中国科学院植物种质创新与特色农业重点实验室

Key Laboratory of Plant Germplasm Enhancement and Specialty Agriculture, Wuhan Botanical Garden, Chinese Academy of Sciences

2014 年报

Annual Report

中国科学院武汉植物园

Wuhan Botanical Garden,Chinese Academy of Sciences

中国科学院植物种质创新与特色农业重点实验室 2014 年报

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一、基本信息

实验室中文名称：中国科学院植物种质创新与特色农业重点实验室

实验室英文名称：Key Laboratory of Plant Germplasm Enhancement and Specialty Agriculture, Wuhan Botanical Garden, Chinese Academy of Sciences

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依托单位：中国科学院武汉植物园

实验室主任：李绍华

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学科与学位点：

	学科 1		学科 2		学科 3	
	名称	代码	名称	代码	名称	代码
学科分类	生物学	0710	林学	0907		
硕士点	植物学	071001	园林植物与观赏园艺	090706		
博士点	植物学	071001				
博士后站	生物学	0710				
研究性质	☐基础研究　☑应用基础研究　☐社会公益性研究 ☐高技术研发					
归口领域	☐化学 ☐数理 ☐地学 ☑生命科学 ☐医学科学 ☐信息 ☐材料 ☐工程					

二、实验室研究方向和发展目标

中国科学院植物种质创新与特色农业重点实验室于2010年1月由中国科学院批准设立，依托单位为中国科学院武汉植物园，5月15日正式挂牌成立。现任学术委员会主任为邓秀新院士，实验室主任为李绍华研究员。

实验室定位：面向国家特色农业植物资源收集保护与可持续利用需求，立足于园林园艺经济植物、能源植物、药用植物、水生经济植物等特色农业资源种质创新与开发利用，系统研究植物濒危机制与保育原理、关键类群的系统发育重建、谱系地理与分子进化，致力于植物资源评价与功能基因发掘、种质创新与新品种培育、功能化合物开发与产业化研究及技术创新，为我国特色农业的快速可持续发展提供理论与技术支撑。

研究方向：

1．特色农业资源植物保育原理：特色农业植物资源遗传评价、核心种质和相应指纹图谱的建立、种质资源迁地保育原理；重要特色农业经济植物的系统发育与保育基因组学；重要农业植物资源遗传多样性分布格局、基因流动态和适应性进化。围绕资源保育与开发利用的共性机理，为特色农业资源植物可持续利用提供理论基础和关键技术支撑。

2．**特色农业资源植物优质和抗性性状的生物学基础：**特色农业资源植物优良品质和特异抗性/耐性的生理生化基础；特种资源植物次生代谢的分子机制；优良品质、特异抗性/耐性相关的重要基因的克隆和生物学功能；重要功能基因的分子标签或紧密连锁分子标记的开发。针对特有的优良品质和抗性/耐性深入开展应用基础研究，阐明其分子和生理生化机制，并为这些优良性状向大田作物的转移提供基因和分子标记资源。

3．特色农业资源植物的种质创新和可持续利用：研究特色资源植物的育种、繁殖、栽培和综合开发利用的技术体系，为特种资源植物的可持续利用提供优良种苗和相应的技术保障。重点培育适应性强并具有自主知识产权的特色资源作物新品种；特色资源植物的高效繁殖和转基因技术；特种资源植物的优质高产和绿色生态栽培技术体系。

发展目标：基于资源植物学、遗传学、基因组学及蛋白组学等学科的原理、研究方法与发展趋势，围绕国家农业产业可持续发展的战略需求，遵循资源收集保护、科学研究与开发利用的“3R 模式”，开展特色园艺植物、能源植物、药用植物、水生经济植物等特色农业资源植物种质资源保护与可持续利用的研究，取得具有国际影响的原创性和前瞻性研究成果，育成具有自主知识产权的特色农作物新品种，促进我国特色农业科学与产业的发展。培养一批高层次人才，建成我国特色农业资源植物种质资源保护与可持续利用研究中心。通过 5-10 年的努力，争取把实验室建设成为国家重点实验室。

学科布置：

序号	研究单元	学术带头人	研　究　方　向
1	植物水分胁迫生物学	产祝龙	草坪草对非生物逆境的抗性应答及分子机制；植物激素和小分子物质诱导植物抗逆性的机理及应用；植物逆境胁迫相互作用的分子机制
2	草坪种质资源学	傅金民	草坪草种质资源评价与种质创新；草坪逆境分子生理机制研究
3	猕猴桃种质资源与育种	龚俊杰	猕猴桃的居群遗传学和进化；猕猴桃重要农艺性状的遗传规律与基因发掘；主要病害的起源与流行机制及其防控；猕猴桃育种
4	植物化学生物学	郭明全	基于功能代谢组学的药用植物资源品质鉴定、植物化学成分的提取分离鉴定、生物活性筛选与功能化合物的作用机理与开发利用
5	果树分子育种学	韩月彭	桃等重要果树果实品质性状形成的分子机理；果树果实品质性状的分子设计育种
6	系统与进化植物学	李建强	植物分子系统学；植物分类学与植物区系地理学
7	园艺作物生物学	李绍华	葡萄种质果实品质特点及遗传规律；葡萄抗逆和果实品质形成的调控机制及其基因的挖掘；转基因改良葡萄果实品质及抗性
8	植物生物技术	李夜光	经济微藻（螺旋藻、红球藻等）优良藻种选育和工业化生产关键技术优化研究；能源微藻资源收集、优良藻种选育和大规模培养技术研究；微藻分类学、系统学研究
9	能源植物基因组学	吕世友	油桐基因组学；油脂代谢途径重要基因的挖掘及功能解析；油桐品种的培育
10	种群遗传学	王　艇	叶绿体进化基因组学；植物重要性状分子解析
11	比较功能基因组学	王　瑛	特种药用植物资源（淫羊藿、枸杞、功能蔬菜）的收集、评价和可持续开发利用；药用植物次生代谢的分子调控机制；跨物种生物信息学数据挖掘
12	水生植物基因组学与遗传育种	杨平仿	莲基因组及其进化；莲重要经济性状的遗传机理及育种；种子休眠与萌发的分子机理
13	天然产物合成生物学	章焰生	资源植物药用化学品质特征及合成调控；资源植物药用化合物的生物合成及关键基因的挖掘

三、工作进展

本年度在研科研课题共 132 项， 合同总经费 15869.4 万元，当年实到经费 2110.3 万元（见附录一），其中 2014 年新增课题 30 项， 新增科研合同经费 3483 万元。在研课题包含：

973 项目	1 项
863 项目	3 项
国家重大专项	2 项
行业重大专项	2 项
科技支撑计划	1 项
国家基金重大项目	1 项
国家基金重点项目	2 项
国家自然科学基金面上和青年基金项目	37 项
国际合作项目	2 项
院重大项目	13 项
省部委项目	13 项
横向合作及其它项目	55 项

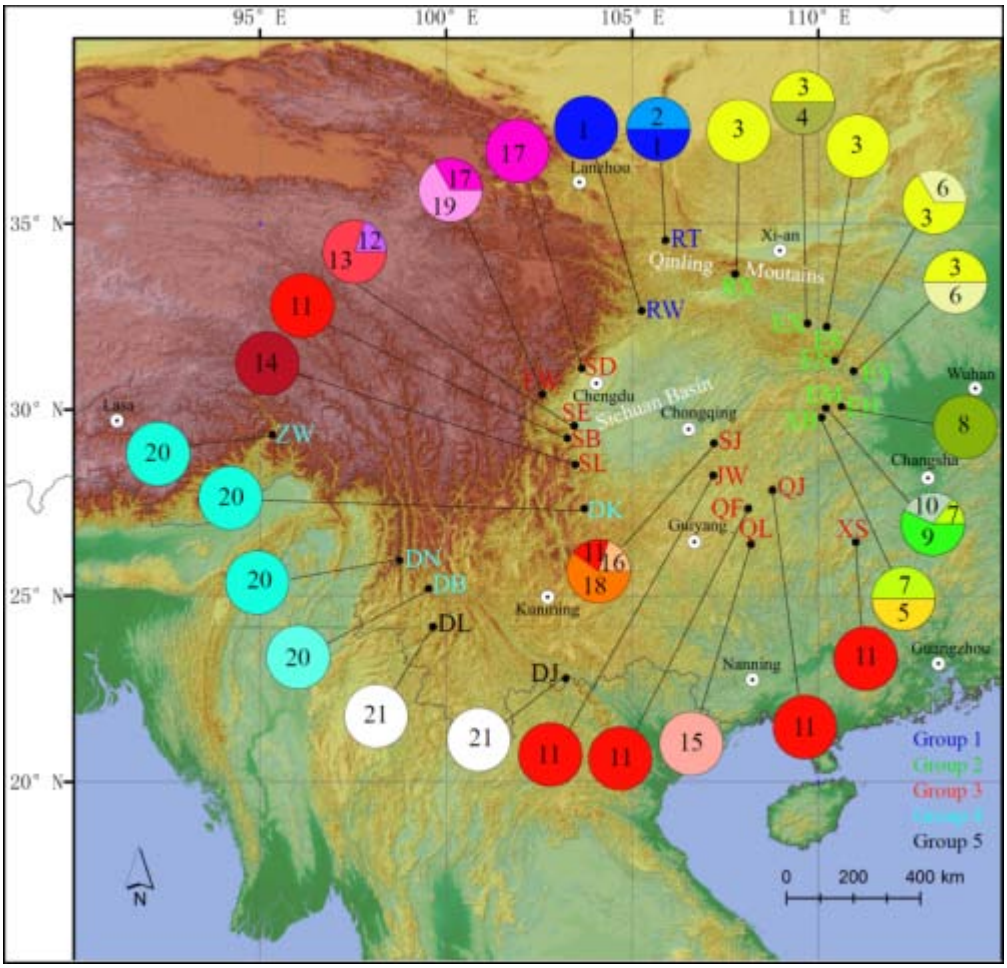
2014 年度获国家自然科学基金资助项目（2015 年开始执行）14 项，其中面上项目 5 项，青年科学基金项目 5 项，国际合作与交流项目 4 项，资助经费总额 888 万元。

（一）特色农业资源植物保育原理

1. 水青树谱系地理学研究

在中国西南地区以及中部地区收集了水青树 27 个居群共 157 个体，并测序了这些个体的 4 个叶绿体基因间区片段（*psbA–trnH*、*rpl32–trnL*、*petA–psbJ* 和 *petL–psbE*）。共发现了 21 个单倍型，用 TCS 软件构建了 21 个单倍型的网状关系图；对 157 个个体的 4 个叶绿体基因片段序列进行了遗传多样性、遗传分化、SAMOVA（spatial analysis of molecular variance）和 AMOVA（analysis of molecular variance）分析，用来探讨水青树的遗传结构。采用 BEAST 软件估算了 21 个单倍型的分化时间，并且通过错配分析（mismatch distribution analysis）来检测水青树居群在历史上是否经历过扩张事件。发现 21 个水青树单倍型中只有 3 个单倍型的分布范围较广，其它单倍型均局限在特定的某个区域。单倍型多样性较高的区域是湖北西部、四川南部和重庆南部。古老单倍型出现在中国西南地区。水青树单倍型的分布具有明显的谱系地理结构（$N_{ST} > G_{ST}$; $P < 0.05$）。SAMOVA 分析将水青树居群划分为 5 个组，这 5 个组与 TCS 鉴定的 5 个进化枝能很好地吻合。

两个独立的居群扩张事件发生在 SAMOVA 鉴定的组 2 和 3，扩张时间分别约为 399 和 311 kya。21 个单倍型的分化时间大约是在 9.6 个百万年之前，然而大多数单倍型的分化时间出现在第四纪。我们认为水青树当前分布格局的形成受到第三纪晚期气候、地质变化以及第四纪冰期事件的双重影响；水青树很有可能在晚第三纪时就已经南迁至中国西南地区；水青树在第四纪冰期时在中国中部地区占据过多处避难所；SAMOVA 鉴定的水青树 5 个组之间不存在或存在很少的基因交流。

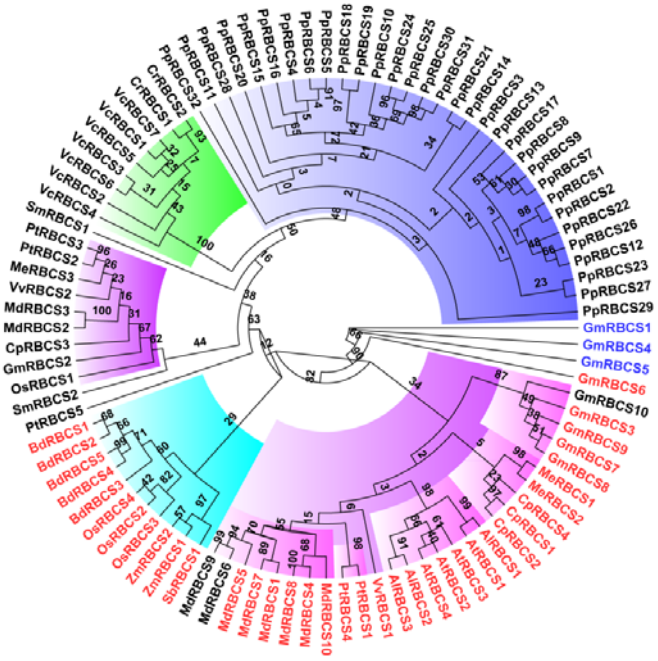


水青树 21 个单倍型分布图

2. 卷柏属 *rbcS* 基因家族分子进化研究

利用刚公布的江南卷柏的全基因组序列，测序得到了深绿卷柏和兖州卷柏的 *rbcS* 基因，分析了卷柏属 *rbcS* 基因家族的分子特征，发现卷柏属 *rbcS* 成员仅有 2 个，且都具有典型的 *rbcS* 基因家族结构域 PF00101。同时利用目前已经公布的 16 种模式植物的全基因组序列，在全序列水平分析了 *rbcS* 基因家族的进化历程。研究发现 *rbcS* 基因家族进化树中存在协同进化的现象。同时发现苔藓植物小立

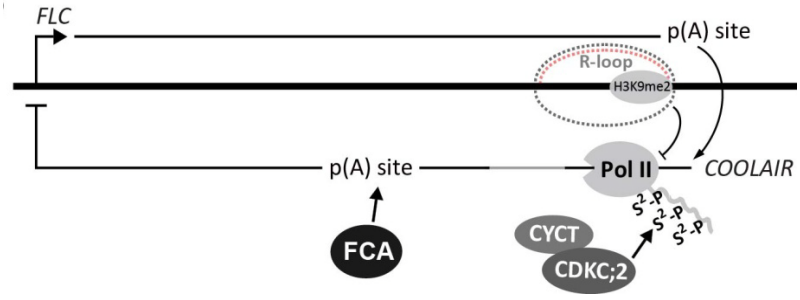
碗蕨中 *rbcS* 基因家族成员数量发生了极大的扩张，而仅有被子植物的 *rbcS* 基因才具有 *rbcS* 基因家族结构域 PF12338 结构域。本研究为进一步研究卷柏及其他植物中 *rbcS* 基因家族分子进化及其功能奠定了良好的基础。



16 种模式植物中 *rbcS* 基因家族进化树

3. 植物开花时间分子调控研究

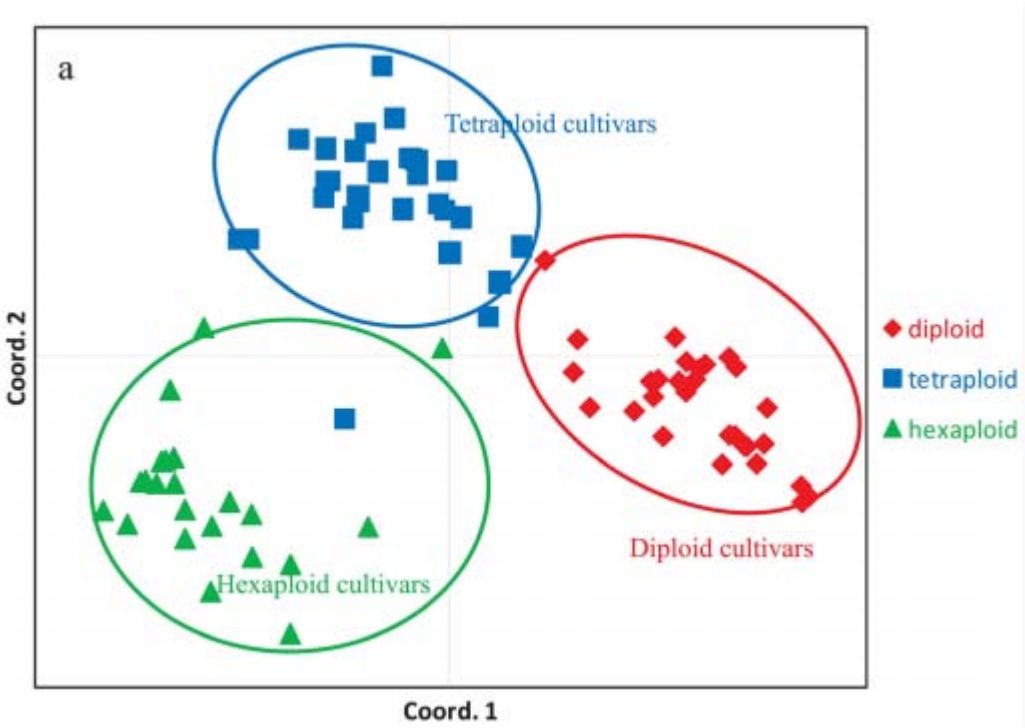
利用染色质免疫共沉淀、液相色谱-串联质谱、双分子荧光互补技术等多种现代分子生物学手段及巧妙的实验设计，成功解析出植物转录延伸因子 P-TEFb 通过调节反义长链非编码 RNA 的转录来定量调控植物开花主要抑制基因 *FLC* 表达的新颖分子机理，这种转录延伸因子通过调节反义长链非编码 RNA 的转录来间接微调功能基因表达的机制可能在真核生物中具有普遍意义，同时深化了对数量性状分子调控的认识。



