasses. St. Augustingrass was an important turfgrass and widely planted in south china but it was limited by the drought stress. To identify the mechanisms underlying drought stress in St. Augustingrass, drought stress-induced changes in protein profile of St. Augustingrass leaves were comprehensively analyzed using iTRAQ (Isobaric Tag for Relative and Absolute Quantification) differential liquid chromatography-tandem mass spectrometry. The research materials included drought-tolerance mutant 12-6 and drought-sensitive CK identified by many years. The different expression proteins were compared from the different drought-tolerance materials. And identify the iTRAQ results with the method of western-blot. Analysis the relationship between the different expression protein and the genes induce by the protein. Make sure the core pathway induced by the different expression protein and built the protein interactions internet in core pathway. And identify protein interactions by bimolecular fluorescence complementation (BiFC). The productions induced by drought stress in core pathway were analyzed by the method of HPLC(high performance liquid chromatography) and GC-MS (chromatography-mass spectrometry). This research was found the molecular mechanism on drought stress from the level of protein, gene and pathway. And the relationships among three levels were verified. It kept the result accuracy and reality. The research was important meaning on improving the germplasm resource and molecular breeding.

Keywords: St. Augustingrass; Drought tolerance; Isobaric Tag for Relative and Absolute; Gene; Pathway



项目组主要成员

编号	姓名	出生年月	性别	职称	学位	单位名称	电话	电子邮件	项目分工	每年工作时间 (月)
1	罗娜	1982.04	女	助理研究员	博士	华南农业大学	02085282180	naluo@scau.edu.cn	项目负责人	10
2	刘天增	1984.10	男	讲师	博士	华南农业大学	02085280228	tzliu1019@163.com	抗逆相关蛋白检测	6
3	赵普艳	1979.01	女	讲师	博士	华南农业大学	020-85280203	375368708@qq.com	响应干旱基因表达分析	6
4	刘颖	1987.12	女	博士生	硕士	华南农业大学	020-85280228	liuying629@st.scau.edu.cn	代谢产物测定	10
总人数		高级		中级		初级		博士后	博士生	硕士生
4				3					1	



经费预算表

(金额单位:万元)

预算编制说明:

1. 在填报本表之前, 请根据项目资助类别认真阅读相关的资助经费管理办法; 经费预算的编制以申请书中的《经费申请表》为基础, 以《国家自然科学基金项目资助批准通知书》中的资助金额为依据;
2. 编制经费预算时, 不考虑不可预见因素和前期投入;
3. 购置与试制仪器设备在5万元以上(包括5万元)时, 须在报告正文中逐项说明用途和必要性。

科目	预算经费	备注(计算依据与说明)
一. 研究经费	19. 2000	
1. 科研业务费	11. 0000	
(1) 测试/计算/分析费	9. 2000	蛋白iTRAQ测定和代谢产物高效液相
(2) 能源/动力费	0	
(3) 会议费/差旅费	1. 0000	参加国内草坪与蛋白相关的会议
(4) 出版物/文献/信息传播事务费	0. 8000	发表文章的审稿费版面费
(5) 其他	0	
2. 实验室材料费	7. 2000	
(1) 原材料/试剂/药品购置费	7. 2000	蛋白提取及qRT-PCR相关试剂
(2) 其他	0	
3. 仪器设备费	1. 0000	
(1) 购置	1. 0000	购置小型仪器
(2) 试制	0	
4. 实验室改装费	0	
5. 协作费	0	
二. 国际合作与交流费	0	
1. 出境国际旅费	0	
2. 境外合作人员来华生活费	0	
3. 来华举办学术会议费	0	
4. 其他	0	
三. 劳务费	3. 6000	直接参加项目研究的研究生、博士后人员的劳务费用
四. 管理费	1. 2000	不得超过预算经费的5%
合 计	24. 0000	
与本项目相关的其他经费来源	国家其他计划资助经费	
	其他经费资助(含部门匹配)	
	其他经费来源合计	



报告正文

研究内容和研究目标按照申请书执行。



国家自然科学基金资助项目签批审核表

<p>我接受国家自然科学基金的资助，将按照申请书、项目批准意见和计划书负责实施本项目（批准号：31401906），严格遵守国家自然科学基金委员会关于资助项目管理、财务等各项规定，切实保证研究工作时间，认真开展研究工作，按时报送有关材料，及时报告重大情况变动，对资助项目发表的论著和取得的研究成果按规定进行标注。</p> <p>项目负责人（签章）： 年 月 日</p>	<p>我单位同意承担上述国家自然科学基金项目，将保证项目负责人及其研究队伍的稳定和研究项目实施所需的条件，严格遵守国家自然科学基金委员会有关资助项目管理、财务等各项规定，并督促实施。</p> <p>依托单位（公章） 年 月 日</p>														
本栏目由基金委填写	<p>科学处审查意见：</p> <p>建议年度拨款计划（本栏目为自动生成，单位：万元）：</p> <table border="1" data-bbox="201 947 1045 1072"><thead><tr><th>年度</th><th>总额</th><th>第一年</th><th>第二年</th><th>第三年</th><th>第四年</th><th>第五年</th></tr></thead><tbody><tr><td>金额</td><td></td><td></td><td></td><td></td><td></td><td></td></tr></tbody></table> <p>负责人（签章）： 年 月 日</p>	年度	总额	第一年	第二年	第三年	第四年	第五年	金额						
	年度	总额	第一年	第二年	第三年	第四年	第五年								
	金额														
<p>科学部审查意见：</p> <p>负责人（签章）： 年 月 日</p>															
本栏目主要用于重大项目等	<p>相关局室审核意见：</p> <p>负责人（签章）： 年 月 日</p>														
	<p>委领导审批意见：</p> <p>委领导（签章）： 年 月 日</p>														

受理编号: c22140500002218

项目编号: 2022A1515011483

文件编号: 粤基金字(2022)3号

广东省基础与应用基础研究基金项目

任务书

项目名称: 基于植物细胞自噬途径的降解体系建立和在抗病毒中应用

项目类别: 广东省自然科学基金-面上项目

项目起止时间: 2022-01-01 至 2024-12-31

管理单位(甲方): 广东省基础与应用基础研究基金委员会

依托单位(乙方): 华南农业大学

通讯地址: 广东省广州市天河区五山路483号

邮政编码: 510642

单位电话: 020-85283435

项目负责人: 罗娜

联系电话: 13450362148



(广东科技微信公众号)



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(受理纸质材料二维码)

广东省基础与应用基础研究
基金委员会
二〇二〇年制

填写说明

一、项目任务书内容原则上要求与申报书相关内容保持一致，不得无故修改。

二、项目承担单位通过广东省科技业务管理阳光政务平台下载项目任务书，按要求完成签名盖章后提交至省科技厅受理窗口。

三、签名盖章说明。请分别在单位工作分工及经费分配情况页、人员信息页、签约各方页等地方按要求签字或盖章，签章不合规或错漏将不予受理。其中，人员信息页要求所有参与人员本人亲笔签名，代签或印章无效，漏签将不予受理。

四、本任务书自签字并加盖公章之日起生效，各方均应负本任务书的法律责任，不应受机构、人事变动影响。

2022A1515011483

一、主要研究内容和要达到的目标

本研究的主要内容:

细胞自噬是维持蛋白稳态,降解蛋白的主要方式。并且相比较泛素-蛋白酶体系统,细胞自噬可以降解大量的细胞内容物,包括大的蛋白复合体、整个细胞器和病毒等。作为重要的降解机制,目前人为利用自噬途径来降解目标物还只局限于动物细胞,植物尚未报导。本研究利用前期构建的既可以识别自噬囊泡又可以识别待降解的目的物的人工衔接蛋白,进一步改造该衔接蛋白,构建基于植物细胞自噬途径的降解体系。本研究通过细胞生物学和分子生物学方法利用烟草瞬转系统检测到该衔接蛋白可改变核定位蛋白TGA5和质膜受体蛋白的BR1的定位;并且可以将过氧化物酶体导入液泡中降解。本研究还进一步验证该衔接蛋白在拟南芥体内的作用。本研究选择两组已经互做的蛋白,并且蛋白的缺失有明显的表型。利用衔接蛋白在植物体内降低目标蛋白PEAM1和BIN2蛋白的含量,从而表现出目标蛋白缺失的表型。本研究还进一步利用上述的降解体系来降解植物体内的葡萄扇叶病毒,从而增强植物抵御病毒的能力。如此一来,本研究可以有针对性的去降解目标物质,如入侵的病毒和重金属等,这将为提高植物抗逆能力、改善农作物产量和品种提供新的思路。

预期目标:

- (1) 通过分子生物学和细胞生物学的方法,构建基于植物细胞自噬途径的降解体系,明确该体系在烟草瞬时表达系统中降解目的蛋白和细胞器是有效的;
- (2) 以遗传学和细胞生物学为主要手段,分析上述体系在拟南芥稳转材料中可以有效地降解目的蛋白,用来研究蛋白功能;
- (3) 以微生物学为手段并结合表型分析来探究上述降解体系在降解葡萄扇叶病毒是有效的。

二、项目预期获得的研究成果及形式

论文及专著情况	国家统计源刊物以上刊物发表论文（篇）		2		科技报告（篇）		1	
	其中被SCI/EI/ISTP收录论文数（篇）		1		培养人才（人）		2	
	专著（册）				引进人才（人）			
专利情况(项)	发明专利		实用新型专利		外观设计专利		国外专利	
	申请	授权	申请	授权	申请	授权	申请	授权
	1							

三、项目进度和阶段目标

(一) 项目起止时间: 2022-01-01 至 2024-12-31

(二) 项目实施进度及阶段主要目标:

开始日期	结束日期	主要工作内容
2022-01-01	2022-12-31	(1)构建拟南芥稳转的表达载体: AIM-GBP-mCherry、PEAMT-GFP、AIM-BZRC82-mCherry和BIN2-GFP载体; (2)烟草瞬时表达系统检测上述载体共定位情况; (3)获得拟南芥纯合稳转的材料; (4)激光共聚焦显微镜观察纯合稳定植株内上述载体的定位情况; (5)开展国内学术交流, 参加国内学术会议2人次;
2023-01-01	2023-12-31	(1)进一步分析上述拟南芥稳转的纯合材料表型; (2)免疫共沉淀分析衔接蛋白是否可以在植物体内识别目标蛋白, 并且导入液泡中; (3)开始撰写论文1至2篇;
2024-01-01	2024-12-31	(1)利用烟草瞬转系统分析葡萄扇叶病毒抗性; (2)免疫共沉淀分析和激光共聚焦显微镜观察AIM-Nb23-mCherry在植物体内的效果; (3)培养研究生1-2名; (4)投稿论文1至2篇

四、项目总经费及省基金委经费预算

1. 省基金委经费下达总额：（大写）壹拾万圆整；（小写）10万元；					
2. 省基金委经费年度下达计划：					
年度	2022 年	年	年	年	年
经费(万元)	10.00				
3. 总经费及省基金委经费开支预算计划：					
经费筹集情况：					(单位：万元)
省基金委经费	自筹资金				合计
	自有资金	贷款	地方政府投入	其它	
10.00	0.00	0.00	0.00	0.00	10.00
政府部门、境外资金及其他资金投入情况说明：					
与本项目相关的其他经费来源			(单位：万元)		
其他计划资助经费：			0.00		
单位配套经费：			0.00		
其他经费资助：			0.00		
其他经费来源合计：					

五、人员信息

项目负责人

姓名	证件号码	年龄	性别	职称	学历	在项目中承担的任务	所在单位	签名
罗娜	230121198204112028	40	女	助理研究员	博士研究生	项目负责人	华南农业大学	罗娜

项目组主要成员

姓名	证件号码	年龄	性别	职称	学历	在项目中承担的任务	所在单位	签名
赵普艳	612128197901018224	43	女	讲师	博士研究生	病菌接菌及植株抗性分析	华南农业大学	赵普艳
潘璐怡	440106199102161822	31	女	实验师	硕士研究生	激光共聚焦显微镜观察蛋白定位情况	华南农业大学	潘璐怡
郑春艳	430524199201074065	30	女	未取得	硕士研究生	烟草瞬时表达和拟南芥农杆菌转化	华南农业大学	郑春艳
麦金燕	440883199807304222	24	女	未取得	本科	载体的构建, 植株根部表型分析	华南农业大学	麦金燕
黄佳睿	513902199804161068	24	女	未取得	本科	烟草瞬时表达载体的构建	华南农业大学	黄佳睿

六、工作分工及经费分配

承担/参与单位名称 (盖章)	工作分工	总经费分摊 (万元)	省基金委经费分配 (万元)
华南农业大学	本项目为华南农业大学独立完成，不涉及经费分摊。	10.00	10.00
合计		10.00	10.00

七、任务书条款

第一条 甲方与乙方根据《中华人民共和国民法典》及国家有关法规和规定，按照《广东省科学技术厅关于广东省基础与应用基础研究基金（省自然科学基金、联合基金等）项目管理的实施细则（试行）》《广东省省级科技计划项目验收结题工作规程（试行）》等规定，为顺利完成（2022）年基于植物细胞自噬途径的降解体系建立和在抗病毒中应用专项项目（文件编号：粤基金字（2022）3号）经协商一致，特订立本任务书，作为甲乙双方在项目实施管理过程中共同遵守的依据。

第二条 甲方的权利义务：

1. 按任务书规定进行经费核拨的有关工作协调。
2. 根据甲方需要，在不影响乙方工作的前提下，定期或不定期对乙方项目的实施情况和经费使用情况进行检查或抽查。
3. 根据《广东省科研诚信管理办法（试行）》等规定对乙方进行科技计划信用管理。

第三条 乙方的权利义务：

1. 确保落实自筹经费及有关保障条件。
2. 按任务书规定，对甲方核拨的经费实行专款专用，单独列账，并随时配合甲方进行监督检查。
3. 经费使用按照广东省级财政科研项目经费使用等有关规定进行管理。
4. 项目依托单位应制定经费使用“负面清单+包干制”内部管理制度并报甲方备案。
5. 使用财政资金采购设备、原材料等，按照《广东省实施〈中华人民共和国招标投标法〉办法》有关规定，符合招标条件的须进行招标。
6. 项目任务书任务完成后，或任务书规定的任务、指标及经费投入等提前完成的，乙方可提出验收结题申请，并按甲方要求做好项目验收结题工作。
7. 若项目发生需要终止结题的情况，乙方须提出终止结题申请，并按甲方要求做好项目终止结题工作。
8. 在每年规定时间内向甲方如实提交上年度工作情况报告，报告内容包含上年度项目进展情况、经费决算和取得的成果等。
9. 按照国家和省有关规定，提交科技报告及其他材料。
10. 利用甲方的经费获得的研究成果，项目负责人和参与者应当注明获得“广东省基础与应用基础研究基金（英文：Guangdong Basic and Applied Basic Research Foundation）（项目编号）”资助或作有关说明。
11. 乙方要恪守科学道德准则，遵守科研活动规范，践行科研诚信要求，不得抄袭、剽窃他人科研成果或者伪造、篡改研究数据、研究结论；不得购买、代写、代投论文，虚构同行评议专家及评议意见；不得违反论文署名规范，擅自标注或虚假标注获得科技计划（专项、基金等）等资助；不得弄虚作假，骗取科技计划（专项、基金等）项目、科研经费以及奖励、荣誉等；不得有其他违背科研诚信要求的行为。
12. 确保本项目开展的研究工作符合我国科技伦理管理相关规定。

第四条 在履行本任务书的过程中，如出现广东省相关政策法规重大改变等不可抗力情况，甲方有权对所核拨经费的数量和时间进行相应调整。

第五条 在履行本任务书的过程中，当事人一方发现可能导致项目整体或部分失败的情形时，应及时通知另一方，并采取适当措施减少损失，没有及时通知并采取适当措施，致使损失扩大的，应当就扩大的损失承担责任。

第六条 本项目技术成果的归属、转让和实施技术成果所产生的经济利益的分享，除双方另有约定外，按国家和广东省有关法规执行。

第七条 根据项目具体情况，经双方另行协商订立的附加条款，作为本任务书正式内容的一部分，与本任务书具有同等效力。

第八条 本任务书一式三份，各份具有同等效力。甲、乙方及项目负责人各执一份，三方签字、盖章后即生效，有效期至项目结题后一年内。各方均应负任务书的法律责任，不应受机构、人事变动的影响。

第九条 乙方必须接受甲方聘请的本项目任务书监理单位的监督和管理。监理单位按照甲方赋予的权利对本项目任务书的履行进行审核、进度调查，对项目任务书变更、经费使用情况进行监督管理及组织项目验收。

说明：1. 本任务书中，凡是当事人约定无需填写的内容，应在空白处划（/）。

2. 委托代理人签订本任务书的，应出具合法、有效的委托书。

八、本任务书签约各方

管理单位（甲方）：

广东省基础与应用基础研究基金委员会（盖章）

法定代表人（或法人代理）：

曾路

（签章）

2022 年 04 月 21 日

依托单位（乙方）：

华南农业大学

（盖章）

法定代表人（或法人代理）：

刘雅红

（签章）

联系人（项目主管）姓名：

阮慧群

（签章）

Email: kjcgxk@scau.edu.cn

电话: 020-85283435 / 15920301530

开户单位名称：

华南农业大学

开户银行名称：

广东广州工行五山支行

开户银行帐号：

3602002609000310520

2022 年 4 月 21 日

联系人（项目负责人）姓名：

罗娜

（签名）

Email: naluo@scau.edu.cn

电话: 13450362148

2022 年 4 月 21 日

项目编号: S2013040014817
资助类别: 博士启动
文件编号: 粤科基办字(2013) 3
号

自由申请
广东省自然科学基金重点项目
博士启动

合 同 书

课题名称: 钝叶草抗旱分子机理的研究

下达单位(甲方): 广东省自然科学基金管理委员会

课题负责人: 罗娜 资助经费: 3(万元)

通信地址: 广州市天河区五山路283号

邮政编码: 510642 联系电话: 13450362148

电子邮件: paluo@scau.edu.cn

依托单位(乙方): 华南农业大学

联系人: 苏第华 联系电话: 85280070

执行年限: 2013年10月至 2015年10月

填表日期: 2013年10月08日

广东省自然科学基金管理委员会

二〇一〇年 制

三、进度和阶段目标:

2013年10月~2014年10月	抗旱相关的生理指标测定，明确干旱所引起的差异表达蛋白位点
2014年11月~2015年10月	差异位点质谱分析和主要差异蛋白对应代谢途径分析，投稿一篇文章
年 月~ 年 月	
年 月~ 年 月	
年 月~ 年 月	

四、项目承担单位、参加单位及项目组成员

承担单位（盖章）：

参与单位1 (盖章):

参与单位2（盖章）：

项目 负责人:

姓 名	证件类型	证件号码	出生日期	职称	学位	所在单位	项目中的分工	签名
罗娜	身份证	230121198204112028	1982年4月11日	助理研究员	博士	华南农业大学	设计实验，指导实验进展，解决关键技术	罗娜

主要研究人员（须如实填写，以便检查核实）：

[illegible]

本合同签约各方

下达单位（甲方）：广东省自然科学基金管理委员会		（盖章）
法定代表人（或法人代理）：		（签章）
		2013年11月4日
依托单位（乙方）：		
法定代表人（或法人代理）：		（盖章）
联系人（项目主管）姓名：		（签章）
电话：020-85280070	乙方开户银行名称：广东广州工行五山支行	
乙方开户单位名称：华南农业大学		
乙方开户银行帐号：3602002609000310520		
		年 月 日
联系人（课题负责人）姓名：		（盖章）
电话：13450362148	E-mail: naluo@scau.edu.cn	
		年 月 日

国家自然科学基金资助项目批准通知

黄晓 先生/女士：

根据《国家自然科学基金条例》规定和专家评审意见，国家自然科学基金委员会（以下简称自然科学基金委）决定资助您申请的项目。项目批准号：32070195，项目名称：拟南芥自噬核心蛋白ATG8在花粉管生长过程中调节囊泡运输的分子机制研究，直接费用：58.00万元，项目起止年月：2021年01月至2024年12月，有关项目的评审意见及修改意见附后。

请尽早登录科学基金网络信息系统（<https://isisn.nsfc.gov.cn>），获取《国家自然科学基金资助项目计划书》（以下简称计划书）并按要求填写。对于有修改意见的项目，请按修改意见及时调整计划书相关内容；如对修改意见有异议，须在电子版计划书报送截止日期前向相关科学处提出。

电子版计划书通过科学基金网络信息系统（<https://isisn.nsfc.gov.cn>）上传，依托单位审核后提交至自然科学基金委进行审核。审核未通过者，返回修改后再行提交；审核通过者，打印纸质版计划书（一式两份，双面打印），依托单位审核并加盖单位公章，将申请书纸质签字盖章页订在其中一份计划书之后，一并将上述材料报送至自然科学基金委项目材料接收工作组。电子版和纸质版计划书内容应当保证一致。**自然科学基金委将对申请书纸质签字盖章页进行审核，对存在问题的，允许依托单位进行一次修改或补齐。**

向自然科学基金委补交申请书纸质签字盖章页、提交和报送计划书截止时间节点如下：

1. **2020年10月14日16点**：提交电子版计划书的截止时间（视为计划书正式提交时间）；
2. **2020年10月21日16点**：提交电子修改版计划书的截止时间；
3. **2020年10月28日16点**：报送纸质版计划书（其中一份包含申请书纸质签字盖章页）的截止时间。
4. **2020年11月18日16点**：报送修改后的申请书纸质签字盖章页的截止时间。



项目批准号	32070195
申请代码	C020101
归口管理部门	
依托单位代码	51064208A0499-0932



国家自然科学基金委员会 资助项目计划书

资助类别：面上项目

亚类说明：

附注说明：

项目名称：拟南芥自噬核心蛋白ATG8在花粉管生长过程中调节囊泡运输的分子机制研究

直接费用：58万元 执行年限：2021.01-2024.12

负责人：黄晓

通讯地址：广东广州市天河区五山路483号华南农业大学生命科学学院北一609

邮政编码：510642 电 话：13660639294

电子邮件：xiaohuang@scau.edu.cn

依托单位：华南农业大学

联系人：倪慧群 电 话：020-85280070

填表日期：2020年09月29日

国家自然科学基金委员会制



国家自然科学基金委员会资助项目计划书填报说明

- 一、项目负责人收到《关于国家自然科学基金资助项目批准及有关事项的通知》（以下简称《批准通知》）后，请认真阅读本填报说明，参照国家自然科学基金相关项目管理办法及《国家自然科学基金资助项目资金管理办法》（请查阅国家自然科学基金委员会官方网站首页“政策法规”栏目），按《批准通知》的要求认真填写和提交《国家自然科学基金委员会资助项目计划书》（以下简称《计划书》）。
- 二、填写《计划书》时要求科学严谨、实事求是、表述清晰、准确。《计划书》经国家自然科学基金委员会相关项目管理部门审核批准后，将作为项目研究计划执行和检查、验收的依据。
- 三、《计划书》各部分填写要求如下：
 - （一）简表：由系统自动生成。
 - （二）摘要及关键词：各类获资助项目都必须填写中、英文摘要及关键词。
 - （三）项目组主要成员：计划书中列出姓名的项目组主要成员由系统自动生成，与申请书原成员保持一致，不可随意调整。如果批准通知中“项目评审意见及修改意见表”中“对研究方案的修改意见”栏目有调整项目组成员相关要求的，待项目开始执行后，按照项目成员变更程序另行办理。
 - （四）资金预算表：根据批准资助的直接费用，按照《国家自然科学基金项目预算表编制说明》填报资金预算表和预算说明书。国家重大科研仪器研制项目、重大项目还应按照预算评审后批复的直接费用各科目金额填报资金预算表、预算说明书及相应的预算明细表。
 - （五）正文：
 1. 面上项目、青年科学基金项目、地区科学基金项目：如果《批准通知》中没有修改要求的，只需选择“研究内容和研究目标按照申请书执行”即可；如果《批准通知》中“项目评审意见及修改意见表”中“对研究方案的修改意见”栏目明确要求调整研究期限和研究内容等的，须选择“根据研究方案修改意见更改”并填报相关修改内容。
 2. 重点项目、重点国际（地区）合作研究项目、重大项目、国家重大科研仪器研制项目：须选择“根据研究方案修改意见更改”，根据《批准通知》的要求填写研究（研制）内容，不得自行降低、更改研究目标（或仪器研制的技术性能与主要技术指标以及验收技术指标）或缩减研究（研制）内容。此外，还要突出以下几点：
 - （1）研究的难点和在实施过程中可能遇到的问题（或仪器研制风险），拟采用的研究（研制）方案和技术路线；
 - （2）项目主要参与者分工，合作研究单位之间的关系与分工，重大项目还需说明课题之间的关联；
 - （3）详细的年度研究（研制）计划。



3. 国家杰出青年科学基金、优秀青年科学基金和海外及港澳学者合作研究基金项目：须选择“根据研究方案修改意见更改”，按下列提纲撰写：
 - (1) 研究方向；
 - (2) 结合国内外研究现状，说明研究工作的学术思想和科学意义（限两个页面）；
 - (3) 研究内容、研究方案及预期目标（限两个页面）；
 - (4) 年度研究计划；
 - (5) 研究队伍的组成情况。
4. 国家自然科学基金基础科学中心项目：须选择“根据研究方案修改意见更改”，应当根据评审委员会和现场考察专家组的意见和建议，进一步完善并细化研究计划，作为评估和验收的依据。按下列提纲撰写：
 - (1) 五年拟开展的研究工作（包括主要研究方向、关键科学问题与研究内容）；
 - (2) 研究方案（包括骨干成员之间的分工及合作方式、学科交叉融合研究计划等）；
 - (3) 年度研究计划；
 - (4) 五年预期目标和可能取得的重大突破等；
 - (5) 研究队伍的组成情况。
5. 对于其他类型项目，参照面上项目的方式进行选择和填写。



简表

项目负责人信息	姓 名	黄晓	性 别	女	出生年月	1976年03月	民 族	汉族
	学 位	博士			职称	副教授		
	是否在站博士后	否			电子邮件	xiaohuang@scau.edu.cn		
	电 话	13660639294			个人网页			
	工 作 单 位	华南农业大学						
	所 在 院 系 所	生命科学学院						
依托单位信息	名 称	华南农业大学					代码	51064208A0499
	联 系 人	倪慧群			电子邮件	kycjkh@scau.edu.cn		
	电 话	020-85280070			网站地址	http://kjc.scau.edu.cn/		
合作单位信息	单 位 名 称							
项目基本信息	项 目 名 称	拟南芥自噬核心蛋白ATG8在花粉管生长过程中调节囊泡运输的分子机制研究						
	资 助 类 别	面上项目				亚 类 说 明		
	附 注 说 明							
	申 请 代 码	C020101:植物结构与功能				C0709:细胞自噬		
	基 地 类 别							
	执 行 年 限	2021.01-2024.12						
	直 接 费 用	58万元						



项目摘要

中文摘要:

细胞自噬是真核细胞中降解胞内物质并促进营养物质循环利用的一种重要代谢机制,在调控植物生长发育和响应逆境胁迫中发挥重要作用。作为该途径的核心蛋白,ATG8在植物中的具体功能仍有待研究。申请人前期创建了拟南芥atg8多重缺失突变体,进一步研究发现该突变体除具有常见的自噬缺陷表型外,还呈现出与已知自噬突变体显著不同的表型:其花粉管生长时的囊泡运输受阻,进而导致结实率明显降低。据此我们提出假设:拟南芥的ATG8蛋白同时具有自噬和非自噬功能,并参与了花粉管生长过程的囊泡运输。本项目将以atg8多重缺失突变体和ATG8脂化缺陷突变的转基因植株为遗传材料,旨在从遗传学角度揭示ATG8的自噬和非自噬功能,并以细胞生物学为主要技术手段阐明ATG8在花粉管生长过程中调节囊泡运输的分子机制。本项目有望揭示ATG8的新功能,且对深入理解植物细胞自噬的形成与调控及探索植物ATG8的非自噬功能具重要启示作用。

Abstract:

Autophagy is a highly conserved degradation pathway in eukaryotes whereby cytoplasmic material is sequestered in vesicles and delivered to lysosome or the vacuole for breakdown. Rapid progress has been made in describing the mechanisms of autophagy regulation and autophagic trafficking using yeast and mammalian cells as models. The ubiquitin-like protein ATG8, identified as an autophagy core protein in its lipidated form, also has been revealed to play several non-autophagic roles. However, the precise roles of ATG8 remain unclear in plants. Our prior work has shown that ATG8 is duplicated in the Arabidopsis genome, and generated several atg8 multi-gene mutants through CRISPR/Cas9 mediated genome editing. Besides phenotypes similar to those well-known atg mutants, the atg8 multi-gene mutants also display distinct phenotypes: Both pollen germination and tube growth are inhibited significantly. Further study shows that in these mutants the vesicle trafficking has been compromised in pollen tubes, and thus inhibits their growth. Therefore, we propose that ATG8 is involved in the regulation of vesicle trafficking during pollen tube growth, and its deletion would lead to subsequent decrease of the tube growth rate. The objectives of this proposal are to (i) reveal the roles of ATG8 in vesicle trafficking during pollen tube growth; (ii) further define the non-autophagic roles of ATG8 involved in plant growth and senescence. Collectively, these experiments will provide deep insight into plant autophagy regulation or vesicle trafficking. This research may also help us better understand the roles of plant ATG8 proteins, particularly their non-autophagic roles.

关键词(用分号分开): 自噬; 囊泡运输; ATG8; 非自噬功能; 花粉管

Keywords(用分号分开): Autophagy; Vesicle trafficking; ATG8; Non-autophagic role; Pollen tube



项目组主要成员

编号	姓名	出生年月	性别	职称	学位	单位名称	电话	证件号码	项目分工	每年工作时间（月）				
1	黄晓	1976.03	女	副教授	博士	华南农业大学	13660639294	440520197603041423	项目负责人	10				
2	罗娜	1982.04	女	助理研究员	博士	华南农业大学	17316802018	230121198204112028	研究ATG8的自噬功能	8				
3	郑萍	1989.11	女	博士后	博士	华南农业大学	17620147537	429005198911196185	研究ATG8蛋白的亚细胞定位与分布	10				
4	郑春艳	1992.01	女	博士生	硕士	华南农业大学	18819266206	430524199201074065	研究ATG8蛋白的亚细胞定位与分布	10				
5	李丹阳	1994.03	女	博士生	硕士	华南农业大学	15931821877	22050219940318082X	不依赖于ATG8脂化的突变体表型鉴定	10				
6	刘易林	1995.08	女	硕士生	学士	华南农业大学	18154718743	450121199508302125	突变体的表型鉴定	10				
7	梁秀霞	1995.07	女	硕士生	学士	华南农业大学	17818520521	450981199507183726	突变体的表型鉴定	10				
8	潘璐怡	1991.02	女	助理实验师	硕士	华南农业大学	13822279183	440106199102161822	ATG8在囊泡运输途径上的定位结构解析	6				
总人数			高级		中级		初级		博士后		博士生		硕士生	
8			1		1		1		1		2		2	



国家自然科学基金项目直接费用预算表（定额补助）

项目批准号：32070195

项目负责人：黄晓

金额单位：万元

序号	科目名称	金额
1	项目直接费用合计	58.0000
2	1、设备费	0.0000
3	(1)设备购置费	0.00
4	(2)设备试制费	0.00
5	(3)设备升级改造与租赁费	0.00
6	2、材料费	25.0000
7	3、测试化验加工费	14.7000
8	4、燃料动力费	0.00
9	5、差旅/会议/国际合作与交流费	3.00
10	6、出版/文献/信息传播/知识产权事务费	1.50
11	7、劳务费	12.80
12	8、专家咨询费	1.00
13	9、其他支出	0.00



预算说明书（定额补助）

（请按照《国家自然科学基金项目预算表编制说明》等的有关要求，对各项支出的主要用途和测算理由，以及合作研究外援资金、单价≥10万元的设备费等内容进行必要说明。）

1. 设备费（0.00万元）：

无需购买设备。

2. 材料费（25.00万元）：**（1）原材料/试剂/药品购置费用（24.00万元）**

- 植物材料种植（5000元/年，4年共2.00万元）：种植用土，不同尺寸育苗盆，植物绑扎线，长竹签，肥料，驱虫剂，黏虫板等。
- 实验室所需常规耗材（17500元/年，4年共7.00万元）：包括移液枪头，移液管，离心管，Eppendorf管，三角锥瓶，试剂瓶，烧杯，细菌培养皿，细胞培养皿，基因枪所需材料（压力爆破片、承载片和停止网），载玻片，盖玻片，锡箔纸，尼龙膜，封口膜和手套等的购置费用。
- 药品试剂（37500元/年，4年共15.00万元）：包括细菌、细胞和植物培养基，分子克隆所需各种酶和试剂盒（Taq酶，高保真扩增酶，各种限制性内切酶，连接酶，小量质粒抽提试剂盒，大量质粒抽提试剂盒，PCR片断回收试剂盒，DNA凝胶回收试剂盒，RNA抽提试剂盒和T载体连接试剂盒等），基因枪所需钨粉，常用细胞药物处理试剂（Wortmannin 和 ConA），聚丙烯酰胺凝胶，复合蛋白酶抑制剂，一般荧光染料（FM4-64 和苯胺蓝），Western blot二抗，核酸和蛋白质 Marker，常规生化试剂和药品（例如蔗糖，琼脂，琼脂糖，苯酚，氯仿，乙醇，丙酮，硫酸，盐酸，Tris，氯化钠，氯化钾，氯化钙，氢氧化钠，硼酸盐，Triton X100，Tween20和牛血清白蛋白等）的购置费用。

（2）其它（1.00万元）

用于购买透射电子显微镜样品制备所需的渗透剂、包埋剂以及样品观察用的镍网和铜网。

3. 测试化验加工费（14.70万元）：**（1）测试分析（3.00万元）：**用于DNA测序、肽链的合成、引物的合成等费用。**（2）仪器使用（11.70万元）：**用于大型仪器的使用费。

- 激光扫描共聚焦电子显微镜(收费：200元/小时，18000元/年，4年共计7.20万元)，
- 透射电子显微镜(收费：200元/小时，6000元/年，4年共计2.40万元)，
- 超薄切片机(收费：45元/小时，2500元/年，4年共计1.00万元)，
- 超速冷冻离心机(收费：20元/小时，2750元/年，4年共计1.10万元)。

4. 燃料动力费（0.00万元）：无。**5. 差旅 / 会议 / 国际合作与交流费（3.00万元）：**

用于课题组成员参加国内或国际会议的注册、交通和住宿费用。拟参加国内会议2次，2人/次：植物生理学大会1次，细胞生物学大会1次。拟参加国际会议1次，1人/次：美国植物生理年会（ASPB）1次。

6. 出版/文献/信息传播/知识产权事务费（1.50万元）：

用于在国际/国内期刊上发表论文的版面费（2篇），文献期刊的查阅、资料查新等费用。

7. 劳务费（12.80万元）：

用于支付课题组参加本项目的硕士研究生（2人）和博士研究生（2人）的劳务费。硕士研究生600元/月/人，博士研究生1000元/月/人，4年40个月，4人合计12.80万元。

8. 专家咨询费（1.00万元）：

邀请同行专家进行评议，咨询费用。

9. 其它支出（0.00万元）：无。



报告正文

研究内容和研究目标按照申请书执行。



国家自然科学基金资助项目签批审核表

我接受国家自然科学基金的资助，将按照申请书、项目批准意见和计划书负责实施本项目（批准号：32070195），严格遵守国家自然科学基金委员会关于资助项目管理、项目资金管理等各项规定，切实保证研究工作时间，认真开展研究工作，按时报送有关材料，及时报告重大情况变动，对资助项目发表的论著和取得的研究成果按规定进行标注。	依托单位科研管理部门：															
	负责人（签章）： 年 月 日															
项目负责人（签章）： 年 月 日	依托单位财务管理部门：															
	负责人（签章）： 年 月 日															
我单位同意承担上述国家自然科学基金项目，将保证项目负责人及其研究队伍的稳定和研究项目实施所需的条件，严格遵守国家自然科学基金委员会有关资助项目管理、项目资金管理等各项规定，并督促实施。																
依托单位（公章） 年 月 日																
本栏目由基金委填写	科学处审查意见：															
	建议年度拨款计划（本栏目为自动生成，单位：万元）：															
	<table><tr><td>年度</td><td>总额</td><td>第一年</td><td>第二年</td><td>第三年</td><td>第四年</td><td>第五年</td></tr><tr><td>金额</td><td></td><td></td><td></td><td></td><td></td><td></td></tr></table>		年度	总额	第一年	第二年	第三年	第四年	第五年	金额						
	年度	总额	第一年	第二年	第三年	第四年	第五年									
金额																
负责人（签章）： 年 月 日																
科学部审查意见：	负责人（签章）： 年 月 日															
	相关局室审查意见：															
本栏目主要用于重大项目等	负责人（签章）： 年 月 日															
	委领导审批意见：															
委领导（签章）： 年 月 日																



国家自然科学基金资助项目批准通知

李发强 先生/女士：

根据《国家自然科学基金条例》和专家评审意见，国家自然科学基金委员会（以下简称自然科学基金委）决定批准资助您的申请项目。项目批准号：31970307，项目名称：拟南芥ABA受体通过选择性细胞自噬降解的分子机制研究，直接费用：58.00万元，项目起止年月：2020年01月至2023年12月，有关项目的评审意见及修改意见附后。

请尽早登录科学基金网络信息系统（<https://isisn.nsfc.gov.cn>），获取《国家自然科学基金资助项目计划书》（以下简称计划书）并按要求填写。对于有修改意见的项目，请按修改意见及时调整计划书相关内容；如对修改意见有异议，须在电子版计划书报送截止日期前向相关科学处提出。

电子版计划书通过科学基金网络信息系统（<https://isisn.nsfc.gov.cn>）上传，依托单位审核后提交至自然科学基金委进行审核。审核未通过者，返回修改后再行提交；审核通过者，打印纸质版计划书（一式两份，双面打印），依托单位审核并加盖单位公章后报送至自然科学基金委项目材料接收工作组。电子版和纸质版计划书内容应当保证一致。向自然科学基金委提交和报送计划书截止时间节点如下：

- 1、提交电子版计划书截止时间为**2019年9月11日16点**（视为计划书正式提交时间）；
- 2、提交电子修改版计划书截止时间为**2019年9月18日16点**；
- 3、报送纸质版计划书截止时间为**2019年9月26日16点**。

请按照以上规定及时提交电子版计划书，并报送纸质版计划书，未说明理由且逾期不报计划书者，视为自动放弃接受资助。

附件：项目评审意见及修改意见表

国家自然科学基金委员会
2019年8月16日



项目批准号	31970307
申请代码	C020407
归口管理部门	
依托单位代码	51064208A0499-0932



国家自然科学基金委员会 资助项目计划书

资助类别：面上项目

亚类说明：

附注说明：

项目名称：拟南芥ABA受体通过选择性细胞自噬降解的分子机制研究

直接费用：58万元 执行年限：2020.01-2023.12

负责人：李发强

通讯地址：广东省广州市天河区五山路483号华南农业大学生命科学学院

邮政编码：510642 电 话：13229912617

电子邮件：fqli@scau.edu.cn

依托单位：华南农业大学

联系人：倪慧群 电 话：020-85280070

填表日期：2019年08月20日

国家自然科学基金委员会制



国家自然科学基金委员会资助项目计划书填报说明

- 一、项目负责人收到《关于国家自然科学基金资助项目批准及有关事项的通知》（以下简称《批准通知》）后，请认真阅读本填报说明，参照国家自然科学基金相关项目管理办法及《国家自然科学基金资助项目资金管理办法》（请查阅国家自然科学基金委员会官方网站首页“政策法规”栏目），按《批准通知》的要求认真填写和提交《国家自然科学基金委员会资助项目计划书》（以下简称《计划书》）。
- 二、填写《计划书》时要求科学严谨、实事求是、表述清晰、准确。《计划书》经国家自然科学基金委员会相关项目管理部门审核批准后，将作为项目研究计划执行和检查、验收的依据。
- 三、《计划书》各部分填写要求如下：
 - （一）简表：由系统自动生成。
 - （二）摘要及关键词：各类获资助项目都必须填写中、英文摘要及关键词。
 - （三）项目组主要成员：计划书中列出姓名的项目组主要成员由系统自动生成，与申请书原成员保持一致，不可随意调整。如果批准通知中“项目评审意见及修改意见表”中“对研究方案的修改意见”栏目有调整项目组成员相关要求的，待项目开始执行后，按照项目成员变更程序另行办理。
 - （四）资金预算表：根据批准资助的直接费用，按照《国家自然科学基金项目预算表编制说明》填报资金预算表和预算说明书。国家重大科研仪器研制项目、重大项目还应按照预算评审后批复的直接费用各科目金额填报资金预算表、预算说明书及相应的预算明细表。
 - （五）正文：
 1. 面上项目、青年科学基金项目、地区科学基金项目：如果《批准通知》中没有修改要求的，只需选择“研究内容和研究目标按照申请书执行”即可；如果《批准通知》中“项目评审意见及修改意见表”中“对研究方案的修改意见”栏目明确要求调整研究期限和研究内容等的，须选择“根据研究方案修改意见更改”并填报相关修改内容。
 2. 重点项目、重点国际（地区）合作研究项目、重大项目、国家重大科研仪器研制项目：须选择“根据研究方案修改意见更改”，根据《批准通知》的要求填写研究（研制）内容，不得自行降低、更改研究目标（或仪器研制的技术性能与主要技术指标以及验收技术指标）或缩减研究（研制）内容。此外，还要突出以下几点：
 - （1）研究的难点和在实施过程中可能遇到的问题（或仪器研制风险），拟采用的研究（研制）方案和技术路线；
 - （2）项目主要参与者分工，合作研究单位之间的关系与分工，重大项目还需说明课题之间的关联；
 - （3）详细的年度研究（研制）计划。



3. 国家杰出青年科学基金、优秀青年科学基金和海外及港澳学者合作研究基金项目：须选择“根据研究方案修改意见更改”，按下列提纲撰写：
 - (1) 研究方向；
 - (2) 结合国内外研究现状，说明研究工作的学术思想和科学意义（限两个页面）；
 - (3) 研究内容、研究方案及预期目标（限两个页面）；
 - (4) 年度研究计划；
 - (5) 研究队伍的组成情况。
4. 国家自然科学基金基础科学中心项目：须选择“根据研究方案修改意见更改”，应当根据评审委员会和现场考察专家组的意见和建议，进一步完善并细化研究计划，作为评估和验收的依据。按下列提纲撰写：
 - (1) 五年拟开展的研究工作（包括主要研究方向、关键科学问题与研究内容）；
 - (2) 研究方案（包括骨干成员之间的分工及合作方式、学科交叉融合研究计划等）；
 - (3) 年度研究计划；
 - (4) 五年预期目标和可能取得的重大突破等；
 - (5) 研究队伍的组成情况。
5. 对于其他类型项目，参照面上项目的方式进行选择和填写。



简表

申请者信息	姓 名	李发强	性 别	男	出生年月	1975年11月	民 族	汉族
	学 位	博士			职称	教授		
	是否在站博士后	否			电子邮件	fqli@scau.edu.cn		
	电 话	13229912617			个人网页			
	工 作 单 位	华南农业大学						
	所 在 院 系 所	生命科学学院						
依托单位信息	名 称	华南农业大学					代码	51064208A0499
	联 系 人	倪慧群			电子邮件	kyc.jhk@scau.edu.cn		
	电 话	020-85280070			网站地址	http://kjc.scau.edu.cn/		
合作单位信息	单 位 名 称							
项目基本信息	项 目 名 称	拟南芥ABA受体通过选择性细胞自噬降解的分子机制研究						
	资 助 类 别	面上项目				亚 类 说 明		
	附 注 说 明							
	申 请 代 码	C020407:植物激素与生长调节物质				C0709:细胞自噬		
	基 地 类 别							
	执 行 年 限	2020.01-2023.12						
	直 接 费 用	58万元						



项目摘要

中文摘要:

脱落酸ABA是植物调控生长发育和响应逆境胁迫的一种关键激素。作为该信号通路中重要的一环,ABA受体的稳定性受到泛素蛋白酶体系统和内吞作用的调控。但能否被细胞内的主要降解途径—细胞自噬调控仍有待研究。我们前期研究发现细胞自噬对ABA受体具有上位效应并介导了它们的自噬降解;进而还发现ABA受体PYL4/7可通过一个ATG8互作蛋白AIP1的介导被液泡降解。由此,我们首次提出ABA受体降解的新机制:通过AIP1的介导,细胞可以选择性自噬降解部分的ABA受体,从而调控该信号通路。本项目拟以生化、细胞生物学和遗传学的方法解析AIP1的功能,从而阐述ABA受体选择性自噬降解的分子机制,还将探讨细胞自噬与内吞作用在调控ABA受体稳定性方面的相互关系。本研究不仅可为深入理解ABA信号通路的调控作出贡献,还能为更好地理解植物自噬对逆境胁迫的响应机制,为植物抗逆性研究及作物改良提供理论参照。

Abstract:

The phytohormone abscisic acid (ABA) plays vital roles in plant development and in abiotic stress responses. Many studies have shown that ABA signaling is regulated at the transcriptional, translational and post-translational levels. Recently, increasing evidence demonstrates that ABA receptors are degraded not only through the ubiquitin-26S proteasome system but also through the endocytic trafficking pathway. However, it is still largely unknown whether ABA receptor can be turnover by autophagy, one of the major intracellular catabolic pathways. Our prior work has found that Arabidopsis autophagy mutants are sensitive to ABA during germination and have better tolerance to drought stress. Further genetic relationship analysis revealed that the key autophagy components, ATG5 and ATG7, likely act epistatically upstream of ABA receptors. In addition, we found that these receptors could be selectively degraded by autophagy mediated by an ATG8 interacting Protein 1 (AIP1). The objectives of this proposal are to (i) further define the impact of autophagy deficiency on ABA signaling transduction; (ii) reveal the mechanism of selective autophagic degradation of ABA receptors mediated by AIP1 protein; (iii) study the relationship between autophagy and endocytosis on the vacuolar degradation of ABA receptors. Collectively, these experiments will help us better understand the role of autophagy in abiotic stress responses in plant, and may also provide us clues for improving crop abiotic stress resistance.

关键词(用分号分开): 受体; 蛋白质降解; 细胞自噬; 内膜运输途径; 逆境胁迫

Keywords(用分号分开): ABA receptor; protein degradation; autophagy; endocytosis; abiotic stress



项目组主要成员

编号	姓名	出生年月	性别	职称	学位	单位名称	电话	证件号码	项目分工	每年工 作时间 (月)				
1	李发强	1975. 11	男	教授	博士	华南农业大学	13229912617	441402197511121015	项目负责人	10				
2	黄晓	1976. 03	女	副教授	博士	华南农业大学	13660639294	440520197603041423	细胞自噬与内吞在调控ABA受体降解方面的相互关系的研究	8				
3	罗娜	1982. 04	女	助理研究员	博士	华南农业大学	13450362148	230121198204112028	自噬缺失对ABA受体亚细胞定位和稳定性的影响	10				
4	胡伟明	1985. 11	男	博士后	博士	华南农业大学	020-85280185	410221198511140810	研究选择性自噬降解ABA受体的分子机理	10				
5	王丽平	1988. 09	女	博士生	硕士	华南农业大学	020-85280185	420821198809250726	协助研究细胞自噬与内吞在调控ABA受体降解方面的相互关系	10				
6	汤芷薇	1997. 04	女	硕士生	学士	华南农业大学	020-85280185	429004199704030367	检测自噬突变体的ABA相关表型	10				
7	刘蓉	1994. 03	女	硕士生	学士	华南农业大学	020-85280185	522101199403174643	ABA受体亚细胞定位和稳定性的影响	10				
总人数			高级		中级		初级		博士后		博士生		硕士生	
7			2		1		0		1		1		2	



国家自然科学基金项目直接费用预算表（定额补助）

项目批准号：31970307

项目负责人：李发强

金额单位：万元

序号	科目名称	金额
1	项目直接费用合计	58.0000
2	1、设备费	0.0000
3	(1)设备购置费	0.00
4	(2)设备试制费	0.00
5	(3)设备升级改造与租赁费	0.00
6	2、材料费	30.0000
7	3、测试化验加工费	12.0000
8	4、燃料动力费	0.0000
9	5、差旅/会议/国际合作与交流费	3.4000
10	6、出版/文献/信息传播/知识产权事务费	2.0000
11	7、劳务费	9.60
12	8、专家咨询费	1.00
13	9、其他支出	0.00



预算说明书（定额补助）

（请按照《国家自然科学基金项目预算表编制说明》等的有关要求，对各项支出的主要用途和测算理由，以及合作研究外拨资金、单价≥10万元的设备费等内容进行必要说明。）

1. 设备费（0万元）：

无需购买设备。

2. 材料费（30万元）：

- 植物材料种植（0.5万/年，4年共2万元）：泥土，花盆，植物束缚带，长竹签，驱虫剂，黏虫纸等。
- 实验室所需常规耗材（3万/年，4年共12万元）：包括枪头，离心管，EP管，三角瓶，锡箔纸，尼龙膜，封口膜，培养皿，载玻片，盖玻片，试剂瓶，手套，细胞培养皿和移液管等的购置费用。
- 药品试剂（4万/年，4年共16万元）：包括细胞，细菌和植物培养基，分子克隆所需各种酶和试剂盒（Taq酶，高保真扩增酶，各种限制性内切酶，连接酶，小量质粒抽提试剂盒，大量质粒抽提试剂盒，PCR片断回收试剂盒，胶回收试剂盒，RNA抽提试剂盒和T载体连接试剂盒等），常用细胞药物处理试剂（Wortmannin, BFA 和 ConA），聚丙烯酰胺凝胶，复合蛋白酶抑制剂，一般荧光染料（FM4-64 和 LysoTracker），Western blot 二抗，核酸和蛋白质Marker，常规生化试剂和药品（例如：蔗糖，琼脂，琼脂糖，苯酚，氯仿，乙醇，丙酮，硫酸，盐酸，Tris，氯化钠，氯化钾，氯化钙，氢氧化钠，硼酸盐，Triton X-100，Tween20和牛血清白蛋白等）的购置费用。

3. 测试化验加工费（12万元）：

- （1）测试分析（5万元）：用于DNA测序，肽链的合成，引物的合成等费用。
- （2）仪器使用（7万元）：用于大型仪器的使用费。
 - 激光共聚焦扫描电子显微镜（收费：300元/小时，1.5万元/年，4年共计6万元），
 - 超速离心机（收费：100元/小时，0.25万元/年，4年共计1万元）。

4. 燃料动力费（0万元）：无。**5. 差旅 / 会议 / 国际合作与交流费（3.4万元）：**

- （1）课题组成员参加国际或国内会议的注册、交通和住宿费用（2.8万元）。
 - 拟参加国内会议2次，2人/次：植物生理学大会1次，细胞生物学大会1次。
 - 拟参加国际会议1次，1人/次：美国植物生理年会（ASPB）1次。
- （2）合作与交流费用（0.6万元）：
 - 邀请中科院遗传所谢旗教授来申请人实验室进行有关ABA受体降解研究的短期学术访问所需费用，包括交通和住宿等费用。0.6万元/人/次。

6. 出版/文献/信息传播/知识产权事务费（2万元）：

用于在国际/国内期刊上发表论文的版面费（2篇），文献期刊的查阅和资料查新等费用。

7. 劳务费（9.6万元）：

用于支付课题组参加项目硕士(2人)和博士(1人)研究生的劳务费。硕士研究生600元/月/人，博士研究生1200元/月/人，4年40个月，合计9.6万元。

8. 专家咨询费（1万元）：

邀请同行专家进行评议，咨询费用。

9. 其它支出（0万元）：无。

项目负责人签字：

科研部门公章：

财务部门公章：



报告正文

研究内容和研究目标按照申请书执行。



国家自然科学基金资助项目签批审核表

<p>我接受国家自然科学基金的资助，将按照申请书、项目批准意见和计划书负责实施本项目（批准号：31970307），严格遵守国家自然科学基金委员会关于资助项目管理、财务等各项规定，切实保证研究工作时间，认真开展研究工作，按时报送有关材料，及时报告重大情况变动，对资助项目发表的论著和取得的研究成果按规定进行标注。</p> <p>项目负责人（签章）： 年 月 日</p>		<p>我单位同意承担上述国家自然科学基金项目，将保证项目负责人及其研究队伍的稳定和研究项目实施所需的条件，严格遵守国家自然科学基金委员会有关资助项目管理、财务等各项规定，并督促实施。</p> <p>依托单位（公章） 年 月 日</p>					
本栏目由基金委填写	<p>科学处审查意见：</p>						
	<p>建议年度拨款计划（本栏目为自动生成，单位：万元）：</p>						
	年度	总额	第一年	第二年	第三年	第四年	第五年
	金额						
	<p>科学部审查意见：</p> <p>负责人（签章）： 年 月 日</p>						
	<p>相关局室审核意见：</p> <p>负责人（签章）： 年 月 日</p>						
本栏目主要用于重大项目等	<p>委领导审批意见：</p> <p>委领导（签章）： 年 月 日</p>						

关于国家自然科学基金资助项目批准及有关事项的通知

刘慧丽 先生/女士：

根据《国家自然科学基金条例》的规定和专家评审意见，国家自然科学基金委员会（以下简称自然科学基金委）决定批准资助您的申请项目。项目批准号：

31870177，项目名称：RopGEF调控拟南芥花序模式的分子机理，直接费用：60.00万元，项目起止年月：2019年01月至2022年12月，有关项目的评审意见及修改意见附后。

请尽早登录科学基金网络信息系统（<https://isisn.nsf.gov.cn>），获取《国家自然科学基金资助项目计划书》（以下简称计划书）并按要求填写。对于有修改意见的项目，请按修改意见及时调整计划书相关内容；如对修改意见有异议，须在计划书电子版报送截止日期前提出。

计划书电子版通过科学基金网络信息系统（<https://isisn.nsf.gov.cn>）上传，由依托单位审核后提交至自然科学基金委进行审核。审核未通过者，返回修改后再行提交；审核通过者，打印为计划书纸质版（一式两份，双面打印），由依托单位审核并加盖单位公章后报送至自然科学基金委项目材料接收工作组。计划书电子版和纸质版内容应当保证一致。向自然科学基金委提交和报送计划书截止时间节点如下：

- 1、提交计划书电子版截止时间为**2018年9月11日16点**（视为计划书正式提交时间）；
- 2、提交计划书电子修改版截止时间为**2018年9月18日16点**；
- 3、报送计划书纸质版截止时间为**2018年9月26日16点**。

请按照以上规定及时提交计划书电子版，并报送计划书纸质版，未说明理由且逾期不报计划书者，视为自动放弃接受资助。

附件：项目评审意见及修改意见表

国家自然科学基金委员会
生命科学部
2018年8月16日

附件：项目评审意见及修改意见表

项目批准号	31870177	项目负责人	刘慧丽	申请代码1	C020102
项目名称	RopGEF调控拟南芥花序模式的分子机理				
资助类别	面上项目	亚类说明			
附注说明					
依托单位	华南农业大学				
直接费用	60.00 万元	起止年月	2019年01月 至 2022年12月		
<p>通讯评审意见：</p> <p><1> 植物茎尖分生组织不断产生新的器官，而且排列有序，这些有序分布的特点由细胞分裂素和生长素共同进行调节。拟南芥组氨酸磷酸转移酶6（AHP6）是细胞分裂素信号途径的负调控因子、又受生长素的调控，而且在器官的发生过程中呈现动态分布。申请书发现RopGTPase的激活因子RopGEF可以与AHP6发生互作，而且RopGEF的突变体也表现出叶序紊乱表型。据此，申请者将从RopGEF与AHP6互作入手，分析小G蛋白在如何协调生长素和细胞分裂素信号，进而调节叶序的发生。研究结果将为理解叶序形成的分子机理增添新的知识。项目书的研究内容充实、技术手段成熟，加上申请者曾完成青年项目“OsRopGEF在根系发育中的功能研究”，对本小G蛋白领域比较熟悉。</p> <p><2> 植物花序模式的调控涉及很多复杂的作用机制，其中生长素及其极性运输是叶序模式的决定因素，但是生长素和细胞分裂素如何协同作用参与叶序模式建立的分子机制仍不清楚。申请人前期的工作发现拟南芥小G蛋白ROP/RAC激活因子RoPGEF突变体花序排列紊乱，并且与AHP6互作。拟通过基因表达模式分析、突变体表型观察、蛋白质相互作用、激素信号途径分析等手段，以期揭示RoPGEF在调控叶序发育过程的分子机制。该研究具有重要科学意义，研究发现了RoPGEF与AHP6具有相互作用，具有一定的创新性，申请人有较好的前期工作积累，有合理的人员组成和项目开展相关的技术平台，为该项目的顺利进行提供了保证。</p> <p><3> RopGEF是重要的信号通路蛋白，在植物中参与很多方面的发育过程。这项研究利用模式植物拟南芥研究RopGEF参与花序形态建立。申请书的整体设计较为合理，实验设计也比较全面。前期研究基础稍显薄弱，申请人在发育表型上已经看到了花序的异常，也发现RopGEF能够与AHP6有蛋白互作，如果能够进一步提供一些RopGEF参与花序建立的机制性实验（比如RopGEF与AHP6的遗传关系，RopGEF的上下游信号通路等），会使得申请书的可行性增强，也会提高科学问题的意义（比如是什么信号在控制花序的发生）。</p> <p>修改意见：</p> <p style="text-align: right;">生命科学部</p> <p style="text-align: right;">2018年8月16日</p>					



项目批准号	31870177
申请代码	C020102
归口管理部门	
依托单位代码	51064208A0499-0932



3 1870177 1006412

国家自然科学基金委员会 资助项目计划书

资助类别：面上项目

亚类说明：

附注说明：

项目名称：RopGEF调控拟南芥花序模式的分子机理

直接费用：60万元 执行年限：2019.01-2022.12

负责人：刘慧丽

通讯地址：广州市五山路 华南农业大学 生命科学学院 南楼513

邮政编码：510642 电 话：020-38297790

电子邮件：liuhuili@scau.edu.cn

依托单位：华南农业大学

联系人：唐家林 电 话：020-85280070

填表日期：2018年08月22日

国家自然科学基金委员会制



国家自然科学基金委员会资助项目计划书填报说明

- 一、项目负责人收到《关于国家自然科学基金资助项目批准及有关事项的通知》（以下简称《批准通知》）后，请认真阅读本填报说明，参照国家自然科学基金相关项目管理办法及《国家自然科学基金资助项目资金管理办法》（请查阅国家自然科学基金委员会官方网站首页“政策法规”栏目），按《批准通知》的要求认真填写和提交《国家自然科学基金委员会资助项目计划书》（以下简称《计划书》）。
- 二、填写《计划书》时要求科学严谨、实事求是、表述清晰、准确。《计划书》经国家自然科学基金委员会相关项目管理部门审核批准后，将作为项目研究计划执行和检查、验收的依据。
- 三、《计划书》各部分填写要求如下：
 - （一）简表：由系统自动生成。
 - （二）摘要及关键词：各类获资助项目都必须填写中、英文摘要及关键词。
 - （三）项目组主要成员：计划书中列出姓名的项目组主要成员由系统自动生成，与申请书原成员保持一致，不可随意调整。如果批准通知中“项目评审意见及修改意见表”中“对研究方案的修改意见”栏目有调整项目组成员相关要求的，待项目开始执行后，按照项目成员变更程序另行办理。
 - （四）资金预算表：根据批准资助的直接费用，按照《国家自然科学基金项目预算表编制说明》填报资金预算表和预算说明书。国家重大科研仪器研制项目、重大项目还应按照预算评审后批复的直接费用各科目金额填报资金预算表、预算说明书及相应的预算明细表。
 - （五）正文：
 1. 面上项目、青年科学基金项目、地区科学基金项目：如果《批准通知》中没有修改要求的，只需选择“研究内容和研究目标按照申请书执行”即可；如果《批准通知》中“项目评审意见及修改意见表”中“对研究方案的修改意见”栏目明确要求调整研究期限和研究内容等的，须选择“根据研究方案修改意见更改”并填报相关修改内容。
 2. 重点项目、重点国际（地区）合作研究项目、重大项目、国家重大科研仪器研制项目：须选择“根据研究方案修改意见更改”，根据《批准通知》的要求填写研究（研制）内容，不得自行降低、更改研究目标（或仪器研制的技术性能与主要技术指标以及验收技术指标）或缩减研究（研制）内容。此外，还要突出以下几点：
 - （1）研究的难点和在实施过程中可能遇到的问题（或仪器研制风险），拟采用的研究（研制）方案和技术路线；
 - （2）项目主要参与者分工，合作研究单位之间的关系与分工，重大项目还需说明课题之间的关联；
 - （3）详细的年度研究（研制）计划。



3. 国家杰出青年科学基金、优秀青年科学基金和海外及港澳学者合作研究基金项目：须选择“根据研究方案修改意见更改”，按下列提纲撰写：
 - (1) 研究方向；
 - (2) 结合国内外研究现状，说明研究工作的学术思想和科学意义（限两个页面）；
 - (3) 研究内容、研究方案及预期目标（限两个页面）；
 - (4) 年度研究计划；
 - (5) 研究队伍的组成情况。
4. 国家自然科学基金基础科学中心项目：须选择“根据研究方案修改意见更改”，应当根据评审委员会和现场考察专家组的意见和建议，进一步完善并细化研究计划，作为评估和验收的依据。按下列提纲撰写：
 - (1) 五年拟开展的研究工作（包括主要研究方向、关键科学问题与研究内容）；
 - (2) 研究方案（包括骨干成员之间的分工及合作方式、学科交叉融合研究计划等）；
 - (3) 年度研究计划；
 - (4) 五年预期目标和可能取得的重大突破等；
 - (5) 研究队伍的组成情况。
5. 对于其他类型项目，参照面上项目的方式进行选择和填写。



简表

申请者信息	姓 名	刘慧丽	性 别	女	出生年月	1975年10月	民 族	汉族
	学 位	博士			职称	助理研究员		
	是否在站博士后	否			电子邮件	liuhuili@scau.edu.cn		
	电 话	020-38297790			个人网页			
	工 作 单 位	华南农业大学						
	所 在 院 系 所	生命科学学院						
依托单位信息	名 称	华南农业大学					代码	51064208A0499
	联 系 人	唐家林			电子邮件	kyc.jhk@scau.edu.cn		
	电 话	020-85280070			网站地址	http://web.scau.edu.cn/kjc/		
合作单位信息	单 位 名 称							
项目基本信息	项 目 名 称	RopGEF调控拟南芥花序模式的分子机理						
	资 助 类 别	面上项目				亚 类 说 明		
	附 注 说 明							
	申 请 代 码	C020102:植物形态发生				C020408:植物的生长发育		
	基 地 类 别							
	执 行 年 限	2019.01-2022.12						
	直 接 费 用	60万元						



项目摘要

中文摘要:

植物侧生器官如叶和花有规律地排列在茎的周围, 形成特定的叶序 (phyllotaxis)。目前的研究认为生长素及其极性运输是叶序模式的决定因素, 同时细胞分裂素与生长素协同作用, 促进器官的形成和叶序模式的稳定。而生长素和细胞分裂素如何协同作用, 共同调控叶序的分子机理仍待完善。拟南芥组氨酸磷酸转移酶蛋白AHP6是新发现的整合两条信号通路的重要调控因子, 在生长素下游协调和平衡生长素和细胞分裂素的活性, 影响花序模式的建立。我们的前期工作发现拟南芥小G蛋白ROP/RAC的激活因子, RopGEF突变体花序排列紊乱, RopGEF成员能与AHP6互作, 表明RopGEF可能是AHP6调控花序模式信号途径的新组分。本项目拟在前期工作基础上, 明确RopGEF与AHP6的互作关系, 解析RopGEF在调控花序发育中的功能, 阐明RopGEF在生长素与细胞分裂协同作用下调控花序模式的分子机理。

Abstract:

Lateral organs such as leaves and flowers are arranged regularly around the stem of a plant, a phenomenon known as phyllotaxis. Plants have evolved sophisticated phyllotaxis to rapidly maximize light capture. Diversity of phyllotaxis in flower is apparently related to different reproductive strategies. Recent tremendous advances have identified the plant hormone auxin as the major regulator of phyllotaxis, and the plant hormone cytokinin, along with auxin, synergistically promote the earliest steps of organ initiation and robustness of the phyllotactic pattern. But the mechanism of the synergistic effect of auxin and CK during the formation of phyllotactic pattern remains elusive. ARABIDOPSIS HISTIDINE PHOSPHOTRANSFER PROTEIN 6 (AHP6), which is identified as important regulator of phyllotactic patterns, acts directly downstream of auxin by integrating and balancing the activity of the cytokinin and auxin. Our previous work found Arabidopsis mutant of the small GTPase RAC/ROP activator, RopGEF, caused aberrant phyllotaxis and members of RopGEF interacted with AHP6 in Yeast 2-Hybrid analysis, indicating that RopGEF might function as signaling component of AHP6 regulated phyllotaxis formation. This project will investigate the function of RopGEF in establishing phyllotactic pattern, identify the interaction between RopGEF member and AHP6, elucidate the role of RopGEF in the synergistical effect of auxin and cytokinin regulated phyllotactic pattern.