CDKC;2 调控 *FLC* 转录模型

4. 猕猴桃种质资源遗传多样性研究

采用 AFLP 分子标记，研究了猕猴桃国家种质资源圃 79 个品种以及来自 6 个不同倍性杂交组合的 122 个 F1 代杂交个体的遗传多样性。研究结果表明，目前猕猴桃不同倍性品种（2 倍体、4 倍体和 6 倍体）的遗传多样性不存在显著差异，这暗示这些直接从自然界选育的猕猴桃品种的遗传多样性并没有出现遗传侵蚀现象；聚类结果表明，猕猴桃品种的聚类基本按照倍性来划分；例如红色猕猴桃品系的品种聚在一起，反应了他们具有相同的遗传背景；此外，对杂交后代不同倍性的个体的遗传多样性分析表明，种间杂交有利于增加后代品种的遗传多样性；研究结果对于猕猴桃资源的保护以及可持续利用提供了理论依据。



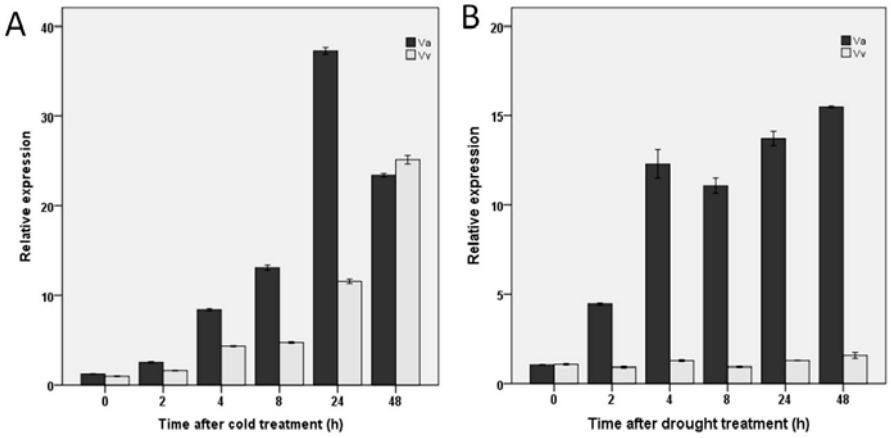
不同倍性品种的聚类分析图

（二）特色农业资源植物优质和抗性性状的生物学基础

1. 葡萄抗寒旱机理研究

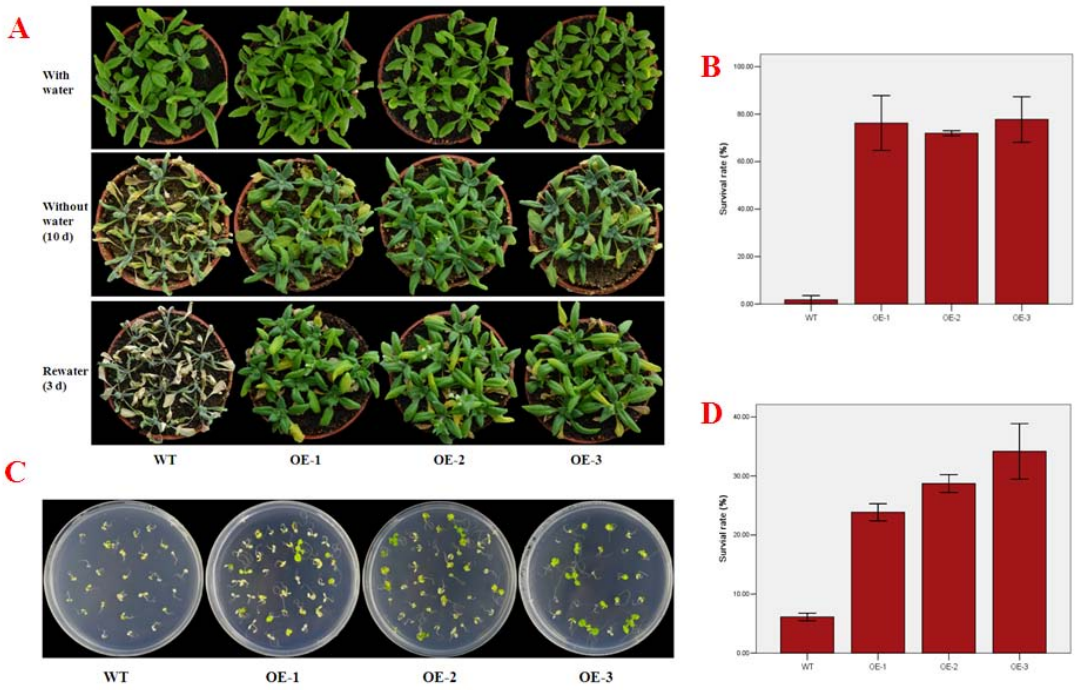
针对我国葡萄主产区冬季寒旱胁迫，以耐寒旱性强的山葡萄（*Vitis amurensis*）为主要研究材料，利用组学和遗传学等手段，深入挖掘山葡萄高耐寒旱机理，以期为定向改良葡萄品种的耐寒旱性提供理论基础和分子元件。通过比较山葡萄和不耐寒旱的欧亚种（*V. vinifera*）‘玫瑰香’葡萄在冷胁迫下的转录组变化差异，鉴定到了一个在山葡萄中能够快速大量的响应冷胁迫基因。通过 BLAST 发现，该基因属于 NAC 转录因子家族成员，命名为 *NAC26*。定量 RT-PCR 分析表明，冷胁迫下 *NAC26* 表达量在山葡萄中迅速增加，其响应速度和程度均高于‘玫瑰

香’葡萄；同时发现 *NAC26* 也响应干旱胁迫，其响应模式在山葡萄和‘玫瑰香’之间存在极大差别，山葡萄中表达量随干旱处理迅速增加，而在‘玫瑰香’葡萄中基本无变化。但编码区分析表明，两种葡萄中 *NAC26* 不存在显著差异。利用山葡萄中的编码序列构建了 35S 启动子驱动的过表达载体并转化拟南芥结果表明，与野生型拟南芥相比，过表达株系具有良好的耐旱能力，复水后野生型基本死亡，而过表达株系的成活率在 80%左右；过表达株系的耐盐能力亦有增强，在 120 mM NaCl 条件下，其成活率为野生型的 4 倍。因此推测 *NAC26* 可能参与贡献了山葡萄的高耐寒旱性。上述研究为揭示山葡萄抗寒机理提供了重要线索。



冷胁迫（A）和干旱胁迫（B）下，NAC26 在山葡萄（Va）和‘玫瑰香’（Vv）葡萄中表达水平的响应模式比较

A：4℃处理不同时间下 NAC26 的表达模式；B：6% PEG 处理不同时间下 NAC26 的表达模式

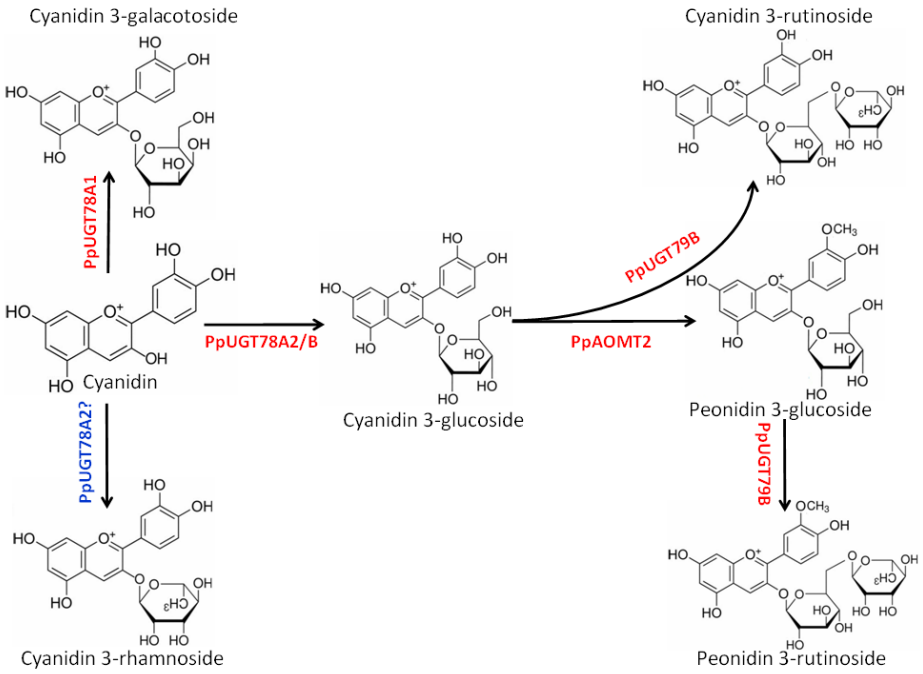


过量表达 *VaNAC26* 可以增强拟南芥植株的耐旱及耐盐能力

A 和 B：野生型（WT）和过量表达 *VaNAC26* 株系（OE-1，OE-2，OE-3）在正常生长、缺水和复水条件下的表型比较（A）及致死率统计（B）；C 和 D: 野生型（WT）和过量表达 *VaNAC26* 株系（OE-1，OE-2，OE-3）在 120 mM NaCl 条件下的生长表型（C）比较和系致死率统计（D）

2. 桃花青素修饰分子机理

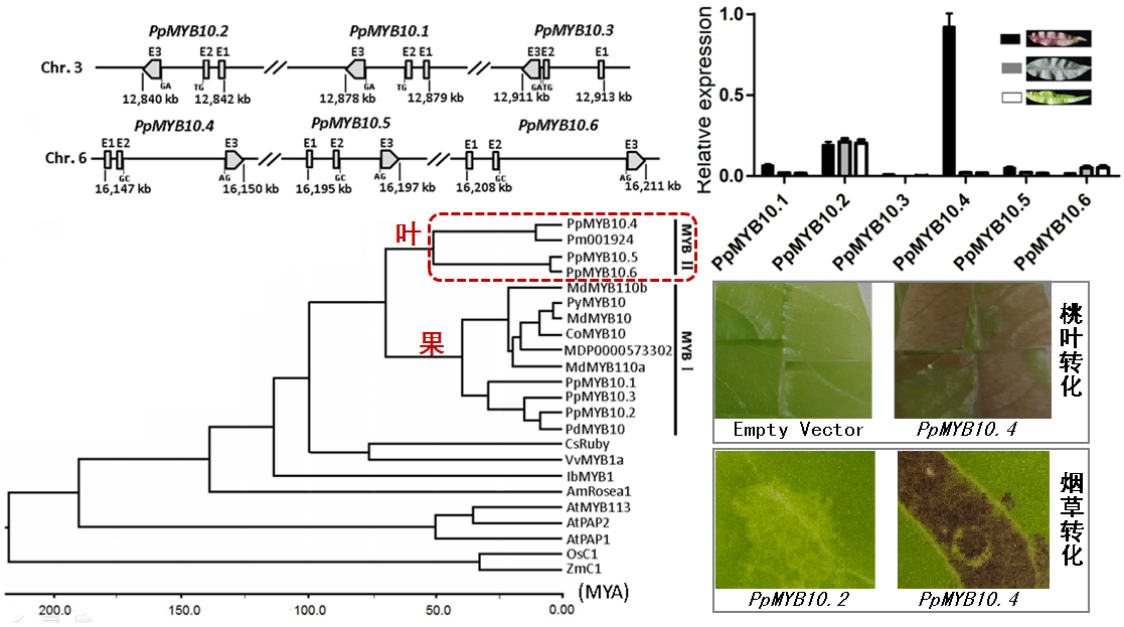
揭示了桃花青苷多样性与糖基化和甲基化修饰有关，获得了参与桃花青素修饰的相关基因，发现了桃糖基化和甲基化基因均经历了复制、功能分化的演变过程，明确了桃花青素修饰的分子机理（图 1），这为今后花青苷组分遗传改良提供了理论依据和改良工具。同时，发现栽培桃在花青素组成方面与新疆桃非常相似，但明显不同于山桃、甘肃桃，这为新疆桃为栽培桃的过渡体假说提供了新的理论依据。



桃花青素修饰示意图

3．桃花青苷着色的关键基因挖掘

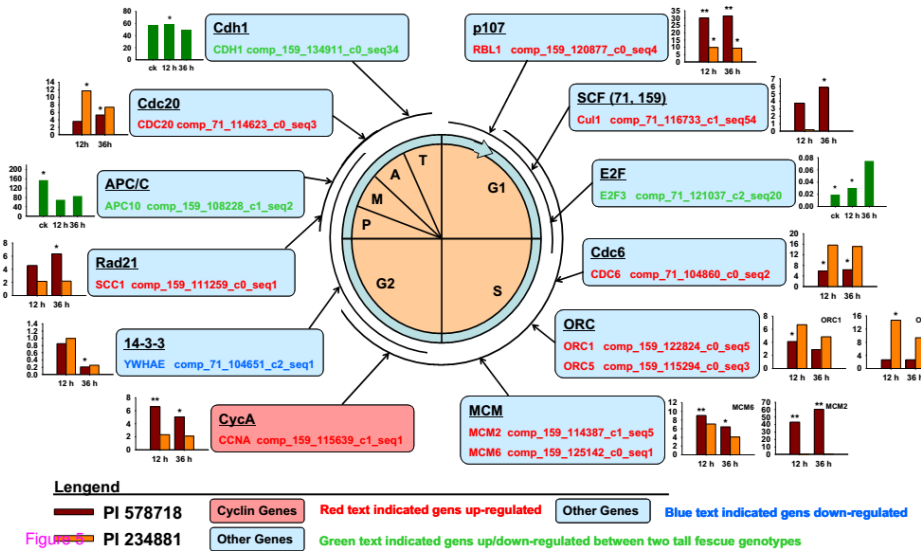
通过红、绿叶转录组比较分析，并结合前人红叶性状基因定位的结果，发掘了控制观赏红叶桃叶片花青苷着色的关键基因 *PpMYB10.4*，该基因在绿色桃叶和烟草叶片中瞬时表达都能促进花青苷积累着色；同时揭示了控制植物花青苷合成的 MYB 调节基因可分成两类，它们拥有共同的祖先，但大约在 7 千万年发生了功能分化，分别调控叶、果花青苷的积累。研究成果弄清了植物花青苷 MYB 调节基因的进化特征，为桃花青素着色的遗传改良提供了理论基础与分子工具。



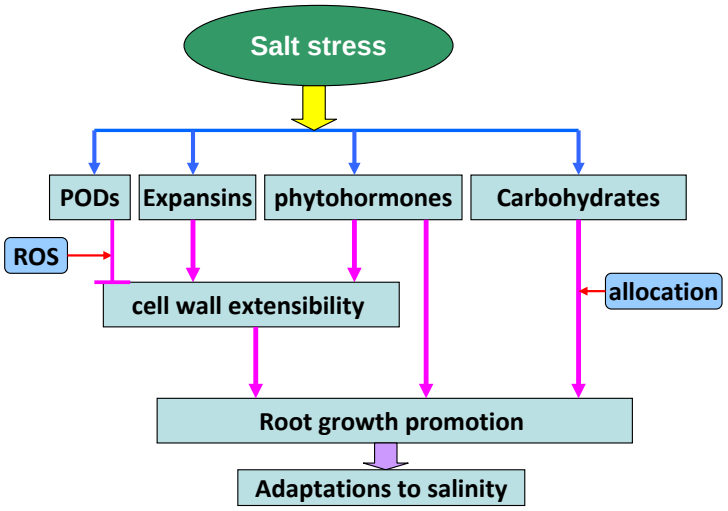
控制桃红叶性状的 *PpMYB10.4* 及其进化、表达特征和瞬时转化功能分析

4. 草坪草耐逆分子生理机制

针对非生物胁迫对草坪草发展利用的限制，利用极端耐逆和敏感高羊茅和狗牙根材料，采用 SSR 标记性状关联分析、RNA-Seq 和 GC-MS 技术，系统研究了草坪草耐逆分子生理机制，发现高羊茅耐高温与 PSⅡ反应中心 PITotal 和 ETRma 活性下降有关，并鉴定了两个耐热指数 HTI 和 Y（Ⅱ）；盐胁迫下糖类和氨基酸类代谢物在狗牙根系中大量富集从而增强耐盐性；并发现 116 和 37 个 SSR 位点（P <5×10^{-4}）分别与耐热和耐镉性状相关联；获得了高羊茅耐高温 Unigene 12,974 条；获得了狗牙根耐低温 Unigene 121,166 条和耐盐 Unigene 103,324 条，狗牙根耐盐能力主要受激素、活性氧和糖代谢相关基因的上调表达调控。获得一株对镉具有极端耐受性的微生物棘孢曲霉真菌（*Aspergillus aculeatus*），棘孢曲霉真菌能够显著抑制镉的狗牙根体内的向上运输从而提高狗牙根的耐镉能力。另外，对选育的耐逆和品质优的草坪草在武汉五里界进行了千亩草坪示范种植，在安陆进行了百亩草坪示范种植。以上研究为培育耐逆和草坪草质量优良的草种提供了大量有用的基因资源和科学基础。