关键词(用分号分开): 分生组织; 信号转导; 叶序; Rop鸟苷酸交换因子

Keywords(用分号分开): meristem; signal transduction; phyllotaxis; RopGEF



项目组主要成员

编号	姓名	出生年月	性别	职称	学位	单位名称	电话	证件号码	项目分工	每年工作时间(月)
1	刘慧丽	1975. 10	女	助理研究员	博士	华南农业大学	020-38297790	370881197510101141	项目负责人	11
2	刘娥娥	1970. 03	女	副研究员	博士	华南农业大学	02085280194	612301197003220640	原核蛋白表达及蛋白互作分析	6
3	罗娜	1982. 04	女	助理研究员	博士	华南农业大学	13450362148	230121198204112028	基因遗传互作分析	6
4	刘太波	1982. 08	男	讲师	博士	华南农业大学	18719139057	430529198208122370	载体构建及基因表达模式分析	6
5	董庆坤	1989. 12	男	博士生	学士	华南农业大学	18819493317	370783198912040910	突变体表型分析, 报告基因共聚焦显微镜观察分析	11
6	张韬	1992. 10	男	硕士生	学士	华南农业大学	38297790	421002199210261835	酵母双杂及BIFC互作分析	10
7	张志伟	1994. 03	男	硕士生	学士	华南农业大学	38297790	440183199403100014	突变体杂交鉴定	10
8	黄劲荣	1994. 11	男	硕士生	学士	华南农业大学	02038297790	450121199411242437	突变体表型分析, 扫描电镜观察	10
总人数		高级		中级		初级		博士后	博士生	硕士生
8		1		3					1	3



国家自然科学基金项目直接费用预算表（定额补助）

项目批准号：31870177

项目负责人：刘慧丽

金额单位：万元

序号	科目名称	金额
1	项目直接费用合计	60.0000
2	1、设备费	0.0000
3	(1)设备购置费	0.00
4	(2)设备试制费	0.00
5	(3)设备升级改造与租赁费	0.00
6	2、材料费	24.0000
7	3、测试化验加工费	16.0000
8	4、燃料动力费	0.00
9	5、差旅/会议/国际合作与交流费	5.00
10	6、出版/文献/信息传播/知识产权事务费	2.00
11	7、劳务费	12.0000
12	8、专家咨询费	1.00
13	9、其他支出	0.00



预算说明书（定额补助）

（请按照《国家自然科学基金项目预算表编制说明》的有关要求，对各项支出的主要用途和测算理由，以及合作研究外拨资金、单价≥10万元的设备费等内容进行必要说明。）

本项目的直接申请费用60万元，未购置10万元以上设备，各项支出的用途及测算说明如下：

1. 项目中材料费预算24万元，主要是用于购买分子生物学的各种试剂盒，如原位杂交试剂盒、载体构建用的质粒提取试剂盒、核酸检测分析用的DNA和RNA提取试剂盒及各种qPCR试剂盒合计10万元。引物合成、常用耗材及培养拟南芥用的植物基质、常规分子生物学实验室枪头、离心管、Miracloth、植物激素、抗生素、Parafilm等每年2万，合计8万元。用于生化实验的各种生化试剂、蛋白电泳及抗体等合计6万元。三项合计24万元。
2. 项目的测试费预算16万元，主要用于两方面：激光共聚焦是本项目必需的分析检测仪器，按200元/小时，150小时/每年，每年约3万元，合计12万元；另外项目需完成基因克隆，各种载体和转基因植物鉴定等核酸检测测序的费用每年1万元，合计4万元，两项共计16万元。
3. 设置差旅/会议费5万元，用于参与项目的课题组人员参加国内会议交流和技术培训的注册费，交通费和住宿费，按每人0.5万元，预计10人次，合计5万元。
4. 出版信息费设置2万元，主要用于论文发表的版面费用，拟发表两篇学术论文，版面费和文献检索费合计约2万元。
5. 另设劳务费12万，主要用于参与项目的4名研究生的劳务费，硕士生3人，0.6万元/人年，博士生1人，1.2万元/人年，合计 $(1.8+1.2) \times 4 = 12$ 万元。
6. 设专家咨询费1万元，用于邀请国内外的专家2-3名讨论项目中的问题，技术咨询等费用。

项目负责人签字：刘慧丽

科研部门公章：

财务部门公章：



报告正文

研究内容和研究目标按照申请书执行。



国家自然科学基金资助项目签批审核表

	<p>我接受国家自然科学基金的资助，将按照申请书、项目批准意见和计划书负责实施本项目（批准号：31870177），严格遵守国家自然科学基金委员会关于资助项目管理、财务等各项规定，切实保证研究工作时间，认真开展研究工作，按时报送有关材料，及时报告重大情况变动，对资助项目发表的论著和取得的研究成果按规定进行标注。</p> <p>项目负责人（签章）： 年 月 日</p>	<p>我单位同意承担上述国家自然科学基金项目，将保证项目负责人及其研究队伍的稳定和研究项目实施所需的条件，严格遵守国家自然科学基金委员会有关资助项目管理、财务等各项规定，并督促实施。</p> <p>依托单位（公章） 年 月 日</p>					
本栏目由基金委填写	<p>科学处审查意见：</p>						
	<p>建议年度拨款计划（本栏目为自动生成，单位：万元）：</p>						
	年度	总额	第一年	第二年	第三年	第四年	第五年
	金额						
	<p>科学部审查意见：</p> <p>负责人（签章）： 年 月 日</p>						
本栏目主要用于重大项目等	<p>相关局室审核意见：</p> <p>负责人（签章）： 年 月 日</p>						
	<p>委领导审批意见：</p> <p>委领导（签章）： 年 月 日</p>						

关于国家自然科学基金资助项目批准及有关事项的通知

卢少云 先生/女士：

根据《国家自然科学基金条例》的规定和专家评审意见，国家自然科学基金委员会（以下简称自然科学基金委）决定批准资助您的申请项目。项目批准号：

31672185，项目名称：狗牙根CIPK5基因调控抗旱性的研究，直接费用：60.00万元，项目起止年月：2017年01月至2020年12月，有关项目的评审意见及修改意见附后。

请尽早登录科学基金网络信息系统（<https://isisn.nsfc.gov.cn>），获取《国家自然科学基金资助项目计划书》（以下简称计划书）并按要求填写。对于有修改意见的项目，请按修改意见及时调整计划书相关内容；如对修改意见有异议，须在计划书电子版报送截止日期前提出。**注意：请严格按照《国家自然科学基金资助项目资金管理办法》填写计划书的资金预算表，其中，劳务费、专家咨询费科目所列金额与申请书相比不得调增。**

计划书电子版通过科学基金网络信息系统（<https://isisn.nsfc.gov.cn>）上传，由依托单位审核后提交至自然科学基金委进行审核。审核未通过者，返回修改后再行提交；审核通过者，打印为计划书纸质版（一式两份，双面打印），由依托单位审核并加盖单位公章后报送至自然科学基金委项目材料接收工作组。计划书电子版和纸质版内容应当保证一致。

向自然科学基金委提交和报送计划书截止时间节点如下：

- 1、提交计划书电子版截止时间为**2016年9月11日16点**（视为计划书正式提交时间）；
- 2、提交计划书电子修改版截止时间为**2016年9月18日16点**；
- 3、报送计划书纸质版截止时间为**2016年9月26日16点**。

请按照以上规定及时提交计划书电子版，并报送计划书纸质版，未说明理由且逾期不报计划书者，视为自动放弃接受资助。

附件：项目评审意见及修改意见

国家自然科学基金委员会
生命科学部
2016年8月17日



项目批准号	31672185
申请代码	C150303
归口管理部门	
依托单位代码	51064208A0499-0932



31672185 1003394

国家自然科学基金委员会 资助项目计划书

资助类别: 面上项目

亚类说明:

附注说明: 常规面上项目

项目名称: 狗牙根CIPK5基因调控抗旱性的研究

直接费用: 60万元

执行年限: 2017.01-2020.12

负责人: 卢少云

通讯地址: 广州市天河区五山路483号

邮政编码: 510642

电话: 020-85282469

电子邮件: lurflab@scau.edu.cn

依托单位: 华南农业大学

联系人: 全锋

电话: 020-85280070

填表日期:

2016年08月23日

国家自然科学基金委员会制

Version: 1.003.394



国家自然科学基金委员会资助项目计划书填报说明

- 一、项目负责人收到《关于国家自然科学基金资助项目批准及有关事项的通知》（以下简称《批准通知》）后，请认真阅读本填报说明，参照国家自然科学基金相关项目管理办法及《国家自然科学基金资助项目资金管理办法》（请查阅国家自然科学基金委员会官方网站首页“政策法规”-“管理办法”栏目），按《批准通知》的要求认真填写和提交《国家自然科学基金委员会资助项目计划书》（以下简称《计划书》）。
- 二、填写《计划书》时要求科学严谨、实事求是、表述清晰、准确。《计划书》经国家自然科学基金委员会相关项目管理部门审核批准后，将作为项目研究计划执行和检查、验收的依据。
- 三、《计划书》各部分填写要求如下：
 - （一）简表：由系统自动生成。
 - （二）摘要及关键词：各类获资助项目都必须填写中、英文摘要及关键词。
 - （三）项目组主要成员：计划书中列出姓名的项目组主要成员由系统自动生成，与申请书原成员保持一致，不可随意调整。如果批准通知中“项目评审意见及修改意见表”中“对研究方案的修改意见”栏目有调整项目组成员相关要求的，待项目开始执行后，按照项目成员变更程序另行办理。
 - （四）资金预算表：按批准资助的直接费用填报资金预算表和预算说明书，其中的劳务费、专家咨询费金额不应高于申请书中相应金额。国家重大科研仪器研制项目、重大项目还应按照预算评审后批复的直接费用各科目金额填报资金预算表、预算说明书及相应的预算明细表。
 - （五）正文：
 1. 面上项目、青年科学基金项目、地区科学基金项目：如果《批准通知》中没有修改要求的，只需选择“研究内容和研究目标按照申请书执行”即可；如果《批准通知》中“项目评审意见及修改意见表”中“对研究方案的修改意见”栏目明确要求调整研究期限和研究内容等的，须选择“根据研究方案修改意见更改”并填报相关修改内容。
 2. 重点项目、重点国际（地区）合作研究项目、重大项目、国家重大科研仪器研制项目：须选择“根据研究方案修改意见更改”，根据《批准通知》的要求填写研究（研制）内容，不得自行降低、更改研究目标（或仪器研制的技术性能与主要技术指标以及验收技术指标）或缩减研究（研制）内容。此外，还要突出以下几点：
 - （1）研究的难点和在实施过程中可能遇到的问题（或仪器研制风险），拟采用的研究（研制）方案和技术路线；
 - （2）项目主要参与者分工，合作研究单位之间的关系与分工，重大项目还需说明课题之间的关联；
 - （3）详细的年度研究（研制）计划。





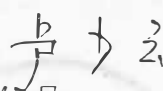
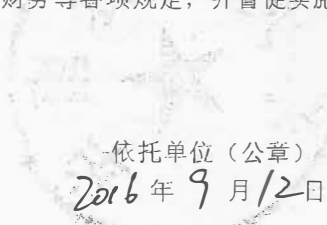
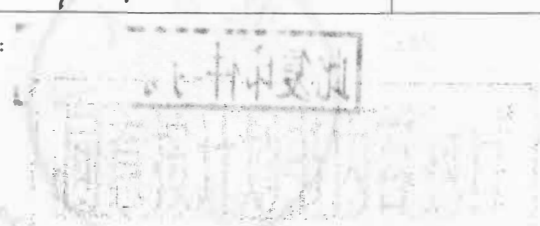

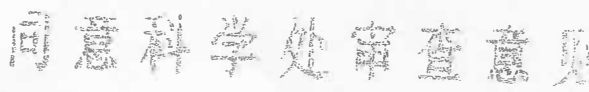

3. 国家杰出青年科学基金、优秀青年科学基金和海外及港澳学者合作研究基金项目：须选择“根据研究方案修改意见更改”，按下列提纲撰写：
 - (1) 研究方向；
 - (2) 结合国内外研究现状，说明研究工作的学术思想和科学意义（限两个页面）；
 - (3) 研究内容、研究方案及预期目标（限两个页面）；
 - (4) 年度研究计划；
 - (5) 研究队伍的组成情况。
4. 对于其他类型项目，参照面上项目的方式进行选择和填写。

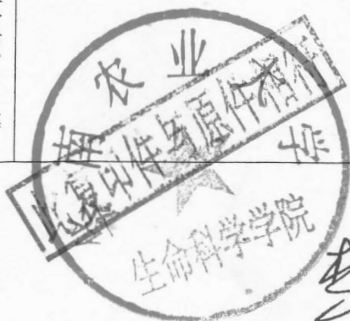


李书气



国家自然科学基金资助项目签批审核表

本栏目由基金委填写	<p>我接受国家自然科学基金的资助，将按照申请书、项目批准意见和计划书负责实施本项目（批准号：31672185），严格遵守国家自然科学基金委员会关于资助项目管理、财务等各项规定，切实保证研究工作时间，认真开展研究工作，按时报送有关材料，及时报告重大情况变动，对资助项目发表的论著和取得的研究成果按规定进行标注。</p> <p>项目负责人（签章）： 2016年9月12日</p>	<p>我单位同意承担上述国家自然科学基金项目，将保证项目负责人及其研究队伍的稳定和研究项目实施所需的条件，严格遵守国家自然科学基金委员会有关资助项目管理、财务等各项规定，并督促实施。</p> <p>依托单位（公章）  2016年9月12日</p>													
	<p>科学处审查意见： </p> <p>建议年度拨款计划（本栏目为自动生成，单位：万元）：</p> <table border="1"><thead><tr><th>年度</th><th>总额</th><th>第一年</th><th>第二年</th><th>第三年</th><th>第四年</th><th>第五年</th></tr></thead><tbody><tr><td>金额</td><td></td><td></td><td></td><td></td><td></td><td></td></tr></tbody></table> <p>负责人（签章）： 2016年10月26日</p>	年度	总额	第一年	第二年	第三年	第四年	第五年	金额						
	年度	总额	第一年	第二年	第三年	第四年	第五年								
	金额														
<p>科学部审查意见： </p> <p>负责人（签章）： 2016年10月28日</p>															
<p>相关局室审核意见：</p> <p>负责人（签章）： 年 月 日</p> <p>委领导审批意见：</p> <p>委领导（签章）： 年 月 日</p>															



李永光



简表

申请者信息	姓 名	卢少云	性 别	女	出生年月	1967年11月	民 族	壮族
	学 位	博士			职 称	教授		
	电 话	020-85282469			电子邮件	turflab@scau.edu.cn		
	传 真	020-85282180			个人网页			
	工 作 单 位	华南农业大学						
	所 在 院 系 所	生命科学学院						
依托单位信息	名 称	华南农业大学					代 码	51064208A099
	联 系 人	全锋			电子邮件	kycjkh@scau.edu.cn		
	电 话	020-85280070			网站地址	http://web.scau.edu.cn/kjc/		
合作单位信息	单 位 名 称							代 码
项目基本信息	项 目 名 称	狗牙根CIPK5基因调控抗旱性的研究						
	资 助 类 别	面上项目				亚 类 说 明		
	附 注 说 明	常规面上项目						
	申 请 代 码	C150303: 观赏园艺学						
	基 地 类 别	亚热带农业生物资源保护与利用国家重点实验室						
	执 行 年 限	2017.01-2020.12						
	直 接 费 用	60万元						





项目摘要

中文摘要(500字以内):

狗牙根是重要的暖季型草坪草, CBL-CIPK信号途径是 Ca^{2+} 信号系统的重要组成部分, 在植物胁迫应答中起重要作用。在已结题项目的后续研究中, 申请人克隆了受ABA、干旱和盐胁迫诱导的狗牙根CdCIPK5基因, 其编码蛋白与水稻OsCIPK5最相似。过量表达CdCIPK5提高水稻抗旱、耐盐性, 干涉表达则降低水稻抗旱、耐盐性。据此, 项目拟将CdCIPK5导入水稻oscipk5突变体, 明确其互补OsCIPK5的功能; 获得过量表达CdCIPK5和RNAi干涉CdCIPK5表达的转基因狗牙根, 阐明CdCIPK5对狗牙根抗旱性的调控作用; 对上调和下调CdCIPK5的转基因狗牙根及其野生型开展RNA-seq分析, 了解CdCIPK5调控下游基因网络和生理生化过程, 阐明CdCIPK5调控抗旱性机理; 筛选和鉴定与CdCIPK5结合的CBL及CdCIPK5的下游靶蛋白, 为揭示CBL-CIPK调控抗旱性分子机理奠定基础。

关键词: 草坪草; 非生物逆境; 水分胁迫; 基因功能; 基因调控

Abstract(limited to 4000 words):

Bermudagrass is a widely applied warm-season turfgrass species, while the calcineurin B-like protein-CBL-interacting protein kinase (CBL-CIPK) signaling pathway is a Ca^{2+} -related pathway that responds strongly to environmental stresses. In our previous investigations, a full length of CdCIPK5 cDNA which showed an induced expression in response to ABA treatment, drought and salinity was cloned. Sequence analysis indicated that CdCIPK5 had a high identity to OsCIPK5. Overexpression of CdCIPK5 resulted in elevated tolerance to drought and salinity, while down-regulation of CdCIPK5 expression led to reduced tolerance to drought and salinity in transgenic rice plants. Based on the observations, this project is planned to investigate the role of CdCIPK5 in regulation of drought tolerance in bermudagrass. To identify the function of CdCIPK5, expression vector of CdCIPK5 driven by OsCIPK5 promoter will be introduced into rice mutant oscipk5 to analyze the phenotypic recovery of the mutant. Transgenic bermudagrass plants overexpressing CdCIPK5 and RNAi plants down-regulating CdCIPK5 will be generated using the methods of Agrobacterium-mediated transformation for evaluation of drought tolerance so as to validate the essential role of CdCIPK5 in drought tolerance. To elucidate the mechanisms of CdCIPK5 in regulation of drought tolerance, transcriptome profiling and the relevant biochemical pathways and physiological processes will be also analyzed using the transgenic bermudagrass in comparison with the wild type. CBL members and downstream target proteins binding to CdCIPK5 will be screened and identified using two-yeast hybrid systems in combination with the methods of bimolecular fluorescence complementation (BiFC) and co-immunoprecipitation (Co-IP). The investigations will provide us with novel understanding of drought tolerance in bermudagrass associated with involvement CBL-CIPK signaling pathway as well as an effective candidate gene used for transgenic improvements of crops or turfgrass species on drought tolerance.

Keywords: turfgrass; abiotic stress; water stress; gene function; gene regulation

项目组主要成员

编号	姓名	出生年月	性别	职称	学位	单位名称	电话	证件号码	项目分工	每年工作时间(月)	
1	卢少云	1967.11	女	教授	博士	华南农业大学	020-85282469	440106196711301866	项目负责人	6	
2	罗娜	1982.04	女	助理研究员	博士	华南农业大学	020-85282469	230121198204112028	生理生化测定	4	
3	罗玉容	1979.12	女	实验师	硕士	华南农业大学	38635008	440783197912180423	生理生化测定	4	
4	黄石连	1989.11	男	博士生	硕士	华南农业大学	020-85282469	372926198911183931	转基因狗牙根分析、基因调控网络分析	10	
5	王琦	1983.04	男	博士生	硕士	华南农业大学	020-85282469	412727198304160533	载体构建、时空表达分析、互作蛋白筛选	10	
6	王晨	1992.07	女	博士生	学士	华南农业大学	020-85282469	341124199207020065	转基因水稻分析、基因调控网络分析	10	
7	彭麒文	1992.09	男	硕士生	学士	华南农业大学	020-85282469	522129199209300032	狗牙根、水稻基因转化	10	
8	高永勇	1993.01	男	硕士生	学士	华南农业大学	020-85282469	622701199301164010	生理生化测定、水稻基因转化	10	
总人数		高级		中级		初级		博士后		博士生	硕士生
8		1		2						3	2



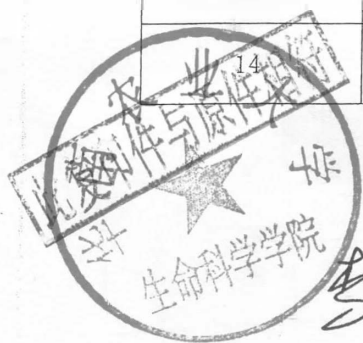
国家自然科学基金项目直接费用预算表（定额补助）

项目批准号：31672185

项目负责人：卢少云

金额单位：万元

序号	科目名称	金额
1	一、项目直接费用	60.0000
2	1、设备费	0.0000
3	(1)设备购置费	0.00
4	(2)设备试制费	0.0000
5	(3)设备改造与租赁费	0.0000
6	2、材料费	25.5000
7	3、测试化验加工费	4.5000
8	4、燃料动力费	0.00
9	5、差旅/会议/国际合作与交流费	12.5000
10	6、出版/文献/信息传播/知识产权事务费	1.5000
11	7、劳务费	15.0000
12	8、专家咨询费	1.00
13	9、其他支出	0.00
14	二、自筹资金	0.00



预算说明书（定额补助）

（请按《国家自然科学基金项目资金预算表编制说明》中的要求，对各项支出的主要用途和测算理由及合作研究外拨资金，单价 ≥ 10 万元的设备等内容进行详细说明，可根据需要另加附页。）

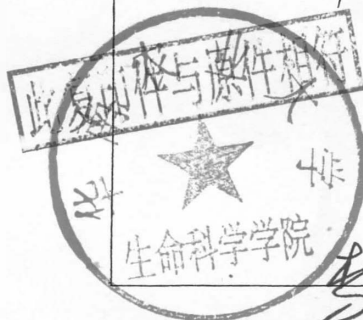
- 1、设备费 0 万元。
- 2、材料费 共计 25.5 万元。购买基因克隆、表达分析、载体构建等所需试剂 5.0 万元、转基因植物及其分子检测所需试剂 5 万元、酵母双杂交试剂盒及免疫共沉淀实验所需生化试剂、试剂盒及抗体等 5 万元、生理生化检测所需试剂及各种常用分析纯化学试剂等 5 万元、实验常用的玻璃器皿及分子操作实验常用的一次性用品等实验室耗材共计 4 万元，以及植物材料培养所需基质、培养盆和肥料等其他实验材料费用 1.5 万元。
- 3、测试化验加工费 共计 4.5 万元。表达谱分析费用约为 4 万元，DNA 测序及引物合成费用约 0.5 万元，委托生物技术公司完成。
- 4、燃料动力费 0 万元。
- 5、差旅/会议/国际合作与交流费 共计 12.5 万元，用于课题组成员及研究生参加国内学术会议、在国内及省内调研等活动的旅差费及 2—3 人次参加国际学术会议。其中，差旅费预算 6 万元，国际合作与交流费预算 6.5 万元。
- 6、出版/文献/信息传播/知识产权事务费 共计 1.5 万元，用于发表论文或科技文献检索、查新等及资料打印、复印、专利申请费等。
- 7、劳务费 共计 15 万元，用于参加项目研究的博士和硕士研究生生活补助。
- 9、专家咨询费 共计 1.0 万元，用于国内外专家 5 人次讲学或指导的咨询费。

项目负责人签字：

李力云

科研部门公章：

财务部门公章：



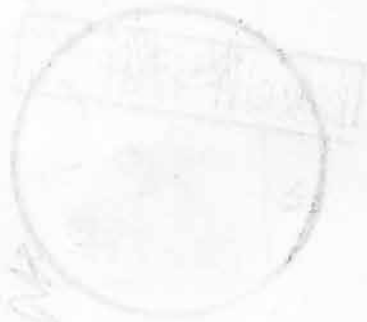
李力云

报告正文

研究内容和研究目标按照申请书执行。



李永光



受理编号: c18140500000820

项目编号: 2018A030313931

文件编号: 粤科规财字(2018)207号



(广东科技微信公众号)



(受理纸质材料二维码)

广东省自然科学基金项目 合同书

项目名称: 拟南芥RopGEF调控叶序模式的功能研究

项目类别: 广东省自然科学基金-自由申请

项目起止时间: 2018-05-01 至 2021-04-30

管理单位(甲方): 广东省自然科学基金管理委员会

依托单位(乙方): 华南农业大学

通讯地址: 广东省广州市天河区五山路483号

邮政编码: 510642

单位电话: 020-38632819

项目负责人: 刘慧丽

联系电话: 020-38297790

广东省科学技术厅
二〇一四年制

一、主要研究内容和要达到的目标

研究目标:

1. 明确RopGEF成员在茎尖分生组织和原基发育过程中的表达模式和蛋白定位。
2. 明确RopGEF成员调控叶序发育的细胞和发育生物学基础。
3. 明确RopGEF与已知调控叶序发育的重要组分AHP6的互作关系。
4. 明确茎尖分生组织中RopGEF成员参与的激素信号途径。

研究内容

1. 根据已有的花序分生组织中基因表达谱的数据,我们发现有两个RopGEF成员特异在茎尖分生组织中表达,进一步我们将采用mRNA原位杂交的方法,详细分析这两个基因在花序分生组织和原基起始发育过程中的表达模式,同时构建pRopGEF-RopGEF-GFP 融合蛋白的转基因植株,利用共聚焦显微镜分析RopGEF成员在花序分生组织和原基起始发育过程中的表达模式。
2. 详细分析RopGEF突变体植株的表型,有的成员没有T-DNA插入突变体,我们采用CRISP/Cas9技术构建敲除突变体。由于RopGEF成员间存在功能冗余,现有的T-DNA插入突变体表型不显著,我们用遗传杂交的方法获得双突变体。利用单突和双突变体定量统计分析叶序紊乱的表型,同时采用扫描电镜观察花序分生组织的结构大小,利用共聚焦显微镜观察分析花序分生组织发育过程中原基起始位置,原基发生次序的情况。
3. 前期工作中我们利用酵母双杂交鉴定发现RopGEF成员能与AHP6互作,并利用双分子荧光互补在原生质体中验证了二者间的互作。进一步我们将诱导原核蛋白用pull-down的方法验证蛋白间的互作,构建RopGEF-TAG载体转化已有的pAHP6- AHP6-GFP植株,进行Co-IP验证。此外由于AHP6是整合生长素和细胞分裂素信号的关键调控因子,我们也将用遗传杂交的方法分析RopGEF突变体和AHP6的在遗传学中的上下游关系。
4. 分析RopGEF成员对两种植物激素生长素和细胞分裂素的响应,本实验室前期的实验发现RopGEF成员参与了生长素信号调控的根发育和胚胎发育过程,我们也发现部分RopGEF成员对细胞分裂素有响应,进一步我们将分析激素处理对RopGEF成员蛋白在原基中的定位和分布的影响。在此基础上分析生长素和细胞分裂素信号的报告基因marker如DR5-VENUS和TCS-GFP在RopGEF突变体中的表达和分布模式变化,检测RopGEF对激素信号响应的作用,明确茎尖分生组织中RopGEF成员参与的激素信号途径。

二、研究成果及形式

论文及专著情况	国家统计源刊物以上刊物 发表论文（篇）		1		科技报告（篇）		0	
	专著（册）		0					
专利情况(项)	发明专利		实用新型专利		外观设计专利		国外专利	
	申请	授权	申请	授权	申请	授权	申请	授权
	0	0	0	0	0	0	0	0
其他	无							

三、项目进度和阶段目标

1. 项目起止时间： 2018-05-01 至 2021-04-30		
2. 项目实施进度及阶段主要目标：		
开始日期	结束日期	主要工作内容
2018-05-01	2019-04-30	<p>2018. 5-2019. 4</p> <p>1. 完成原位杂交实验，分析基因的表达模式，获得pRopGEF-RopGEF-GFP融合蛋白的转基因植株，分析蛋白的定位和分布模式。进一步分析两种植物激素对RopGEF蛋白定位或表达的影响。</p> <p>2. 鉴定双突变体植株，完成叶序的表型分析和茎尖分生组织的结构观察分析。</p> <p>3. 构建获得RopGEF融合蛋白标签载体，并诱导原核蛋白，获得标签载体的转基因植株。</p>
2019-05-01	2020-04-30	<p>1. 完成 RopGEF和 AHP6 蛋白体外和体内互作验证实验。</p> <p>2. 获得RopGEF突变体和ahp6突变体遗传杂交的材料，确定遗传学上互作关系。</p> <p>3. 获得双突变体与生长素和细胞分裂素报告基因的杂交植株，分别分析生长素报告基因DR5-VENUS和细胞分裂素TCS-GFP在突变体茎尖分生组织中的表达是否改变。</p>
2020-05-01	2021-04-30	整理实验结果，分析数据，撰写论文。

四、项目总经费及省科技厅经费预算

1. 省科技厅经费下达总额：（大写）壹拾万圆整；（小写）10万元；					
2. 省科技厅经费年度下达计划：					
年度	2018 年	年	年	年	年
经费(万元)	10.00				
3. 总经费开支预算计划：					
经费筹集情况：					(单位：万元)
省科技厅经费	自筹资金				合计
	自有资金	贷款	地方政府投入	其它	
10.00	0	0	0	0	10.00
政府部门、境外资金及其他资金投入情况说明：	无				

经费预算			(单位: 万元)	
	总投入经费		省科技厅经费	
支出经费	经费额	用途说明	经费额	用途说明
基建费:	0	无		
1、直接费用:	9.50	用于项目涉及的实验材料和测试费等的支出	9.50	用于项目涉及的实验材料和测试费等的支出
(1) 设备费:	0	0	0	0
(2) 材料费:	4.00	购买实验所用的分子生物学和生化试剂、耗材及植物材料培养所需的基质等	4.00	购买实验所用的分子生物学和生化试剂、耗材及植物材料培养所需的基质等
(3) 测试化验加工外协费:	3.00	基因测序, 共聚焦显微镜的测试费, 扫描电镜的制样使用费用等	3.00	基因测序, 共聚焦显微镜的测试费, 扫描电镜的制样使用费用等
(4) 燃料动力费:	0	无	0	无
(5) 差旅费/会议费/国际合作与交流费:	0	无	0	无
(6) 出版/文献/信息传播/知识产权事务费:	0.50	发表文章所需版面费及文献检索等费用	0.50	发表文章所需版面费及文献检索等费用
(7) 劳务费:	2.00	参加课题的研究生的劳务费	2.00	参加课题的研究生的劳务费
(8) 人员费:	0	无	0	无
(9) 专家咨询费:	0	无	0	无
(10) 直接费用其他支出:	0	无	0	无
(11) 科技金融服务体系其他费用:	0.00	无	0.00	无
①信用评级补贴:	0	无	0	无
②大赛场租:	0	无	0	无
③特派员奖励与补贴:	0	无	0	无
2、间接费用:	0.50	无	0.50	无
(1) 间接成本:	0	无	0	无
(2) 管理成本:	0.50	无	0.50	无
(3) 绩效支出:	0	无	0	无
合计:	10.00	无	10.00	无

五、人员信息

项目负责人								
姓名	证件号码	年龄	性别	职称	学历	在项目中承担的任务	所在单位	签名
刘慧丽	370881197510101141	43	女	助理研究员	博士研究生	项目负责人	华南农业大学	

项目组主要成员								
姓名	证件号码	年龄	性别	职称	学历	在项目中承担的任务	所在单位	签名
罗娜	230121198204112028	36	女	助理研究员	博士研究生	蛋白互作及表达谱数据分析	华南农业大学	
刘太波	430529198208122370	36	男	讲师	博士研究生	突变体表型分析	华南农业大学	
董庆坤	370783198912040910	29	男	未取得	本科	基因功能分析，蛋白互作及功能验证	华南农业大学	
邹益	511002199001080036	28	男	未取得	本科	互作蛋白的筛选及功能分析	华南农业大学	
张晓晶	142602199109260025	27	女	未取得	硕士研究生	突变体杂交及表型分析	华南农业大学	
张志伟	440183199403100014	24	男	未取得	本科	载体构建及基因表达模式分析	华南农业大学	
黄劲荣	450121199411242437	24	男	未取得	本科	植物材料培养及载体构建	华南农业大学	

六、依托单位与合作单位的合作协议

承担/参与单位名称 (盖章)	工作分工	总经费分摊 (万元)	省科技厅经费分配 (万元)
华南农业大学		10.00	10.00
	合计	10.00	10.00

2018A030313931

七、合同条款

第一条 甲方与乙方根据《中华人民共和国合同法》及国家有关法规和规定，为顺利完成（2018）年拟南芥RopGEF调控叶序模式的功能研究 专项项目（文件编号： 粤科规财字〔2018〕207号）经协商一致，特订立本合同，作为甲乙双方在项目实施管理过程中共同遵守的依据。

第二条 甲方的权利义务：

1. 按合同书规定进行经费核拨的有关工作协调。
2. 根据甲方需要，在不影响乙方工作的前提下，定期或不定期对乙方项目的实施情况和经费使用情况进行检查或抽查。
3. 根据《广东省科技计划项目信用管理办法(试行)》对乙方进行科技计划信用管理。

第三条 乙方的权利义务：

1. 确保落实自筹经费及有关保障条件。
2. 按合同书规定，对甲方核拨的经费实行专款专用，单独列账，并随时配合甲方进行监督检查。
3. 使用财政资金采购设备、原材料等，按照《广东省实施〈中华人民共和国招标投标法〉办法》有关规定，符合招标条件的须进行招标。
4. 项目实施完成或实施到一定程度，须按照《广东省省级科技计划项目结题管理的实施细则（试行）》提出验收或终止结题的申请，并按甲方要求做好项目结题工作。
5. 在每年规定时间内向甲方如实提交上年度工作情况报告，报告内容包含上年度项目进展情况、经费决算和取得的成果等。
6. 按照国家和省有关规定，提交科技报告及其他材料。

第四条 在履行本合同的过程中，如出现广东省相关政策法规重大改变等不可抗力情况，甲方有权对所核拨经费的数量和时间进行相应调整。

第五条 对分年度拨款（滚动资助）项目，甲方有权利根据项目研究进展或中期考核情况变更或中止项目后续资助经费数额。

第六条 在履行本合同的过程中，当事人一方发现可能导致项目整体或部分失败的情形时，应及时通知另一方，并采取适当措施减少损失，没有及时通知并采取适当措施，致使损失扩大的，应当就扩大的损失承担责任。

第七条 本项目技术成果的归属、转让和实施技术成果所产生的经济利益的分享，除双方另有约定外，按国家和广东省有关法规执行。

第八条 根据项目具体情况，经双方另行协商订立的附加条款，作为本合同正式内容的一部分，与本合同具有同等效力。

第九条 本合同一式三份，各份具有同等效力。甲、乙方及课题负责人各执一份，三方签字、盖章后即生效，有效期至项目结题后一年内。各方均应负合同的法律责任，不应受机构、人事变动的影响。

第十条 乙方必须接受甲方聘请的本项目合同监理单位的监督和管理。监理单位按照甲方赋予的权利对本项目合同的履行进行审核、进度调查，对项目合同变更、经费使用情况进行监督管理及组织项目验收。

说明：1. 本合同书中，凡是当事人约定无需填写的内容，应在空白处划（/）。

2. 委托代理人签订本合同书的，应出具合法、有效的委托书。

2018A030313931

八、本合同签约各方

管理单位（甲方）：广东省自然科学基金管理委员会（盖章）		
法定代表人（或法人代理）：_____（签章）		
年 月 日		
依托单位（乙方）：华南农业大学（盖章）		
法定代表人（或法人代理）：陈晓阳_____（签章）		
联系人（项目主管）姓名：郑鹏_____（签章）		
Email: kjcgxk@scau.edu.cn		
电话：020-85283435 / 13560344902		
开户单位名称：华南农业大学		
开户银行名称：广东广州工行五山支行		
开户银行帐号：3602002609000310520		
年 月 日		
联系人（课题负责人）姓名：刘慧丽（签名）		
Email: liuhuili@scau.edu.cn		
电话：020-38297790		
年 月 日		

受理编号: c24140500001897

项目编号: 2024A1515011671

文件编号: 粤基金字(2024)7号

广东省基础与应用基础研究基金项目

任务书

项目名称: 基于自噬改造及基因编辑以创制籽粒低镉水稻的研究

项目类别: 广东省自然科学基金-面上项目

项目起止时间: 2024-01-01 至 2026-12-31

管理单位(甲方): 广东省基础与应用基础研究基金委员会

依托单位(乙方): 华南农业大学

通讯地址: 广东省广州市天河区五山路483号

邮政编码: 510642

单位电话: 020-85283435

项目负责人: 黄晓

联系电话: 13660639294



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广东省基础与应用基础研究
基金委员会
二〇二〇年制

五、人员信息

项目负责人								
姓名	证件号码	年龄	性别	职称	学历	在项目中承担的任务	所在单位	签名
黄晓	440520197603041423	48	女	副教授	博士研究生	项目负责人	华南农业大学	黄晓

项目组主要成员								
姓名	证件号码	年龄	性别	职称	学历	在项目中承担的任务	所在单位	签名
罗娜	230121198204112028	42	女	助理研究员	博士研究生	负责酵母和水稻部分的实验	华南农业大学	罗娜
董坤田	371122199403080914	30	男	未取得	硕士研究生	负责拟南芥部分的实验	华南农业大学	董坤田
郭怡帆	410728199908255217	25	男	未取得	本科	负责Cd结合自噬衔接蛋白表达水稻的农艺性状调查及镉含量分析	华南农业大学	郭怡帆
苏健宇	445321199905055299	25	男	未取得	本科	负责确定拟南芥Cd结合自噬衔接蛋白的亚细胞定位及分析植株对Cd吸收、转运和积累	华南农业大学	苏健宇