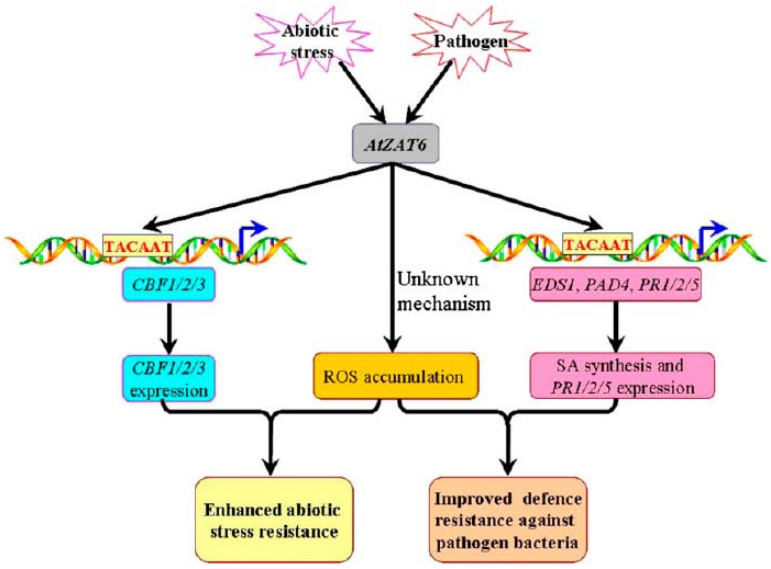
高温诱导的细胞分裂和凋亡相关基因 RNA-Seq 分析



RNA-Seq 技术揭示狗牙根应答盐胁迫的信号途径

5. 植物应答外界胁迫信号途径上游转录因子功能的解析

在植物胁迫反应过程中，众多的转录因子承上启下，起着至关重要的作用。通过大规模基因芯片分析，鉴定了一个受多种非生物胁迫诱导表达的转录因子 *AtZAT6* 和 *AtHAP5A*。*AtZAT6* 可以正调控植物对非生物胁迫(包括干旱、盐和冷害)的抗性。利用染色质免疫共沉淀技术，发现 *AtZAT6* 直接结合 *AtCBF1-3* 启动子区的 TACAAT 元件并正调控这些基因的表达。而 *AtHAP5A* 作为转录因子可以直接结合 CCAAT 元件并调控下游 *AtXTH21* 基因，并通过调控活性氧代谢和脱落酸(ABA)的敏感性参与植物对冷冻害胁迫应答。相关研究结果深化了人们对于植物应答冷害分子机制的认识，具有重要的理论意义。

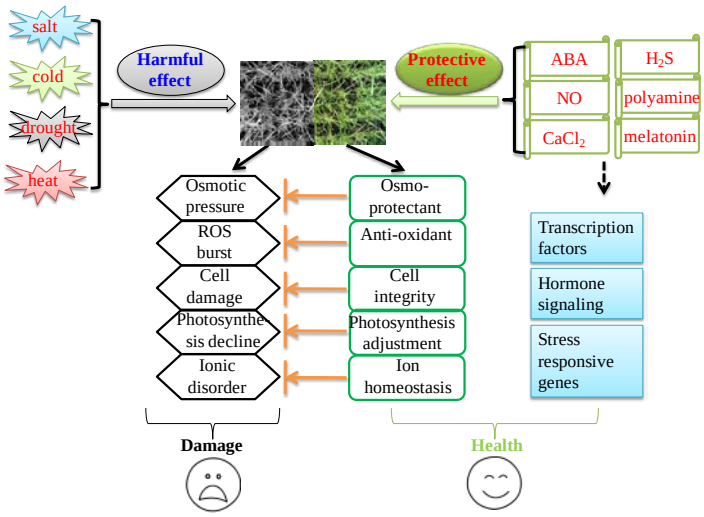


ZAT6 调控下游基因的表达和植物抗性

外界胁迫诱导了锌指蛋白转录因子 ZAT6 的表达。ZAT6 进一步结合到下游 CBF 和 PR 基因启动子的 TACAAT 区，调控相应基因的表达，从而提高植物对非生物胁迫和生物胁迫的抗性

6. 外源植物激素提高植物抗性的机理

研究发现叶片衰老可以诱导植物体内褪黑素的合成，而叶片衰老和外源褪黑素处理均下调转录因子 *IAA17* 基因的表达。*IAA17* 直接参与调控衰老相关基因(SEN4 和 SAG12)的表达是褪黑素抑制叶片衰老所必需的。同时发现冷害可以诱导植物体内褪黑素的积累和 *AtZAT6* 基因的表达。证实内源褪黑素影响了 *AtZAT6* 的表达，后者通过调控 *CBF1-3* 基因的表达来提高植物对冷胁迫的抗性。另外通过外源褪黑素处理，发现可以诱导狗牙根中 2361 个基因的上调和 1572 个基因的下调，包括 *bZIP*、*DREB*、*ERF*、*MYB* 等转录因子以及与 ABA、JA 信号传导途径相关的基因。褪黑素处理可以调控活性氧代谢、胁迫相关基因的表达和多种代谢物的积累。上述结果为进一步利用外源小分子物质来提高植物的抗逆性提供了理论依据。



外源小分子物质处理诱导植物抗逆性的机理

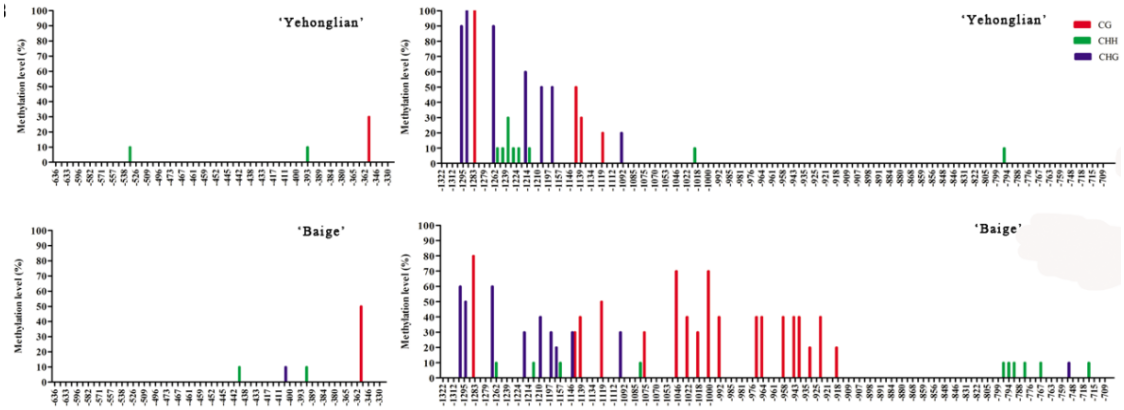
非生物胁迫诱导植物体内产生活性氧，并导致离子毒害、渗透势的改变、细胞的损伤，并损害光合作用。外源小分子物质和植物激素处理可以激活下游的代谢通路，缓解胁迫给植物带来的损伤，提高植物的抗逆性。

（三）特色农业资源植物的种质创新和可持续利用

1. 莲

1）荷花花色的遗传机理研究

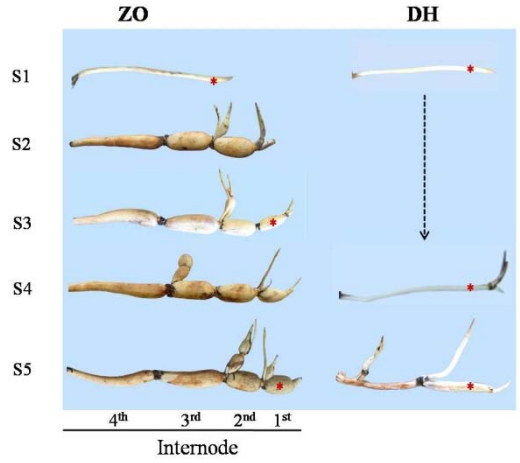
以红色和白色的花莲品种为亲本进行杂交， F2 代群体红白花色的分离比率约为 3:1，该结果显示红白花色是一个受单基因控制的质量性状。在此基础上对红色和白色的品种进行了比较蛋白质组学研究。结果显示 *ANS* 基因表达的差异可能是导致红白花色差异的主要因素。基因序列分析显示在红色和白色的品种中该基因及其启动子序列不存在差异。对启动子序列的甲基化分析显示 MyB 转录因子结合区域附件的一段序列的甲基化强度在白色品种中高于红色品种。这种差异引起的表观遗传调控可能是引起荷花红白花色的主要原因。



红色（上）与白色（下）荷花品种 *ANS* 基因启动子序列的甲基化

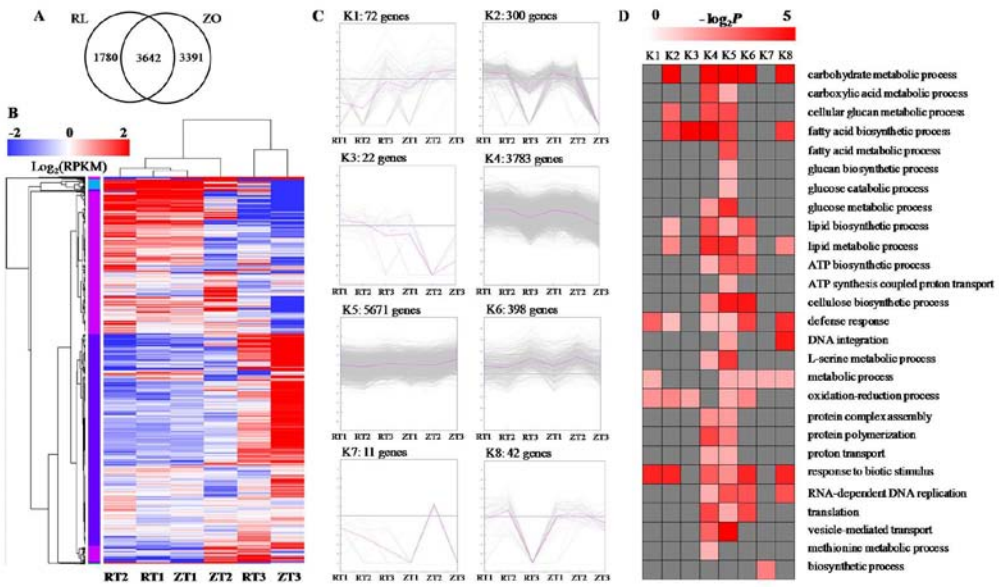
2）莲藕地下茎膨大的调控机理研究

分别对地下茎膨大的藕莲品种和地下茎不膨大的花莲品种的地下茎发育的不同时期进行转录组研究，结果显示在地下茎膨大的品种中差异表达的基因数量远远多于地下茎不膨大的数目。进一步的分析显示光周期、植物激素等因素可能参与了对地下茎膨大的调控。此外，还分别以这两种材料为父母本进行杂交，获得 F1 代植株后自交获得了 F2 代群体。将用于进一步的研究膨大的遗传规律。以上研究结果对于莲的育种实践具有重要的指导意义。



地下茎的发育不同阶段

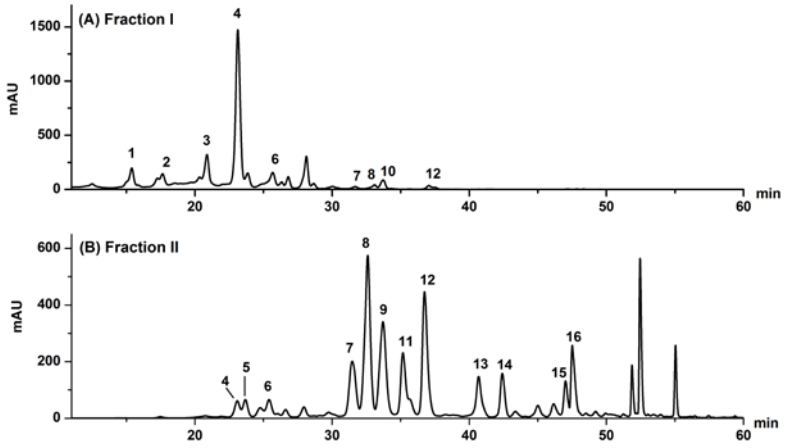
膨大（ZO）与不膨大品种（DH）



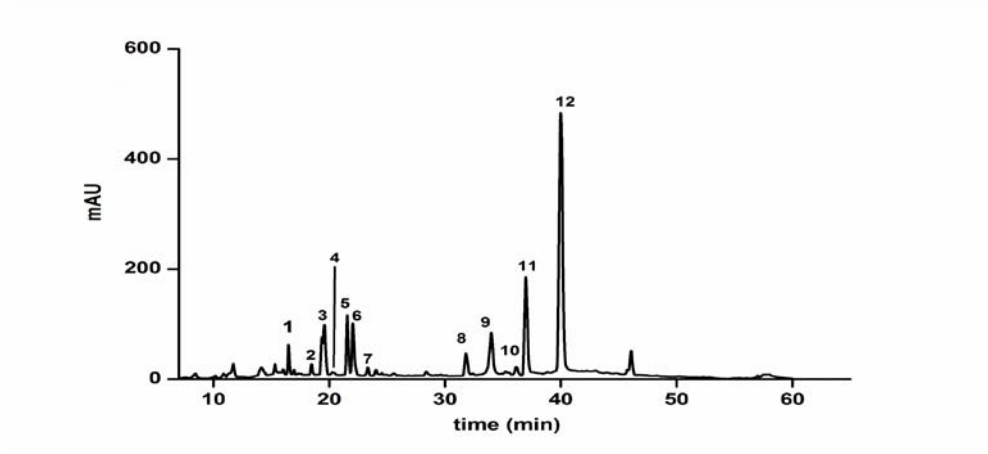
地下茎发育过程中两种不同品种中的差异表达基因图

3）莲中功能化合物分析

利用离线二维色谱和质谱技术对莲中的黄酮类和生物碱类成分进行了系统的分析研究。从莲子心中分析检测到至少 16 个黄酮类成分，其中 8 个为首次从莲中发现；分析检测到至少 12 个生物碱类成分，其中有 8 个为首次发现。



莲子心中的黄酮类成分色谱图

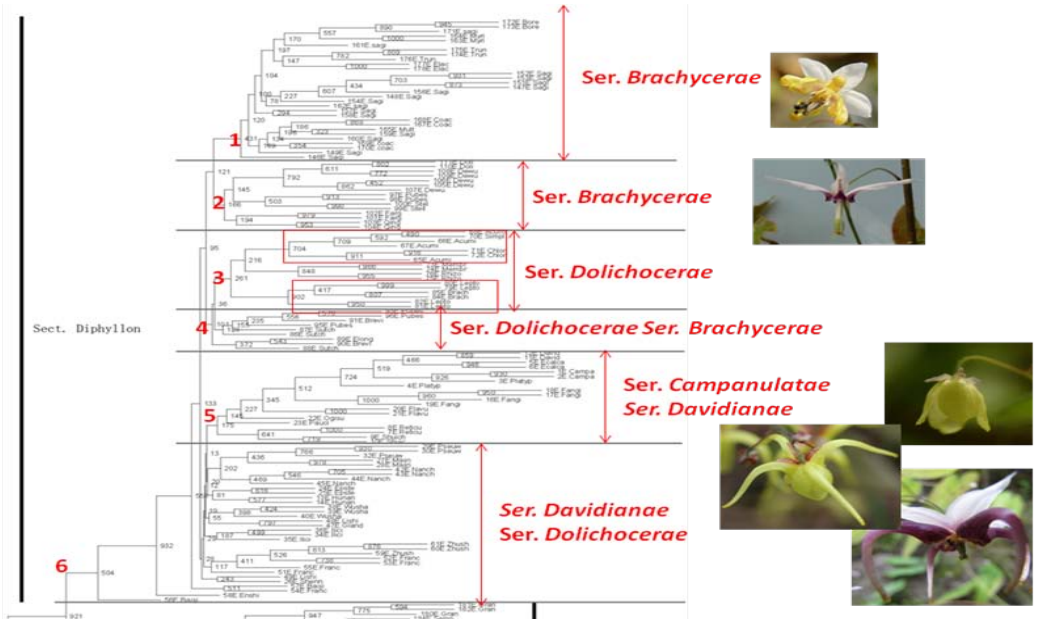


莲子心中的生物碱类成分色谱图

2. 药用植物

- 淫羊藿

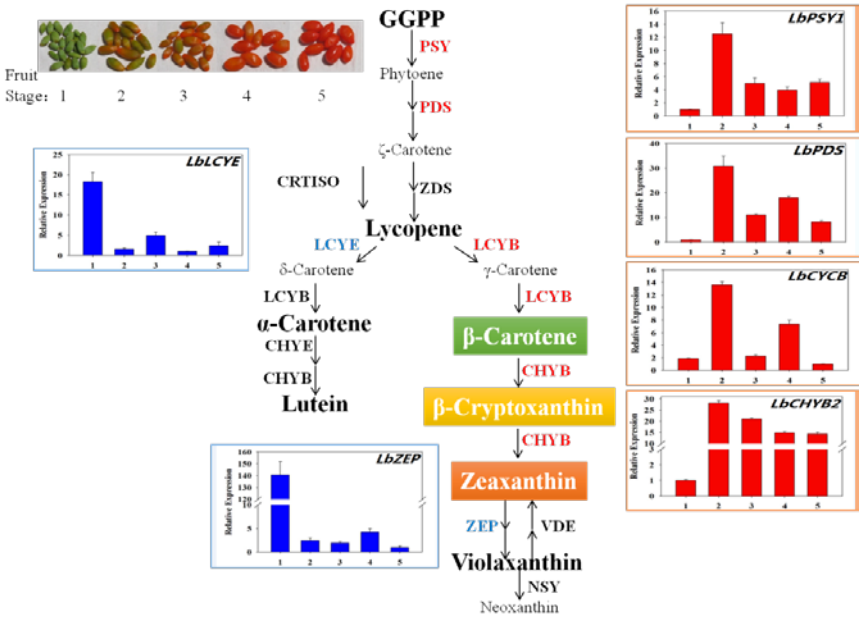
收集淫羊藿属所有物种 DNA 材料近 300 余份，植物活体材料近 2000 株。对淫羊藿属进行了系统全面的分类学修订，并进行了分子系统发育重建。确认中国淫羊藿属植物 42 种；描述了两个新种，将 8 种、3 变种处理为新异名；纠正了前人对 5 个单叶物种的描述，重新探讨了它们的系统位置；修订了 8 个物种的花被片特征，其中 4 个种重新进行了属下归类；为 4 种、1 变种指定了新模式。以淫羊藿属 58 个物种和 3 个变种共 144 个样本为研究对象，利用 AFLP 技术开展系统分类学研究。该结果首次很好的支持了经典分类学的研究结果，同时相对于以前分子系统学研究对于中国淫羊藿属属下系统关系不能给予任何启示，本研究中国类群聚为 5 个与花部特征密切相关的类群，首次证实了中国淫羊藿属属下系统关系与花部特征密切相关。