八、本任务书签约各方

管理单位（甲方）：

广东省基础与应用基础研究基金委员会（盖章）

法定代表人（或法人代理）：

曾晓

（签章）



依托单位（乙方）：华南农业大学

法定代表人（或法人代理）：薛红卫

联系人（项目主管）姓名：倪慧群

Email: kjcgxk@scau.edu.cn

电话：020-85283435 / 15920301530

开户单位名称：华南农业大学

开户银行名称：广东广州工行五山支行

开户银行账号：3602002609000310520



2024年05月22日

联系人（项目负责人）姓名：黄晓

（签名）

黄晓

Email: xiaohuang@scau.edu.cn

电话：13660639294

2024年5月23日



科技风

KE JI FENG

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基于科教融合的 “植物生理学”课程教学的改革与探索

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摘要:“植物生理学”是农科植物生产类专业的必修课,课程知识点较多,实用性强,用于指导农业生产实践。在课程教学中探索科教融合的模式,在理论课和实验课教学中均将科研工作融入教学中,更是在实验中增加了教师自己的科研成果。科研促进教学,教学反哺于科研,全面培养学生的科研思维,激发他们主动学习的能力和创新思维能力。

关键词:科教融合;植物生理学;人才培养

一、概述

目前,我国已经进入了高质量发展的新阶段,提升科技能力非常关键。国家一直提倡“科教兴国”的发展战略,《国家中长期教育改革和发展规划纲要(2010—2020)》进一步指出,高等教育要支持学生参与科学研究,强化实践教学环节,促进科研与教学的实质融合^[1]。教学和科研是高等学校两项基本职能,科研促进教学,教学反哺于科研,科研与教学达到双赢。科教融合教学模式有利于加强高等教育与科学研究相互融合在一起,这样可以从根本上促进创新型人才的培养,更好地实现科技、教育、人才一体化^[2]。科研融合一方面将科研理念应用于育人理念中,将科学成果转化为教学范例,培养学生创新精神;另一方面科学研究可以提供学生前沿的专业知识^[3]。

华南农业大学是国家“双一流”建设高校,农业科学和生命科学为学校的优势学科。而“植物生理学”是我校植物生产类相关专业如农学、植保、园艺、林学等的专业基础课,也是生物科学与生物技术的专业课。每年修课人数超过1400人,覆盖面较广。近年来,“植物生理学”教学团队积极开展基于科教融合理念的课程改革,将教师科研工作中的部分内容融入教学之中,启发学生的科研思维,培养他们的分析以及综合利用所学知识解决实际问题的能力。教学团队成员都主持或参与多项国家级和省级的科研项目,积累了丰富的科研经验,这样才有利于把最新的科研成果融汇于课堂上,科研与教学互动,学生可以接触到最新的科技成果,这些是我们做好科教融合工作的底气和保障。

二、科教融合在理论课教学中的体现

(一)加强知识点研究背景的学习

传统的教学模式通常重视知识本身的传授,忽视对知识点相应的科研过程的介绍,学生不知道是如何发现问题、分析问题、解决问题、得出结论的。而科教融合教学模

式会将学科重要事件的研究背景加以较详细地阐述。如卡尔文进行CO₂固定时的具体思路、步骤和技术手段;胚芽鞘向光性实验的思路和方法;光敏色素发现过程的思路及所用的技术。这不仅有利于加强学生对理论课知识的整体理解,更主要的是慢慢渗透了如何分析、解决问题的科研思维,提高学生对科研的兴趣。

(二)读书报告

理论课的内容相对枯燥,且绝大多数的时间以教师“填鸭式”教学模式为主,如果能调动学生学习的积极性,让学生带着有趣味性的科学问题主动学习吸收知识,对内容的理解和分析问题的深度都会高于被动学习,也会更容易应用于农业生产实践中。

调动学生的积极性具体做法是:在教学平台如雨课堂和QQ教学群中^[4],提供部分相关的资料,要求他们检索、阅读和讨论,针对涉及的知识点或者问题进行更深入的研究性学习,并完成小组的读书报告。教师根据报告内容、讲述情况以及回答问题的情况综合给出分数,作为平时成绩的一部分。如在果实发育部分,学生做的汇报:(1)乙烯在苹果成熟中的作用;(2)类YABBY转录因子在番茄驯化过程中的作用;(3)果实开裂的调控;(4)影响番茄果实大小的关键数量性状位点;(5)番茄种质中SUN、OVATE、LC、FAS的分布及其与果实形状多样性的关系等。

理论课激素信号转导部分,学生的读书报告内容有:(1)ABA信号传导在植物抗逆性方面的研究进展;(2)生长素生物合成、转运和信号转导中的可逆蛋白磷酸化;(3)拟南芥乙烯信号通路;(4)GA的细胞信号转导及其突变体研究。(5)生长素的信号转导和转录调控等。

读书汇报对知识点进行总结,其难度高于课堂讲授的部分,在完成过程中不仅能培养学生的自主学习能力和科研兴趣,锻炼了表达能力的同时,还有利于良好师生关系

的建立。

(三) 学术讲座

华南农业大学学校的科研氛围浓厚,经常举办国内外专家的学术讲座,教师会将相关的信息转发给学生,要求他们参加已经学习过的知识点的相关讲座,了解学科的最新进展和发展方向、学习学术专家们的学术思想,对他们以后考研目标的选择有很大帮助。对于这些最新的研究成果,教师也会在上课时,进行简单讲解讲座的逻辑思路。

(四) 鼓励学生参与教师的科研项目中

我们会鼓励大二的学生参与教师的科研项目,使学生了解科学问题的研究思路,提高学生的创新意识。同时,学生参与教师科研项目研究也会促进项目的进度,形成良性循环,互相促进。这样,学生也会在做研究中积累经验,参加一些国家和省级项目申报。

三、科教融合在实验课教学中的体现

实验课教学是教学的重要组成部分,学生通过理解实验目的、根据实验流程亲自动手操作、认真观察实验现象、分析实验结果,都可以培养学生熟练的实验技能与学生严谨细致、独立创新的科学态度。同时,实验课操作性强,容易给学生留下深刻的印象,有利于学生更好地掌握的理论知识^[5]。以前,我校的“植物生理学”实验主要包括验证性实验和综合性实验两大类,都是常规性实验。为了增强实验课的趣味性,提高学生参与的热情,我们探索科教融合模式下的实验课,具体内容如下:

(一) 科教融合的模式一

1. 实验一:香蕉的催熟过程以及冷害、青皮熟现象观察

实验原理:刚采收的香蕉不能立即食用,放置一段时间后,其色香味才符合人们的食用要求,这一过程称为后熟。后熟可以分为自然后熟和人工催熟。自然后熟的过程缓慢,成熟不均匀,颜色也不均匀,影响水果的品质;人工催熟可达到快速均匀成熟。香蕉是呼吸跃变型果实,当环境乙烯浓度达到或超过阈值时,香蕉果实合成大量的乙烯,呼吸速率迅速提高,启动了果实的后熟过程。香蕉催熟就是人为提高贮藏环境的乙烯浓度,加快后熟进程,因乙烯利溶液处理操作简单,而普遍被广泛使用。

香蕉对温度非常敏感,温度较高会加速乙烯的产生和香蕉的后熟,但温度超过 28℃ 时,香蕉果皮叶绿素降解慢,出现果肉成熟,而果皮中叶绿素不能降,仍然保持青色的青皮熟现象。另外,香蕉的适宜贮藏温度为 11~13℃,温度较低时香蕉容易产生冷害,影响后熟和品质风味。实验目的筛选乙烯利起作用的最适条件。

实验步骤:将已冲洗干净的香蕉果实分别放入不同浓度的乙烯利溶液中浸泡 2 分钟,取出晾干。用聚乙烯包装袋分别密封包装 4 个处理的香蕉果实,其中对照没有浸泡乙烯利的果实,分别放在 4℃、15℃、20℃、30℃ 培养箱里。24h 后打开密封包装袋通风透气,稍封袋口或者去除包装,

做进一步观察,每天记录香蕉果实出现软熟的时间和外观症状。

该实验综合了理论课知识乙烯催熟、果实成熟中色香味的变化、叶绿素的降解等方面的内容。学生通过自己的实验验证了所学知识点,尤其是香蕉的冷害和青皮熟特性更贴近生产实践,学生可以用自己理论课学到的知识来指导生产实践。

2. 实验二:植物花青素苷的理化性质分析

实验原理:花色素苷主要存在于液泡中,具有水溶性的天然植物色素,存在植物的液泡中,赋予果蔬、花卉五彩缤纷的颜色的物质基础。花色素苷在食品化工上都起着重要作用。花色素苷是一种糖苷,其非糖部分称为花色素。在植物的花、茎、叶、根等器官的细胞液中都存在花色素苷。

花色素苷的纯化分离主要根据其理化性质,主要是可溶于水和带正电荷、在低 pH 值下较稳定等特性。本实验设计采用 0.5mol/L 的 HCl 处理花瓣花色素苷,通过 Amberlite XAD-7 对在低 pH 值下带正电荷的花色素苷有较强的吸附力,对其他不带电荷的杂质如糖类、有机酸等吸附力较弱,这些杂质可通过洗涤除去,被吸附的花色素苷可以采用甲醇洗脱,最后达到对花色素苷初步纯化的目的。

花色素苷在不同的 pH 值下呈现不同的颜色,主要原因是在不同的 pH 值下结构不同。在强酸条件下(pH<3)时,花色素苷会呈现红色,主要存在形式为盐阳离子;随着 pH 值升高至弱酸(pH 范围 4~5),主要存在形式变成非离子型,其结构会转化为假碱式花色素苷;在碱性条件下(pH>7),花色素苷存在形式为阴离子,颜色为蓝紫色的。

不同的 pH 值决定盐离子的比例,比如在 pH 值为 1 花色素苷颜色较 pH 值为 3.5 更红,主要原因为 pH 值为 1 的盐离子浓度较高。具体分析花色素苷的结构,可以采用光谱分析的方法。

实验步骤:

(1)花色素苷的提取方法:10g 玫瑰花瓣剪碎,加入 100mL 0.5mol/L 的 HCl 中浸提过夜,过滤得玫瑰花色素苷粗提取液。

(2)装填 Amberlite XAD-7 树脂吸附层析柱:将 Amberlite XAD-7 树脂用蒸馏水溶胀后,慢慢地倒入层析柱,使装填物均匀地自然下降。

(3)玫瑰花色素苷的初步纯化:将玫瑰花色素苷粗提取液上 Amberlite XAD-7 树脂吸附层析柱,上完样后,本实验采用适量的蒸馏水冲洗柱子,目的是去除杂质。接着用定量 0.1% HCl 甲醇反复洗脱柱子,用离心管收集红色的洗脱液,最后为了获得高浓度、高纯度的花色素苷,本实验采用减压旋转蒸发仪浓缩和除去甲醇。

(4)不同 pH 值处理下玫瑰花色素苷的光谱分析:本实验采用 0.4mol/L KCl/HCl、pH1.0、0.4mol/L 柠檬酸、磷酸氢二钠 pH 值为 3.0、5.0、7.0 的缓冲液稀释玫瑰花色素苷浓缩液至 50mL,平衡 30min 后,用紫外可见分光光度计

测定不同 pH 值下的可见光吸收光谱。

该实验综合了花色素苷的提取、吸附层析、旋转蒸发浓缩、吸收光谱分析等技术,以及观察到不同 pH 值条件下花色素苷颜色的变化情况。本实验涉及的技术都很实用,可以进一步应用于生活实践中,比如生活中对果酱色素的鉴定。

3. 实验三:超薄等电聚焦电泳测定种子纯度

实验原理:在聚丙烯酰胺凝胶中加入载体两性电解质以达到在两极形成连续、稳定和线性的 pH 梯度。根据蛋白质具有不同的等电点,进入加有载体两性电解质的聚丙烯酰胺凝胶后,在电场的作用下,蛋白质电泳图谱是根据蛋白质停留在其等电点相等的 pH 值处(聚焦),经染色后显示出不同位置的条带。该电泳方法可用于作物品种真实性和种子纯度的鉴定。

实验步骤:

(1)蛋白质的提取:取水稻、番茄、辣椒等农作物种子 50 粒,去除种皮后用种子粉碎机逐粒粉碎,分别置于离心管中,加入 PBS 提取液,室温下涡旋 10 分钟提取,离心,取上清液用于电泳。

(2)凝胶的制备:根据不同植物材料设计不同的凝胶配方。称取一定量的尿素、牛磺酸,加入一定量的聚丙烯酰胺母液、两性电解质、TEMED、过硫酸铵,拌器上混匀。本实验采用拍打技术进行制胶片,首先在聚酯胶片中间滴入一定量的凝胶液,然后将玻璃板放到聚酯胶片上,通过玻璃板的两个长边上贴有厚度为 0.15mm 的胶布保证制出的凝胶厚度均一,即为 0.15mm。室温下聚合约 45min 后,用一小刀插入玻璃板之间将两块玻璃板分开,然后小心将聚酯胶片从玻璃板上揭起,即为制好的凝胶。

(3)点样:将制好的凝胶放于经预冷的水平平板电泳槽冷却板上,将经阳极和阴极电解液充分浸润的滤纸条放在凝胶上,然后平行放置上 52 孔点样带,一定要注意点样带与凝胶完全密封。吸取一定量的蛋白质提取液加入点样孔中。对照提取液,还需要一个泳道点 marker。

(4)电泳:250V 下,电泳 70min。

(5)固定:将电泳胶片小心取下,放入固定液中固定 20min。

(6)染色、脱色后结果观察:观察电泳条带的情况,计算出所试种子的纯度,或与标准样品相比较鉴定作物品种的真实性。

种子的纯度鉴定是种子生产中非常重要的一环。本实验应用超薄等电聚焦电泳技术,胶薄,实验用的试剂少、省钱且清晰度高,结果的准确率高,需要的样品量少,适合单个种子的检测。实验的关键是超薄电泳胶的制作,在教师示范后都能成功。

以上的三个实验,每一个内容都比普通实验指导书上的更综合、更贴近生产实践,更能培养学生的兴趣。在经过多轮的实践后,我们将修订后相关的内容编入了本校出

版的植物生理实验指导中^[6],较好地完成了科学研究应用于教学过程中的转化。

(二)科教融合的模式二

各位任课教师的科研和教学经验都很丰富,实验药品和仪器也较为齐全。本科生可以在大学二年级时候申请进入任课教师的实验室,首先跟随师兄、师姐完成一些基础的实验操作,逐渐建立科研思维、接触科研项目,在教师的指导下学会查阅相关文献,拟订实验方案并实施完成一个项目。完成这些项目,对学生的科研思维、动手能力、应用知识的能力等各个方面能力的综合提升效果非常明显。科研成果可以作为毕业论文,也可以参加大学生创新创业训练项目的校级和省级比赛。

结语

科教融合为“植物生理学”课程改革和学生培养拓展了新思路。将前沿发展动态以及教师的相关科研成果融入教学中,无论是理论课的读书报告、听学术讲座,还是实验课教师课题都鼓励学生进课题组,参加科研项目,激发学生学习兴趣,变被动学习为主动学习。今后,我们还将提炼更多的科研成果融入教学中,注重科研教学相互融合,培养学生实践能力与创新能力。

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实验设计与生物化学实验课相结合的上课模式的探讨

——以糖酵解中间产物的鉴定为例

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摘要:生物化学是农业院校的基础理论课。生物化学的学习有助于理解其他课程, 并且可以推动学生对生命科学的热爱。作为生物化学的辅助-生物化学实验课起着重要作用。目前生物化学实验课存在的问题学生过度依赖于老师, 被动学习。本研究探讨了实验设计与生物化学实验课结合的上课模式, 利用实验设计的思维激发学生主动学习, 培养学生科研创新的思维。本研究以实验课“糖酵解中间产物的鉴定”为例, 详细分析了该节课与实验设计结合的切入点, 进一步分析实验设计在生物化学实验的作用及展望。

关键词:实验设计; 生物化学实验; 糖酵解

生物化学实验课是在生物化学理论课的基础上, 可以激发学生对生命科学的热爱, 也为学生进行本科生毕业设计奠定基础。面对新世纪对研究人员创新能力的要求, 要对本科生培养独立设计实验的能力, 本实验课共包含五个实验, 蛋白质含量的测定、维生素C含量的测定、糖酵解中间产物的测定和酵母菌蔗糖酶的提取及比活力的测定。这些实验涵盖了理论课的各个章节, 为进一步理解理论课, 升华了理论课的内容。但是传统的实验课, 实验老师主要按照实验书的内容写好板书, 学生就是按部就班的按照板书的内容来操作, 教学目标是以提高动手能力和学习测定方法为主, 根本对每一步为什么这么设计缺少思考, 无法保证每个学生可以理解实验中的重点与难点。长久以来, 学生会变得依赖老师, 学习方式变成为被动学习, 不会思考问题。

实验设计来源于科学实验与统计学的结合, 其具有周密性和科学性。设计过程中一直在回答三个问题: 为什么做、怎么做、做到什么程度的问题。学生以后进入课题组, 独立做毕业论文阶段, 是否能获得准确可靠的数据, 实验设计起着重要作用。因此针对学生高年级的毕业设计和科研能力的提高, 要培养学生独立设计实验的能力。本研究就是探讨实验设计与生物化学实验课相结合的上课模式。

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1 实验设计与生物化学实验课相结合的上课模式

设计实验首先要明确为什么做, 就是本实验的目的, 比如糖酵解中间产物的鉴定这个实验。首先引导学生理论课内容糖降解的图, 糖酵解后丙酮酸将在无氧气条件下产生酒精、在有氧条件下进入三羧酸循环产生大量CO₂。中间产物的鉴定就要明确代谢通路要停止在哪里? 为什么要停止在这里及其后续的验证问题。

提出假设, 假设是对可能存在的条件的一种假设, 这种假设可以为寻找事实做了基础。设计假设是实验设计方法融于生物化学实验。合理设计假设, 是实验成功与否的根本。培养学生大胆的质疑能力, 提出自己的意见。根据本研究的实验目的, 糖酵解中间产物的测定。并且糖酵解是一个不可逆的反应过程, 只要反应启动, 就会经过一系列的酶促反应, 产生ATP供能。中间产物包括葡萄糖-6-磷酸、果糖-6-磷酸、果糖1,6-二磷酸、二羟基丙酮酸、甘油醛-3-磷酸、1,3-二磷酸甘油酸、3-磷酸甘油酸、2-磷酸甘油酸、磷酸烯醇式丙酮酸和烯醇式。观察这些中间产物都是以磷酸化合物的形式存在的, 并且二羟基丙酮酸和甘油醛-3-磷酸属于丙糖类可以用显色反应(2,4-二硝基苯肼)来验证其是否存在。进一步引导学生观察一下糖酵解过程中涉及的主要的酶包括己糖激酶、磷酸葡萄糖异构酶、磷酸果糖激酶、醛缩酶、甘油醛3-磷酸脱氢酶、磷酸甘油酸激酶二磷酸甘油酸变位酶、烯醇化酶、丙酮酸激酶。通过查阅文献知道碘乙酸可以抑制甘油醛3-磷酸脱氢酶活性, 造成二羟基丙酮酸和甘油醛-3-磷酸的积累(图1)。同学们马上提出来假设糖酵解代谢终止在这一步。

继续回答实验设计中的怎么做的的问题, 根据上

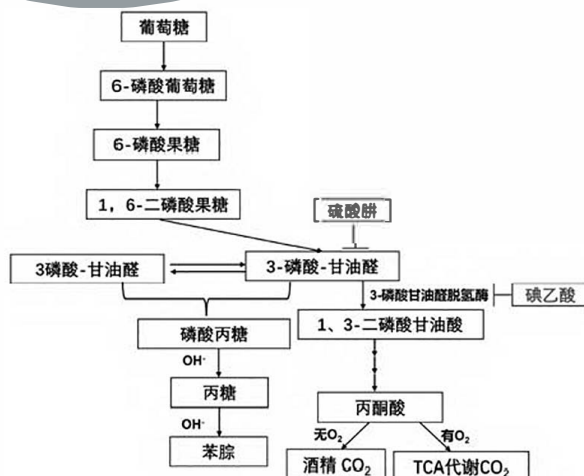


图1 糖酵解中间产物测定

注:其中碘乙酸是专一抑制3-磷酸甘油醛脱氢酶的活性;硫酸脒为稳定剂,可防止3-磷酸-甘油醛挥发。

述假设,引导学生老师互动形式设计实验。本实验处理为利用碘乙酸和硫酸脒,使其中间产物终止在二羟基丙酮酸和甘油醛-3-磷酸。实验设计中要设立作为比较标准的对照处理。根据研究的目的和内容,可选择不同形式的对照。其中包括空白对照、正向对照、负向对照、自身对照等。本研究中根据实验目的,启发学生设计了两个对照,一个是空白对照,糖酵解过程顺利进行,为了验证能够显色的物质不是反应的终产物丙酮酸;一个是负对照,三氯醋酸是一种蛋白酶的变性剂,能让酵母中所有酶失活,使糖酵解不能进行或者很小程度进行,也是为了后续验证反应底物是不能起显色反应的。

实验方案中还要注意比较间的唯一差异原则。是指在设计实验中,要保证只有处理单因素的不同,其他所有的条件应当相同。比如本实验中,要保证实验中加入的试剂都一致。单一差异是加入的时间不同,导致最后只有产物不同。引导同学发现问题,表1描述的处理条件是否为单一的因素差异?学生会发现不是,因此反应1.5小时后,会在表2继续补加试剂。补加的试剂为三氯醋酸、碘乙酸和硫酸脒。

做到什么程度的问题,反应必须要适时终止。要根据前期的预实验,合理的终止实验反应。

2 实验设计在生物化学实验中的作用

第一,增强学生参与的热度,增强学生的好奇心。生物化学的理论课知识点难度很大,枯燥无味,学生很难马上完全理解接受。传统的实验教学一般是以老师灌输为主,因此学生只是按照步骤一步一步模仿的做,根部不知道每一步为什么这么做,结果就不能很好的辅助理论课学习。而实验设计根据实验目的和原理,带着问题来做实验,每一步都是来验

表1 发酵过程观察

长试管编号	10%三氯乙酸(mL)	0.002mol/L. 碘乙酸(mL)	0.56mol/L. 硫酸脒(mL)	5%葡萄糖(mL)	37℃保温45 min,观察气泡,并记录
1	1	0.5	0.5	5	
2	0	0.5	0.5	5	
3	0	0	0	5	

表2 终止发酵和补加试剂

长试管编号	10% 三氯乙酸 (mL)	0.002mol/L. 碘乙酸 (mL)	0.56mol/L. 硫酸脒 (mL)
1	1	0.5	0.5
2	0	0.5	0.5
3	0	0	0

证一个预期的结论。学生还可以推测出来预期的实验现象,增强学生的参与热度。并且为学生以后独立的解决科学问题,怎么设计实验奠定基础。

第二,增强学生独立思考的能力,开发学生探究问题的能力^[2]。学生会自己设计假设,然后通过查找文献来独立思考怎么验证这个假设。刚开始,我们对于刚刚升入大二的学生,也担心他们会畏惧,面对问题不知道怎么着手。后来经过一段时间引导,学生开始探索性设定不同的假设,然后根据假设设计合理的对照。这个过程可以增强学生独立思考的能力,并且可以通过讨论,增强学生小组间的合作。

第三,增强学生的逻辑思维能力。实验设计过程中一定要遵循唯一差异原理,因此在整个实验过程中,学生可以自己检验实验的合理性,增强学生辩证的思维能力。这种方式可以突破传统的教师为主的实验课,让学生成为主体。并且为以后进入课题组,面对新的课题,都可以合理地设计实验。

第四,增强学生分析实验结果的能力。传统的实验报告就是抄实验书上的实验原理、实验仪器和用品、实验材料和试剂、实验步骤、结果与计算。本次改革后的实验报告包括以下部分,实验原理,实验步骤、实验结果、实验结果的讨论。实验步骤是按照流程图的方式描绘,实验结果讨论是对结果和不同组间之间分析和比较,并且查阅文献进行比较不同方法间的差异。

第五,增强了学生和老师之间的交流,以前的实验教学模式,学生只是填鸭式的接受,因此对实验步骤只是照葫芦画瓢。本次改革增强了实验设计的内容,赋予学生更多自主探索的实践,让学生可以进行思想风暴,让学生更加注重理论课的学习,在理论课的基础上提出合理的问题,这种教学方式有利于培养拔尖人才^[3]。老师会根据学生的问题慢慢引导,让学生体会到发现问题,验证问题的乐趣。

3 实验设计与生物化学实验课相结合的上课模式的展望

(下转第39页)

Analysis of Ideological and Political Education in the Construction of Fishery Specialty Based on the Idea of to Foster Virtue Through Education

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Abstract: Who to train, how to train and for whom are the fundamental problems of education. Taking the cultivation of aquatic products professionals as the goal, combined with the ideological and political characteristics of various courses of aquatic products majors, this paper puts forward to build an all-round and multi-angle education system by correcting students' and parents' professional prejudice, enhancing their professional understanding, understanding the professional development trend and prospects, earnestly doing the first lesson, grasping the ideological and political aspects of each professional course, grasping the practice link, and carrying out employment guidance, so as to cultivate students' spirit of knowing and loving agriculture.

Keywords: Ideological and political theories teaching in all course; fisheries; speciality; educating people

(上接第36页) 首先实验设计是科学研究的基础,把实验设计的思想贯穿于生物化学实验课上,实验设计被称为行之有效的教学方法和载体(4)。让学生从本科生就培养理性的逻辑思维,使他们可以更好地理解理论知识。为培养学生以后从事科学研究做的铺垫。

其次实验设计融于生物化学实验课,不仅有利于学生对生物化学实验课的学习,还有利于对于其他实验课的学习,培养了学生探索的精神,具有创新精神,注重培养学生的能力、提出问题、分析问题和解决问题的能力。

最后在生物化学实验课上建立实验设计的思想可以运用于毕业论文设计。农业院校的大学,要求本科生毕业要独立完成一项毕业设计。这个毕业设计是对学生大学期间学的知识的综合考核。并且毕业设计中的实验要设计合理,有科学价值。因此实验设计起着重要的作用。

4 结论

本研究探讨了实验设计与生物化学实验课相结合的上課模式,激发学生学习生物化学实验的兴趣,并且改变传统的教学模式,让学生从“要我学”,变成以学生为主体的“我要学”模式,培养学生科学的思维能力、创新能力。

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Discussion on the Model of Combining Experimental Design and Biochemistry Experiment – taking the Identification of Glycolysis Intermediates as an Example

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Abstract: Biochemistry is a basic theoretical course in agricultural colleges. The study of biochemistry contributes to the understanding of other courses and can promote students' love of life sciences. As an assistant to biochemistry, the biochemistry experiment course plays an important role. At present, the problem of biochemistry experiment course is that students over-rely on teachers and learn passively. This study explores the teaching mode of combining experimental design and biochemistry experiment class, using the thinking of experimental design to stimulate students' active learning and good for students' thinking of scientific research and innovation. Taking the experimental course "Identification of glycolysis intermediates" as an example, this study analyzed the breakthrough point of combining the course with experimental design in detail, and further analyzed the role and prospect of experimental design in biochemical experiments.

Keywords: Experimental design; Biochemical experiment; Glycolysis

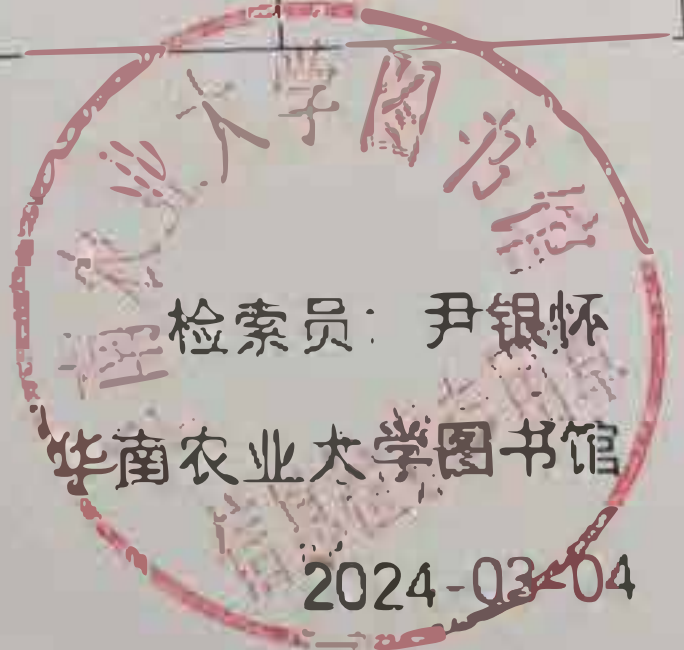
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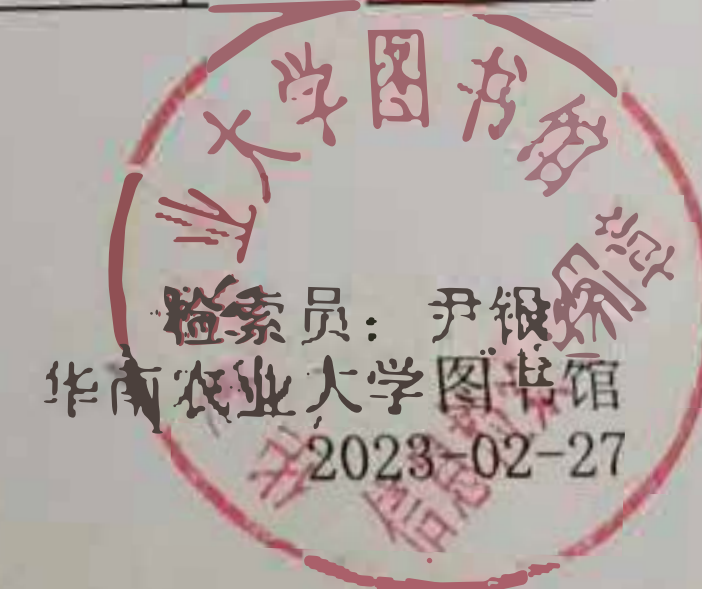
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2	Engineered ATG8-binding motif-based selective autophagy to degrade proteins and organelles in planta	New Phytologist 出版年: 2022 JAN 2023 卷期: 237 2 页码: 684-697 文献类型:	第一作者	T2类	华南农业大学	SCI	IF2-year=10.323 IF5-year=10.768 (2021)	生物学 1区 Top期刊: 是 (2022)

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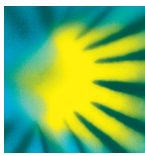
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Cover Legend

White blister rust infection caused by the oomycete pathogen *Albugo candida*, showing pustulous symptoms on the stem of oilseed mustard *Brassica juncea*. Image courtesy of Eric B. Holub. (Redkar et al., pp. 532–547).

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Methods

Engineered ATG8-binding motif-based selective autophagy to degrade proteins and organelles *in planta*

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Summary

- Protein-targeting technologies represent essential approaches in biological research. Protein knockdown tools developed recently in mammalian cells by exploiting natural degradation mechanisms allow for precise determination of protein function and discovery of degrader-type drugs. However, no method to directly target endogenous proteins for degradation is currently available in plants.
- Here, we describe a novel method for targeted protein clearance by engineering an autophagy receptor with a binder to provide target specificity and an ATG8-binding motif (AIM) to link the targets to nascent autophagosomes, thus harnessing the autophagy machinery for degradation.
- We demonstrate its specificity and broad potentials by degrading various fluorescence-tagged proteins, including cytosolic mCherry, the nucleus-localized bZIP transcription factor TGA5, and the plasma membrane-anchored brassinosteroid receptor BRI1, as well as fluorescence-coated peroxisomes, using a tobacco-based transient expression system. Stable expression of AIM-based autophagy receptors in Arabidopsis further confirms the feasibility of this approach in selective autophagy of endogenous proteins.
- With its wide substrate scope and its specificity, our concept of engineered AIM-based selective autophagy could provide a convenient and robust research tool for manipulating endogenous proteins in plants and may open an avenue toward degradation of cytoplasmic components other than proteins in plant research.

Introduction

Technologies that disrupt protein expression are essential tools for the analysis of gene function and have revolutionized both animal and plant research. Nucleic acid-based methods represented by Clustered Regularly Interspaced Short Palindromic Repeat (CRISPR) or RNA interference are widely used for the interruption of protein expression at the DNA and RNA levels, respectively (Elbashir *et al.*, 2001; Knott & Doudna, 2018). These tools have changed the ways of genetic manipulation with their simplicity and versatility. By contrast, no general technologies have been developed to selectively recognize and degrade proteins although they are highly desired both for basic research and for the discovery of degrader-type drugs (Wu *et al.*, 2020).

To develop techniques that probe protein function more directly, biologists have exploited natural cellular degradation systems, such as the ubiquitin–proteasome system (UPS) and the macroautophagy (hereafter referred to as autophagy)–lysosome system, to eliminate proteins of interest (POIs), either their variants, oligomers, or their aggregates; Ding *et al.*, 2020; Verma *et al.*, 2020). For example, PROteolysis-TArgeting Chimera (PROTAC), a UPS-dependent approach, recruits E3 ligases through chimeric small molecules to target specific proteins and trigger their degradation (Verma *et al.*, 2020). However, owing to the intrinsic limitation of the UPS and only few E3 applicable for PROTACs, the approach is largely restricted to a limited set of cytoplasmic proteins and has limited capability to eliminate protein aggregates and nonprotein molecules. Contrary to

UPS-dependent approaches, autophagy-based technologies degrade their substrates via lysosome or the vacuole and have a broader range of targets. Moreover, they are applicable for multiple organisms due to the evolutionary conservation of the autophagy–lysosome system (Ding *et al.*, 2020).

During autophagy, cells remove unwanted or dysfunctional cytoplasmic components by sequestering them into double membrane-bound vesicles termed autophagosomes and then delivering them to vacuoles (plants and fungi) or lysosomes (animals) for degradation (Galluzzi *et al.*, 2017; Marshall & Vierstra, 2018; Yang *et al.*, 2020). Although best described as a starvation response, autophagy plays a vital role in maintaining cellular homeostasis by removing defective proteins, protein aggregates, and damaged or superfluous organelles through various selective autophagy pathways (Kirkin & Rogov, 2019; Johansen & Lamark, 2020).