基于 AFLP 的淫羊藿属物种的系统分类学研究

- 枸杞

深入研究了玉米黄素在宁夏枸杞果实中大量积累的机制：宁夏枸杞果实中有色体的发育为类胡萝卜素的积累提供了储存空间；体外酶功能研究证实了宁夏枸杞中三种重要类胡萝卜素合成酶（有色体特异性的 PSY1、CYC-B 和 CRTR-B2）的催化功能；通过对宁夏枸杞中类胡萝卜素生物合成相关基因的表达分析，发现类胡萝卜素生物合成相关基因转录水平的调控很好的解释了宁夏枸杞果实中玉米黄素的大量积累。

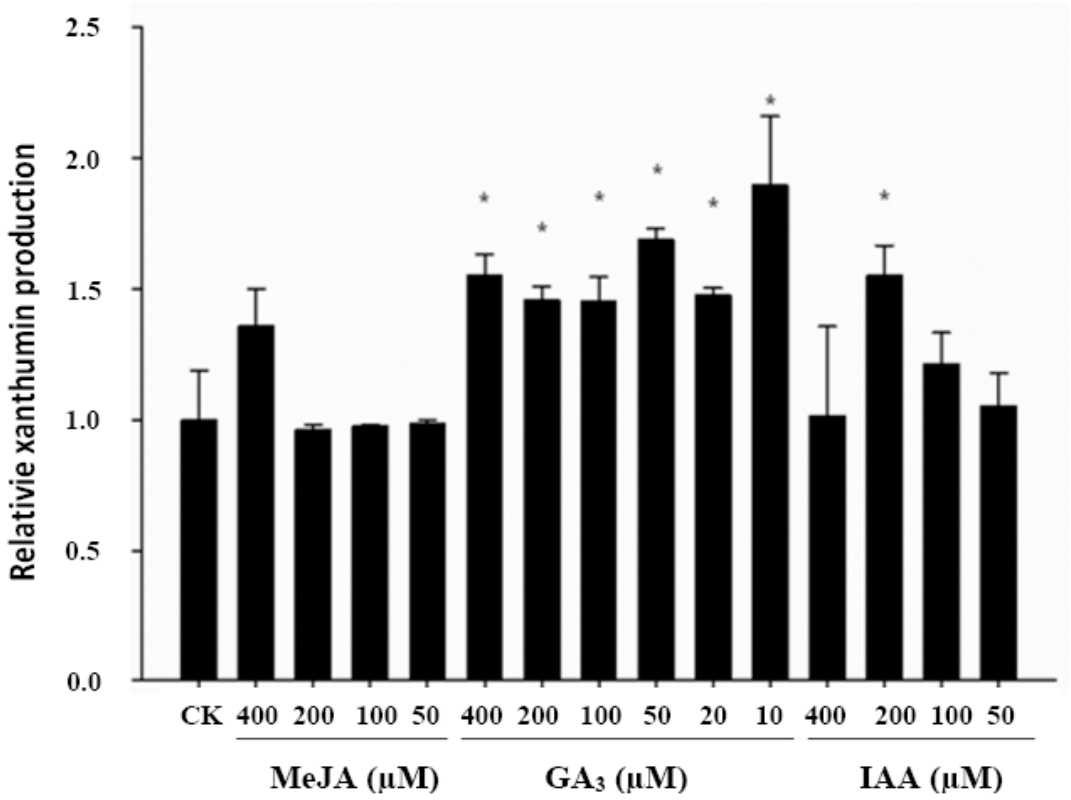


类胡萝卜素生物合成相关基因转录水平的调控是宁夏枸杞果实中玉米黄素大量积累的主要原因

随着果实的发育成熟，玉米黄素上游的生物合成基因（DXS2、PSY1、PDS、ZDS、CRTISO、CYC-B 和 CRTR-B2）的表达量明显增加，导致了玉米黄素的大量积累；LCY-E 和 ZEP 的表达量随着果实的发育明显降低，导致宁夏枸杞成熟果实中没有检测到 α -胡萝卜素分支类胡萝卜素及玉米黄素下游类胡萝卜素的积累。

- 苍耳

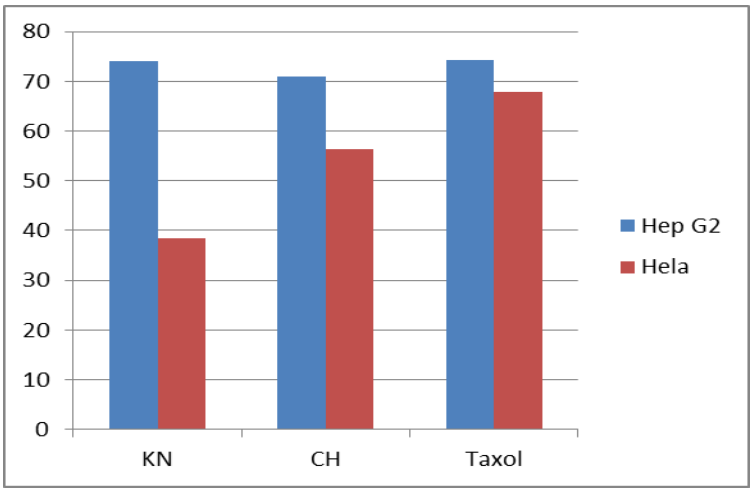
采用 LC-MS 分析方法研究了不同外源激素对苍耳素生物合成的影响，结果发现赤霉素显著促进了苍耳素的生物合成，进一步解析了赤霉素促进苍耳素合成的机理，结果显示赤霉素诱导了苍耳素合成相关基因的上调表达而对苍耳植物腺体细胞发育无任何影响。上述实验结果为激素调控苍耳素合成提供了科学依据。



赤霉素显著促进了苍耳素的生物合成

- 仙茅

建立并比较了中国和非洲仙茅提取分离馏分的指纹图谱，并测试了其相应的抗肿瘤活性；已经成功分离鉴定出其单体化合物 4 个，希望得到的单体化合物 15 个。

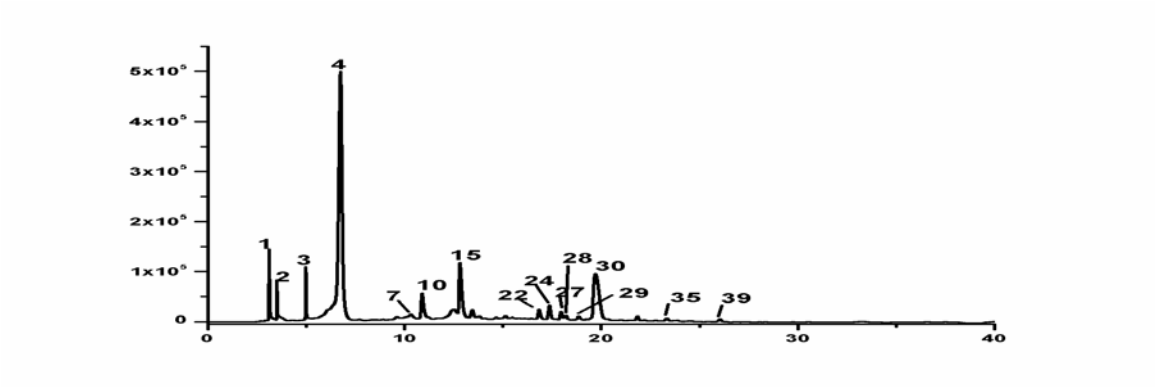


中国和非洲仙茅提取分离馏分抗肿瘤活性的差异

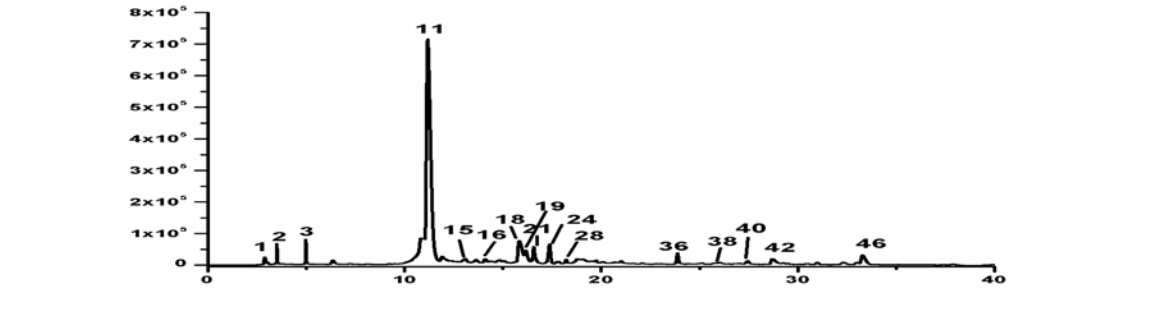
KN-Kenya,CH-China; Hep G2 人肝癌细胞，Hela,人子宫颈癌细胞

- 石松

从中国和非洲的石松中共分离检测到 48 个生物碱类成分，初步分析出具有显著产地差异的生物碱 9 个，有 10 多个生物碱为首次从石松中发现。



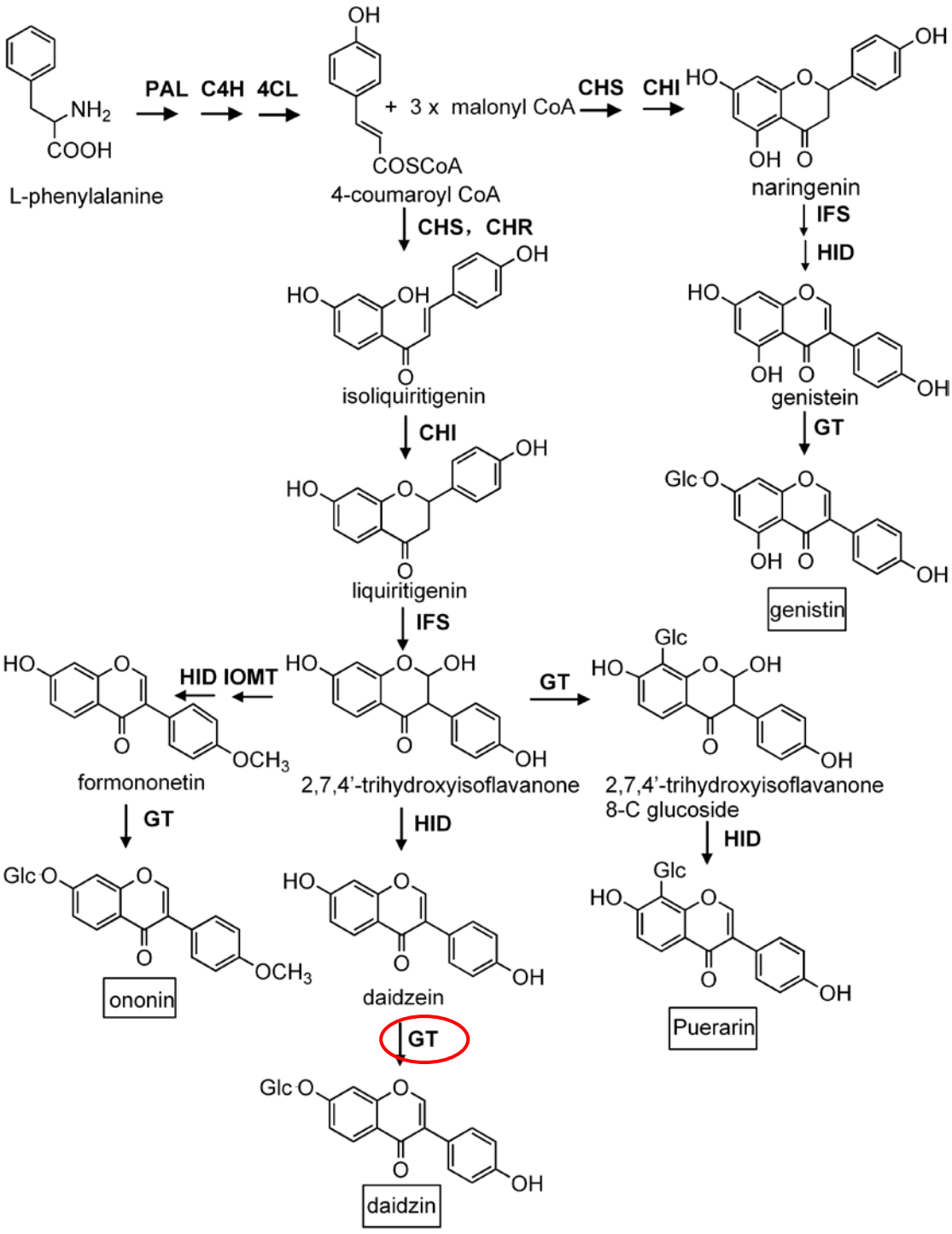
湖北石松中的生物碱类成分指纹图谱



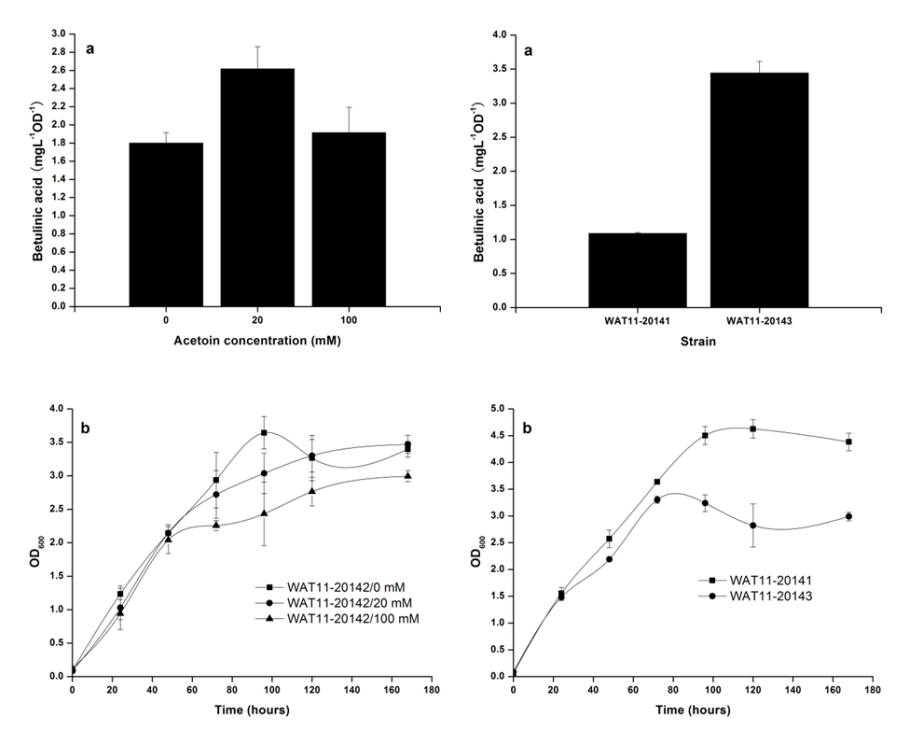
非洲石松中的生物碱类成分指纹图谱

- 其他药用植物

成功构建了野葛根与叶的转录组文库并进行了系统分析；利用上述数据库，挖掘了药用化合物大豆苷合成关键糖基转移酶基因；以旋覆花为研究材料，分离并进一步功能分析了催化倍半萜内酯形成的关键 P450 基因；优化了植物药用化合物白桦脂酸生物合成途径。



大豆苷合成关键糖基转移酶基因的分离(其中红圈表示所挖掘的关键糖基转移酶基因)

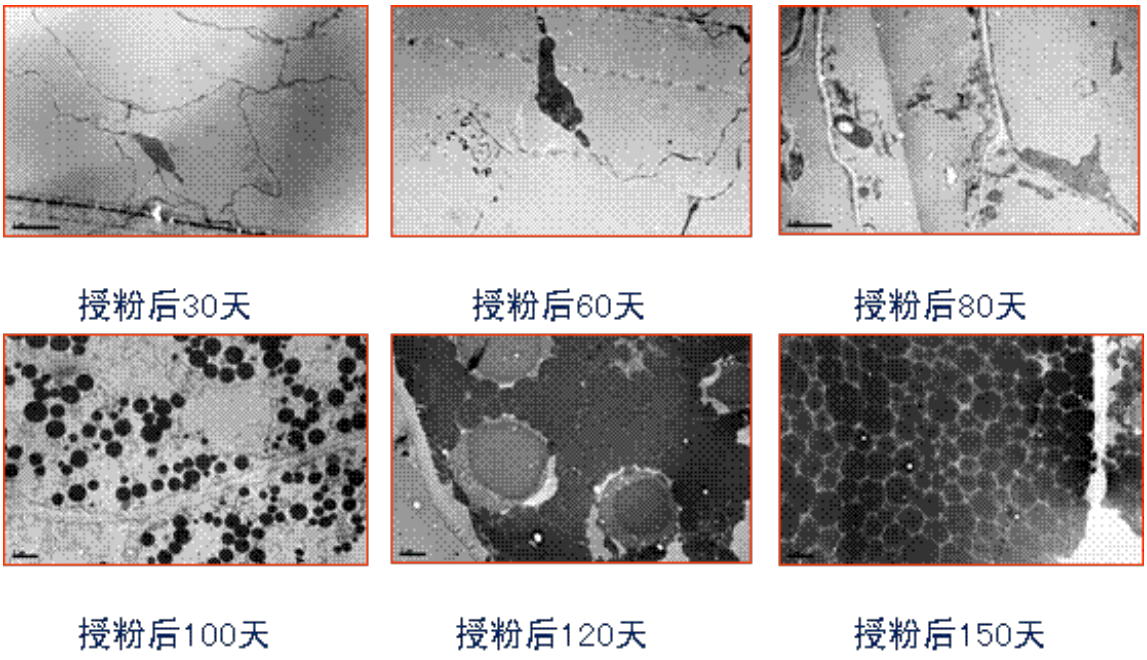


氧离子与辅酶因子供应浓度显著影响白桦脂酸的生物合成

3. 能源植物

- 油桐

与中科院基因组所的胡松年研究员的课题组合作，对油桐基因组进行了测序拼接工作。初步组装情况为：500 bp 的 Scaffold 有 67,411 个；总长度为 1.17G bp；N50 为 153 K。利用投射电镜观察了油桐种子发育过程中油脂的积累，发现在授粉后 80 天仍然没有油体的积累，从授粉后 100 天开始进入迅速积累过程，授粉后 150 天，细胞内充满油体。含油量测定也验证了这一现象。确定了桐油提取的样品处理条件；建立了桐油脂肪酸组分测定的 GC 分析方法。完成了油桐群体近 400 份种子脂肪酸组分及含油量的测定。目前筛选到几份种仁含油量达 60%以上的基因型以及桐酸含量低于 70%的基因型，有待进一步验证。分析了不同基因型桐油放置多年后的表型；发现桐油表型差异与脂肪酸组分变异存在一定关系。上述研究为油桐油脂代谢相关基因分子机理工作做好了前期准备，具有重要的理论价值。

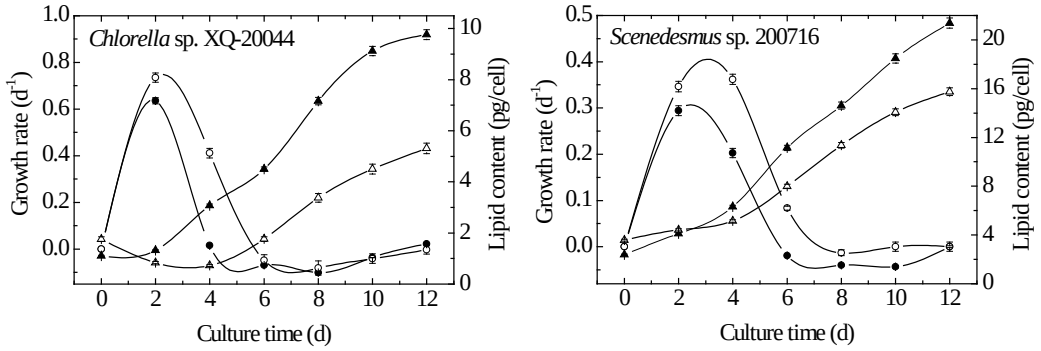


油桐种子不同发育时期油脂的积累

- 微藻

1）探明分批培养绿藻的“二步法”产油特性

利用环形培养池模拟系统，在不同氮源浓度条件下对一株小球藻和一株栅藻进行了分批培养。在培养前期，两株微藻细胞迅速分裂，达到最大生长速率，而总脂含量在 20%左右且基本保持稳定；培养后期细胞停止分裂，但生物质干重仍然持续增加，同时总脂含量显著增高，生物质干重的增加主要来自细胞内油脂的积累。表明绿藻分批培养生产油脂的过程具有两步法特征：第一步是细胞生产阶段，微藻细胞迅速分裂从而增加细胞数量、产生大量生物质；第二步是油脂生产阶段，细胞分裂终止，胞内油脂开始积累。鉴于氮源胁迫是诱导绿藻油脂积累的最主要因素，推测绿藻产油可能受氮源阈值（范围）控制，培养液中氮源浓度高于阈值（范围）时细胞分裂旺盛，不积累油脂；低于阈值（范围），则细胞开始积累油脂。如果存在这一阈值（范围），通过调控氮源浓度即能建立“一步法”培养模式。如果不存在氮源阈值（范围），两步法培养模式则是提高油脂产率的最佳途径。



比生长速率及单细胞油脂含量随培养时间的变化

数据点代表两次培养实验的平均值 ○ 比生长速率（0.3g L^{-1} 硝酸钠）● 比生长速率（0.1g L^{-1} 硝酸钠）▵ 单细胞总脂含量（0.3g L^{-1} 硝酸钠）▲ 单细胞总脂含量（0.1g L^{-1} 硝酸钠）

2）建立小球藻 *Chlorella*“一步法”产油模式，成功实现同步生长和产油

国内外培养产油微藻均采用分批培养模式，细胞生长和油脂积累分两步先后完成，油脂积累时细胞生长受到严重抑制，从而不可避免地降低了油脂产率，成为微藻生物柴油研发中的一个技术难题。本研究在恒定的温度、光照强度、pH 值、CO_2 供应等条件下在柱式光反应器中对一株具有应用潜力的产油微藻-蛋白核小球藻 *Chlorella pyrenoidosa* XQ-20044 进行连续培养，当稀释速率为 0.48-1.44 d^{-1} 时，小球藻以 0.48-1.44d^{-1} 的生长速率达到稳定生长状态（Steady-state），胞内三酰基甘油的含量显著提高，即迅速生长的小球藻细胞亦可积累油脂，调控这一同步生长和油脂积累的关键因素是氮源（硝态氮）比输入速率。油脂产率最高达到 144.93 mg $L^{-1}d^{-1}$，与相同条件下分批培养相比，油脂产率提高了 48%，脂肪酸组成没有显著差异。研究结果证明微藻“一步法”产油的可行性和高效性，为提高微藻产油效率开辟了一条新的途径。

小球藻 *C. pyrenoidosa* XQ-20044 在连续培养和分批培养时生长和油脂积累的比较

Culture mode	Dilution rate (d^{-1})	Nitrate consumed (mM/8d)	Biomass productivity (mg $L^{-1}d^{-1}$)	Lipid content (% DW)	Lipid productivity (mg $L^{-1}d^{-1}$)	C16+C18 (% total FFA)
Continuous	0.48	2.71	417.81±26.88	34.69±0.68	144.93±5.16	93.68±1.15
Continuous	0.72	4.07	482.40±13.15	25.51±1.06	124.25±5.65	95.22±0.97
Continuous	0.96	5.42	539.87±30.73	23.32±1.31	125.89±8.50	93.47±2.04
Continuous	1.44	8.13	641.52±24.37	19.97±063	128.12±4.28	92.12±1.42
Batch	/	2.71	215.81±8.08	44.07±6.04	96.28±4.55	93.92±1.17

四、科研产出

本年度实验室共发表科研论文 94 篇（见附录二），其中 SCI 论文 82 篇，以重点实验室为第一作者/共同第一作者或通讯作者的 SCI 论文 66 篇（含 Top 30%34 篇，Top 10% 12 篇）；主编论著 2 部；授权专利 9 项，申请专利 9 项。作为第二单位参与完成的“毒品原植物 DNA 检测技术及应用”项目获公安部科学技术奖二等奖。

五、人员信息

1. 队伍建设

重点实验室现有固定人员 66 人（见附录三），包括研究员 16 人（其中 10 人入选中国科学院“百人计划” ），副研究员 22 人，助理研究员 24 人，获博士学位的人数占固定人员总数的 87.88 %。

人才引进：

副研究员：邓显豹

青年博士：潘程、丁俊、李明、孙延霞

2. 研究生培养情况

现有博士生导师 14 人，硕士生导师 29 人，在读研究生 87 人（见附录四），其中博士 35 人（含 1 名外籍留学生），硕士 52 人（含 4 名外籍留学生）。本年度毕业研究生 27 人，其中博士 14 人，硕士 13 人，在站博士后 3 人，本年度出站博士后 1 人。研究生培养取得的成绩：

1 名研究生获“院长优秀奖”；1 名研究生获“朱李月华优秀博士生奖”； 1 名研究生获“保罗生物科技优秀学生奖”； 1 名研究生获院“优秀博士论文”

16 名研究生分获院“优秀毕业生”、 “三好学生”或“优秀学生干部”荣誉称号；

2 名研究生获教育部“国家奖学金”；

2 名研究生分获湖北省“优秀博士论文”和“优秀硕士论文”；

4 名研究生获武汉教育基地“优秀毕业生”荣誉称号。

导师获奖情况：

李绍华研究员获中国科学院“优秀研究生指导教师奖”；

傅金民研究员获中国科学院“朱李月华优秀教师奖”。

六、合作与交流

大力开拓广泛的国际合作渠道，提高研究水平。目前实验室与美国康奈尔大学、美国伊利诺伊大学、美国肯塔基大学、美国克莱姆森大学、法国波尔多大学、新西兰植物和食品研究所、澳大利亚昆士兰大学、日本筑波大学等国外高校或科研院所建立了长期合作关系。本年度共有科研人员 20 人次赴国外参加国际会议或开展合作交流。邀请 9 人次来实验室进行学术交流。邀请国内外专家来室讲学 17 人次，其中来自国外单位的专家 6 人次，来自国内单位专家 11 人次（见附录五）。

1. 中-非联合研究中心对非工作进展顺利

7 月 29 日-8 月 1 日， 应肯尼亚乔莫•肯尼亚塔农业与技术大学（Jomo Kenyatta University of Agriculture and Technology）的邀请，中科院代表团访问肯尼亚，与非方合作伙伴共同举行了“中-非联合研究中心”建设工作对接会，并以“中-非联合研究中心”的名义与肯尼亚国家博物馆、马赛马拉大学签署了合作协议。

8 月 2 日-8 月 6 日，应坦桑尼亚渔业研究所的邀请，中科院代表团访问坦桑尼亚，并与坦桑尼亚渔业研究所签署了合作协议。为拓展“中-非联合研究中心”在东非地区的合作伙伴网络，代表团还访问了坦桑尼亚国家公园管理局。

9 月 28 日-30 日，肯尼亚马赛马拉大学董事会主席 Okumu John Joseph、名誉校长 Ngunjiri Philip Gichonge、常务副校长 Nadolo Mary Khakoni 等一行 5 人应中-非联合研究中心邀请参观访问了武汉植物园。中方研究人员分别就植物天然化合物开发、药用植物与能源植物利用及生物多样性保护作了研究报告。常务副校长 Nadolo Mary Khakoni 女士代表马赛马拉大学表达了该校在上述领域，尤其是资源植物开发利用与市场开拓方面，与武汉植物园进行长期稳定合作的愿望。代表团本次访华与武汉植物园就双方未来的合作研究领域、合作方式和人才培养等实施方案进行了深入商讨。

10 月 13 日-14 日，肯尼亚高等教育与科技部司长 Areba Nyang'ate、肯尼亚 JKUAT 校长 Mabel Imbuga、副校长 Romanus O. Odhiambo、中-非中心肯方主任 Robert W. Gituru、校董事会财务总监 Bertha Dena Joseph 等一行 5 人访问了武汉植物园。访问期间主要就双方在项目建设期间需要承担的义务进行了深入磋商，包括仪器免税通关、环境评价批准、植物的调查与收集等方面，双方还就现代农业研究与示范区的用地规模及建设内容进行了深入的讨论。

10 月 25 日-26 日，陪同坦桑尼亚总统访华的坦国家公园管理局局长 Allen Kijazi 访问了武汉植物园。中-非联合研究中心执行主任王青锋向 Allen Kijazi 详细介绍了武汉植物园的机构设置、科学研究和公共服务。Allen Kijazi 也介绍了

坦桑尼亚国家公园管理局的基本情况，并热忱欢迎中国科学家赴坦桑尼亚就生物多样性调查与保护、生物资源有效利用、水环境污染等开展合作研究，希望能给坦桑尼亚面临的环境保护与旅游开发矛盾提供科学的管理经验。Allen Kijazi 的此次来访加强了中心与坦桑尼亚相关部门的合作交流，扩展了中心在东非的合作平台，有助于提升中心在国际上的影响力。

七、仪器设备

按照科学院有关实验室公共平台建设的要求，实验室高度重视现有平台的维护与共享。同时，实验室平台建设也得到了科学院和全园的大力支持，科学院本年度支持购买的双向电泳成像及分析系统、多功能激光成像仪、磷屏成像分析系统、多通道固相萃取系统、全自动快速溶剂萃取仪、流式细胞仪、激光粒度粒形分析仪、质谱仪等已陆续到位并投入使用，这些设备和设施的添置将为实验室相关科研工作的开展提供更加有利的保障。

目前，实验室 5 万元以上仪器设备共 98 台（套），设备总值 3300 余万元（见附录六）。

八、2014 年度大事记

3 月，王瑛研究员当选为第十五届中国植物学会药用植物及植物药专业委员会副主任委员。

4 月，王瑛研究员获 2014 年度国家留学基金委公派留学项目资助。

4 月 29 日，重点实验室举行 2014 年首场学术交流会，相关学科组博士研究生作了 11 场学术报告，谢燕和邓娇获优秀报告奖。

5 月，杨平仿、谷超、闫娟 3 位科研人员获 2014 年度中国科学院公派出国留学计划项目资助。

5 月 7 日，重点实验室召开第一届学术委员会第五次会议，委员们就平台建设、人才引进和下一步研究计划等提出了建议。

12 月 8 日，重点实验室举行 2014 年第二场学术交流会，4 位学科组长作了学术报告，科研人员及研究生共 90 余人参加了会议。

九、附录

附录一 在研项目（经费单位：万元）

1. 国际合作项目

序号	来源	项目名称	总经费	本年实到经费	开题时间	结题时间	负责人
1	日本丸善制药株式会社	甘草资源调查和优良品种培育	152	5	2010-12-1	2016-4-30	陈建军
2	意大利	新品种授权商业开发	1000	187.5	2005-8-1	2028-12-1	龚俊杰
合计	--	--	1152	192.5	--	--	--

2. 国家及部委项目

序号	类别	项目名称	总经费	本年实到经费	开题时间	结题时间	负责人
1	国家重大专项	泛喜马拉雅地区植物综合考察与植物志编研	20	0.05	2013-6-1	2017-5-30	李建强
2	国家重大专项	中国产油微藻调查	105	14	2012-5-1	2017-4-30	李夜光
3	973	重要园艺作物果实品质形成机理与调控	317	15	2011-1-1	2015-12-31	李绍华
4	863	若干植物源化合物的人工合成体系构建	45.5	8.75	2012-12-1	2015-12-31	章焰生
5	863	利用微藻清洁制备生物燃料关键技术	50	0	2013-1-1	2016-12-31	李夜光
6	863	微藻生物固碳关键技术与产品开发	45	13.5	2014-1-1	2016-12-31	王中杰
7	国家基金重大	芒草的驯化性状评价和群体遗传学分析	30	5	2012-1-1	2015-12-31	李建强
8	国家基金重点	野生二粒小麦抗锈病和耐逆境基因的挖掘研究	220	0	2011-1-1	2015-12-31	傅金民
9	国家基金重点	葡萄种质资源抗旱寒评价及其抗性基因挖掘与利用	300	0	2012-1-1	2016-12-31	李绍华

10	基金委其他基金项目	药用植物箭叶淫羊藿类黄桐异戊烯转移酶基因的克隆与功能研究	23	9.2	2013-1-1	2015-12-31	黄文俊
11	基金委其他基金项目	远缘嫁接中枸杞砧木对番茄接穗的生长发育和果实品质的影响	23	9.2	2013-1-1	2015-12-31	吕海燕
12	基金委其他基金项目	箭叶淫羊藿叶片发育过程中花青素苷和黄酮类醇苷代谢分支的分子调控	82	0	2013-1-1	2016-12-31	王　瑛
13	基金委其他基金项目	狗牙根适应盐胁迫的要/冠异速生长特性及机理研究	23	0	2012-1-1	2014-12-31	胡龙兴
14	基金委其他基金项目	高羊茅铅富集关键基因的发掘与功能解析	25	10	2013-1-1	2015-12-31	李惠英
15	基金委其他基金项目	野生狗牙根抗寒关键基因发掘和功能解析	80	0	2013-1-1	2016-12-31	傅金民
16	基金委其他基金项目	桃 CHI 基因启动子区一个插入片段参与调控叶片花青素合成的机制研究	33.7	0	2012-1-1	2014-12-31	周　莹
17	基金委其他基金项目	桃 endoPG 基因簇控制果肉溶质和离核性状遗传的分子机制研究	25	10	2013-1-1	2015-12-31	谷　超
18	基金委其他基金项目	苹果果实酸度 QTLs 区域液泡膜 H+-ATPase 及相关调节基因的关联分析和功能研究	80	48	2014-1-1	2017-12-31	韩月彭
19	基金委其他基金项目	猕猴桃维生素 C 遗传机理及 Vc 代谢关键基因发掘	25	0	2012-1-1	2015-12-31	李大卫
20	基金委其他基金项目	温度影响红肉猕猴桃呈色的色素降解机制研究	60	6	2012-1-1	2015-12-31	王彦昌
21	基金委其他基金项目	孑遗植物鹅掌揪分布范围限制的进化机制研究	75	0	2013-1-1	2016-12-31	姚小洪
22	基金委其他基金项目	雌雄异株猕猴桃种间杂交高密度遗传图谱构建	23	13.8	2014-1-1	2016-12-31	张　琼
23	基金委其他基金项目	中华猕猴桃复合体及其近缘种的分子谱系地理学研究	80	48	2014-1-1	2017-12-31	李作洲