The specificity of selective autophagy is governed by a diversity of autophagy receptors, which tether cargoes and nascent autophagosomes by interacting with membrane-anchored autophagy-related protein 8 (ATG8). Autophagy receptors typically contain an ATG8-interacting motif (AIM) with a core consensus sequence, W/F/Y-X-X-L/I/V (Noda *et al.*, 2008, 2010). In addition to canonical AIMs, some receptors can bind ATG8 via a ubiquitin-interacting motif-like sequence (Marshall *et al.*, 2019). Autophagy receptors thus recognize their cargoes directly or in a ubiquitin-dependent manner (Kirkin & Rogov, 2019; Johansen & Lamark, 2020). Although our understanding of plant selective autophagy is far from complete, a growing number of studies have advanced our appreciation of its broad substrate range, including individual or aggregated proteins, macromolecular complexes (such as proteasomes and ribosomes), organelles (such as mitochondria, peroxisomes, and chloroplasts), and even non-proteinaceous biomolecules (such as porphyrins; Marshall & Vierstra, 2018; Stephani & Dagdas, 2020).

Inspired by the very nature of selective autophagy, biologists have developed several biological research methods that mimic the action of the autophagy receptor to promote degradation (Ding *et al.*, 2020); for example, the autophagosome-tethering compound ATTEC is a small molecule with a similar function to the autophagy receptor, which links ATG8 and mutant Huntington protein to mediate the latter's degradation (Z. Li *et al.*, 2019). Similarly, the autophagy-targeting chimera molecule AUTAC contains a specific binder to provide target specificity and a degradation tag (guanine derivative) that triggers the K63 polyubiquitination of targets to initiate selective autophagy (Takahashi *et al.*, 2019). Moreover, the AUTOPhagy-Targeting Chimera (AUTOTAC) employs bifunctional molecules consisting of a target-binding ligand and an autophagy receptor p62-binding moiety to induce the sequestration and degradation of targets (Ji *et al.*, 2022). These degradation systems not only complement current genetic methods for altering the levels of proteins but are also applicable to various nonproteinaceous cargoes such as lipid droplet given the wide substrate scope of selective autophagy (Fu *et al.*, 2021). However, similar methods are not currently available in plant research.

To explore autophagy to knock down plant POIs, we designed an AIM-based autophagy receptor to harness selective autophagy

of POIs in plants as a test. The concept of our idea consists of an AIM, a specific binder that targets the POI, and a fluorescent protein for tracing (Fig. 1a). The target(s) of the AIM-based autophagy receptor can be customized by modifying/substituting the binder to interact with a specific POI. We reasoned that the receptor, when expressed transiently or stably *in planta*, would specifically bind its target via the binder and, through the interaction between the AIM and ATG8, deliver the receptor–target complex to the vacuole for selective degradation.

Materials and Methods

Vector construction

The following constructs originate from these publications: GFP-ATG8a (Thompson *et al.*, 2005), BRI-GFP (Friedrichsen *et al.*, 2000), and BIN2-GFP (Peng *et al.*, 2008). To generate the *AIM-mCherry* construct, the sequence encoding the AIM fragment (amino acids 651–675) of AtNBR1 was PCR-amplified using the primers Li1204 and Li1205, and the mCherry fragment was PCR-amplified from the *mCherry-ATG8a* (Suttangkakul *et al.*, 2011) construct using the primers Li1206 and Li1312 (all primers are listed in supporting information Table S1). An overlapping PCR approach was then used to generate the AIM-mCherry fragment, which was cloned into the pEGAD vector (accession no. AF218816) between the *AgeI* and *BamHI* restriction sites using ClonExpress II One-Step Cloning Kit (Vazyme Biotech Co., Nanjing, China) to create the *pEGAD-AIM-mCherry* construct. The *pEGAD-mAIM-mCherry* was generated by PCR mutagenesis with the primer pairs Li1480 + Li302 and Li1481 + Li301 to introduce two point mutations (W661A/I664A) in the AIM sequence. To generate the *pEGAD-PB1-AIM-mCherry* vector, the sequence encoding the PB1 fragment (amino acids 7–96) was PCR-amplified from AtNBR1 using the primers Li1500 and Li1501 and then cloned into the *pEGAD-AIM-mCherry* vector between the *AgeI* and *BamHI* sites. To generate the *pEGAD-FYVE-AIM-mCherry* construct, an FYVE fragment was PCR-amplified from an FYVE domain-containing *Arabidopsis* transgenic line PIPLINE#18 (P18Y, ABRC# CS2105611; Simon *et al.*, 2014) and inserted into the vector *pEGAD-AIM-mCherry* between the *AgeI* and *EcoRI* restriction sites. To generate the *pEGAD-3XAIM-mCherry* vector, a 2XAIM fragment was synthesized by Tsingke Biotechnology Co. (Beijing, China) and inserted between the *AgeI* and *EcoRI* restriction sites of the vector *pEGAD-AIM-mCherry*. The vector *pEGAD-mCherry* was developed by double enzyme digestion of the *pEGAD-AIM-mCherry* with *EcoRI* and *HindIII* enzymes, followed by filling in and blunted by DNA polymerase and ligated with T4 ligase.

To develop the *pEGAD-nCBL1-GFP-ATG8a* plasmid, the nCBL1-GFP-ATG8a fragment was first produced using three-primer PCR with the primers Li1961, Li1962, and Li0023 (the ratio of Li1961 to Li1962 equals 20 : 1) and then cloned into the *AgeI* and *BamHI* restriction sites of the pEGAD vector.

To develop GFP-binding protein (GBP)-containing vectors, a plant codon-optimized GBP allele was synthesized by Tsingke Co. based on the amino acid sequences of GBP (Rothbauer *et al.*, 2006) and then inserted into the *pEGAD-AIM-mCherry* and

pEGAD-mAIM-mCherry vectors between the *HindIII* and *SpeI* sites by restriction enzyme digestion and T4 ligation. TGA5 cDNA was PCR-amplified from *Arabidopsis* cDNA with the primers Li1536 and Li1522 and then inserted into the vector

pEGAD between the *EcoRI* and *BamHI* sites to generate the *pEGAD-GFP-TGA5* plasmid. PEX3 cDNA was PCR-amplified from *Arabidopsis* cDNA with the primers Li1802 and Li1083, then introduced into the *pEGAD* vector between the *AgeI* and *AvrII*

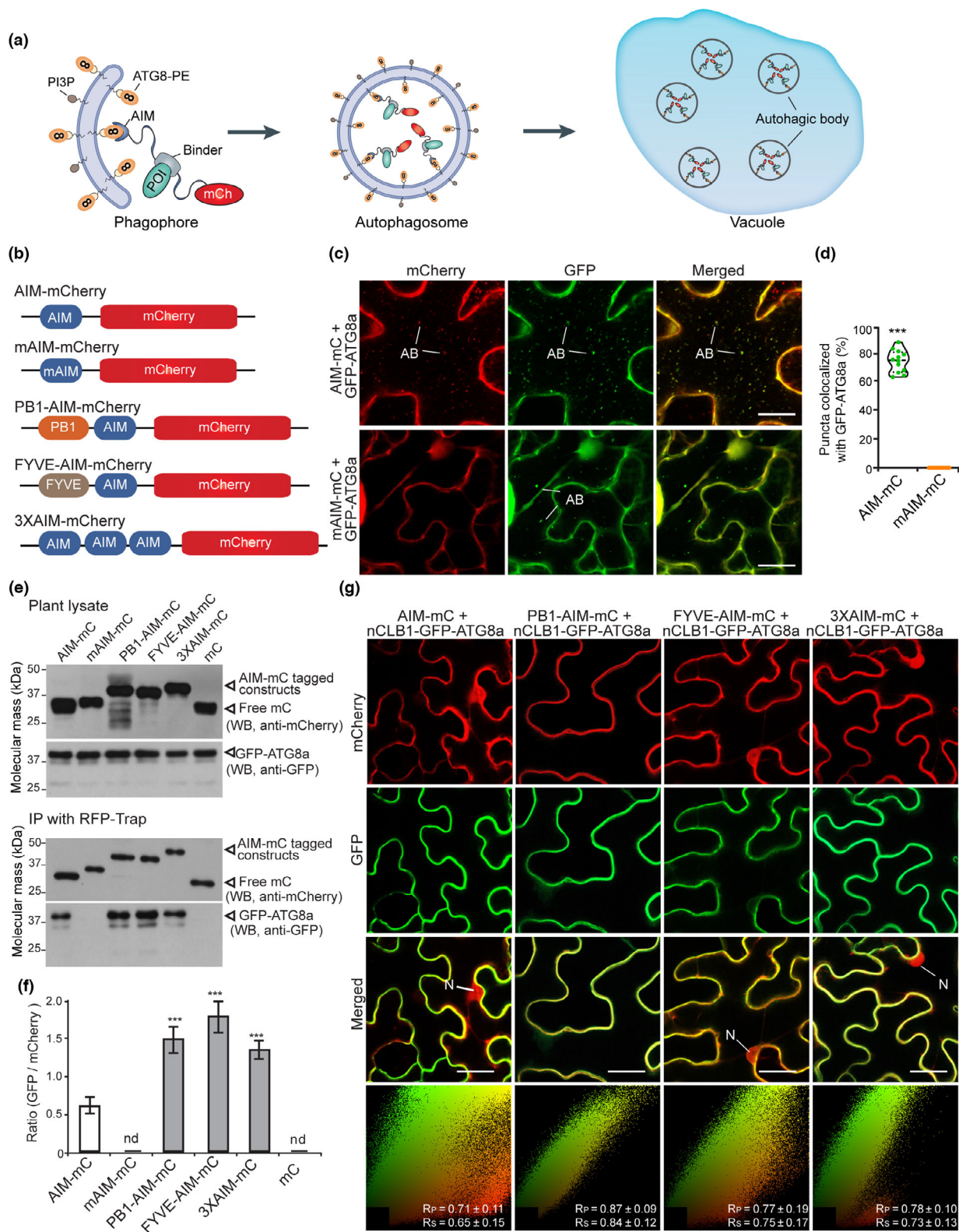


Fig. 1 Development of an ATG8-interacting motif (AIM)-based autophagy receptor for protein degradation. (a) Diagram of an AIM-based autophagy receptor. The autophagy receptor binds to a protein of interest (POI) via a specific binder and tethers it to expanding autophagosomes through the AIM. 8, ATG8; mC, mCherry fluorescent protein. (b) Constructs containing the AIM and additional motifs that facilitate autophagic degradation. mAIM, mutated AIM; PB1, an oligomerization domain derived from *Arabidopsis* AtNBR1 protein; FYVE, a PI(3)P-binding domain; 3XAIM, triple repeats of AIM. (c) Representative confocal images showing the colocalization of AIM-mCherry with the autophagy marker GFP-ATG8a in the vacuole. *Nicotiana benthamiana* leaf epidermal cells were co-infiltrated with GFP-ATG8a and AIM-mCherry (AIM-mC) or mAIM-mCherry (mAIM-mC) and then analyzed with confocal microscopy 36 h after agroinfiltration followed by a 16-h incubation with 1 μ M of the autophagy inhibitor concanamycin A (ConA). AB, autophagic body; Bars, 10 μ m. (d) Quantification of (m)AIM-mCherry puncta colocalizing with autophagy marker GFP-ATG8a. Images ($n = 12$) similar to those shown in (c) were collected to calculate the percentage of (m)AIM-mCherry puncta showing fluorescence at the green channel. In the violin plots, each individual value is represented by a dot, and three lines (from the bottom to the top) in each plot show the location of the lower, the median, and the upper quartiles, respectively. Asterisks indicate that the ratio of AIM-mCherry puncta colocalizing with GFP-ATG8a is significantly different from the value of mAIM-mCherry according to the *t*-test. ***, $P < 0.001$. (e) Co-immunoprecipitation assay showing interactions between ATG8 and various AIM-containing proteins. Total proteins were extracted from *Nicotiana benthamiana* leaves co-infiltrated with GFP-ATG8a and various AIM-containing constructs, followed by immunoprecipitation with RFP-Trap, after which they were detected with the indicated antibodies. (f) Quantification of co-immunoprecipitated GFP-ATG8a with various AIM-containing peptide ratios using densitometric scans of the immunoblots shown in (e). Bars represent the mean (\pm SD) of three biological replicates. Columns marked with asterisks represent means that are significantly different from AIM-mCherry control according to the *t*-test. ***, $P < 0.001$. nd, not detectable. (g) Confocal imaging showing an nCBL1-GFP-ATG8a-based co-translocation assay and its targeting of various AIM-mCherry constructs to the plasma membrane when co-expressed. nCBL1-GFP-ATG8a was co-expressed together with various AIM-mCherry constructs in *N. benthamiana* leaf epidermal cells for 36 h and then analyzed using confocal microscopy. Bars, 10 μ m. The bottom of each row shows the corresponding scatterplot obtained using the PSC colocalization plug-in in IMAGEJ. The overlapping percentage of the fluorescent signals is indicated by the linear Pearson correlation coefficient (Rp) and the nonlinear Spearman correlation coefficient (Rs). Values are presented as mean \pm SD; $n \geq 15$ optical section images from three biological replicates. N, nucleus.

sites, followed by replacing green fluorescent protein (GFP) with YFP to generate the *pEGAD-PEX3-YFP* construct. To develop the mCerulean-tagged peroxisome marker, mCerulean-PTS1 was produced by PCR amplified from mCerulean-containing plasmid with the primers Li1575 and Li1733 and then introduced into the *pEGAD* vector between the *Age*I and *Bam*HI sites.

To create the *AIM-BZR1C82-mCherry* and *mAIM-BZR1C82-mCherry* vectors, a sequence encoding the C82 fragment of BZR1 (amino acids 255–336) was PCR-amplified from *Arabidopsis* cDNA using the primers Li1959 and Li1960 and introduced into the *pEGAD-AIM-mCherry* and *pEGAD-mAIM-mCherry* vectors, respectively, between the *Hind*III and *Spe*I sites. The *AIM-BZR1C82-mCherry* fragment was then PCR-amplified from the *pEGAD-AIM-BZR1C82-mCherry* vector with the primers Li1959 and Li1960, and the AIM-mCherry fragment was replaced within the *pEGAD-PB1-AIM-mCherry* vector by *Hind*III and *Spe*I double enzyme digestion to create the *pEGAD-PB1-BZR1C82-mCherry* vector.

Plant materials and growth conditions

Wild-type *Arabidopsis thaliana* (L.) Heynh ecotype Col-0 was used as the wild-type control; the *defective primary root 2* (*dpr2*) mutant (Zou *et al.*, 2019), transgenic lines *PEAMT1-GFP* (Zou *et al.*, 2019), and *BIN2-GFP* (Peng *et al.*, 2008) were described previously. All *Arabidopsis* seeds were surface-sterilized using the vapor phase method and stratified by incubating with water at 4°C for 2 d. The seeds then germinated on 1 \times Murashige and Skoog (MS) solid medium (4.3 g l⁻¹ MS basal salts, 1% (w/v) sucrose, 0.05% (w/v) MES, pH 5.7, and 0.7% (w/v) agar) at 22°C under a long-day (LD) (16 h : 8 h, light : dark) photoperiod. When reaching the four-leaf stage, seedlings were transferred to soil and grown at 22°C under LD conditions. *Nicotiana benthamiana* was grown at 25°C, 60% humidity and under an LD photoperiod.

To generate transgenes expressing *AIM-GBP-mCherry* or *mAIM-GBP-mCherry*, the sequencing-verified constructs were

respectively delivered into the *Agrobacterium tumefaciens* strain GV3101 and then transformed into the *PEAMT1-GFP dpr2* background via the floral dip method (Clough & Bent, 1998). Homozygous *PEAMT1-GFP dpr2* plants expressing *AIM-GBP-mCherry* or *mAIM-GBP-mCherry* were obtained in T3 generation by antibiotic resistance and fluorescence microscopy observation. The protein levels of AIM-GBP-mCherry and mAIM-GBP-mCherry were determined by western blot with anti-mCherry antibodies. Using the same approach, *AIM-BZR1C82-mCherry*, *PB1-AIM-BZR1C82-mCherry*, *mAIM-BZR1C82-mCherry*, and *AIM-mCherry* were respectively transformed into the *BIN2-GFP* transgenic background to generate transgenes expressing various BZR1C82-mCherry-tagged proteins.

The free GFP (or mCherry) release assays were conducted as previously described (Huang *et al.*, 2019). One-week-old seedlings expressing *PEAMT1-GFP* and *AIM-GBP-mCherry* or *mAIM-GBP-mCherry* grown on MS solid medium were transferred to MS liquid medium lacking nitrogen and incubated under continuous light for 16 h.

Root length measurement

The root lengths of transgenes in the *PEAMT1-GFP dpr2* background were measured according to the method described previously (Zou *et al.*, 2019). Briefly, seeds were stratified at 4°C in distilled water for at least 6 d after surface sterilization, then germinated on 1/2 MS solid medium, and grown vertically. After 8 d, seedlings were imaged, and the root length was determined using IMAGEJ software (<https://imagej.nih.gov/>). The experiments were repeated three times with similar results.

Transient expression in *N. benthamiana*

In planta transient expression was performed by leaf agroinfiltration as described previously (Schornack *et al.*, 2009). All 35 S promoter-based *A. tumefaciens* T-DNA binary constructs were

introduced into the *Agrobacterium* strain GV3101. Overnight *Agrobacterium* cultures were harvested and adjusted to $OD_{600\text{ nm}} = 0.8$ with infiltration medium (10 mM $MgCl_2$, 5 mM 2-(N-Morpholino)ethanesulfonic acid (MES), pH 5.6, and 100 μ M acetosyringone) before agroinfiltration. The viral silencing suppressor P19-containing plasmid pCB301-p19 (Win & Kamoun, 2004) was also co-infiltrated to increase protein expression and signal intensity. Leaf disks were examined 36–48 h after infiltration by confocal fluorescence microscopy. For concanamycin A (ConA) treatment, leaves were infiltrated with 1 μ M ConA (SC-202111; Santa Cruz Biotechnology, Santa Cruz, CA, USA) solution 36 h after *Agrobacterium* infiltration. The infiltrated leaves were then detached, placed into Petri dishes containing several layers of wet filter paper, and placed in darkness. Leaf disks were examined 16 h after ConA infiltration by confocal fluorescence microscopy.

Fluorescence confocal microscope imaging and analysis

The confocal imaging of stable transgenic lines expressing (*m*) *AIM-GFP-mCherry* in the *PEAMT1-GFP dpr2* background and (*m*) *AIM-BZR1C82-mCherry* in the *BIN2-GFP* background was conducted as described previously (Suttangkakul *et al.*, 2011; Huang *et al.*, 2019). Briefly, 6-d-old seedlings grown on MS solid medium were transferred to fresh MS liquid medium with or without ConA, and the images of the cell fluorescence within the root elongation zone were acquired. For infiltrated tobacco leaves, several leaf squares (0.5 × 0.5 cm) surrounding the infiltration point were cut and mounted in tap water. Confocal fluorescent microscopy was performed on a Zeiss LSM 800 laser scanning confocal microscope (Carl Zeiss, <https://www.zeiss.com>). For imaging of the coexpression of GFP/YFP and mCherry constructs, excitation wavelengths of 488 nm for GFP/YFP and 543 nm for mCherry were used alternatively in the multitrack mode of the microscope with line switching. For imaging of coexpression of mCerulean, YFP and mCherry constructs, excitation lines of 458 nm for mCerulean, 514 nm for YFP, and 543 nm for mCherry were used. Scanning was performed by sequential frame switching to prevent signal bleed-through.

Quantitative analysis of confocal microscope images was performed by using IMAGEJ (NIH) as described by Kim *et al.* (2021). Briefly, quantification of colocalization between mCherry puncta with GFP-ATG8A-positive vesicles was performed by using the Comdet plug-in for IMAGEJ (<https://imagej.net/plugins/spots-colocalization-comdet>). Fluorescence colocalization analysis was performed using the PSC colocalization plug-in, and Pearson correlation coefficients were calculated as described previously (French *et al.*, 2008). Quantification of mCherry puncta in tobacco leaves was performed by using whole frame of fluorescence image (106.59 μ m × 106.59 μ m).

Co-immunoprecipitation analysis

The Co-IP assay for testing the interactions between various AIM-containing proteins and ATG8 was performed as previously described (H. Li *et al.*, 2019). mCherry-tagged AIM-containing

constructs and GFP-ATG8a were co-expressed transiently in *N. benthamiana* leaves, and samples were harvested 30 h after infiltration. 0.5 g samples were homogenized in 2 ml lysis buffer (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1 mM $MgCl_2$, 20% glycerol, 0.2% NP-40, and 1× protease inhibitor cocktail from Roche) for protein extraction. Cell lysates were clarified by centrifugation at 13 000 *g* at 4°C for 10 min and then mixed with anti-RFP antibody coupled agarose beads (rtma-10; ChromoTek, Munich, Germany) for 2 h at 4°C with gentle rotation. After incubation, the beads were washed three times with wash buffer (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1 mM $MgCl_2$, 20% glycerol, and 0.01% NP-40), and the bound proteins were eluted by boiling in 2× SDS sample buffer, subjected to SDS-PAGE separation, and detected by immunoblot with anti-GFP (ab290; 1 : 3000; Abcam, Cambridge, UK) or anti-mCherry (B1153; 1 : 3000; Biodragon, Beijing, China) antibodies. Protein levels were quantified densitometrically using TOTALLAB™ software as previously described (Huang *et al.*, 2019).

Protein isolation and immunoblot analyses

Total protein was extracted from infiltrated tobacco leaves or *Arabidopsis* seedlings by homogenizing in SDS-PAGE sample buffer containing 10% (v/v) 2-mercaptoethanol. The homogenates were vortexed for 5 min, boiled at 100°C for 5 min, and clarified at 13 000 *g* for 10 min. The supernatants were then separated by SDS-PAGE and transferred onto polyvinylidene difluoride membranes (IPVH00010; Millipore) for immunoblot analysis. Antibodies against ATG8 were as described (Thompson *et al.*, 2005). Antibodies against BIN2, GFP, histone H3, and mCherry were purchased from Youke Biotechnology (Shanghai, China) (YKZPK72), Roche Applied Science (Indianapolis, IN, USA) (11814460001), Abcam (ab1791), and Biodragon (B1153), respectively. Blots were developed using BeyoECL Plus kit (P0018S; Beyotime, Shanghai, China) or BeyoECL Star kit (P0018AS; Beyotime), according to the manufacturer's instructions.

Expression analysis of ATG genes

Total RNA was isolated by using the Plant RNAout kit (160906-50; Tiandz Inc., Beijing, China) from 7-d-old seedlings grown on MS medium and was converted to cDNA via HiScript® II Q RT SuperMix for qPCR Kit (R222-01; Vazyme Biotech Co. Ltd) with oligo(dT)20 primers and then used as templates for quantitative RT-PCR with gene-specific primers (Table S1). All PCR reactions were carried out on a Bio-Rad CFX96™ real-time PCR system using ChamQ Universal SYBR qPCR Master Mix (Q711; Vazyme Biotech Co. Ltd). *Arabidopsis UBQ10* was used as an internal control, and the relative expression level of each ATG gene was determined using the $2^{-\Delta\Delta C_t}$ method. Three biological replicates were performed for each gene.

Statistical analysis

Data reported in this study are mean ± SD of three independent experiments unless otherwise indicated. The significance of the

differences between groups was determined by a two-tailed Student's *t*-test. *P*-values of < 0.05 or < 0.01 were considered significant. The quantification of PEAMT1-GFP-labeled puncta was statistically analyzed by one-way ANOVA followed by Tukey *post hoc* test, and quantifications of root length and rosette size were statistically analyzed using a nonparametric Kruskal–Wallis test.

Results

Principle of AIM-based autophagy receptors

Using a tobacco (*N. benthamiana*)-based transient expression system, we first tested whether our proposed AIM (derived from the *Arabidopsis* autophagy receptor AtNBR1; Svenning *et al.*, 2011; Fig. 1b), when fused to the nonautophagy substrate mCherry, could efficiently target mCherry to the vacuole. As expected, numerous puncta were readily detected in the vacuole of *AIM-mCherry*-expressing cells when treated with ConA, a specific inhibitor of vacuolar-type ATPases (Dröse *et al.*, 1993; Dettmer *et al.*, 2006), whereas the untagged *mCherry* control was predominantly diffused in the cytosol and nucleus regardless of ConA treatment (Fig. S1). We then confirmed using colocalization studies that the puncta were autophagic bodies, by co-expressing *AIM-mCherry* and the autophagic marker *GFP-ATG8a*; most vacuolar puncta were labeled with both fluorescent proteins (Figs 1c, S2, S3). Mutations of the core hydrophobic residues in an AIM impair its ATG8-binding capability (Svenning *et al.*, 2011). Accordingly, mCherry fused with mutated AIM (mAIM-mCherry) was mainly distributed in the cytosol (Figs 1c, S1, S2). Together, the data demonstrated that tagging an AIM to a nonautophagy substrate/peptide confers autophagic degradation to the latter.

To optimize the targeting efficiency of the AIM-containing peptide, we generated the following three constructs by adding extra domains (Fig. 1b): PB1-AIM-mCherry contains the Phox and Bem1 (PB1) domain of AtNBR1 to promote its polymerization; FYVE-AIM-mCherry possesses the membrane-tethering domain FYVE (Fab-1, YGL23, Vps27, and EEA1), which serves as an additional anchor to tether the receptor to the autophagosomal membranes; and, 3XAIM has a triplicate AIM to improve its binding strength. By independently co-expressing each construct with GFP-ATG8a, we determined that all these three constructs had higher ATG8-binding affinity than AIM-mCherry using Co-IP, while neither mCherry nor mAIM-mCherry showed any interaction with ATG8, which was consistent with our microscopic observations (Fig. 1e,f).

Subsequently, we applied a Calcineurin B-like protein 1 (CBL1)-driven plasma membrane co-translocation assay to test the targeting efficiency of these constructs in a plant cellular environment (Baticic *et al.*, 2008). nCBL1-GFP-ATG8a (GFP-ATG8a fused to the C terminus of CBL1) was anchored to the plasma membrane by the lipidation modification on CBL1, causing the co-translocation of AIM-containing proteins but not free mCherry or mAIM-mCherry due to the interaction between ATG8 and AIM motif (Fig. S4). When expressed individually, the subcellular localizations of various AIM-containing peptides

were similar to that of AIM-mCherry, with the exception of FYVE-AIM-mCherry, which was also found in some rapidly moving vesicular structures in addition to the cytosol, indicating that the addition of extra domain(s) did not affect the vacuolar targeting of AIM-mCherry (Figs S5, S6; Video S1). When co-expressed with nCBL1-GFP-ATG8a, these three peptides all displayed improved co-translocation with nCBL1-GFP-ATG8a compared to that with AIM-mCherry (Fig. 1g), with PB1-AIM-mCherry having the highest targeting efficiency. Taken together, these data suggest that AIM triplication or the addition of extra domains can be used to enhance the binding to ATG8, although the addition of the FYVE domain could mistarget ATG8 to certain nonautophagic vesicles.

AIM-based autophagy receptors can target diverse GFP-tagged substrates

We next examined whether a nonautophagic substrate could be targeted by an engineered autophagy receptor through protein–protein interactions. GFP-binding protein is the variable domain of a llama (*Lama glama*) heavy-chain antibody that can bind with high affinity to GFP and its variant YFP, but not CFP (Rothbauer *et al.*, 2006; Schornack *et al.*, 2009). We created a GBP-containing receptor (AIM-GBP-mCherry) to knock down GFP-tagged proteins (Figs 2a, S7). We first tested this system using TGA5 (Kim & Delaney, 2002), a nucleus-localized bZIP transcription factor from *Arabidopsis*. When expressed in tobacco, the GFP-TGA5 signal localizes exclusively in the nucleus regardless of ConA treatment, suggesting that it is not an autophagy substrate (Fig. S8a). When co-expressed with *AIM-GBP-mCherry* but not *mAIM-GBP-mCherry*, GFP-TGA5-labeled puncta accumulated in the vacuole, colocalizing with AIM-GBP-mCherry upon ConA treatment, indicating the latter's capability to deliver a GFP fusion protein to the vacuole (Figs 2b, S8c). In addition to the GFP-TGA5 fusion, another representative, the plasma membrane-anchored brassinosteroid (BR) receptor Brassinosteroid insensitive 1 (BRI1)-GFP, also showed autophagic degradation in the presence of AIM-GBP-mCherry (Figs 2d, S8b,d). We then further confirmed that the GFP-TGA5 and BRI1-GFP fusions were targeted for autophagic turnover using the free GFP release assay, an approach that measures autophagy-dependent vacuolar transport (Marshall & Vierstra, 2018). As shown in Fig. 2c, free GFP derived from GFP-TGA5 is readily detected when co-expressed with AIM-GBP-mCherry, but not with mAIM-GBP-mCherry regardless of ConA, indicating that GFP-TGA5 can be degraded through autophagy mediated by AIM-GBP-mCherry. Free GFP released from BRI1-GFP could be detected both in AIM-GBP-mCherry and in mAIM-GBP-mCherry samples, but accumulated more in AIM-GBP-mCherry samples (Fig. 2e). The accumulation of free GFP in mAIM-GBP-mCherry samples could be due to endocytosis-mediated degradation as endocytosis is a well-known mechanism for BRI1 activity regulation (Geldner *et al.*, 2007). The confocal and immunoblot results clearly demonstrate that the AIM-GBP-mCherry receptor can promote the autophagic degradation of GFP fusion proteins.

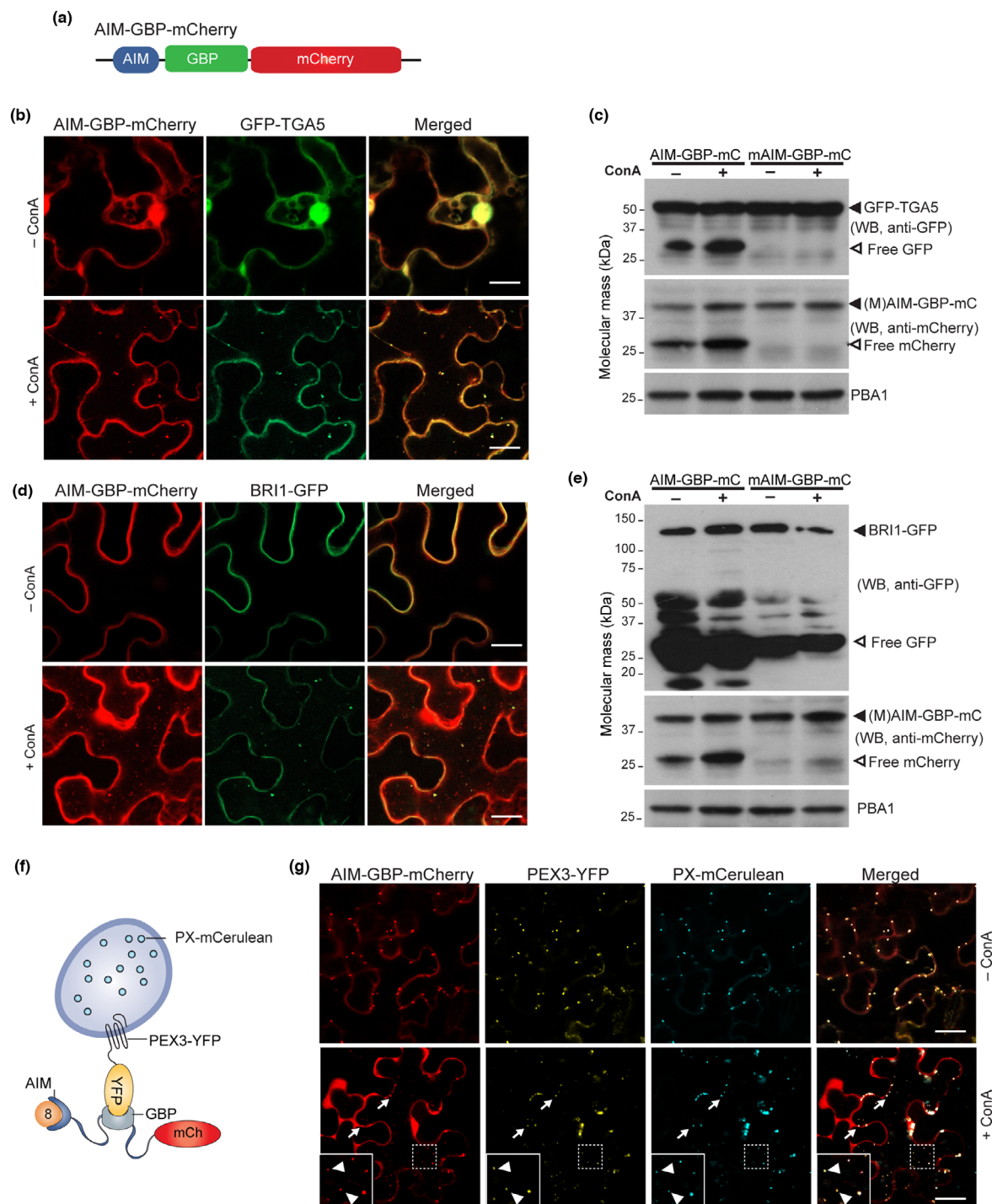


Fig. 2 GFP-binding protein-containing autophagy receptor is sufficient for directing GFP/YFP-tagged proteins and peroxisomes to the vacuole for degradation. (a) Diagram of the AIM-GBP-mCherry construct. GBP, GFP-binding protein. (b, d) AIM-GBP-mCherry targets GFP-tagged TGA5 (a nucleus-localized bZIP transcription factor; (b) and BRI1 (plasma membrane-anchored brassinosteroid receptor; (d) to the vacuole. *Nicotiana benthamiana* leaf epidermal cells were co-infiltrated with AIM-GBP-mCherry and GFP-TGA5 or BRI1-GFP, then analyzed using confocal microscopy 36 h after agroinfiltration and subsequent 16-h incubation with 1 μ M concanamycin A (+ConA) or DMSO (-ConA). Bars, 10 μ m. (c, e) Immunoblot analysis showing that autophagic receptor AIM-GBP-mCherry promotes autophagic degradation of GFP-TGA5 and BRI1-GFP. Total proteins were extracted from *N. benthamiana* leaves in (b, d) and immunoblotted with anti-GFP or anti-mCherry antibodies. 20 S proteasome subunit PBA1 was used to confirm near-equal protein loading. (f) Illustration of the AIM-GBP-mCherry and PEX3-YFP constructs for promoting pexophagy. PEX3-YFP anchors to the peroxisomal membrane with its C-terminal YFP exposed to the cytosol, where it is recognized by AIM-GBP-mCherry for autophagic degradation. (g) Confocal imaging showing that AIM-GBP-mCherry directs PEX3-YFP-anchored peroxisomes to the vacuole. *Nicotiana benthamiana* leaf epidermal cells were co-infiltrated with PEX3-YFP, AIM-GBP-mCherry, and PX-mCherry (peroxisome matrix marker), then analyzed using confocal microscopy 36 h after agroinfiltration and subsequent 16-h incubation with 1 μ M ConA. Insets show 2.5 \times magnifications of the colocalized signals of the three proteins (outlined by the white dash box). Arrows indicate peroxisomes in the cytosol, while arrowheads indicate peroxisomes in the vacuole lumen. Bars, 10 μ m.