24	基金委其他基金项目	软枣猕猴桃野生种质的遗传多样性及果实品质的关联解析	15	15	2014-1-1	2015-12-30	王彦昌
25	基金委其他基金项目	拟南芥蜡质合成 CER16 的功能解析	80	48	2014-1-1	2017-12-31	吕世友
26	基金委其他基金项目	葛根素生物合成途径关键糖基转酶基因的克隆与功能分析	55	5.5	2012-1-1	2015-12-31	章焰生
27	基金委其他基金项目	3'-甲氧基葛根素生物合成途径中关键甲基转移酶基因的克隆与功能分析	23	13.8	2014-1-1	2016-12-31	黎　佳
28	基金委其他基金项目	倍半萜药用化合物苍耳素生物合成关键 P450 基因的分离与功能分析	80	48	2014-1-1	2017-12-31	章焰生
29	基金委其他基金项目	猕猴桃属种复合体的综合分类学研究	55	5.5	2012-1-1	2015-12-31	李新伟
30	基金委其他基金项目	安息香科的系统发育基因学和花形态发生研究	80	48	2014-1-1	2017-12-31	王恒昌
31	基金委其他基金项目	东亚特有植物水青树的谱系地理学研究	80	48	2014-1-1	2017-12-31	李建强
32	基金委其他基金项目	基于高密度遗传图谱的葡萄果实白藜芦醇含量 QTL 定位	60	6	2012-1-1	2015-12-31	汪　念
33	基金委其他基金项目	葡萄高密度遗传图谱构建及果实糖酸含量 QTL 定位	85	51	2014-1-1	2017-12-31	李绍华
34	基金委其他基金项目	中国芒抽穗期与分蘖相关基因等位变异与功能标记开发研究	22	0	2012-1-1	2014-12-31	赵　华
35	基金委其他基金项目	一氧化氮介导植物对干旱胁迫的反应机理研究	25	10	2013-1-1	2015-12-31	施海涛
36	基金委其他基金项目	美洲黑杨对冬季水淹及后期恢复的性别特异性响应	83	0	2013-1-1	2016-12-31	杨　帆

37	基金委其他基金项目	拟南芥ADRM3和ADRM7参与调控ABA信号转导和干旱胁迫应答的分子机制	23	13.8	2014-1-1	2016-12-31	王艳平
38	基金委其他基金项目	拟南芥 ABA 受体相互作用蛋白的鉴定和功能解析	80	48	2014-1-1	2017-12-31	产祝龙
39	基金委其他基金项目	微藻产油、固碳、脱流、除硝等一体化模式研究	80	0	2013-1-1	2016-12-31	李夜光
40	基金委其他基金项目	萝卜源 Ogura 细胞质雄性不育新恢复基因座新恢复基因 Rfo2 的克隆及功能解析	22	0	2012-1-1	2014-12-31	汪志伟
41	基金委其他基金项目	解析蕨类植物叶绿体基因组高变区的进化式样、过程和机制	80	48	2014-1-1	2017-12-31	王　艇
42	基金委其他基金项目	水稻柱头与花粉识别机理的蛋白质组学研究	23	0	2012-1-1	2015-12-31	王　坤
43	基金委其他基金项目	水稻线粒体定位基因 OsB12D1 在种子萌发中的功能及作用机理研究	23	0	2012-1-1	2015-12-31	何冬丽
44	基金委其他基金项目	莲花期候选基因关联分析与开发	22	8.8	2013-1-1	2015-12-31	杨　美
45	基金委其他基金项目	水稻种子萌发过程中参与赤霉素信号传递的关键转录因子挖掘与功能分析	84	0	2013-1-1	2016-12-31	杨平仿
46	基金委其他基金项目	鹅掌揪柱头与花粉识别过程的转录组分析及关键基因挖掘	27	16.2	2014-1-1	2016-12-31	李　明
47	行业性重大专项	油桐良种规模繁育技术体系研究示范	20	6	2013-1-1	2016-12-30	傅金民
48	行业性重大专项	国家猕猴桃种质资源圃	270	0	2010-10-1	2018-12-31	龚俊杰
49	科技支撑计划	主要果树新品种选育	78	14	2013-1-1	2017-12-31	李绍华
50	院重大项目	生态系统固碳现状、速率、机制和潜力	120	0	2011-1-1	2015-12-31	傅金民

51	院重大项目	中国科学院现代农业示范与区域创新集群计划	18	12	2013-1-1	2015-12-31	傅金民
52	院重大项目	非洲草坪草种质资源收集与评价	100	30	2013-1-1	2015-12-31	傅金民
53	院重大项目	考古遗存典型农作物-野生植物鉴定	140	13.3	2011-1-1	2015-12-1	韩月彭
54	院重大项目	咖啡野生种质资源的收集与保育	100	30	2013-1-1	2015-12-31	韩月彭
55	院重大项目	非洲油脂植物调查、收集与成分分析	60	20	2013-1-1	2015-12-31	吕世友
56	院重大项目	中华大典.生物典.植物分类编纂	20	0	2010-1-1	2015-12-31	李建强
57	院重大项目	新一代能源作物芒草的驯化生物学	40	15	2012-1-1	2015-12-30	闫　娟
58	院重大项目	中国科学院现代农业示范与区域创新集群计划	120	0	2013-1-1	2015-12-31	李绍华
59	院重大项目	中国植物园联盟	160	100	2013-1-1	2016-12-31	李绍华
60	院重大项目	非洲植物天然化合物合作开发研究	100	40	2013-1-1	2015-12-31	郭明全
61	院重大项目	天然药用植物系列复合天然功能保健品研发	50	10	2013-5-9	2017-5-8	郭明全
62	院重大项目	非洲和亚洲地区水稻生物和非生物胁迫抗性基因资源的转录组学和蛋白质组学比较研究	220	50	2013-1-1	2015-12-31	杨平仿
63	部委项目	中药（民族药）标准化研究	35	10.1	2013-1-1	2015-12-31	王　瑛
64	部委项目	湖南重金属超富集草坪草的选育与示范	40	25.2	2011-1-1	2014-12-31	李惠英
65	部委项目	果树果实重要品质改善的遗传机制与分子改良	173	0	2011-1-1	2015-12-31	韩月彭
66	部委项目	遗传分析仪基因型检测相关配套关键技术的开发与应用	50	0	2012-11-26	2015-12-31	韩月彭
67	部委项目	基于高密度遗传图谱的猕猴桃糖酸性状的 QTL	40	10	2012-1-1	2015-12-31	姚小洪

		定位					
68	部委项目	典型县域猕猴桃产业化示范	470	218	2014-1-1	2016-5-30	钟彩虹
69	部委项目	能源植物油桐的重要功能基因挖掘及种质资源创新	260	0	2013-12-1	2016-12-31	吕世友
70	部委项目	狗牙根适应逆境环境的分子机制	260	50	2013-4-19	2016-4-19	产祝龙
71	部委项目	植物对逆境胁迫应答的分子机制研究	40	10	2014-1-1	2017-12-31	施海涛
72	部委项目	药用植物化学生物学	260	0	2013-12-1	2017-12-31	郭明全
73	部委项目	植物雄性不育研究	40	10	2012-1-1	2015-12-31	汪志伟
74	部委项目	植物繁殖过程中种子形成与萌发的分子机理	260	0	2011-1-1	2014-12-31	杨平仿
75	部委项目	植物药用化合物合成途径功能基因的挖掘	260	0	2011-1-1	2014-12-31	章焰生
合计	--	--	6801.2	1317.7	--	--	--

3. 横向合作及其他项目

序号	类别	项目名称	总经费	本年实到经费	开题时间	结题时间	负责人
1	自主部署	非洲特色植物资源的研究	140	56	2014-1-1	2016-12-31	郭明全 产祝龙
2	自主部署	川东-鄂西植物多样性形成及维持机制	120	0	2011-9-1	2015-8-30	李建强
3	自主部署	莲生物学与遗传育种	170	68	2014-1-1	2016-12-31	杨平仿
4	研究所自选	果树分子育种	77	0	2008-11-1	2015-12-31	韩月彭
5	研究所自选	猕猴桃种质资源圃成果推广	50	0	2012-5-1	2017-12-31	龚俊杰
6	研究所自选	油桐新种质遗传张良	70	0	2013-1-1	2016-12-31	吕世友
7	研究所自选	天然药物生物合成学	77	0	2010-1-1	2015-12-31	章焰生
8	研究所自选	植物分典编纂 PP	20	0	2010-9-1	2014-12-31	李建强
9	研究所自选	草坪草-狗牙根对水分胁迫的应答机制	70	0	2011-11-1	2015-12-31	产祝龙

10	研究所自选	药用植物化学与功能代谢组学	70	0	2012-8-1	2015-8-30	郭明全
11	研究所自选	资源植物繁殖生物学	77	0	2010-1-1	2015-12-31	杨平仿
12	其他国家费	重要果树基因资源发掘与创新的关键技术合作研发	100	14	2011-1-1	2015-12-31	韩月彭
13	其他国家费	猕猴桃种质资源收集、编目、更新与利用	78	30	2012-1-1	2015-12-31	钟彩虹
14	其他国家费	中国喜马拉雅地区蒿属（Artemisia)分类修订	10	0	2012-6-1	2015-12-31	李晓东
15	地方政府委托	枸杞新品种培育的应用基础研究	60	10	2014-1-1	2015-12-31	王　瑛
16	地方政府委托	枸杞新品种中科绿川 1 号标准化生产及产业化技术提升	108	50	2014-1-1	2015-6-30	王　瑛
17	地方政府委托	兴山县低效生态公益森改造一期项目	20	6	2012-8-2	2015-12-31	廖　燎
18	地方政府委托	猕猴桃特色资源收集与新品种选育研究	86.5	10	2011-11-1	2015-12-30	王彦昌
19	地方政府委托	中国猕猴桃溃疡病菌的流行机制研究	6	0	2013-1-1	2015-12-31	李　黎
20	地方政府委托	六盘水地区猕猴桃产业合作	200	0	2013-1-1	2018-12-31	钟彩虹
21	地方政府委托	湖北野生植物资源调查	55	28	2013-11-1	2015-12-31	王恒昌
22	地方政府委托	杨树用于三峡水库消落区生态防护林建设的关键技术研究	30	0	2012-1-1	2015-12-31	杨　帆
23	地方政府委托	秭归县三峡水库消落区治理一期规划设计	27.64	0	2012-3-1	2015-12-31	杨　帆
24	企业委托	特种功能蔬菜推广种植	35	0	2010-10-1	2016-9-30	王　瑛
25	企业委托	中科绿川 1 号枸杞及第二代升级品种生产技术服务	75	0	2013-1-1	2016-12-31	王　瑛
26	企业委托	宜昌年产 3 亿株现代化	77.5	0	2010-9-1	2015-12-30	廖　燎

		种苗工厂项目					
27	企业委托	万州区生态农业园建设实施规划	20	0	2010-8-1	2015-2-28	廖　燎
28	企业委托	三峡库区设施农业园及生态农业园设计	810	0	2010-9-1	2015-12-31	廖　燎
29	企业委托	三峡库区巴东县溪丘湾生态茶叶示范园建设	27	0	2011-1-1	2015-12-31	廖　燎
30	企业委托	兴山生态农业园可研	55.5	0	2012-1-1	2015-12-31	廖　燎
31	企业委托	合作建设成都猕猴桃资源基因库	120	0	2009-8-20	2014-8-20	钟彩虹
32	企业委托	猕猴桃新品种中试及开发	100	0	2011-1-1	2016-12-31	钟彩虹
33	企业委托	猕猴桃科研开发-华夏联诚果业	490	0	2011-11-10	2021-11-10	钟彩虹
34	企业委托	红肉猕猴桃新品种东红	950	0	2012-2-24	2032-2-24	钟彩虹
35	企业委托	猕猴桃新品种及配套栽培技术	128	88.6	2012-11-27	2015-11-27	钟彩虹
36	企业委托	黄肉新品种金圆的开发技术研究	830	0	2013-2-1	2033-2-28	钟彩虹
37	企业委托	猕猴桃新品种新技术中试	30	0	2013-7-1	2018-7-30	钟彩虹
38	企业委托	神农架猕猴桃种质技术服务	100	20	2014-6-1	2019-12-30	王彦昌
39	企业委托	湖南省花垣县猕猴桃产业示范基地开发合作	164	16.4	2014-7-1	2017-6-30	钟彩虹
40	企业委托	都江堰市国际猕猴桃博览馆布展项目	20	20	2014-6-2	2015-12-31	李大卫
41	企业委托	红昇猕猴桃品种示范技术服务	550	15	2014-8-1	2019-12-31	王彦昌
42	企业委托	天然保健品研发	24	0	2013-4-1	2016-4-30	章焰生
43	企业委托	免疫调节天然药用植物种植和研发中心建设	50	10	2013-1-1	2017-12-31	李长福
44	企业委托	猕猴桃的维生素深加工工艺的分析和优化	40	20	2014-1-1	2016-12-31	章焰生
45	企业委托	微生物合成抗癌化合物白桦脂酸	60	20	2014-7-7	2017-12-30	章焰生
46	企业委托	三峡库区后续工程生态	100	0	2012-3-1	2015-12-31	王 勇

		农业一期实施规划					
47	企业委托	黄鹤楼（红坪）百草园适栽植物栽培与示范	112	0	2008-1-1	2014-12-30	王　庆
48	企业委托	功能蔬菜和神农金菊的种植	50	10.8	2012-4-26	2017-12-31	王　庆
49	企业委托	红球藻中试及规模化养殖	60.8	0	2006-11-15	2016-11-14	李夜光
50	企业委托	微藻生物柴油成套技术开发	475	0	2010-1-1	2015-12-31	李夜光
51	企业委托	微藻生物能源示范工程-红球藻培养	50	0	2012-5-1	2014-12-31	李夜光
52	企业委托	黄石猕猴桃开发项目	187.8	88.6	2012-12-27	2015-12-27	钟彩虹
53	其他委托	新型鲜食枸杞品种及采后保鲜技术示范	14.5	5.8	2013-1-1	2015-12-31	王　瑛
54	其他委托	宁夏耐盐碱草及葡萄筛选	28	0	2011-11-1	2015-12-31	傅金民
55	其他委托	猕猴桃新品种研究	360	12.9	2005-7-1	2015-12-31	龚俊杰
合计	--	--	7916.2	600.1	--	--	--

4. 2014 年获批的国家自然基金项目

序号	项目类别	项目名称	批准经费	开始时间	结题时间	负责人
1	青年科学基金	蕨类植物叶绿体 RNA 编辑及其适应性进化研究	26	2015-1-1	2017-12-31	高　磊
2	青年科学基金	拟南芥 CER9 基因参与调节种子油脂合成的机理研究	24	2015-1-1	2017-12-31	李荣俊
3	青年科学基金	鬼伞属真菌中抗肿瘤 guanacastane 类二萜及其作用机制研究	25	2015-1-1	2017-12-31	刘源振
4	青年科学基金	基于谱系地理学的濒危植物银鹊树的保护生物学研究	24	2015-1-1	2017-12-31	田　华
5	青年科学基金	低温响应转录因子 CdCAMTA1 在野生狗牙根抗寒中的功能及其抗寒分子机理研究	25	2015-1-1	2017-12-31	陈　良

6	面上项目	高羊茅耐热相关 miRNA 及耙基因的发掘与功能解析	85	2015-1-1	2018-12-31	傅金民
7	面上项目	萝卜细胞质雄性不育育性恢复新机制的分子解析	85	2015-1-1	2018-12-31	汪志伟
8	面上项目	桃三个 MADS-box 基因调控果实发育其分子机理的研究	86	2015-1-1	2018-12-31	谷　超
9	面上项目	WRKY28 和 WRKY43 调控葡萄耐寒的分子机理研究	85	2015-1-1	2018-12-31	辛海平
10	面上项目	结合连锁分析和关联分析定位莲花期和地下茎QTL及候选基因鉴定	85	2015-1-1	2018-12-31	杨　美
11	国际合作与交流	苹果果实糖酸性状的全基因组关联分析及其遗传调控网络研究	278	2015-1-1	2019-12-31	韩月彭
12	国际合作与交流	基于 SSR 遗传图谱的狗牙根叶片质地 QTL 定位	20	2015-1-1	2016-12-31	傅金民
13	国际合作与交流	具有生物相容性的环境刺激响应的聚合物纳米管在抗癌药物控释体系中的应用	20	2015-1-1	2016-12-31	郭明全
14	国际合作与交流	莲的基因挖掘与功能分析	20	2015-1-1	2015-12-31	韩月彭
合计			888			

附录二 科研产出

1. 发表论文情况（按第一作者姓氏拼音排序）

1) Chen K, Sun XY, Amombo E, Zhu Q, Zhao ZJ, Chen L, Xu QG, Fu JM. High correlation between thermotolerance and photosystem II activity in tall fescue. **Photosynthesis Research** 2014, 122: 305-314 (SCI, IF: 3.185)
2) Chen YY, Bao ZX, Qu Y, Li W, Li ZZ. Genetic diversity and population structure of the medicinal orchid *Gastrodia elata* revealed by microsatellite analysis. **Biochemical Systematics and Ecology** 2014, 54 :182-189 (SCI, IF: 1.17)
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2. 专著

黄宏文.The Genus Actinidia, a World Monograph, 北京，科学出版社

黄宏文主编，副主编：钟彩虹、张琼、朱元朝、龚俊杰.　猕猴桃研究进展 VII,2014.7，北京，科学出版社

3. 获奖

王瑛研究员参与完成的“毒品原植物 DNA 检测技术及应用”项目获公安部科学技术奖二等奖（武汉植物园为第二完成单位）

4. 专利

授权专利 9 项，申请专利 9 项

序号	类别	名　称	专利号/申请号	发明人
1	授权	一种低盐诱导处理提高水稻和苜蓿及狗牙根抗盐和抗旱性的方法	ZL201310096011.X	产祝龙、施海涛
2	授权	一种多胺诱导处理提高狗牙根抗盐和抗旱的方法	ZL201310095350.6	产祝龙、施海涛

3	授权	一种调控植物类黄酮合成的基因及应用	ZL201310062733.3	王瑛、黄文俊、孙伟、吕海燕
4	授权	一种生物农药增效剂	ZL201310616674.X	王庆、董静洲、王瑛、张华峰、雷灿
5	授权	岩土边坡快速生态恢复方法	ZL201310221702.8	傅金民、娄燕宏
6	授权	一种提高多年生黑麦草愈伤组织再生率的方法	ZL201310284048.5	傅金民、郭慧娟、娄燕宏
7	授权	一种干旱诱导处理提高水稻和苜蓿及狗牙根抗盐和抗旱性的方法	ZL201310094573.0	产祝龙、施海涛
8	授权	利用草坪草-微生物联合修复镉污染土壤的方法	ZL201310538654.5	傅金民、罗宏基、娄燕宏
9	授权	利用耐镉真菌在镉污染土壤中进行生态修复的方法	ZL201310538617.4	傅金民、罗宏基、娄燕宏
10	申请	一种高效分离和测定草坪草内源激素的方法	201410074332.4	傅金民、胡龙兴、娄燕宏
11	申请	同步分离测定草坪草内源脱落酸、赤霉素和生长素的方法	201410074685.4	傅金民、胡龙兴、娄燕宏
12	申请	一种高效分离和测定草坪草内源细胞分裂素的方法	201410074317.X	傅金民、胡龙兴、娄燕宏
13	申请	拟南芥抗逆相关基因 AtZAT6 及制备方法和应用	201410246461.7	产祝龙、施海涛
14	申请	一种十数樟组织培养快繁方法	201410393484.0	吕世友、张玲玲
15	申请	用于猕猴桃杂交群体雌雄性别鉴定的 SSR 分子标记 A001	201410525014.5	黄宏文、张琼、钟彩虹、龚俊杰、刘春燕
16	申请	用于猕猴桃杂交群体雌雄性别鉴定的 SSR 分子标记 A002	201410524999.X	黄宏文、张琼、钟彩虹、龚俊杰、刘春燕
17	申请	用于猕猴桃杂交群体雌雄性别鉴定的 SSR 分子标记 A003	201410523043.8	张琼、黄宏文、刘春燕、钟彩虹、龚俊杰
18	申请	高产优质猕猴桃园建立的高垄栽培法	201410630810.5	钟彩虹、黄宏文、姜正旺、张鹏、龚俊杰

附录三 人员信息

1. 第一届学术委员会

姓　名	职　称	工作单位	室内职务
傅廷栋	教授、院士	华中农业大学	名誉主任
邓秀新	教授、院士	华中农业大学	主任
彭良才	教授、长江学者	华中农业大学	委员
匡汉晖	教授、长江学者	华中农业大学	委员
戴思兰	教授	北京林业大学	委员
何光源	教授	华中科技大学	委员
张本刚	研究员	中国医学科学院	委员
丁文军	教授	中国科学院大学生命科学学院	委员
李来庚	研究员	中国科学院上海植物生理生态研究所	委员
吴国江	研究员	中国科学院华南植物园	委员
陈　凡	研究员	中国科学院遗传与发育生物学研究所	委员
何舜平	研究员	中国科学院水生生物研究所	委员
李绍华	研究员	中国科学院武汉植物园	委员
张全发	研究员	中国科学院武汉植物园	委员
王　瑛	研究员	中国科学院武汉植物园	委员
王　艇	研究员	中国科学院武汉植物园	委员
傅金民	研究员	中国科学院武汉植物园	委员
韩月彭	研究员	中国科学院武汉植物园	委员
杨平仿	研究员	中国科学院武汉植物园	秘书

2. 重点实验室固定人员名单（相同职称按姓氏拼音排序）

序号	姓名	性别	出生年月	最后学位	所学专业	职务	职称	岗位种类
1	产祝龙	男	1975.9	博士	植物学		研究员	研究
2	傅金民	男	1961.12	博士	园艺学	重点实验室副主任	研究员	研究
3	龚俊杰	女	1957.1	硕士	农学		研究员	研究
4	郭明全	男	1975.10	博士	化学		研究员	研究
5	韩月彭	男	1968.11	博士	作物遗传育种	重点实验室副主任	研究员	研究
6	李建强	男	1954.11	博士	植物学		研究员	研究

7	李绍华	男	1957.9	博士	园艺园林	重点实验室主任	研究员	研究
8	李夜光	男	1962.5	硕士	植物学		研究员	研究
9	吕世友	男	1974.10	博士	植物分子生物学		研究员	研究
10	王恒昌	男	1967.3	博士	植物学		研究员	研究
11	王　庆	女	1955.10	学士	药学		研究员	研究
12	王　艇	男	1969.3	博士	生物化学		研究员	研究
13	王　瑛	女	1973.10	博士	植物遗传学		研究员	研究
14	吴金清	男	1963.6	硕士	植物学		研究员	研究
15	杨平仿	男	1975.7	博士	植物蛋白质组学		研究员	研究
16	章焰生	男	1972.12	博士	植物学		研究员	研究
17	陈　良	男	1981.2	博士	植物分子遗传学		副研究员	研究
18	邓显豹	男	1975.8	博士	园艺		副研究员	研究
19	高　磊	男	1981.5	博士	植物学		副研究员	研究
20	谷　超	男	1985.8	博士	发育生物学		副研究员	研究
21	胡龙兴	男	1982.7	博士	园艺学		副研究员	研究
22	姜正旺	男	1965.6	学士	果树学		副研究员	研究
23	李惠英	女	1977.3	博士	植物学		副研究员	研究
24	李晓东	男	1966.11	博士	植物学		副研究员	研究
25	李新伟	男	1974.10	博士	植物学		副研究员	研究
26	李作洲	男	1967.5	博士	植物学		副研究员	研究
27	施海涛	男	1984.12	博士	发育生物学		副研究员	研究
28	汪志伟	男	1978.12	博士	种群遗传学		副研究员	研究
29	王　坤	男	1981.12	博士	遗传学		副研究员	研究
30	王彦昌	男	1973..9	博士	农学		副研究员	研究
31	王艳平	女	1983.1	博士	发育生物学		副研究员	研究
32	辛海平	男	1980.2	博士	发育生物学		副研究员	研究
33	闫　娟	女	1982.10	博士	植物学		副研究员	研究
34	杨　帆	男	1979.6	博士	植物学		副研究员	研究
35	杨　美	女	1981.8	博士	植物遗传学		副研究员	研究
36	姚小洪	男	1975.11	博士	植物学		副研究员	研究

37	张燕君	女	1980.9	博士	植物学		副研究员	研究
38	钟彩虹	女	1968.2	博士	植物学		副研究员	研究
39	陈方方	女	1982.2	博士	作物生物技术		助理研究员	研究
40	陈建军	男	1979.12	博士	植物学		助理研究员	研究
41	陈　丽	女	1982.10	博士	植物学		助理研究员	研究
42	丁　俊	女	1988.2	博士	分析化学		助理研究员	研究
43	丁　奕	女	1986.10	博士	水生生物学		助理研究员	研究
44	何冬丽	女	1977.11	博士	藻类遗传与生物技术		助理研究员	研究
45	胡　涛	男	1981.10	博士	植物学		助理研究员	研究
46	黄文俊	男	1981.5	博士	植物学		助理研究员	研究
47	黎　佳	女	1982.2	博士	微生物学		助理研究员	研究
48	李大卫	男	1983.5	博士	植物学		助理研究员	研究
49	李　黎	女	1985.12	博士	微生物学		助理研究员	研究
50	李　明	男	1982.10	博士	植物学		助理研究员	研究
51	李荣俊	男	1979.4	博士	发育生物学		助理研究员	研究
52	廖　燎	男	1984.7	博士	园林植物与观赏园艺		助理研究员	研究
53	刘源振	男	1985.10	博士	天然产物化学		助理研究员	研究
54	卢　洋	男	1981.7	博士	植物学		助理研究员	研究
55	潘　程	男	1983.3	博士	植物遗传学		助理研究员	研究
56	孙延霞	女	1984.2	博士	植物学		助理研究员	助研
57	王　鲁	男	1976.12	博士	发育生物学		助理研究员	研究
58	王　欣	男	1982.3	博士	遗传学		助理研究员	研究
59	王中杰	男	1984.8	博士	水生生物学		助理研究员	研究
60	游　均	男	1983.11	博士	生物化学与分子生物学		助理研究员	研究
61	张玲玲	女	1985.3	博士	植物学		助理研究员	研究
62	张　琼	女	1981.1	博士	植物学		助理研究员	研究
63	耿亚洪	女	1962.6	本科	经济管理学		高级实验师	技术
64	孟爱平	女	1968.7	硕士	植物学		高级工程师	技术
65	李长福	女	1971.12	硕士	昆虫学		工程师	技术
66	梁　琼	女	1975.5	博士	植物学		处长	管理

3. 重要人才情况

序号	人员姓名	荣誉称号	获得年份
1	李绍华	中国科学院“百人计划”	2003 年
2	王　瑛	中国科学院“百人计划”	2004 年
3	王　艇	中国科学院“百人计划”	2005 年
4	韩月彭	中国科学院“百人计划”	2008 年
5	傅金民	中国科学院“百人计划”	2008 年
6	杨平仿	中国科学院“百人计划”	2010 年
7	章焰生	中国科学院“百人计划”	2010 年
8	产祝龙	中国科学院“百人计划”	2011 年
9	郭明全	中国科学院“百人计划”	2012 年
10	吕世友	中国科学院“百人计划”	2013 年

4. 国内外学术组织任职情况

序号	姓名	学术组织名称	职务	任职时间
1	产祝龙	中国草学会草业生物技术委员会	理事	2014-
		中国植物生理与分子生物学学会植物生物技术及其产业化分会	理事	2014-
2	龚俊杰	中国园艺学会猕猴桃分会	副理事长	2005-
		湖北省林木品种审定委员会观赏植物组	副组长	2009-2014
		湖北省暨武汉市植物学会	常务理事	2010-
		湖北省园艺学会	理事	2010-
3	韩月彭	湖北省遗传学会	理事	2009-
4	李绍华	国际生物多样性计划中国委员会	委员	2010-2014
		中国科学院生物多样性委员会	委员	2010-2014
		湖北省植物学会、武汉市植物学会	理事长	2008-
		中国园艺学会	常务理事	2005-
		中国园艺学会桃分会	常务理事	2005-
		中国农学会葡萄分会	常务理事	2006-
		中国园艺学会李杏分会	副理事长	2001-
		11TH International Conference on Grapevine Breeding and Genetics	主席	2010-2014
5	李晓东	国际自然保护联盟（IUCN）物种保护专业委员会	专家组成员	2006-
6	李夜光	中国海洋湖泊学会	理事	2012-

		中国藻类学会	常务理事	2011-
7	王　庆	中国植物学会第十五届药用植物和植物药专业委员会	委员□	2014-2019
8	王　艇	中国花卉协会蕨类植物分会第四届理事会	理事	2010-2014
9	王　瑛	中国植物学会药用植物和植物药专业委员会	副主任	2013-
		湖北省植物学会	理事	2007-
		湖北省细胞生物学会	理事	2009-
10	杨平仿	Asia Oceania Agricultural Proteomics Organization (AOAPO)	Council Member	2011-
		中国生物化学与分子生物学会蛋白质组学专业委员会	委员	2011-
		中国植物学会种子科学与技术专业委员会	委员	2011-
11	章焰生	中国科学院大学药学专业硕士培养教指委员会	委员	2012-
12	钟彩虹	中国园艺学会猕猴桃分会	代理秘书长	2012-

5. 国内外学术期刊任职情况

序号	姓名	学术期刊名称	职　务	任职时间
1	产祝龙	Frontiers in Plant Science	客座副主编	2014-
		PLoS ONE	编委	2014-
2	郭明全	The Scientific World Journal	编委	2011-
		Asian Journal of Chemistry	编委	2008-
3	傅金民	Ecotoxicology	编委	2010-
4	韩月彭	Plant Molecular Biology Reporter	编委	2008-
		Canadian Journal of Plant Science	编委	2010-
		PLoS ONE	编委	2013-
5	李绍华	Journal International des Sciences de la Vigne et du Vin	编委	2011-
		《园艺学报》	副主编	2006-
		《果树科学》	副主编	2006-
		《植物科学学报》	主编	2010-
		《广西植物》	编委	2011-
6	王　艇	PLoS ONE	编委	2012-
7	杨　帆	BioMed Research International	编委	2013-
8	杨平仿	PLoS ONE	编委	2014-

附录四 人才培养

1. 出站博士后

陈　柯（指导教师：傅金民）

2. 毕业研究生学位和论文情况

序号	姓 名	性别	攻读学位	所学专业	指导教师	论文题目
1	李吉涛	男	博士	植物学	李绍华	葡萄抗寒相关基因 DREB1E、CBF4、ICE1 和 HOS1 的克隆及功能分析
2	肖　贡	男	博士	植物学	王　瑛	枸杞和淫羊藿重要次生代谢途径的生物信息学研究
3	胡　蝶	女	博士	植物学	李建强	苜蓿属的分子系统学研究— 兼论花苜蓿的谱系地理学问题
4	温小斌	男	博士	植物学	李夜光	产油微藻的分离、筛选及高效产油培养模式研究
5	杨爱红	女	博士	植物学	黄宏文	孑遗植物鹅掌楸的居群遗传结构与谱系地理格局研究
6	程　钧	男	博士	植物学	韩月彭	桃花青苷合成及修饰相关基因的鉴定与分析
7	杜志敏	女	博士	植物学	傅金民	施肥、刈割对三峡库区草地生产力、生物多样性及碳氮储量的影响
8	韩　超	男	博士	植物学	杨平仿	水稻种子萌发的蛋白质组学研究
9	李　晶	男	博士	植物学	章焰生	抗 HIV 化合物白桦脂酸的微生物合成的研究
10	李　明	男	博士	植物学	杨平仿	柱头与花粉相互作用的蛋白质组学研究
11	任　景	女	博士	植物学	傅金民	野生二粒小麦遗传多样性评价及条锈病抗性基因位点挖掘与分子定位
12	孙小艳	女	博士	植物学	傅金民	高羊茅种质资源耐热生理评价及分子遗传基础研究
13	孙延霞	女	博士	植物学	李建强	东亚特有植物水青树叶绿体基因组与谱系地理学研究
14	马娟娟	女	博士	植物学	韩月彭	桃类胡萝卜素代谢研究

15	姜　斌	男	硕士	植物学	王　艇	稻属 AA 型植物叶绿体基因组的适应性进化研究
16	王应丽	女	硕士	植物学	王　瑛	淫羊藿类黄酮糖基转移酶基因的克隆及功能分析
17	张　虎	男	硕士	植物学	李夜光	能源微藻资源调查及优良藻种筛选
18	沈笑飞	男	硕士	植物学	王　瑛	黑果枸杞类黄酮代谢重要结构基因和调控基因的克隆及功能验证
19	刘瑞杰	女	硕士	园林植物与观赏园艺	产祝龙	莲应答盐胁迫的生理生化研究和莲 CIPK 基因的克隆分析
20	王莉娜	女	硕士	园林植物与观赏园艺	李绍华	WRKY 基因家族的鉴定及其在葡萄冷胁迫中的功能分析
21	魏国超	男	硕士	园林植物与观赏园艺	韩月彭	原花青素合成及低温促进花青素积累的分子机理研究
22	赵状军	男	硕士	园林植物与观赏园艺	傅金民	高羊茅应答高温胁迫的代谢产物分析
23	刘淑倩	女	硕士	生物工程	傅金民	狗牙根遗传转化体系的构建
24	沈　佳	女	硕士	生物工程	章焰生	酿酒酵母中萜烯类化合物合成调节基因的挖掘及鉴定
25	王　欣	女	硕士	生物工程	杨平仿	水稻授粉过程中雌蕊 microRNA 的挖掘与表达模式研究
26	赵　双	女	硕士	生物工程	韩月彭	苹果内在品质性状的分子遗传机理研究
27	王传德	男	硕士	生物工程	汪志伟	萝卜新细胞质雄性不育系的败育特征及育性恢复位点分子解析

3. 博士后研究人员

Sornkanok Vimolmangkang （指导教师：韩月彭）

汪　巍（指导教师：韩月彭）

刘艳丽（指导教师：杨平仿）

4. 在读博士研究生

年级	姓　名	导师姓名	专业	年级	姓　名	导师姓名	专业
2009	李文彬	黄宏文	植物学	2013	冯　涛	王恒昌	植物学
2009	王淑慧	王　瑛	植物学	2013	金　蕊	产祝龙	植物学
2010	胡伟明	王　瑛	植物学	2013	李缘君	章焰生	植物学
2010	王　博	王　艇	植物学	2013	刘春燕	黄宏文	植物学
2011	方林川	李绍华	植物学	2013	刘翠霞	李绍华	植物学
2011	杨路路	王　瑛	植物学	2013	权文利	产祝龙	植物学
2011	周　晖	韩月彭	植物学	2013	田永强	郭明全	植物学
2012	孙小明	李绍华	植物学	2013	杨贤鹏	吕世友	植物学
2012	杜　奎	李夜光	植物学	2013	袁阳阳	李绍华	植物学
2012	刘永亮	王　瑛	植物学	2013	张　慧	杨平仿	植物学
2012	A.B.M. Khaldun	王　瑛	植物学	2014	范吉标	傅金民	植物学
2012	李　佳	王　艇	植物学	2014	陈桂林	郭明全	植物学
2012	邓　娇	杨平仿	植物学	2014	黄龙雨	杨平仿	植物学
2012	谢　燕	傅金民	植物学	2014	李林懋	吕世友	植物学
2012	叶甜甜	产祝龙	植物学	2014	闫明慧	王恒昌	植物学
2012	苟君波	章焰生	植物学	2014	殷明珠	产祝龙	植物学
2012	朱洺志	郭明全	生态学	2014	张郎郎	李绍华	植物学
2013	马百全	韩月彭	植物学				

5. 在读硕士研究生

年级	姓　名	导师姓名	专业	年级	姓　名	导师姓名	专业
2012	董　霞	郭明全	植物学	2013	赵婷婷	李新伟	植物学
2012	范荣艳	章焰生	植物学	2013	钟　宝	产祝龙	植物学
2012	符子阳	杨平仿	植物学	2013	Erick Amombo	傅金民	植物学
2012	陶　珂	王　艇	植物学	2013	Collins Otieno Ogutu	韩月彭	植物学
2012	薛定磊	王　瑛	植物学	2013	方　庭	韩月彭	园林植物与观赏园艺
2012	闫明科	姚小洪	植物学	2013	高　翔	吕世友	园林植物与