To test whether the GBP-containing receptor can perceive a larger GFP-tagged substrate, such as a membrane-bound organelle, we labeled a peroxisome with YFP at the cytoplasmically exposed C terminus tail of *Arabidopsis* PEX3 (peroxisomal biogenesis factor 3; Soukupova *et al.*, 1999), a peroxisomal membrane protein (Fig. 2f). The PEX3-YFP signal was observed as ring-like structures surrounding PX-mCerulean, a peroxisomal luminal mCerulean marker protein appended with the well-characterized peroxisomal targeting signal 1 (PTS1; Fig. S9). When co-expressed, AIM-GBP-mCherry colocalized with PEX3-YFP on rapidly moving punctate structures resembling peroxisomes, in addition to its accumulation in the cytosol (Fig. S10a). Higher-resolution microscopic imaging revealed that the fluorescent pattern of AIM-GBP-mCherry mostly overlapped with that of PEX3-YFP. By contrast, AIM-mCherry remained in the cytosol when co-expressed with PEX3-YFP (Fig. S10b). These results strongly suggest that the AIM-GBP-mCherry receptor can attach to peroxisomes through the interaction between GBP and PEX3-YFP.

To determine whether AIM-GBP-mCherry targets PEX3-YFP-coated peroxisomes for vacuolar degradation, we further co-expressed *AIM-GBP-mCherry*, *PEX3-YFP*, and *PX-mCerulean*. Whereas most of the three fluorescent proteins colocalized in the cytosol as punctate structures before the ConA treatment, some colocalized puncta of these fluorescent proteins appeared in the vacuole upon ConA treatment (Fig. 2g). In addition, confocal time-lapse imaging revealed that the colocalized puncta of AIM-GBP-mCherry and PEX3-YFP exhibited two distinct types of movement: one characterized by rapid movement along the plasma membrane and the other by random movement, which is typical for autophagic bodies within the vacuolar lumen (Video S2). By contrast, coexpression of *mAIM-GBP-mCherry* with *PEX3-YFP* and *PX-mCerulean* failed to detect the colocalized puncta of mAIM-GBP-mCherry and PEX3-YFP in the vacuole (Fig. S11). Taken together, the above data demonstrate that the AIM-GBP-mCherry receptor can bind YFP-coated peroxisomes and mediate their autophagic degradation.

AIM-based autophagy receptors mediate GFP fusion protein degradation in transgenic plants

Having confirmed that AIM-GBP-mCherry could mediate the autophagic degradation of GFP fusion proteins when expressed transiently, we next asked whether this approach could be applied to stably expressed GFP fusions. For this purpose, we chose the *Arabidopsis* mutant *defective primary root 2* (*dpr2* or *peamt1*) carrying the rescue transgene *PEAMT1-GFP* (Zou *et al.*, 2019). *PEAMT1* encodes phosphoethanolamine N-methyltransferase 1, an enzyme essential for the biosynthesis of the phospholipid phosphatidylcholine. The loss of *PEAMT1* affected the reactive oxygen species- and auxin-regulated cell differentiation in the root apical meristem and caused a short primary root phenotype (Zou *et al.*, 2019). We reasoned that, if the expression of *AIM-GBP-mCherry* could knock down *PEAMT1-GFP*, the loss-of-function phenotypes of *dpr2* would be phenocopied. We compared the primary root lengths of *PEAMT1-GFP dpr2* to those of

PEAMT1-GFP dpr2 in which *AIM-GBP-mCherry* or *mAIM-GBP-mCherry* was introduced, along with wild-type (WT) and *dpr2* seedlings. As expected, transgenic lines expressing *mAIM-GBP-mCherry* showed a normal primary root length, whereas lines expressing *AIM-GBP-mCherry* showed short primary root phenotype similar to the *dpr2* mutant (Fig. 3a,b), albeit to a slightly lesser extent.

To determine whether the phenocopy of the *dpr2* mutant is caused by the autophagic degradation of *PEAMT1-GFP* mediated by AIM-GBP-mCherry, we first applied confocal microscopy imaging to track the subcellular localization of *PEAMT1-GFP*. It was mainly localized in the cytosol of root cells and showed no obvious change in subcellular localization upon ConA treatment (Fig. S12); however, *PEAMT1-GFP*-labeled puncta were readily detected in the vacuoles of *PEAMT1-GFP AIM-GBP-mCherry dpr2* seedlings when treated with ConA, whereas they were absent in similarly treated *PEAMT1-GFP mAIM-GBP-mCherry dpr2* seedlings (Fig. 3c,d). We then used free GFP release assay to test whether the *PEAMT1-GFP* fusion was targeted for autophagic degradation. While we detected fused GFP forms in all tested lines using an immunoblot analysis, we observed free GFP forms only in seedlings expressing *AIM-GBP-mCherry*, not in *PEAMT1-GFP dpr2* or seedlings expressing *mAIM-GBP-mCherry* (Fig. 3e). Moreover, a strong accumulation of free GFP was seen when autophagy activity is induced by nitrogen starvation (Fig. 3e). From the above experiments, we concluded that AIM-GBP-mCherry is indeed able to mediate the autophagic degradation of *PEAMT1-GFP*, thus phenocopying the loss-of-function mutant *dpr2*.

AIM-based autophagy receptors can be used to degrade endogenous BIN2 protein

As described in the previous section, in principle, it should be possible to degrade nongenetically modified native proteins instead of GFP fusions by replacing the GBP with alternative binders that have a high affinity to the target POIs. To this end, we decided to target *Arabidopsis* Brassinosteroid insensitive 2 (BIN2), a GSK3-like kinase that inhibits BR downstream signal transduction by phosphorylating the transcription factors BRI1-EMS suppressor 1 (BES1) and Brassinazole resistant 1 (BZR1), thus affecting plant architecture, including rosette size, petiole length, and plant stature (Li & Nam, 2002). The protein abundance of BIN2 is tightly regulated by the UPS (Peng *et al.*, 2010; Zhu *et al.*, 2017). To degrade endogenous BIN2 via the autophagy pathway, we designed an autophagy receptor bearing the C82 fragment (amino acids 255–336), a BIN2-binding sequence from BZR1 (Peng *et al.*, 2008). A transient expression experiment showed that the designed constructs *AIM-BZR1C82-mCherry* and *PB1-AIM-BZR1C82-mCherry*, but not *mAIM-BZR1C82-mCherry*, could target BIN2 for autophagic degradation (Fig. S13).

We then created transgenic plants by expressing the *AIM-BZR1C82-mCherry* construct in a *BIN2-GFP* line, which displays a similar but weaker BR signaling-deficient phenotype than that of gain-of-function *bin2-1* mutant due to overexpression of BIN2-GFP (Peng *et al.*, 2008; Fig. 4a). For control and

comparison, we also created transgenic plants expressing *PB1-AIM-BZR1C82-mCherry*, *mAIM-BZR1C82-mCherry*, or *AIM-mCherry* individually. As expected, the resulting transgenic plants expressing *AIM-mCherry* showed no changes in their rosette

phenotypes, whereas the majority of transgenic plants expressing *AIM-BZR1C82-mCherry* or *PB1-AIM-BZR1C82-mCherry* produced bigger rosettes with visible petioles and flatter leaves than the *BIN2-GFP* control (Fig. S14). Notably, *c.* 30% of transgenic

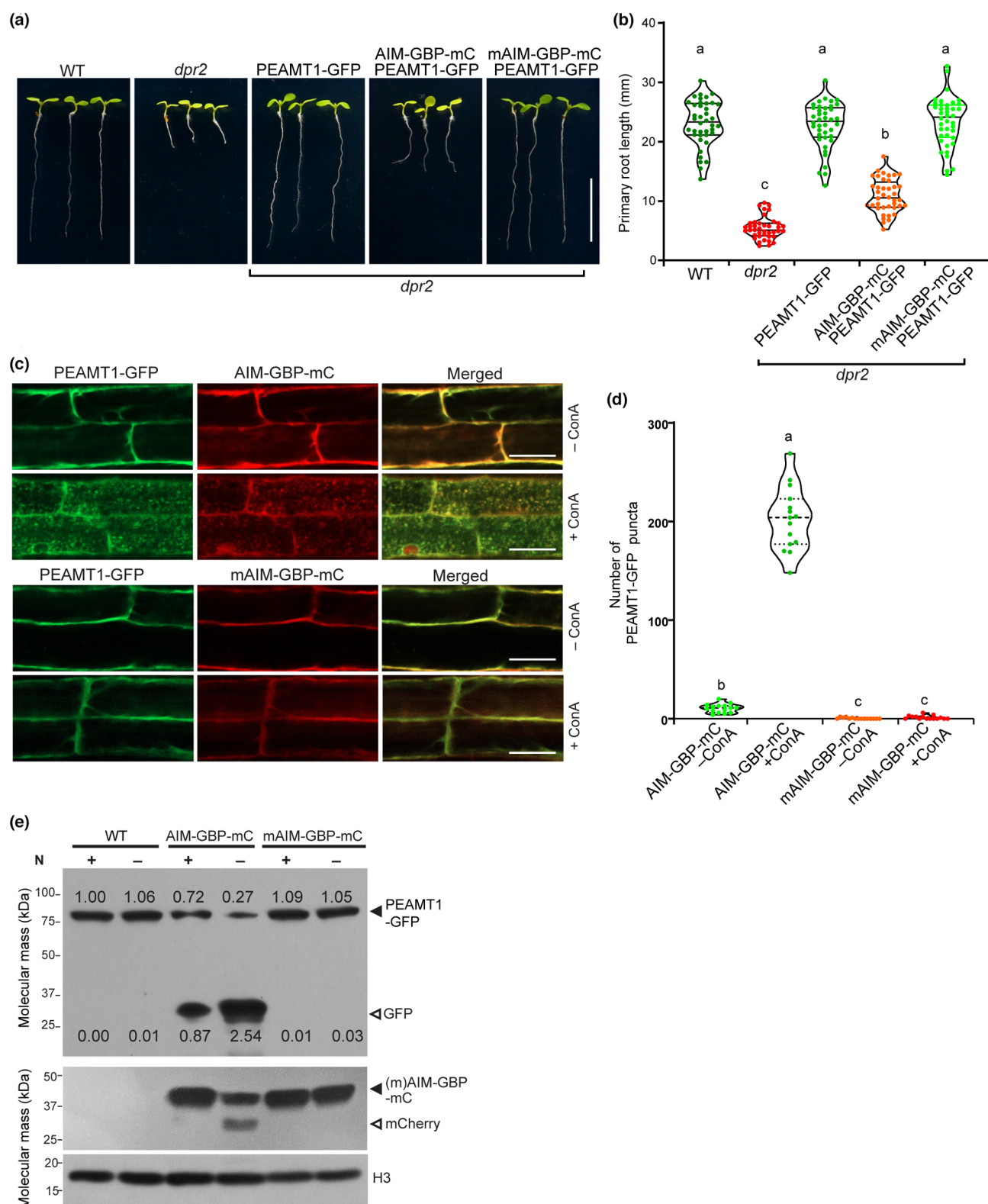


Fig. 3 GFP-binding protein-containing peptide directs PEAMT1-GFP to the vacuole for autophagic degradation. (a, b) Root phenotype of representative *Arabidopsis* plants expressing *AIM-GBP-mCherry* (AIM-GBP-mC) or *mAIM-GBP-mCherry* (mAIM-GBP-mC) in the *PEAMT1-GFP dpr2* background. For each construct, three transgenic lines with comparable levels of mCherry protein were selected for further phenotypic analysis. Root lengths of these representative lines, as well as those of the wild-type (Col-0), *dpr2* mutant, and *PEAMT1-GFP dpr2* rescued line, were measured at 8 d after germination and shown in (b). Violin plots show the distribution of primary root length ($n > 30$ seedlings per genotype from three independent experiments). Each individual value is represented by a dot, and three lines (from the bottom to the top) in each plot show the location of the lower, the median, and the upper quartiles, respectively. Data are mean \pm SD. Different letters indicate statistically significant differences ($P < 0.05$), as determined with a nonparametric Kruskal–Wallis test. Bar, 1 cm. (c, d) Deposition of PEAMT1-GFP-containing vesicles in the vacuole requires a functional GBP-containing autophagy receptor. Plants co-expressing *PEAMT1-GFP* together with *AIM-GBP-mCherry* or *mAIM-GBP-mCherry* were grown for 6 d on MS solid medium and transferred to MS liquid medium containing 1 μ M concanamycin A (ConA) for an additional 24 h. Root cells were imaged using confocal fluorescence microscopy. The quantification of PEAMT1-GFP-labeled puncta is shown in (d). Images ($n = 15$) were collected to calculate the number of PEAMT1-GFP puncta per frame (mean \pm SE). In the violin plots, each individual value is represented by a dot and three lines (from the bottom to the top) in each plot show the location of the lower, median, and upper quartiles, respectively. Different letters indicate statistically significant differences ($P < 0.05$), as determined using one-way ANOVA followed by Tukey's test. Bars, 10 μ m. (e) Nitrogen starvation promotes autophagic degradation of PEAMT1-GFP in plants expressing a functional GBP-containing autophagy receptor. One-week-old plants expressing *PEAMT1-GFP* alone, or co-expressing *PEAMT1-GFP* with *AIM-GBP-mCherry* or *mAIM-GBP-mCherry*, were grown on MS solid medium and then transferred to fresh MS liquid medium or medium lacking nitrogen (–N) for an additional 24 h. Total seedling protein extracts were immunoblotted with anti-GFP or anti-mCherry antibodies. Histone H3 was used to confirm near-equal protein loading. Numbers above bands of PEAMT1-GFP and below bands of free GFP indicate the ratio of the band to H3, normalized such that the ratio from the wild-type under nitrogen-rich conditions (WT, +) was set to 1.0.

plants expressing these two constructs had a rosette width comparable with wild-type seedlings. Four representative transgenic plants expressing *AIM-BZR1C82-mCherry* with varying phenotypes are shown in Fig. 4(a). Unexpectedly, about half of the plants expressing *mAIM-BZR1C82-mCherry* also showed improved rosette phenotypes, albeit to a lesser degree than *AIM-BZR1C82-mCherry* plants (Figs 4b, S14). A previous study by Nolan *et al.* (2017) has shown that autophagy can modulate BR signaling in plants through autophagy receptor DSK2-mediated degradation of BES1. Therefore, we examined the endogenous autophagy activity in the transgenes by qRT-PCR analysis of multiple *ATG* genes and immunoblot analysis of ATG8 proteins. As shown in Fig. S15, the expression levels of multiple *ATG* genes and the abundance of ATG8 proteins are comparable to those of wild-type control, indicating that the endogenous autophagy activity of these transgenes is not altered, and the recovery phenotype observed in *mAIM-BZR1C82-mCherry* plants was likely caused by interference with BIN2 by the C82 fragment within it.

We then examined the protein abundance of endogenous BIN2, BIN2-GFP, and *AIM-BZR1C82-mCherry* in the above four representative *AIM-BZR1C82-mCherry*-expressing plants using western blotting. As shown in Fig. 4(c), plants with bigger rosettes (#4) accumulated much higher levels of mCherry fusion proteins than those exhibiting *bin2-1* phenotypes (#1 and #2). Consistent with this result, plants with larger rosettes had significantly lower levels of BIN2-GFP or endogenous BIN2 than smaller plants. A similar immunoblot analysis performed with *mAIM-BZR1C82-mCherry* plants revealed that overexpression of *mAIM-BZR1C82-mCherry* does not change the protein levels of BIN2-GFP (Fig. S16). Microscopy observation of root cells also detected numerous puncta decorated with BIN2-GFP in the vacuole of seedlings expressing *AIM-BZR1C82-mCherry*, but not in *mAIM-BZR1C82-mCherry* plants (Fig. 4d), indicating the vacuolar degradation of BIN2-GFP mediated by *AIM-BZR1C82-mCherry*. Taken together, these data demonstrate the capability and specificity of BIN2-targeting peptides (*AIM-BZR1C82-mCherry* and *PB1-AIM-BZR1C82-mCherry*)

in the autophagic degradation of their endogenous binding partner BIN2.

Discussion

In this study, we established a new targeting method for degrading diverse fluorescently tagged or native proteins *in vivo* using the engineered AIM-based autophagy receptors. The efficient knockdown of various tagged proteins in the tobacco-based transient expression system (Figs 2b,c, S8) and in stable transgenic lines (Fig. 3), as well as the native BIN2 protein (Fig. 4), demonstrates the feasibility and versatility of this AIM-based method, providing proof-of-concept evidence for using it to efficiently degrade endogenous cytosolic proteins *in planta*.

Specificity is the most important property of a protein-targeting system. For our AIM-based autophagy receptors, their specificity is largely dependent on the specificity and affinity of the interaction between the binder and the POI, such as GBP and GFP. Besides the fluorescently tagged or native proteins tested in our study, AIM-based autophagy receptors may also be used for selectively targeting mutant protein variants or post-translationally modified proteins by utilizing specific binders. In addition, combined with other regulatory layers, such as tissue specificity or inducibility by small chemicals or environmental stresses, the method could also be applied to study protein functions in selected tissues or in certain growth stages. More intriguingly, because of the wide substrate range of selective autophagy, the method might be applicable to various cytosolic cargoes such as protein aggregates, organelles, DNA/RNA molecules, and even invading pathogens in plants. It has been demonstrated conceptually by using chimera AUTAC molecules to remove dysfunctional mitochondria (Takahashi *et al.*, 2019), by using ATTECs for targeting lipid droplets in mammalian cells (Fu *et al.*, 2021) and by using AIM-based degrader to degrade fluorescently tagged peroxisomes in this study (Fig. 2e), which is not possible using current nucleic acid-based methods.

The efficacy of AIM-based autophagy receptors also depends on their binding ability to ATG8. We optimized binding by

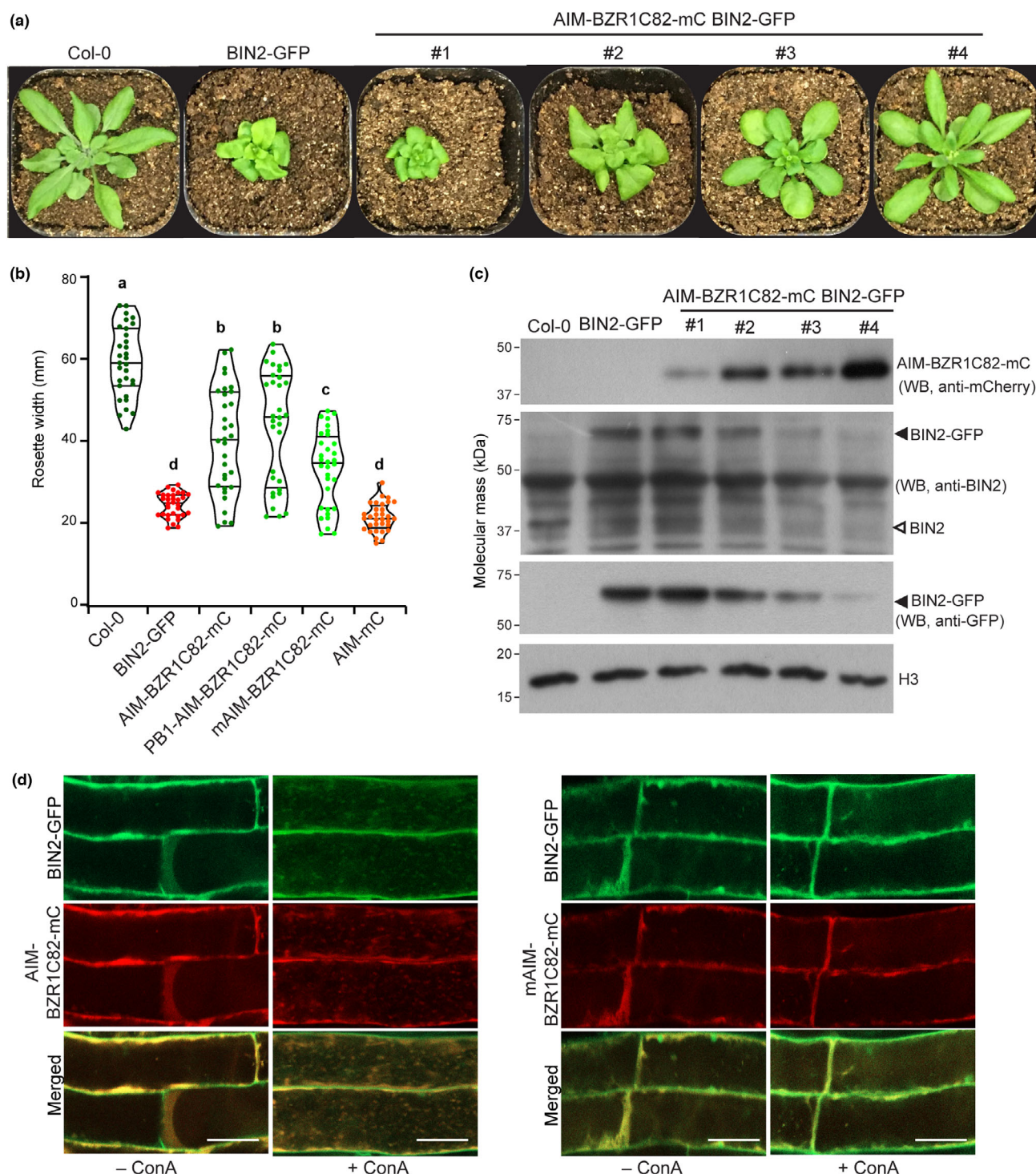


Fig. 4 ATG8-binding motif-based peptide targets BIN2 to the vacuole for autophagic degradation. (a) Four representative transgenic *Arabidopsis* plants expressing AIM-BZR1C82-mCherry in a BIN2-GFP transgenic background with varying phenotypes. Transgenic plants were grown along with the wild-type (Col-0) and BIN2-GFP under long-day (LD) conditions for 4 wk. (b) Rosette width of plants expressing AIM-BZR1C82-mCherry, PB1-AIM-BZR1C82-mCherry, mAIM-BZR1C82-mCherry, and AIM-mCherry in a BIN2-GFP background. For each construct, 32 T₁ seedlings were grown alongside Col-0 and BIN2-GFP under LD conditions for 4 wk, after which rosette width was measured. In the violin plots, each individual value is represented by a dot, and three lines (from the bottom to the top) in each plot show the location of the lower, median, and upper quartiles, respectively. Data are mean \pm SD. Different letters indicate statistically significant differences ($P < 0.05$), as determined with a nonparametric Kruskal–Wallis test. (c) Western blot analysis of AIM-BZR1C82-mCherry, BIN2-GFP, and endogenous BIN2 protein accumulation in the four transgenic lines shown in (a). Total protein extracts were separated using SDS-PAGE and analyzed by immunoblotting with anti-BIN2, anti-GFP, or anti-mCherry antibodies. Histone H3 was used to confirm near-equal protein loading. (d) Deposition of BIN2-GFP-containing vesicles in the vacuole requires functional AIM-BZR1C82 constructs. Plants co-expressing BIN2-GFP together with AIM-BZR1C82-mCherry or mAIM-BZR1C82-mCherry were grown for 6 d on MS solid medium and transferred to MS liquid medium containing 1 μ M concanamycin A (ConA) for an additional 24 h. Root cells were imaged using confocal fluorescence microscopy. Bars, 10 μ m.

AIM triplication and the introduction of additional anchor domains, such as PB1 and FYVE. And we showed that all these approaches could enhance the binding to ATG8 (Fig. 1c,g), which is consistent with the observation described by Stolz *et al.* (2017). However, the addition of an FYVE domain can mistarget ATG8 to certain nonautophagic vesicles (Fig. S5; Video S1). Another optimization approach worth trying is the introduction of negatively charged amino acid residues close to the AIM to improve its binding toward ATG8, which has been reported previously (Wild *et al.*, 2011). Phage display has been used to isolate specific binding peptides toward ATG8 in mammalian cells (Stolz *et al.*, 2017) and could be adapted to isolate new peptides that possess *bona fide* AIM with better affinity to plant ATG8 than that of AtNBR1.

The apparent limitation of this method is that it cannot degrade proteins involved in the autophagy machinery or vacuolar/lysosomal integrity. In addition, the proteins that reside in the endoplasmic reticulum, Golgi apparatus, or other endomembrane organelles generally are involved in co-translational translocation and seem not to be the potential cargo for our method as they are not accessible by autophagy machinery. However, these problems could be partially addressed by harnessing the UPS using a similar strategy. High-affinity binders targeting specific cargoes are vital for the development of AIM-based autophagy receptors. Here we have successfully designed the GBP-containing receptor for targeting GFP-tagged proteins and the BZR1C82-bearing receptor for targeting BIN2 and proven their efficacy. However, there are still many plant proteins and cargoes that lack high-affinity binders, which limits the applicability of AIM-based receptors in plant research. This limitation may be overcome by using methods such as phage display and peptide arrays, which can aid in the discovery of appropriate binders. Nanobodies, like GBP, are also beneficial in this context, because they have a low molecular weight and small size, and can be easily generated and effectively optimized (Wang *et al.*, 2021).

In summary, this study provides a novel tool for perturbing protein levels in plants by harnessing the autophagic degradation pathway. Because it relies on autophagy, the AIM-based degrader could degrade soluble and aggregated proteins, organelles, and other cytosolic substrates. We therefore envisage that combining DNA- and RNA-targeting approaches with our method will advance the decoding of protein functions in basic and applied plant research.

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

Competing interests

None declared.

Author contributions

FL, QX and NL designed the experiments. NL, DS, ZT, JM and FL performed most of the experiments. L-ZT, LL, CG and YQ contributed materials and technical information. FL, QX, XH, NL, LL and CG analyzed the data. FL, QX, XH and NL wrote the manuscript with input from all authors.

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Data availability

The authors declare that all data supporting the findings of this study are available within the paper and its [Supporting Information](#) or are available from the corresponding author upon reasonable request.

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Fig. S1 ATG8-binding motif-containing peptide sorts fused mCherry fluorescent protein to the vacuole.

Fig. S2 Coexpression of ATG8-binding motif-mCherry with autophagy marker GFP-ATG8a in *Nicotiana benthamiana* leaf epidermal cells.

Fig. S3 Representative confocal images showing cellular localizations of mCherry together with GFP-ATG8a.

Fig. S4 nCBL1-GFP-ATG8a is a plasma membrane-anchored protein.

Fig. S5 Subcellular localization of various ATG8-binding motif-mCherry peptides.

Fig. S6 Coexpression of various ATG8-binding motif-mCherry constructs with autophagy marker GFP-ATG8a in *Nicotiana benthamiana* leaf epidermal cells.

Fig. S7 Subcellular localization of ATG8-binding motif-GBP-mCherry protein.

Fig. S8 Subcellular localizations of TAG5-GFP and BRI1-GFP after ConA treatment.

Fig. S9 Peroxisomal localization of PEX3-YFP.

Fig. S10 Peroxisomal membrane protein PEX3-YFP tethers autophagy receptor ATG8-binding motif-GBP-mCherry to peroxisomes.

Fig. S11 Mutated autophagy receptor mAIM-GBP-mCherry fails to direct PEX3-YFP-anchored peroxisomes to the vacuole.

Fig. S12 Subcellular localization of PEAMT1-GFP fusion protein.

Fig. S13 ATG8-binding motif-BZR1C82 peptides target BIN2-GFP fusion to the vacuole for degradation.

Fig. S14 Overexpression phenotypes of various ATG8-binding motif-BZR1C82-mCherry constructs.

Fig. S15 Analysis of the expression levels of representative *ATG* genes and ATG8 protein levels in plants expressing various BZR1C82-mCherry constructs.

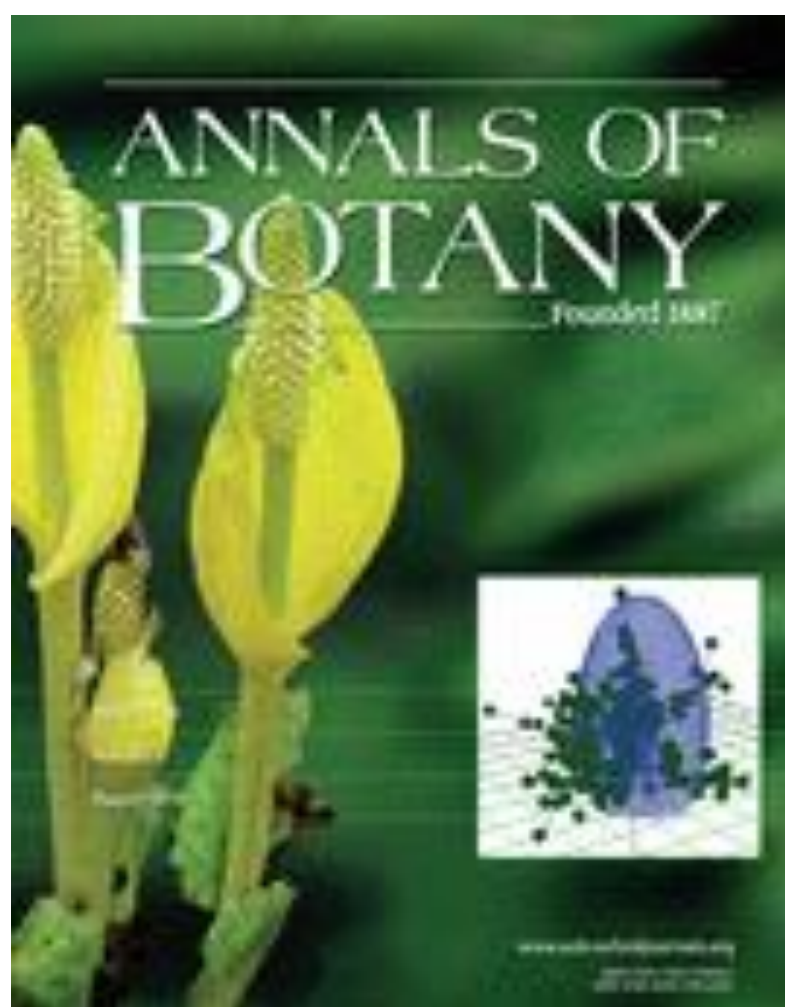
Fig. S16 mAIM-BZR1C82-mCherry peptide does not affect the protein level of BIN2-GFP.

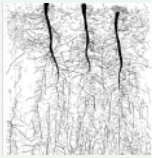
Table S1 Oligonucleotide primers used in this study.

Video S1 Time-lapse imaging of a tobacco leaf cell expressing FYVE-ATG8-binding motif-mCherry.

Video S2 Time-lapse imaging of a tobacco leaf cell expressing ATG8-binding motif-GBP-mCherry and PEX3-YFP under ConA treatment.

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Root system architecture of oilseed rape under two phosphate availabilities

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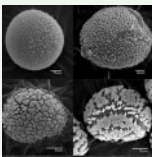
One important adaptation of plants to Phosphorus (P) deficiency is to alter root system architecture (RSA) to increase P acquisition from the soil, but soil-based observations of RSA are technically challenging, especially in mature plants. **Pan *et al.*** (pp. 173–184) developed a new large *Brassica*-rhizotron system to study RSA dynamics of *B. napus* in soils at low and high P. RSA parameters measured in polycarbonate plate roots were empirically consistent with analyses of excavated roots. Given that root senescence is likely to occur earlier at low P, crop P deficiency is likely to affect late water and nitrogen uptake.



Organellar phylogenomics of *Sphagnum*

doi:10.1093/aob/mcw086

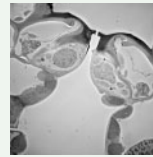
Sphagnum (peatmoss) is rapidly developing as a model for ecological genomics research. Twenty or more species of *Sphagnum* often co-exist within peatlands and sort themselves relative to abiotic niche gradients. Approximately one third of all terrestrial carbon is currently bound up in *Sphagnum*-dominated peatlands. Fundamental to the utility of peatmosses for ecological research is a phylogenetic framework for the genus, but relationships among major clades within *Sphagnum* have been difficult to resolve. **Shaw *et al.*** (pp. 185–196) present a phylogenomic analysis for *Sphagnum* based on mitochondrial and plastid genome sequences and resolve clades suggesting that interspecific ecological differentiation and the evolution of traits that impact global climate evolved early in diversification of peatmosses.



Spore settling velocity to assess moss dispersal rates

doi:10.1093/aob/mcw092

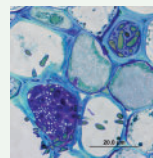
The settling velocity of diaspores is a key parameter influencing dispersal ability in wind-dispersed plants but remains largely undocumented in bryophytes. **Zanatta *et al.*** (pp. 197–206) measured this parameter for nine species using a fall tower design combined with a high-speed camera and determine that, while settling velocity can be estimated by spore diameter in most of the cases, some spores show important departures from theoretical expectations. The authors investigate the potential causes of this divergence by taking specific variation in spore shape and surface roughness into account and suggest that this affects the balance between density and drag.



The remarkable stomata of horsetails (*Equisetum*)

doi:10.1093/aob/mcw094

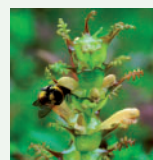
Horsetails are an ancient group of land plants that possess many unusual features, including the structure and development of the apertures (stomatal pores) in the epidermis. In addition to a symmetric pair of guard cells, stomata in *Equisetum* are delimited by an overlying pair of neighbour cells with characteristic vault-like radiating thickenings. Stomatal development involves a well-defined series of asymmetric and symmetric mitoses. The results of **Cullen and Rudall** (pp. 207–218) contribute to our understanding of the diverse patterns of stomatal development in land plants. They add to a considerable catalogue of highly unusual traits of horsetails - one of the most evolutionarily isolated land-plant taxa.



Silicon enhances cellular defences in root tissues of cotton after pathogen inoculation

doi:10.1093/aob/mcw095

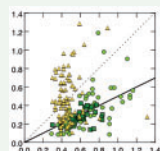
Silicon has been shown to enhance the resistance of plants to fungal and bacterial pathogens, however the underlying mechanisms have not been studied in detail in most plants. **Whan *et al.*** (pp. 219–226) compare cellular and biochemical modifications in root tissue of two cotton cultivars grown in media amended with soluble potassium silicate, and inoculated with a vascular wilt pathogen, *Fusarium oxysporum* f. sp. *vasinfectum*. Cellular defences, including deposition of electron dense material and accumulation of phenolic compounds, were more rapidly induced and more intense after Si treatment in a cultivar with greater inherent resistance to the pathogen.



Conflicting selection on floral traits

doi:10.1093/aob/mcw097

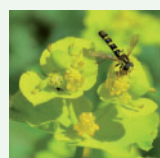
Floral traits attracting pollinators may also attract seed predators; however, evidence for conflicting selection on such traits remains scarce. One could expect that such selection generated by pollinators and seed predators varies geographically. Examining multiple populations across a geographic mosaic of environments and floral variation, **Sun *et al.*** (pp. 227–237) investigate female reproductive success in a bumblebee-pollinated subalpine herb, *Pedicularis rex*, in which tubular flowers are subtended by cupular bracts holding rain water. They found that plants experienced conflicting selection: those with flowers that were more exerted beyond the water-holding bracts were better pollinated, but also suffered more seed predation.



A meta-analysis of leaf nitrogen distribution within plant canopies

doi:10.1093/aob/mcw099

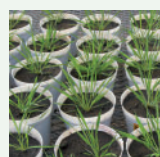
Leaf N is generally highest in top leaves and decreases with depth in leaf canopies. Although such a gradient contributes to efficient use of canopy N for photosynthetic production, the gradient of leaf N is suboptimal in actual canopies. **Hikosaka *et al.*** (pp. 239–247) present a meta-analysis of the vertical gradient of leaf N which reveals that in most canopies except for wheat, the slope of the leaf N was half of the optimal slope.



Plants with similar colours attract different pollinators.

doi:10.1093/aob/mcw103

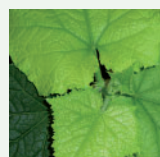
Different pollinator groups (bees, ants, wasps, flies, beetles and butterflies) preferentially visit flowers of certain colours. Interestingly, these colour preferences match the predictions of the pollination syndrome theory. However, flowers with similar colours do not attract similar pollinator assemblages. This is due to the fact that most flower species are pollinator generalist. **Reverté *et al.*** (pp. 249–257) conclude that although pollinator colour preferences seem to condition plant-pollinator interactions, the selective force behind these preferences has not been strong enough to mediate the appearance and maintenance of tight colour-based plant-pollinator associations.



Brachypodium peroxidase and drought tolerance

doi:10.1093/aob/mcw104

The peroxidase (POD) family shows functional diversity. **Luo *et al.*** (pp. 259–270) demonstrate that *Brachypodium distachyon* ecotypes have distinct specific POD isozymes. Two POD genes are closely associated with whole-plant response to drought, which may lead to natural variations of drought tolerance. The role of specific POD genes in differentiating *Brachypodium* accessions with contrasting drought tolerance could be associated with the general fitness of *Brachypodium distachyon* during evolution.

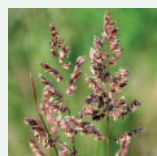


Silicon enhances leaf remobilization of iron in cucumber

doi:10.1093/aob/mcw105

Recently it has been demonstrated that Si nutrition can alleviate Fe deficiency chlorosis in cucumber (*Cucumis sativus*) by enhancing acquisition and root-to-shoot translocation of Fe. Here,

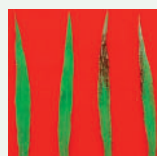
Pavlovic *et al.* (pp. 271–280) show that Si induces Fe mobilization in older (sink) leaves and increases its retranslocation to younger (source) leaves. In older leaves, Si enhanced expression of NAS1 transcripts responsible for an increased tissue concentration of Fe chelator nicotianamine (NA). This was paralleled by an increased expression of the YSL1 transporter and hence more efficient movement of Fe-NA complex from source to sink leaves via the phloem.



Breeding system diversification and evolution in Poa

doi:10.1093/aob/mcw108

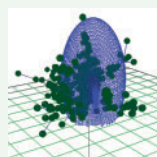
Species of *Poa* show an exceptional diversity in breeding systems represented by the occurrence of hermaphroditism to dioecism, with gynodioecy very frequent among species in South America. **Giussani *et al.*** (pages 281–303) contribute to the understanding of the evolutionary patterns in *Poa* associated with species sexuality and their current distribution. Divergence dating provides a temporal context to the evolution of breeding systems. Infrageneric taxonomic categories in New World *Poa* supersect. *Homalopoa* can be accommodated to the phylogenetic results in correlation with reproductive system and geography.



Silicon moderated K-deficiency-induced leaf chlorosis

doi:10.1093/aob/mcw111

Silicon (Si) has been widely reported to alleviate the plant nutrient deficiency, but the alleviating effect of Si on potassium (K) deficiency and its underlying mechanism are poorly understood. **Chen *et al.*** (pp. 305–315) investigate the influence of Si on putrescine (Put) metabolism under K deficiency in *Sorghum bicolor*, and find that Si application could reduce K-deficiency-induced Put accumulation by inhibiting Put synthesis and could decrease H₂O₂ production via Put oxidation. Decreased H₂O₂ accumulation contributes to the alleviation of cell death, thereby alleviating K-deficiency-induced leaf chlorosis and necrosis. The results indicate that Si application could alleviate the K deficiency-induced leaf chlorosis by decreasing H₂O₂ via inhibiting Put synthesis and oxidation.

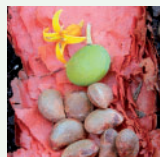


Simulation of shoot and fruit growth in apple

doi:10.1093/aob/mcw085

Modeling approaches which reveal how a plant allocates carbohydrates to the different organs are of major interest since carbohydrate allocation determines many growth processes. In this study, **Pallas *et al.*** (pp. 317–330) describe a modeling approach allowing the simulation of carbohydrate allocation and

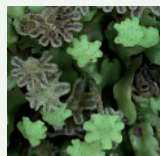
organ growth in apple trees by coupling two models, MappleT and QualiTree. The coupled model was appropriate to simulate growth characteristics at the tree and organ scales. This modeling approach shows the necessity of accurately simulating the impact of distances between sources and sinks as well as shoot ontogenetic characteristics, in order to represent the observed growth variability within tree architecture.



Breaking dormancy in *Persoonia longifolia* endocarps

doi:10.1093/aob/mcw100

Germination of species with hard woody indehiscent endocarps has troubled seed scientists for many years. To understand how germination is regulated in such species **Chia *et al.*** (pp. 331–346) show that the conditions required for dormancy loss and germination are effectively revealed through burial trials matched with environmentally relevant laboratory experiments on a representative species, *Persoonia longifolia*, from southern Western Australia. This approach allowed the authors to identify the key drivers of dormancy loss through a comprehensive series of experiments executed over five years which revealed that warm plus cold stratification is required to break physiological seed dormancy in *P. longifolia*.



Sex differences and plasticity in dehydration tolerance

doi:10.1093/aob/mcw102

Plants have a multitude of strategies for drought survival. Dehydration tolerance (DhT), is one such strategy that provides an opportunity to identify key genes, physiological traits, and ecological factors that could be used to reduce crop and species loss from drought. Here, **Marks *et al.*** (pp. 347–356) demonstrated that *Marchantia inflexa*, a tropical liverwort, has moderate DhT. Interestingly, *M. inflexa* has the capacity to acclimate to different environmental moisture levels, suggesting that DhT is plastic in this species. The authors demonstrate that

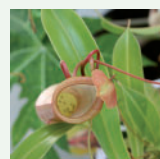
males are less DhT than females, which may explain the female biased sex ratios observed in this and many bryophyte species.



Summer dormancy and seasonal growth in California perennial grasses

doi:10.1093/aob/mcw109

Superior drought survival is expected to result in reduced productivity and competitive ability, but this expectation has seldom been tested in herbaceous perennial species. **Balachowski *et al.*** (pp. 357–368) quantified seasonal growth, functional traits, and drought survival strategies in eight California perennial grasses. Contrary to common assumptions, they found that summer dormancy, an adaptation to Mediterranean-type climates, was associated with greater springtime productivity and more competitive functional traits. The authors conclude that, because summer dormancy confers greater functional similarity to exotic annual species currently invading California grasslands, native summer dormant taxa may play an increasingly important ecological role in the future of these ecosystems.



Prey-induced responses in carnivorous *Nepenthes*

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Carnivorous plants of the genus *Nepenthes* catch and digest prey, mainly arthropods, in their pitcher traps in order to obtain additional nutrients such as nitrogen and phosphate. The digestive character of the pitcher fluid is well known; other features of the fluid are less well understood, in particular the induction and regulation of its composition. Here, **Yilamujiang *et al.*** (pp. 369–375) study the induction of both phytohormones and digestion-related genes in the pitcher of *Nepenthes alata*. The authors demonstrate that insect prey as well as chitin is able to induce first jasmonate phytohormones which in turn can induce genes for digestive enzymes such as a protease, nepenthesin, or a chitinase suggesting a defined signaling pathway.

Specific peroxidases differentiate *Brachypodium distachyon* accessions and are associated with drought tolerance traits

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● **Background and Aims** *Brachypodium distachyon* (*Brachypodium*) is a model system for studying cereal, bioenergy, forage and turf grasses. The genetic and evolutionary basis of the adaptation of this wild grass species to drought stress is largely unknown. Peroxidase (POD) may play a role in plant drought tolerance, but whether the allelic variations of genes encoding the specific POD isoenzymes are associated with plant response to drought stress is not well understood. The objectives of this study were to examine natural variation of POD isoenzyme patterns, to identify nucleotide diversity of *POD* genes and to relate the allelic variation of genes to drought tolerance traits of diverse *Brachypodium* accessions.

● **Methods** Whole-plant drought tolerance and POD activity were examined in contrasting ecotypes. Non-denaturing PAGE and liquid chromatography–mass spectrometry were performed to detect distinct isoforms of POD in 34 accessions. Single nucleotide polymorphisms (SNPs) were identified by comparing DNA sequences of these accessions. Associations of *POD* genes encoding specific POD isoenzymes with drought tolerance traits were analysed using TASSEL software.

● **Key Results** Variations of POD isoenzymes were found among accessions with contrasting drought tolerance, while the most tolerant and susceptible accessions each had their own unique POD isoenzyme band. Eight POD genes were identified and a total of 90 SNPs were found among these genes across 34 accessions. After controlling population structure, significant associations of *Bradi3g41340.1* and *Bradi1g26870.1* with leaf water content or leaf wilting were identified.

● **Conclusions** *Brachypodium* ecotypes have distinct specific POD isoforms. This may contribute to natural variations of drought tolerance of this species. The role of specific *POD* genes in differentiating *Brachypodium* accessions with contrasting drought tolerance could be associated with the general fitness of *Brachypodium* during evolution.

Key words: *Brachypodium distachyon*, drought tolerance, gene and trait association, natural variation, peroxidase, single nucleotide polymorphism.

INTRODUCTION

Brachypodium distachyon (*Brachypodium*) is a temperate, monocot wild grass species and it possesses all the qualities to make it an excellent model organism (Garvin *et al.*, 2008; Bevan *et al.*, 2010). It has diploid ecotypes containing five chromosomes, easily distinguishable chromosomes ($2n = 10$), small genome size (approx. 300 Mbp), self-fertility, small physical status, short life cycle and a simple growth requirement (Draper *et al.*, 2001). This species is phylogenetically closer to some economically important food and bioenergy crops than is rice (*Oryza sativa*) (Draper *et al.*, 2001), thus providing a powerful tool for studying functional and ecological genomics aimed at improving grain, forage and bioenergy crops. To date, genetic and genomic resources for *Brachypodium* have been developed, including the entire genomic sequence (Vogel *et al.*, 2010), a genetic linkage map (Garvin *et al.*, 2010), a high-efficiency transformation system (Vogel *et al.*, 2006; Păcurar *et al.*, 2008; Vogel and Hill, 2008), T-DNA mutants (Bragg

et al., 2012; Thole *et al.*, 2012) and genome diversity (Gordon *et al.*, 2014). All these tools and resources are valuable for examining the genetic and evolutionary basis of complex traits such as abiotic stress tolerance.

Antioxidant metabolism plays a role in drought tolerance of the plants. As one of the reactive oxygen species, hydrogen peroxide (H_2O_2) is a by-product of biological reactions in the plant cell. Adverse environmental conditions such as drought stress can promote excess accumulation of H_2O_2 , potentially leading to oxidative damage to protein, DNA and lipids (Apel and Hirt, 2004). The degree of lipid peroxidation, indicated by the level of malondialdehyde (MDA) content, often increased under drought stress in plant species (Zhang and Kirkham, 1996; Jiang *et al.*, 2010; Xu *et al.*, 2011; Liu and Jiang, 2015). Removal of H_2O_2 is accomplished by the action of several antioxidant enzymes at different cellular locations. Of the antioxidative enzymes, peroxidase (POD) catalyses the reduction of H_2O_2 to water, followed by subsequent oxidation of small molecules (Smith and Veitch, 1998). Drought stress often induces POD

activity in many plant species (Jung, 2004; Sofo *et al.*, 2005; Upadhyaya *et al.*, 2008; Selote and Khanna-Chopra, 2010; Ying *et al.*, 2015). The tolerant genotype of maize (*Zea mays*) exhibited lower accumulation of MDA and H₂O₂ content related to increasing activities of POD and other antioxidant enzymes under water stress conditions (Moussa and Abdel-Aziz, 2008). Increases in POD activities were also found at the vegetative and flowering stages of seven species within the genus *Avena* under drought stress, coupled with an increased level of lipid peroxidation (Pandey *et al.*, 2010). However, POD activity also remained unchanged in some annual and perennial grass species exposed to drought stress (Zhang and Kirkham, 1996; Zhang and Schmidt, 1999; Fu and Huang, 2001; Bian and Jiang, 2009; Jiang *et al.*, 2010). Although two cultivars of Kentucky bluegrass (*Poa pratensis*) did not differ in POD activities under drought stress, the drought-tolerant cultivar exhibited significantly higher POD activities compared with the sensitive one after rewatering (Xu *et al.*, 2011). The results demonstrated complex responses of POD to drought stress, influenced by plant species, cultivar, stress intensity and duration.

In spite of their role in detoxification, PODs have remarkable catalytic versatility involved in a broad range of physiological and developmental processes, including initiation of seed germination (Morohashi, 2002), cellular growth and cell wall loosening (Cosgrove, 2001), cell wall cross-linking (Passardi *et al.*, 2004b), lignification and suberization (Lopez-Serrano *et al.*, 2004), differentiation (Kim *et al.*, 2004), senescence (Ranieri *et al.*, 2000) and plant–pathogen and plant–insect interactions (Delannoy *et al.*, 2003; Gulsen *et al.*, 2010a). Existing as isoenzymes in plant species, the class III plant POD is a haem-containing glycoprotein encoded by a large number of superfamily genes in land plants (Welinder, 1992a, b; Hiraga *et al.*, 2001). For example, the *Arabidopsis* genome contains 73 genes encoding POD (Tognoli *et al.*, 2002), while rice has 138 (Passardi *et al.*, 2004a). The homology between paralogues in a plant ranges from 30 to 100 %, but very close orthologues exist even between evolutionarily distant plants (Bakalovic *et al.*, 2006). More recently, Wang *et al.* (2015) identified 119 non-redundant POD genes in maize, which were divided into 18 groups based on their phylogenetic relationships. Although plant PODs are a family of related proteins possessing highly conserved domains, grasses contain some unique POD clusters not seen in dicot plants, represented by *Arabidopsis* (Duroux and Welinder, 2003). By using *POD* gene polymorphism, genotypic diversity has been examined among *Cynodon* accessions (Gulsen *et al.*, 2009), apple (*Malus domestica*) germplasm (Gulsen *et al.*, 2010b), and Buffalograss (*Bouteloua dactyloides*) and other perennial grass species (Gulsen *et al.*, 2007). Peroxidase profiling also reveals genetic linkage between *POD* gene clusters and basal host and non-host resistance to rusts and mildew in barley (*Hordeum vulgare*) (González *et al.*, 2010). These results demonstrate that the *POD* gene family can be used to study genotypic diversity and evolutionary relationships on an intra- and inter-specific basis. Senescence- and drought-related changes in two *POD* isoforms in leaves of *Ramonda serbica* have been observed (Veljovic-Jovanovic *et al.*, 2006). Although research results have revealed the functional diversity of PODs, the role of a specific *POD* isoenzyme in drought tolerance is not clearly determined, especially at the population level for a grass species.

Natural populations are often collected from a wide range of geographical locations that have enormous diversity, which can maximize the potential of populations to withstand and adapt to biotic and abiotic environmental changes (Jump *et al.*, 2009). Natural *Brachypodium* accessions broadly group into winter and spring annuals, and this may impact plant responses to environments. Luo *et al.* (2011) found that 57 *Brachypodium* natural accessions varied considerably in whole-plant responses to drought stress and they were classified into most tolerant, moderately tolerant, susceptible and most susceptible groups. Comparative analysis of the cold acclimation and freezing tolerance capacities of seven diploid *Brachypodium* accessions revealed only a limited capacity to develop freezing tolerance when compared with winter varieties of temperate cereals such as wheat (*Triticum aestivum*) and barley (Colton-Gagnon *et al.*, 2014). In addition to stress tolerance, Schwartz *et al.* (2010) reported that *VRN* (vernalization regulator) and a portion of the phenotypic variation of flowering time and vernalization was associated with changes in expression of orthologues of *VRN* genes in *Brachypodium* accessions. Variation of phenotypic traits such as plant height, growth habit, stem density, flowering time, seed weight and cell wall composition were also observed among inbred lines (Tyler *et al.*, 2014). Natural populations of *Brachypodium* exhibit distant genetic distance patterns (Vogel *et al.*, 2009); however, the mechanisms of environmental adaptation during evolution are not well understood in *Brachypodium*. For example, it is not well understood whether some candidate genes encoding *POD* contribute to variable drought responses of natural accessions.

Although it can be challenging to determine the functions of a large number of isoenzymes in a single plant species, accumulation of information on individual genes should lead to a better understanding of the role of *POD* in natural variation of stress tolerance and related physiological processes. It is important to know whether allelic variation of genes encoding the specific isoenzymes is associated with plant response to stressful environments. For example, PODs appear to be associated with plant disease resistance based on the presence or absence of isozymes in resistant vs. susceptible varieties. Despite these associations, there is no evidence that the allelic variation of *POD* directly determines the level of disease resistance (González *et al.*, 2010). Therefore, the objectives of this study were to examine natural variation of the *POD* isoenzyme, to identify nucleotide diversity of *POD* genes and to relate the allelic variation of genes to traits of diverse *Brachypodium* accessions. Knowledge obtained about *Brachypodium* natural variation of PODs increases our understanding of stress resistance among grass species or ecotypes within a species that have evolved in different geographic and adaptive conditions.

MATERIALS AND METHODS

Plant materials and growing conditions

For examining drought tolerance and isoenzymes, drought-tolerant (T-9) and intolerant (B2C) accessions of *Brachypodium* were propagated with the tillers in plastic pots containing a sandy-loam soil with a pH of 6.9 in a greenhouse at Purdue University, West Lafayette, IN, USA. Each pot had the same volume of soil and number of plants. Seedlings were

grown in a greenhouse with a 12 h photoperiod at average temperatures of 23/20 °C (day/night). The average intensity of photosynthetically active radiation (PAR) was approx. 415 $\mu\text{mol m}^{-2} \text{s}^{-1}$ during the experiment. Plants were watered every 2 d and fertilized once a week with a soluble fertilizer (N-P₂O₅-K₂O, 24-8-16) (Scotts Inc., Marysville, OH, USA) and micro-nutrients at the rate of approx. 0.25 g nitrogen L⁻¹. No nutrient deficiency symptoms were observed for any of the plants. The selection of these two accessions was based on our previous study of whole-plant drought response of *Brachypodium* (Luo *et al.*, 2011). Subsequently, 34 accessions were selected for detecting POD isoenzyme pattern, population structure, and gene and trait association (Table 1), based on maximization of the geographic and genetic diversity available in these materials (Vogel *et al.*, 2009) as well as phenotypic variation in drought responses (Luo *et al.*, 2011). With the exception of B21, B3-1 and B2-3 from Iraq, all accessions were from Turkey, which lies in the centre of the natural range of *Brachypodium* and contains all of the environments *Brachypodium* inhabits, including coastal regions, hot interior deserts and cold northern highlands (Vogel *et al.*, 2009). Growth conditions of the 34 *Brachypodium* accessions were the same as the conditions for growing T-9 and B2C described above.

Drought treatment

All pots of T-9 and B2C were kept well watered until drought stress was initiated. Drought stresses started on 6 April 2010 after grasses were grown for 21 d from tiller propagation on 16 March 2010. Drought stress was imposed by withholding water from the grasses until permanent wilting (the leaves were no longer rehydrated at night and in the morning) occurred to the plants, especially for the intolerant plants. Drought stress ended on 12 April 2010, at 7 d after stress was initiated. Leaves

and roots were harvested at the end of drought treatment and kept frozen (−80 °C) for further analysis.

The experiment was a randomized complete block design with four replicates. The well-watered and drought-stressed grass pots were arranged randomly within a block. Data were analysed for the significance of the treatments for a given measurement using Statistical Analysis System (version 9.1; SAS Institute, Cary, NC, USA). The means of the treatments were separated using the Least Significant Difference (LSD) at a 0.05 significant level.

Whole-plant measurements

Leaf wilting was visually rated on a scale of 0 (no observable wilting) to 3 (severely wilted or almost dead) (Luo *et al.*, 2011). Leaf photochemical efficiency was determined by measuring chlorophyll fluorescence (F_v/F_m) in the dark on randomly selected leaves in each pot using a fluorescence meter (OS-30P, OPTI-Sciences, Hudson, NH, USA). At the end of drought stress (7 d), approx. 3 g of leaf and 5 g of soil were collected, and leaf water content (LWC) and soil water content (SWC) were measured according to the equation: $WC = (f. wt - d. wt) / f. wt \times 100$, where f. wt is fresh weight and d. wt is dry weight for leaf or soil samples, respectively.

Assay of hydrogen peroxide and malondialdehyde

The H₂O₂ content was determined according to the method of Bernt and Bergmeyer (1974) with some modifications. A 0.1 g powder sample of leaf or root was homogenized in 0.4 mL of 100 mM sodium phosphate buffer (pH 6.8), and extracts were then centrifuged at 16 000 g for 15 min at 4 °C. A 0.17 mL aliquot of supernatant was added to 0.83 mL of peroxidase reagent containing 83 mM sodium phosphate (pH 7.0), 0.005 % (w/v) *o*-dianisidine and 40 μg peroxidase mL⁻¹. The mixture was incubated at 30 °C for 10 min, and 0.17 mL of 1 N perchloric acid was added to stop the reaction. The absorbance was read at 436 nm. The H₂O₂ concentration was calculated by using a standard curve with known concentration.

Malondialdehyde has been widely used as a convenient biomarker for lipid peroxidation of omega-3 and omega-6 fatty acids because of its facile reaction with thiobarbituric acid (TBA) (Esterbauer and Cheeseman, 1990; Ayala *et al.*, 2014). The concentration of MDA was determined to assess the degree of lipid peroxidation using the method of Dhindsa *et al.* (1981) with minor modification. An 80 mg leaf or root sample was homogenized in 1.2 mL of 1 % trichloroacetic acid. The mixture was centrifuged at 10 000 g for 10 min. A 0.5 mL aliquot of supernatant was added to 2 mL of reaction solution (20 % trichloroacetic acid containing 0.5 % TBA). The mixture was heated at 95 °C for 30 min, quickly cooled in an ice bath, and then centrifuged at 10 000 g for 10 min. The absorbance was read at 532 and 600 nm.

POD activity and isoenzyme detection

For leaf enzyme extraction, 0.1 g of ground powder sample was homogenized with 1.2 mL of extraction buffer [50 mM potassium phosphate, 1 mM EDTA, 1 % polyvinylpyrrolidone

TABLE 1. *Experiment identification (ID) and drought tolerance (DT) of Brachypodium distachyon accessions used in this study*

ID	Abbreviation	Accession*	DT†	ID	Abbreviation	Accession	DT
34	T-9	Tek-9	T	19	B30-1	Bd30-1	S
31	T-10	Tek-10	T	17	B2K	BdTR2K	S
4	B1-1	Bd1-1	T	16	B2D	BdTR2D	S
27	G-5	Gaz-5	M	26	B9K	BdTR9K	S
8	B13A	BdTR13A	M	22	B7B	BdTR7B	S
9	B13E	BdTR13E	M	5	B10C	BdTR10C	S
1	A-10	Adi-10	M	10	B13M	BdTR13M	S
2	A-14	Adi-14	M	13	B21	Bd21	S
3	A-2	Adi-2	M	28	Kh-1	Kah-1	S
32	T-2	Tek-2	M	29	Kh-3	Kah-3	S
33	T-7	Tek-7	M	30	Koz-4	Koz-4	S
77	B11C	BdTR11C	M	15	B2C	BdTR2C	MS
6	B11A	BdTR11A	M	14	B2B	BdTR2B	MS
24	B8I	BdTR8I	M	25	B9B	BdTR9B	MS
23	B8C	BdTR8C	M	18	B3-1	Bd3-1	MS
21	B3R	BdTR3R	M	12	B2-3	Bd2-3	MS
20	B3J	BdTR3J	M	11	B18-1	Bd18-1	MS

*B21, B3-1 and B2-3 are from Iraq; all other accessions are from Turkey (Vogel *et al.*, 2009).

†T, M, S and MS indicate the most tolerant, moderately tolerant, susceptible and most susceptible accessions, respectively.

Results were based on a study by Luo *et al.* (2011).

(PVP), pH 7.8). For root enzyme extraction, 0.12 g of powder sample was homogenized with 0.72 mL of the same extraction buffer. The mixtures were centrifuged at 15 000 g for 25 min at 4 °C and the supernatant was collected. Protein content was determined according to Bradford (1976). POD activity was determined by following changes in absorbance at 470 nm (Bian and Jiang, 2009). Details of the methods were described by Zhang and Kirkham (1996).

Electrophoretic separation of isoenzymes was performed using non-denaturing PAGE described by Laemmli (1970), except that SDS was omitted; 8 % stacking and 3 % resolving polyacrylamide gels were used with running buffer at pH 8.5. Equal amounts of protein (30 µg) for the leaf samples were loaded. Native gels were stained for POD activity according to the method of Srivastava and Van Huystee (1977) with some modifications. Briefly, gels were soaked in 100 mM potassium phosphate buffer (pH 6.5) for 15 min and then stained in 12.5 mM guaiacol containing 12 mM H₂O₂.

Protein identification by mass spectrometry

Two mass spectroscopy techniques, matrix-assisted laser desorption (MALDI) (Baumgarner et al., 2013) and liquid chromatography-electrospray ionization (ESI) tandem mass spectrometry (LC-ESI-MS/MS) (Park et al., 2012), were used for protein identification. Briefly, distinct isoenzymes of POD from the selected drought-tolerant and susceptible accessions were recovered from a native PAGE gel. The bands were excised from the gel and processed in the Purdue University Proteomics core facility in the Bindley Biosciences Center for in-gel digestion (Jiménez et al., 1998) and then analysed by MADLI and LC-ESI-MS/MS analysis. The digested peptide solution was spotted on the plate, and peptide identification was performed using MALDI time-of-flight/time-of-flight (TOF/TOF) on an AB 4800 Plus Analyzer (Applied Biosystems) (Baumgarner et al., 2013). MALDI data were analysed using GPS Explorer™ Software (Applied Biosystems). Searches were performed using MS and MS/MS data against databases for *Brachypodium*, rice, *Arabidopsis* and green plants from the National Center for Biotechnology Information (NCBI). In addition, the digested peptides were extracted and separated in a nano-HPLC system (Agilent 110, Agilent Technologies, San Jose, CA, USA), coupled with an ESI hybrid linear ion trap quadrupole Orbitrap mass spectrometer (ESI-LTQ-Orbitrap XL) (Park et al., 2012). Peptides were separated with a C18 reversed phase ZORBAX 300SB-C18 analytical column (150 mm × 75 µm, 3.5 µm) from Agilent. The peptides were eluted from the column with a linear gradient of 5–40 % buffer B (acetonitrile/0.1 % formic acid) in buffer A (water/0.1 % formic acid) over 35 min at a rate of 0.3 µL min⁻¹ followed by a gradient of 40–95 % B for 40–55 min. The column was equilibrated with 5 % buffer B (acetonitrile/0.1 % formic acid) for 55–70 min. The MS spectra were acquired in positive mode in a range from 300 to 2000. The MS/MS spectra were acquired in a data-dependent acquisition mode in which the full MS scan (resolution 30 000) was followed by four MS/MS scans for the most abundant molecular ions and fragmented by collision-induced dissociation (CID) using a normalized collision energy of 35 %. Peptide data (MS/MS) information from LTQ-Orbitrap

were analysed using the AB 4800 Plus/Protein Pilot software (Applied Biosystems) against the SwissProt database. Peptides with similarity values >95 % were considered toward identification of a given protein, with a minimum of two peptides required for positive identification.

DNA extraction and sequencing

Primers were designed to amplify genomic fragments for each gene (Table 3). Genomic DNA of the 34 accessions was extracted using a modified hexadecyltrimethylammonium bromide protocol (Doyle and Doyle, 1990) and purified using a PureLink™ Quick Gel Extraction Kit (Invitrogen, Carlsbad, CA, USA). PCR amplification of the gene fragment was conducted with one cycle at 95 °C for 4 min, followed by 35 cycles of 95 °C for 30 s, 53 °C for 30 s, 72 °C for 60 s, and a final extension step at 72 °C for 5 min. Bands of the expected size were cut from the gel and recovered using a ZR-96 Zymoclean™ Gel DNA Recovery Kit (Zymo Research Corporation, Irvine, CA, USA). The recovered DNA was used for sequencing PCRs with a BigDye Terminator kit with one cycle at 96 °C for 1 min, followed by 35 cycles of 96 °C for 10 s, 50 °C for 5 s and 60 °C for 4 min. After the clean up with ethanol/sodium acetate solution, the purified DNA was sequenced on an ABI 3730XL sequencer (Applied Biosystems Inc., Foster City, CA, USA) at the Genomics Core Facility at Purdue University.

Nucleotide diversity (π) and linkage disequilibrium (LD)

DNA sequence data from 29–34 accessions were aligned by DNASTAR (DNASTAR, Inc., Madison, WI, USA), depending on the quality of sequencing results for the individual genes (Table 4). π, LD and neutrality tests were estimated using DnaSP5.0 (Rozas and Rozas, 1999). The nucleotide level was assayed by nucleotide polymorphism (θ) (Watterson, 1975) and π (Nei, 1987). Values from Tajima’s D test were separated by the standard deviation of π and θ_w and reflected the differences between the number of singletons and total number of mutations (Tajima, 1989). Decay of LD with distance in base pairs between sites within the same gene was evaluated by non-linear regression. The LD descriptive statistic *r*² (Hill and Robertson, 1968) was calculated using TASSEL software (Bradbury et al., 2007). Neighbor-Joining trees were created based on haplotype sequences for each candidate gene using nucleotide number of differences as a distance measure and were calculated with TASSEL software.

TABLE 2. Effects of 7 d drought stress on soil moisture content (SWC), leaf water content (LWC) and chlorophyll fluorescence (F_v/F_m) of *Brachypodium distachyon*

Accession	Treatment	SWC (%)	LWC (%)	F _v /F _m
T-9	Control	19.5 ± 0.62a*	77.3 ± 0.45a	0.81 ± 0.002a
	Drought	5.5 ± 0.34b	59.3 ± 4.46b	0.81 ± 0.003a
B2C	Control	18.7 ± 1.01a	76.0 ± 0.33a	0.82 ± 0.005a
	Drought	4.4 ± 0.19b	46.2 ± 8.92b	0.78 ± 0.017b

Values are mean ± s.d.
*Means followed by the same letter within a column for a given line were not significantly different at *P* < 0.05.

Association analysis of single nucleotide polymorphisms (SNPs) with traits

The population structure was assessed using 46 simple sequence repeats (SSRs) (Vogel *et al.*, 2009) by STRUCTURE software (Pritchard *et al.*, 2000); the detailed procedure was described previously (Yu *et al.*, 2013). Individual SNPs with each trait were tested with the simple linear model and population structure implemented model using TASSEL software (Bradbury *et al.*, 2007). SNP markers with minor allele frequency <5 % were filtered from the association analysis. The SNPs that passed $P < 0.05$ were deemed significant.

Gene expression

Real-time quantitative reverse transcription-PCR (qRT-PCR) was conducted to examine gene expression in the control and drought-stressed leaf samples of T-9 and B2C. Briefly, total RNA was isolated with a Direct-zolTM RNA MiniPrep Kit (Zymo Research Corporation) and then used for reverse transcription with an iScriptTM cDNA Synthesis Kit (Bio-Rad, Hercules, CA, USA). Reaction mixtures were incubated for 5 min at 25 °C, 30 min at 42 °C and 5 min at

85 °C. qRT-PCR was performed with a CFX96 TouchTM Real-Time PCR Detection System using an iTaqTM Universal SYBR[®] Green Kit (Bio-Rad), with reaction for 3 min at 95 °C followed by 40 amplification cycles of 10 s at 95 °C and 1 min at 60 °C. Primers for amplification were as follows: for *Bradi3g41340.1*, Forward 5'-GCAGCAACCTG GAGAAGAT-3' and reverse 5'-GGCAGTCGTGGAAGAA GAG-3'; for *Bradi1g26870.1*, Forward 5'-TCTTCGGTTCT CTCCTTCCT-3' and reverse 5'-ATCGCAGGCTTCCT TGTT-3'. The transcript level of *S*-adenosylmethionine decarboxylase (*SamDC*) was used as a housekeeping gene (Hong *et al.*, 2008). The method of $2^{-\Delta\Delta CT}$ (Livak and Schmittgen, 2001) was used to calculate the expression level under drought-stressed conditions relative to that under control conditions. The analysis included three biological replicates (three pots) and three technical replicates for each accession under control and drought stress conditions.

RESULTS

Whole-plant response to drought stress

The SWC dropped from 19.5 to 5.5 % for T-9 and from 18.7 to 4.4 % for B2C under drought stress (Table 2). The reductions in

TABLE 3. Primers for gene amplification in *Brachypodium distachyon*

Gene	Forward primer	Reverse primer
<i>Bradi1g41900.1</i>	5'-CATCCGCATCTTCTTCCACGA-3'	5'-TTGACCATCCGGCAATTCCT-3'
<i>Bradi2g04490.1</i>	5'-ACTGTCCTGATGCCGAGGATA-3'	5'-TTGAAGAAGCTCGCGTCGAA-3'
<i>Bradi3g41340.1</i>	5'-AGGAGATCAAGGCCAAGCTCAA-3'	5'-TACACGATCGAGAACCAGATGGA-3'
	5'-TGTTTCCTTCCCTTTTTTCTC-3'	5'-GCTTGGACAGACAGTCGATTA-3'
	5'-ACGAGATTAGTTAAAGCCAAGG-3'	5'-GTTACGCTCCCTCATCC-3'
<i>Bradi1g63060.1</i>	5'-AGCTGAGCTGGAACCATCAA-3'	5'-CAGTCGCAAATGCTCCACAA-3'
	5'-TGTCCTCAACTATTGGCATC-3'	5'-TTCTCAAAACCTGGTGCTG-3'
	5'-AAGTAGAACATGCCTCCTCGAT-3'	5'-GGTAGGGTGAGGTCCACAAAAA-3'
<i>Bradi1g26870.1</i>	5'-TCGCGAATCCATTCTCCGA-3'	5'-AAACACCTCCCGTGCATTGT-3'
	5'-GTGCCCTACTGGTCGCTCAA-3'	5'-GGAGCGAAGTAGCACGCGAGTA-3'
<i>Bradi4g32800.1</i>	5'-TCCAGTTCCATGACTGCTTCGT-3'	5'-AAGGCATTCTGGTCCTTGCT-3'
<i>Bradi5g00690.1</i>	5'-AAATCGCCATTGGCCCGTAT-3'	5'-TACTGGTTGTGCAACCGGAAC-3'
<i>Bradi1g65820.2</i>	5'-TTTACCTCCAGTAGCAGCCAT-3'	5'-TTCCAAGAGCAACCAACCCA-3'

TABLE 4. Gene length, sequence length, single nucleotide polymorphism (SNP) and SNP frequency in the whole length and coding region of peroxidase genes

Gene	<i>n</i>	Whole length (bp)	CDS for gene (bp)	Sequencing length (bp)	CDS for sequencing (bp)	Sequencing SNP number	Coding SNP number	Whole SNP frequency	Coding SNP frequency
<i>Bradi1g41900.1</i>	34	1332	1122	804	737	8	5	101	147
<i>Bradi2g04490.1</i>	29	1608	981	1060	557	14	12	76	46
<i>Bradi3g41340.1</i>	33	2911	1008	1184	151	3	0	395	—
<i>Bradi1g63060.1</i>	32	3176	966	1782	424	12	5	149	85
<i>Bradi1g26870.1</i>	34	1449	1047	1271	1032	9	7	141	147
<i>Bradi4g32800.1</i>	30	1483	1044	841	624	18	10	47	62
<i>Bradi5g00690.1</i>	33	1365	693	670	373	8	5	84	75
<i>Bradi1g65820.2</i>	34	3166	753	1566	395	18	0	87	—
Mean	32	2061	952	1147	537	11	6	135	94

n, the number of accessions for which a certain gene could be sequenced; whole length, the genome sequence of the gene; CDS for gene, the coding region of a gene; sequencing length, the entire sequenced length for a gene in this study; CDS for sequencing, the sequenced length for the coding region in this study; sequencing SNP number, number of SNPs discovered in the entire sequenced length; coding SNP number, number of SNPs discovered in the sequenced length for the coding region; whole SNP frequency, calculated by the entire sequenced length/SNP, stands for the average SNP per bp length; coding SNP frequency, the sequenced length for the coding region/SNP, stands for the average SNP per bp length.

SWC under drought stress was >70 % for both accessions, compared with their respective controls. The LWC was significantly reduced from 77.3 to 59.3 % for T-9 and from 76.0 to 46.2 % for B2C after 7 d of drought stress. Drought stress did not change chlorophyll fluorescence (F_v/F_m) for T-9, but significantly decreased F_v/F_m for B2C, compared with the control (Table 2).

The production of H₂O₂ and MDA

Leaf H₂O₂ concentrations were increased by 1.8-fold for T-9 and 2.5-fold for B2C but remained unchanged in the roots for both accessions under drought stress (Fig. 1). The MDA concentrations were increased by 2.8- and 3.5-fold in the leaves for T-9 and B2C, respectively, compared with their controls; but remained unchanged in the roots for both accessions under drought stress (Fig. 1).

Enzyme activity and isoenzyme stain

Compared with the control, drought stress increased leaf POD activities by 2.0-fold for T-9 and 1.6-fold for B2C, but did not affect root POD activities in both accessions (Fig. 1). Based on the non-denaturing PAGE protein gel, drought stress did not induce new anionic POD isoenzymes (pH 8.5) in either

the tolerant accession T-9 or the susceptible B2C (Fig. 2). Both T-9 and B2C had their one own unique isoenzyme not shown in the other. Across the other 32 accessions, the drought-tolerant accessions T-2, T-7, T-10, Bd1-1, B8C and B8I had the same POD isoenzyme patterns as T-9, while the drought-susceptible accessions such as BD18-1, B2B, B9B and B7B had similar isoenzyme patterns to B2C (Fig. 3). Two cationic POD isoenzymes (pH 4.5) were also detected, but the patterns were the same among accessions (data not shown).

Protein and SNP identification

Protein sequences revealed that isoenzyme 1 in T-9 contained four different proteins (Bradi1g41900.1, Bradi2g04490.1, Bradi3g41340.1 and Bradi1g63060.1), while isoenzyme 2 in B2C also contained four different proteins (Bradi1g26870.1, Bradi4g32800.1, Bradi5g00690.1 and Bradi5g65820.2)

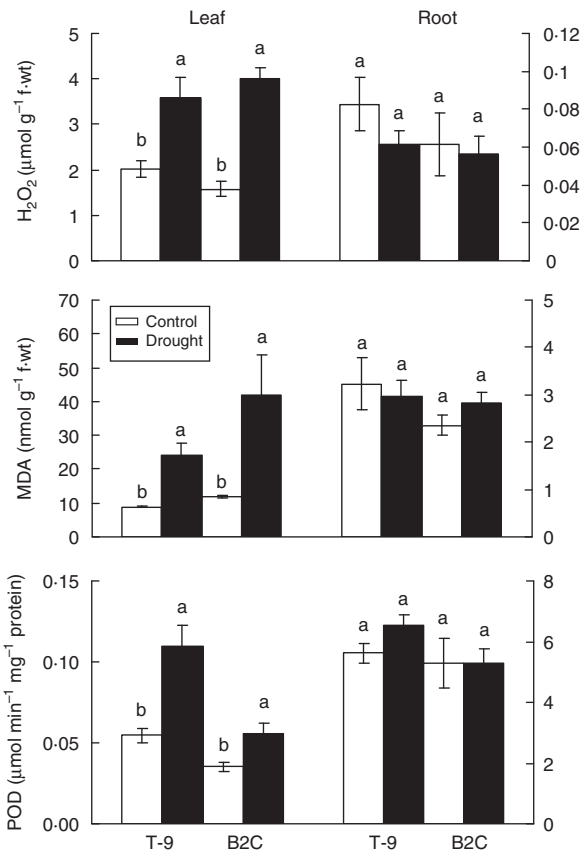


Fig. 1. Effects of 7 d drought stress on the concentration of hydrogen peroxide (H₂O₂) and malondialdehyde (MDA) and activities of peroxidase (POD) in the leaves and roots of *Brachypodium distachyon*. Means followed by the same letter within the leaves or roots for a given line are not significantly different at $P < 0.05$. Vertical bars show \pm s.d. T-9, Tek-9; B2C, BdTR2C.

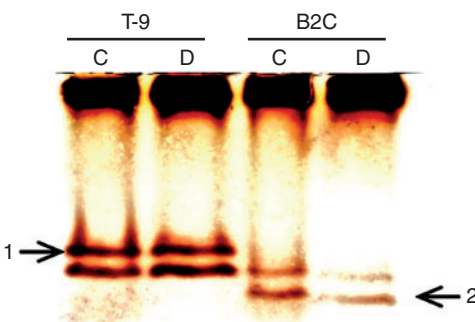


Fig. 2. Electrozymogram showing the peroxidase profile of protein extracts from leaves of *Brachypodium distachyon* under well-watered control (C) and drought stress (D) conditions. Arrows indicate unique bands shown in the drought-tolerant T-9 (isoenzyme 1) and susceptible B2C (isoenzyme 2) accessions. T-9, Tek-9; B2C, BdTR2C.

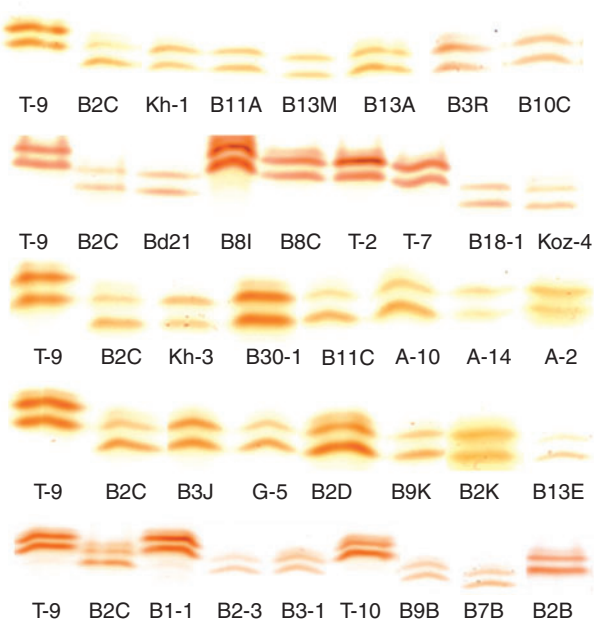


Fig. 3. The total activity stain for peroxidase in the leaves of *Brachypodium distachyon* under well-watered conditions for accessions used in the study.

(Supplementary Data Tables S1 and S2). Genes of *Bradi1g41900.1*, *Bradi2g04490.1*, *Bradi5g41340.1*, *Bradi1g26870.1*, *Bradi4g32800.1* and *Bradi5g00690.1* were identified through MALDI-TOF/TOF, while genes of *Bradi6g3060.1* and *Bradi6g5820.2* were identified by LTQ-Orbitrap (Tables S1 and S2). The genes encoding these proteins were identified and partially sequenced in genotypes with contrasting drought tolerance (Table 4). The total length of the sequences ranged from 670 to 1782 bp among eight genes, with a mean of 1147 bp, and covered approx. 41–88 % of the whole length of the genes (Table 4). The sequences of coding regions ranged from 151 to 1032 bp among the eight genes, with a mean of 546 bp. A total of 90 SNPs were detected in the eight *POD* genes, ranging from three (*Bradi3g41340.1*) to 18 SNPs (*Bradi4g32800.1* and *Bradi5g65820.2*). The coding regions consisted of 44 SNPs ranging from 0 (*Bradi3g41340.1* and *Bradi5g65820.2*) to 12 (*Bradi2g04490.1*). Across all the genes, the average SNP frequency was 1/135 bp, ranging from one SNP per 46 bp (*Bradi4g32800.1*) to one SNP per 395 bp (*Bradi3g41340.1*). The coding regions had an average SNP frequency of 1/94 bp (Table 4).

π and LD

Across eight genes, the values of π ranged from 0.00 (*Bradi3g41340.1* and *Bradi5g65820.2*) to 0.0077 (*Bradi4g32800.1*) and Watterson's θ_w ranged from 0.00 (*Bradi3g41340.1* and *Bradi5g65820.2*) to 0.0061 (*Bradi4g04490.1*), with a mean value of 0.0028 for π and

0.0019 for θ_w , respectively (Table 5). The π values spanned from 0.00 (*Bradi3g41340.1*) to 0.0055 (*Bradi5g00690.1*) of synonymous areas with an average of 0.0019, and ranged from 0.0011 (*Bradi1g26870.1*) to 0.0095 (*Bradi3g041340.1*) of non-synonymous areas with an average of 0.0042 (Table 5). The average π synonymous/non-synonymous ratio was 0.81 (Table 5). The haplotype number varied from four (*Bradi3g41340.1*) to 16, with an average of 9.1 across genes. Haplotype diversity ranged from 0.51 (*Bradi1g41900.1*) to 0.91 (*Bradi4g32800.1*), with a mean value of 0.65. Through the neutrality test, positive Tajima's D values were shown in *Bradi3g41340.1*, *Bradi4g32800.1*, *Bradi5g00690.1* and *Bradi5g65820.2*, while negative Tajima's D values were found in *Bradi1g41900.1*, *Bradi2g04490.1*, *Bradi3g63060.1* and *Bradi1g26870.1*.

Population structure and LD

Two genetic population structures (G1 and G2) were found in 33 genotypes assessed by 46 SSR markers (B30-1 was not included due to missing SSR information) (Fig. 4). The subgroups were identified based on likelihood plots of the models, stability of grouping patterns across different runs, and germplasm information (Vogel *et al.*, 2009; Yu *et al.*, 2013). G1 consisted of T-9, T-10, B1-1, T-2, T-7, B8I, B8C and B7B accessions with better drought tolerance, except for B7B, while G2 contained other accessions varying in drought tolerance but including the most susceptible materials. Based on our previous trait data in these accessions (Luo *et al.*, 2011), G1 had an average value of 0.78, 56.3 and 30.6 % for leaf wilting, percentage

TABLE 5. Nucleotide diversity, haplotype and neutrality test of peroxidase genes in *Brachypodium distachyon*

Gene	π	θ_w	π_{syn}	$\pi_{non-syn}$	$\pi_{syn/non-syn}$	H	Hd \pm s.d.	Neutral test	Tajima's D
<i>Bradi1g41900.1</i>	0.0003	0.0011	0.0009	0.0025	0.366	6	0.51 \pm 0.089	-0.529	-0.603
<i>Bradi2g04490.1</i>	0.0031	0.0061	0.0011	0.0022	0.498	15	0.82 \pm 0.074	-1.847	-1.564
<i>Bradi3g41340.1</i>	0.0000	0.0000	0.0000	0.0095	0.000	4	0.49 \pm 0.091	1.060	0.856
<i>Bradi1g63060.1</i>	0.0026	NA	0.0032	0.0014	2.331	9	0.61 \pm 0.097	0.041	-2.139
<i>Bradi1g26870.1</i>	0.0015	0.0025	0.0012	0.0011	1.055	7	0.52 \pm 0.093	-2.197	-1.057
<i>Bradi4g32800.1</i>	0.0077	NA	0.0030	0.0086	0.352	16	0.91 \pm 0.037	1.363	0.250
<i>Bradi5g00690.1</i>	0.0073	NA	0.0055	0.0029	1.884	7	0.68 \pm 0.059	0.380	1.009
<i>Bradi1g65820.2</i>	0.0000	0.0000	0.0002	0.0053	0.0398	9	0.68 \pm 0.075	1.066	1.522
Mean	0.0028	0.0019	0.0019	0.0042	0.812	9.1	0.65 \pm 0.077	-0.083	-2.156

π and θ_w , nucleotide diversity; π_{syn} and $\pi_{non-syn}$, synonymous and non-synonymous nucleotide diversity, respectively; H, number of haplotypes; Hd, haplotype diversity; NA, not available.

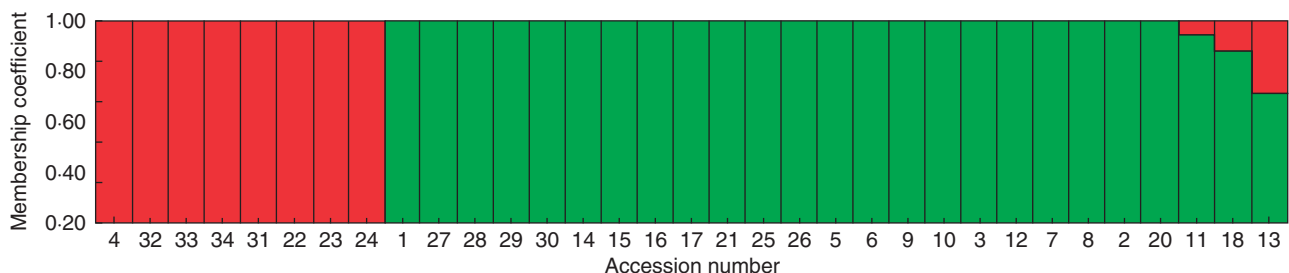


FIG. 4. Hierarchical organization of genetic relatedness of 33 *Brachypodium* genotypes. Numbers on the x-axis represent each individual accession. Numbers on the y-axis indicate the membership coefficient for each individual accession. The colour of the bar indicates the two groups identified through the STRUCTURE program (G1, red; G2, green).

reduction of LWC (R-LWC) and F_v/F_m ($R-F_v/F_m$), which was lower than 1.3, 76.7 and 56.7 % for leaf wilting, R-LWC and $R-F_v/F_m$ observed in G2, respectively.

Linkage disequilibrium estimates r^2 plotted against pairwise distances between SNPs. Across genes, slow LD decay was found in *Brachypodium* (Fig. 5). Overall, LD decay extended to > 1.2 kb with an r^2 higher than 0.6. Similarly, LD decay of combined *Bradi1g41900.1*, *Bradi2g04490.1*, *Bradi3g41340.1* and *Bradi3g63060.1* extended to 1.2 kb with an r^2 of 0.6, while *Bradi1g26870.1*, *Bradi4g32800.1*, *Bradi5g00690.1* and *Bradi5g65820.2* expressed LD decay exceeding the distance of 1.2 kb with an r^2 higher than 0.6 (data not shown).

Association of genes with traits

After controlling population structure, significant associations of *Bradi3g41340.1* and *Bradi1g26870.1* with traits were identified (Table 6). Specifically, SNPs at loci 1087 and 1584 from *Bradi3g41340.1* were associated with R-LWC, with nucleotide changes of A/T and GT. Similarly, SNPs at loci 617, 708, 806, 1164 and 1181 from *Bradi1g26870.1* were associated with R-LWC, with nucleotide changes of A/T, C/T, A/G, C/T

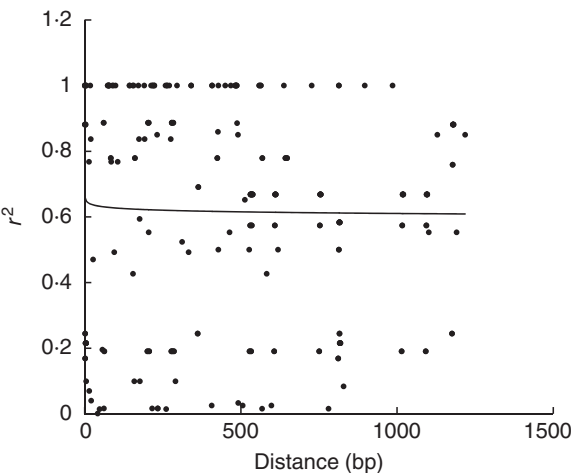


FIG. 5. Pattern of linkage disequilibrium (LD) in eight *POD* genes in *Brachypodium distachyon*. The r^2 values for pairwise LD are plotted against physical distance.

TABLE 6. Association of peroxidase genes with traits and nucleotide changes in *Brachypodium distachyon*

Gene	Trait	Locus	Nucleotide change
<i>Bradi3g41340.1</i>	R-LWC	1087	A/G
	R-LWC	1574	G/T
<i>Bradi1g26870.1</i>	R-LWC	617	A/G
	R-LWC	708	C/T
	R-LWC	806	A/G
	R-LWC	1164	C/T
	R-LWC	1181	G/T
	Wilting	1164	C/T

Wilting, leaf wilting under drought; R-LWC, percentage reduction of leaf water content under drought compared with the control.
Leaf wilting and LWC data were based on a study by Luo et al. (2011).

and G/T. One SNP at locus 1164 from *Bradi1g26870.1* was also associated with leaf wilting. Compared with using the simple linear model, this was an elimination of approx. 87 % significant associations by using the population structure implemented model (data not shown).

Gene expression

The expression of two significant genes of *Bradi3g41340.1* and *Bradi1g26870.1* was analysed in the leaves of both accessions under the non-stress and drought conditions (Fig. 6). Relative to their unstressed control, drought stress significantly increased expression levels of *Bradi3g41340.1* and *Bradi1g26870.1* in both accessions. Specifically, expression of *Bradi3g41340.1* was significantly increased by 4.8-fold for T-9 and 3.1-fold for B2C. For *Bradi1g26870.1*, expression was increased by 5.6-fold for T-9 and 4.7-fold for B2C (Fig. 6). However, differences in fold change were not significant between T-9 and B2C. In addition, by using a reference gene as a control, the absolute expression levels of both genes were not significantly different between the two accessions under the unstressed control and drought stress (data not shown).

Phylogenetic tree

The Neighbor-Joining tree revealed certain relationships among the accessions based on sequences of eight genes. Drought-tolerant accessions such as T-2, T-9, T-7, T-10, B1-1, B8I and B8C were close to each other according to sequences

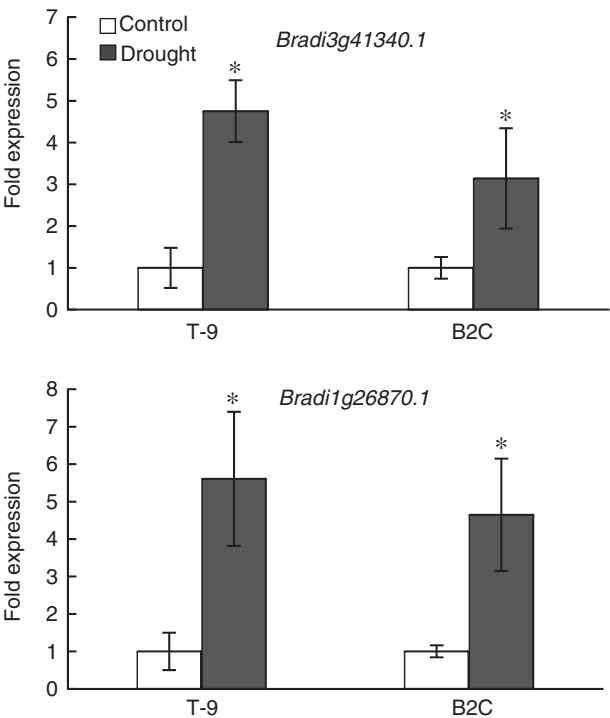


FIG. 6. Effects of 7 d of drought stress on gene expression by using real-time quantitative reverse transcription-PCR in the control and drought-stressed leaf samples of T-9 and B2C. T-9, Tek-9; B2C, BdTR2C. Data were normalized in comparison with the control. *, significant at $P < 0.05$.

of *Bradi3g41340.1*. The results were generally consistent with grouping revealed by population structure using SSR markers (Fig. 7). By using sequences of *Bradi1g26870.1*, grouping was somewhat different from the results from *Bradi3g41340.1*.

DISCUSSION

Lesser reductions in LWC and F_v/F_m found in T-9 compared with B2C exposed to 7 d of drought stress indicated that T-9 was more drought tolerant than B2C. The results were consistent with a study in *Brachypodium* by Luo et al. (2011) who supported that more reductions in LWC and F_v/F_m were observed in B2C. Drought treatment was less severe in this experiment compared with that described by Luo et al. (2011). This could be the main reason for the relatively small decreases in LWC, and particularly F_v/F_m , in the present study when compared with previous results. However, the trends in drought responses of the two accessions were similar between the two studies. A more drought-tolerant perennial ryegrass (*Lolium perenne*) also showed higher LWC and F_v/F_m in the field (Yu et al., 2013), suggesting that drought-tolerant grass plants maintain adequate water status and physiological activity.

Moreover, the drought-tolerant T-9 also showed more increased POD activities and lower H_2O_2 and MDA concentrations, relative to the control. Similar results of increased POD activity were found in the drought-tolerant maize (Moussa and Abdel-Aziz, 2008). However, POD activity also remained unchanged in some grass species under drought stress (Zhang

and Kirkham, 1996; Bian and Jiang, 2009; Jiang et al., 2010). Changes in POD activity under drought stress may depend on the species, variety, duration and intensity of the stress. At the transcript level, expression of the two significant genes, *Bradi3g41340.1* and *Bradi1g26870.1*, was increased in T-9 and B2C under drought stress, relative to their control (Fig. 6). The results were consistent with the increased POD activities found in these two accessions under drought stress (Fig. 1). The increases in enzyme activity and gene expression indicated that enhanced POD plays a role in drought tolerance of *Brachypodium*, which could be associated with reducing oxidative injury. In addition, some POD genes were up-regulated and some were down-regulated in maize due to drought stress (Wang et al., 2015). Higher activities of POD and gene expression were also found in plants exposed to other abiotic stresses such as cold, salt stress, heavy metals and pathogen invasion (Hiraga et al., 2000; Sharma and Dubey, 2004; Azevedo Neto et al., 2006; Xu et al., 2008).

Drought stress could also affect POD isozymes in some plants species. Dehydration and senescence caused disturbance in the redox homeostasis of *Ramonda* leaves, while inducing different POD anionic and cationic isoenzymes (Veljovic-Jovanovic et al., 2006). In *Arabidopsis*, the intensity of two POD isoenzymes increased in drought-stressed young and mature leaves, especially a strong induction in one isoenzyme in mature leaves (Jung 2004). Some PODs were stress-responsive isoenzymes in the roots of wheat (*Triticum aestivum* L.) (Selote and Khanna-Chopra, 2010). In this study, T-9 and B2C showed their own unique isoenzyme of POD under normal conditions.

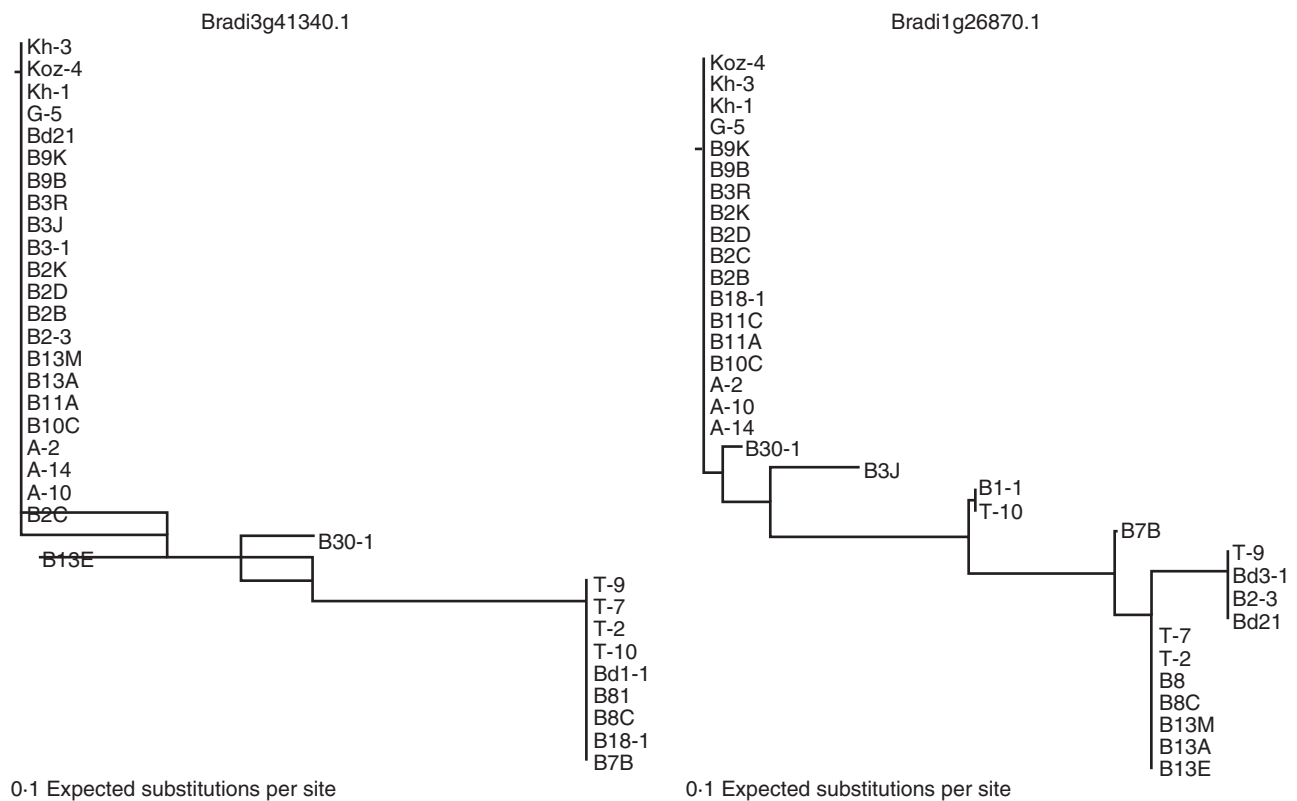


Fig. 7. Neighbor-Joining trees representing gene haplotype relationships in *Brachypodium distachyon*.

Although drought stress did not induce new bands of POD, these specific POD isoenzymes can generally distinguish the more drought-tolerant accessions from the others (Table 1; Fig. 3). The results indicated that the POD isoenzyme could differentiate *Brachypodium* accessions with contrasting drought tolerance. Among those POD isoenzymes, two candidate genes of *Bradi3g41340.1* and *Bradi1g26870.1* encoding specific PODs were significantly associated with drought tolerance traits. Furthermore, the grouping of the drought-tolerant accessions by sequences of *Bradi3g41340.1* was generally consistent with grouping revealed by population structure using genome-wide SSR markers. The results illustrated relationships among certain accessions by POD sequences, which could separate the most drought-tolerant accessions from the other accessions.

The average SNP frequency in eight *POD* genes observed in this study was one SNP every 135 bp in whole sequencing length. It was very similar to that of other antioxidant genes in *Brachypodium* (Luo et al., 2012). However, the average SNP frequency (1 SNP per 180 bp) in *Bradi1g41900.1*, *Bradi2g04490.1*, *Bradi3g41340.1* and *Bradi3g63060.1* initially found in the tolerant accession was only half compared with *Bradi1g26870.1*, *Bradi4g32800.1*, *Bradi5g00690.1* and *Bradi5g65820.2* shown in the susceptible accession. Moreover, the significantly associated genes of *Bradi3g41340.1* and *Bradi1g26870.1* had even lower SNP frequency, suggesting that SNP frequency in *POD* genes varied widely in one species. It indicated that SNP frequency may be influenced by selection, mutation, mating system, effective population size and demography (Nei, 1987). Mean nucleotide diversity ($\pi = 0.0028$, $\theta_w = 0.0019$) of *POD* genes was also similar to the values found in other antioxidant genes in *Brachypodium* (Luo et al., 2012) and in balsam poplar (*Populus balsamifera*) (Olson et al., 2010). The range of π values in *Brachypodium* was similar to the range of those of other genes tested in 34 ecotypes of arabidopsis (Lin et al., 2008). The value of θ_w denotes the number of polymorphic segregating sites (Watterson, 1975). The difference between π and θ_w found in *Bradi1g41900.1*, *Bradi2g04490.1* and *Bradi1g26870.1* could reflect the degree of non-equilibrium conditions in the genetic history of the population. Tajima's D measures the standardized differences between π and θ_w ; negative D values might indicate an excess of low-frequency polymorphisms in *Bradi1g41900.1*, *Bradi2g04490.1*, *Bradi3g63060.1* and *Bradi1g26870.1*, whereas positive values indicated an excess of intermediate polymorphisms in *Bradi3g41340.1*, *Bradi4g32800.1*, *Bradi5g00690.1* and *Bradi5g65820.2*. The ratio between $\pi_{\text{non-syn}}$ and π_{syn} is a strong indicator of selection (Li, 1997), varying greatly in these *POD* genes in *Brachypodium*. A higher $\pi_{\text{non-syn}}$ value in *Bradi3g41340.1* suggested a lack of selection of pressure during evolution in maintaining a high level of polymorphisms.

In summary, POD activity increased in the leaf of *Brachypodium* under drought stress, to a greater extent in the tolerant accession T-9, along with lesser accumulation of MDA and H_2O_2 . Variations of POD isoenzymes were found among accessions with contrasting drought tolerance, while the most tolerant and susceptible accessions had their unique POD isoenzyme band. Eight *POD* genes were identified and a total of 90 SNPs were found across 34 accessions. Nucleotide diversity of *POD* genes ranged from 0.00 to 0.0077 with a mean of 0.0028,

and the average synonymous/non-synonymous π ratio was 0.81. After controlling population structure, significant associations of *Bradi3g41340.1* and *Bradi1g26870.1* with R-LWC or leaf wilting were identified. The results suggested a role for specific *POD* genes in differentiating *Brachypodium* accessions with contrasting drought tolerance, which could be associated with general fitness of *Brachypodium* during evolution.

SUPPLEMENTARY DATA

Supplementary data are available online at www.aob.oxfordjournals.org and consist of the following. Table S1: the proteins identified by using MALDI-TOF/TOF MS in Tek-9 and BdTR2C *Brachypodium distachyon* accessions. Table S2: the proteins identified by LC-ESI-LTQ-Orbitrap XL in Tek-9 and BdTR2C *Brachypodium distachyon* accessions

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