							观赏园艺
2012	杨　力	产祝龙	植物学	2013	郭少剑	李绍华	园林植物与观赏园艺
2012	李菲菲	李建强	植物学	2013	户正荣	傅金民	园林植物与观赏园艺
2012	柴风梅	李绍华	园林植物与观赏园艺	2013	刘　训	产祝龙	生物工程
2012	韩春宇	产祝龙	园林植物与观赏园艺	2013	尚风琴	郭明全	生物工程
2012	郑红玉	韩月彭	园林植物与观赏园艺	2013	付　丹	郭明全	生物工程
2012	成章敏	产祝龙	生物工程	2014	李金珠	产祝龙	植物学
2012	蒋晓明	郭明全	生物工程	2014	李　玲	杨平仿	植物学
2012	陶焕平	李夜光	生物工程	2014	李晓华	章焰生	植物学
2012	周　晨	章焰生	生物工程	2014	刘　奥	王　瑛	植物学
2012	傅金磊	杨平仿	生物工程	2014	邱子栋	郭明全	植物学
2012	马持衡	吕世友	生物工程	2014	唐　萍	姚小洪	植物学
2012	李虹侠	吕世友	生物工程	2014	王宙雅	吕世友	植物学
2013	陈姗姗	王　艇	植物学	2014	徐　准	王　艇	植物学
2013	杜刘稳	王　瑛	植物学	2014	许　岩	李夜光	植物学
2013	刘　婷	郭明全	植物学	2014	郑　斌	闫　娟	植物学
2013	Flora Didii Saleri	郭明全	植物学	2014	王生位	王　瑛	植物学
2013	彭新安	李夜光	植物学	2014	彭　倩	韩月彭	园林植物与观赏园艺
2013	Rebecca Njeri Damaris	杨平仿	植物学	2014	赵亭亭	辛海平	园林植物与观赏园艺
2013	王　琼	杨平仿	植物学	2014	毕傲月	傅金民	生物工程
2013	闫春林	黄宏文	植物学	2014	刘秀林	吕世友	生物工程

6. 研究生获奖一览表

序号	获奖名称	获奖人员	指导教师
1	中国科学院“院长优秀奖”	王莉娜	李绍华
2	中国科学院“朱李月华优秀博士生奖”	孙延霞	李建强
3	中国科学院大学“保罗生物科技优秀学生奖”	谢　燕	傅金民
4	中国科学院“优秀博士论文”	陈　莎	李绍华
5	中国科学院大学“优秀毕业生”	王莉娜	李绍华
6	中国科学院大学“三好学生”	叶甜甜	产祝龙
7	中国科学院大学“三好学生”	朱洺志	郭明全
8	中国科学院大学“三好学生”	刘春燕	黄宏文
9	中国科学院大学“三好学生”	孙小明	李绍华
10	中国科学院大学“三好学生”	王　博	王　艇
11	中国科学院大学“三好学生”	邓　娇	杨平仿
12	中国科学院大学“三好学生”	杨贤鹏	吕世友
13	中国科学院大学“三好学生”	范吉标	傅金民
14	中国科学院大学“三好学生”	程　钧	韩月彭
15	中国科学院大学“三好学生”	冯　涛	王恒昌
16	中国科学院大学“三好学生”	温小斌	李夜光
17	中国科学院大学“三好学生”	杨路路	王　瑛
18	中国科学院大学“三好学生”	李　晶	章焰生
19	中国科学院大学“优秀学生干部”	杜　奎	李夜光
20	中国科学院大学“优秀学生干部”	韩春宇	产祝龙
21	教育部“国家奖学金”	谢　燕	傅金民
22	教育部“国家奖学金”	韩春宇	产祝龙
23	湖北省“优秀博士论文”获得者	陈　莎	李绍华
24	湖北省“优秀硕士论文”获得者	罗宏基	傅金民
25	武汉教育基地“优秀毕业生”	刘瑞杰	产祝龙
26	武汉教育基地“优秀毕业生”	任　景	傅金民
27	武汉教育基地“优秀毕业生”	杨爱红	黄宏文
28	武汉教育基地“优秀毕业生”	李　明	杨平仿

附录五 合作与交流

1. 出访项目

2013 年 11 月 13 日-2014 年 11 月 11 日，受国家留学基金委和湖北省晨光计划联合资助，李惠英副研究员赴美国德州理工大学，进行拟南芥耐热相关分子机制的合作研究。

1 月 3 日-11 日，应肯尼亚乔莫•肯尼亚塔农业与科技大学邀请，郭明全研究员和吕世友研究员赴肯尼亚开展野外考察。

5 月 13 日-22 日，应美国农业部农业研究中心邀请，李绍华研究员赴美国访问美国农业部农业研究中心葡萄遗传研究组，并顺访美国肯塔基大学，洽谈第 11 届葡萄遗传与育种会议的组织事宜。

5 月 20 日-28 日，应美国罗格斯大学邀请，傅金民研究员赴美国开展草坪种质资源收集评价的合作交流。

6 月 13 日-23 日，应美国质谱学会的邀请，郭明全研究员赴美国参加“第 62 届美国质谱学会年会”，在会上作题为“ Comparative Metabolomic Studies on Two Chinese Podophyllum Plants”的报告；并应邀顺访美国国立卫生研究院，就已开展的合作研究进行汇报。

6 月 21 日-28 日，应第 5 届国际应用藻类学会大会组委会邀请，李夜光研究员赴澳大利亚参加“第 5 届国际应用藻类学会大会”，并在会上作题为 “Enhanced Lipid Production In *Chlorella pyrenoidosa* By Continuous Culture”的墙报交流。

7 月 1 日-15 日，应肯尼亚乔莫•肯尼亚塔农业与科技大学邀请，郭明全、吕世友和杨平仿研究员赴肯尼亚开展野外考察。

7 月 1 日-8 月 31 日，应英国伦敦邱园植物标本馆和奥地利维也纳大学植物标本馆邀请，李建强研究员赴英国及奥地利执行猕猴桃科和葫芦科植物标本研究任务。

7 月 28 日-8 月 5 日，应肯尼亚塔农业与科技大学邀请，韩月彭、傅金民和龚俊杰研究员赴肯尼亚开展联合研究和野外考察。

8 月 16 日-23 日，应第 29 届国际园艺学大会秘书处的邀请，王瑛研究员赴澳大利亚参加“29 届国际园艺学大会和国际药用和香料植物大会”，并作了题为 “Biosynthesis of carotenoids in Chinese medicinal plant–*Lycium barbarum*” 的分会邀请报告和 “Biosynthesis of Major Bioactive Compounds in the Medicinal Plant *Epimedium sagittatum*” 墙报宣传报告。

8 月 30 日-9 月 4 日，应德国汉堡大学邀请，杨平仿研究员赴德国参加“首届国际植物蛋白质组组织年会”，并在会上作题为“水稻蛋白质组学研究”的口头报告。

10 月 16 日-22 日，应智利国家农业研究协会邀请，李绍华研究员赴智利开展中智葡萄育种与遗传合作研究。

11 月 1 日-2015 年 1 月 31 日，应日本农业食品产业技术综合研究机构•作物研究所邀请，李明助理研究员赴日本开展水稻种子细胞核蛋白质组合作研究。

11 月 19 日-12 月 5 日，应美国洛杉矶儿童医院邀请，郭明全研究员赴美国开展“儿童白血病

的天然药物筛选”合作研究。

12 月 12 日-2015 年 3 月 9 日，受中国科学院公派出国高级访问学者计划资助，杨平仿研究员赴奥地利维也纳大学，开展莲蛋白质组学合作研究。

2. 来访项目

4 月 3 日-4 日，应王瑛研究员的邀请，韩国首尔大学教授 Tae-Jin Yang 与 Jin-Hoe Huh 一行 5 人来重点实验室，就药用植物分子遗传学和生物信息学研究开展学术交流。

5 月 6 日，应韩月彭研究员的邀请，美国佛罗里达大学教授柑橘研究与教育中心 Fred Gmitter Jr 教授来重点实验室，就柑橘黄龙病危害机理及抗病品种选育开展学术交流。

7 月 3 日-5 日，应傅金民研究员的邀请，美国弗吉尼亚理工大学张训忠副教授来重点实验室，就草坪逆境生理研究开展学术交流。

7 月 13 日，应产祝龙研究员的邀请，美国马里兰大学朱建华教授来重点实验室，就植物抗逆分子机制研究开展学术交流。

11 月 20 日，应产祝龙研究员的邀请，美国德州大学圣安东尼奥健康医学中心细胞生物学系 Russel J. Reiter 教授来重点实验室，就植物褪黑素的研究开展学术交流。

3. 学术报告

序号	时间	报告人	报告人单位	报 告 题 目
1	4 月 3 日	Tae-Jin Yang	首尔大学	Ginseng genome sequencing & NGS-based approach for under-studied plants
2	4 月 3 日	Jin-Hoe Huh	首尔大学	Active DNA demethylation for epigenetic gene regulation in plants
3	4 月 25 日	秦源	福建农林大学	Cross-talk between male and female during double fertilization
4	5 月 6 日	Fred Gmitter Jr	美国佛罗里达大学柑橘研究与教育中心	Genetic and Genomic Tools in the Fight Against Citrus Huanglongbing in Florida
5	7 月 4 日	张训忠	美国弗吉尼亚理工大学作物和土壤环境科学研究中心	草坪和能源草抗旱耐盐分子生理机制研究进展
6	7 月 13 日	朱建华	美国马里兰大学	Recent progress in molecular characterization of abiotic stress responses in plants
7	7 月 24 日	钱伟强	北京大学	植物中 DNA 去甲基化的分子机制

8	7 月 24 日	黄朝锋	南京农业大学	Molecular Mechanisms of Aluminum Resistance in Higher Plants
9	7 月 24 日	滕年军	南京农业大学	菊花远缘杂交生殖障碍机理及育种进展
10	10 月 28 日	白明义	山东大学	激素调控植物适应性的分子机制研究
11	10 月 28 日	周传恩	山东大学	蒺藜苜蓿复叶发育机制研究
12	10 月 29 日	程仲毅	杭州景杰生物科技有限公司	An Integrated Quantitative Proteomics for Dissecting Protein Post-translational Modification (PTM) and Histone Epigenetic Code
13	11 月 14 日	徐晖	广州中医药大学	岭南药用植物的转录组学研究
14	11 月 14 日	孙宇辉	武汉大学药学院	Unusual Acetylation-elimination in the Formation of Tetronate Antibiotics
15	11 月 14 日	胡学博	华中农业大学	Anti-inflammatory Drug Discovery Platform by Engineered Yeast
16	11 月 20 日	Russel J. Reiter	美国德州大学圣安东尼奥健康医学中心	The problem with light at night: the association with cancer
17	11 月 28 日	唐惠儒	复旦大学	植物代谢组学新技术及挑战

4. 开放课题

序号	课题名称	起止时间	经费（万元）	负责人	依托学科组
1	禾本科抗病基因的综合分布图的构建及禾本科不同抗病基因家族的进化规律的研究	2011.1-2012.12	3	陈炯炯	植物应用基因组学
2	苹果 MdMYB10 同源基因的克隆与功能鉴定研究	2011.1-2012.12	3	郑丹曼	果树分子育种学
3	金银花中绿原酸生物合成与转化机理研究	2011.1-2012.12	3	付春华	天然药物生物合成学
4	节水常绿草坪种质筛选及温度胁迫抗性机理研究	2011.1-2012.12	3	徐庆国	草坪种质资源学

5	细胞质雄性不育恢复基因 *Rfo* 的起源及适应性进化	2011.1-2012.12	3	刘海舟	种群遗传学
6	猕猴桃高维生素 C 种质创新及鉴定	2011.1-2012.12	3	刘义飞	植物保育遗传学
7	淫羊藿类胡萝卜素代谢基因表达和化学成分积累的相关性研究	2011.1-2012.12	3	张颖颖	比较功能基因组学
8	商陆中逆境相关基因 *PaNAC* 的克隆与功能分析	2011.1-2012.12	3	吴亮其	园艺作物生物学
9	川东-鄂西篦子三尖杉重要功能基因的分子适应性进化和共进化研究	2012.9-2014.8	3	王鹏良	种群遗传学
10	莲藕淀粉代谢相关基因的克隆与功能分析	2012.9-2014.8	3	张丽瑶	果树分子育种学
11	不同花色荷花品种的比较蛋白质组研究	2012.9-2014.8	3	赵　勇	资源植物繁殖生物学
12	调控荷花瓣型发育的 microRNA 发掘及其功能解析	2013.9-2015.8	3.5	徐迎春	资源植物繁殖生物学
13	莲功能性成分的代谢组学研究	2013.9-2015.8	3.5	焦丽丽	植物化学生物学

附录六 仪器设备

序号	资产名称	型号规格	价格（万元）	数量
1	PCR	mastercycle5333	9.4	15
2	核酸提取仪	Fastprep220	6.9	1
3	超纯水系统	Direct 8	7.1	3
4	显微镜	奥林巴斯	11.4	3
5	紫外分光光度计	PE-LAMBDA45	18.4	1
6	果实色度仪	美能达 CR-300	6.8	1
7	凝胶成像仪	ALPHA-IS2200	10.9	3
8	测序电泳仪	165-3804	6.6	2
9	梯度 PCR 仪	Mastercycler pro	7.2	3
10	电泳仪		6.1	2
11	超高速离心机		22.1	1
12	冷冻离心机		11.1	6
13	离心机	5810R	9.2	4
14	冰箱	KB240	6.2	1
15	液相色谱质谱仪	TSQ Quantum Access MAX	151.3	1
16	稳定同位素质谱仪	Delta V Advantage	179.8	1
17	等离子体质谱仪、快速液相色谱仪	X series	141.3	1
18	便携式光合作用仪	LI-6400 XTP	31.1	1
19	便携式调制叶绿素荧光仪	PAM-2500	29.3	1
20	流式细胞仪	Cyflow Space	28.3	1
21	定量 PCR 仪	CFXconnect	19.4	1
22	离心浓缩系统	refrigerated centrivap	11.8	1
23	气相色谱质谱联用仪	7890A+5975C	97.9	1
24	扫描电子显微镜	Quanta250	104.4	1
25	土壤碳通量系统	LGR908-0011	50.2	1
26	梯度气象监测系统	G2301、ZENO	140.3	1
27	涡动相关分析系统	PICARRO G2311-f;COASTAL ZENO	89.3	1
28	酶标仪	MK3	5.4	1
29	超微量核酸检测仪		5.4	1
30	双向电泳仪	PROTEINi12IEF system	12.9	1

31	天平	XP6	18.3	1
32	实时荧光定量 PCR 仪	Stepone Plus	22.0	2
33	岛津高效液相色谱仪	LC-20AT	27.1	1
34	实时荧光定量 PCR 检测系统	7500 FAST	39.7	1
35	水果品质无损检测仪	K-BA100R	16.6	2
36	遗传分析仪	3730	164.5	1
37	多功能细胞分析系统	CyFlow Cube8	35.7	1
38	微波消解仪	ETHOS ONE	22.5	1
39	蛋白质等电聚焦仪及大型垂直电泳槽	PRTOEAN i12 IEF	21.7	1
40	高效液相色谱仪	1260	53.3	1
41	凝胶成像系统	Gel Doc XR +	6.7	1
42	总有机碳总氮分析仪	Vario TOC	35.4	1
43	荧光差异凝胶（DIGE）分析软件	DeCyder	9.9	1
44	微电极系统	MM-Meter	59.4	1
45	全自动化学分析仪	Eassychem Plus	28.3	1
46	紫外可见分光光度计	ND8000	30.3	1
47	氨基酸分析仪	ICS 5000+	68.5	1
48	高通量溶剂蒸发工作站	EZ-2 Plus system	32.7	1
49	多功能全波长酶标仪	M200 PRO	32.2	1
50	全自动连续流动分析仪	Flowsys	29.6	1
51	激光捕捉显微分离系统	ArcturusXT	116.4	1
52	液相色谱仪	1100	40.0	1
53	CH_4 同位素碳分析仪 912-0023		68.8	1
54	土壤 CO_2 通量分析仪	912-0023	56.4	1
55	土壤碳氮循环监测系统	BaPS	32.1	1
56	双向电泳成像及分析系统	GS-800	21.3	1
57	冷冻真空干燥仪	LABCONCO	39.1	1
58	多功能激光成像仪	FLA9500	105.3	1
59	磷屏成像分析系统	FLA7000	73.7	1
60	多通道固相萃取系统	GX-274　ASPEC	38.1	1
61	全自动快速溶剂萃取仪	ASE 350	37.2	1
62	流式细胞仪	BD Accuri C6	53.2	1
63	激光粒度粒形分析仪	Mastersizer 3000	39.8	1
64	质谱仪	TripleTOF 5600	296.4	1

附录七 论文选编

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REGULAR PAPER

High correlation between thermotolerance and photosystem II activity in tall fescue

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Abstract Heat stress affects a broad spectrum of cellular components and metabolism. The objectives of this study were to investigate the behavior of Photosystem II (PSII) in tall fescue (*Festuca arundinacea Schreb*) with various thermotolerance capacities and to broaden our comprehension about the relationship between thermotolerance and PSII function. Heat-tolerant and heat-sensitive accessions were incubated at 24 °C (control) and 46 °C (heat stress) for 5 h. The fluorescence transient curves (OJIP curves), slow Chl fluorescence kinetic, and light response curve were employed to study the behavior of PSII subjected to heat stress. After heat stress, performance index for energy conservation from photons absorbed by PSII antenna until the reduction of PSI acceptors (PI_{Total}), the value of electrons produced per photon (a), and the maximal rate of electron transport (ETR_{max}) of heat-tolerant accessions were lower than those of heat-sensitive accessions. Relatively lower reactive oxygen species (ROS) contents were detected in heat-tolerant accessions. Simultaneously, there was a significant decline in the quantum yield of photochemical energy conversion in PS II (Y(II)), probability that a PSII Chl molecule functions as reaction center (γ_{RC}), and the increase of quantum yield for non-regulated non-photochemical energy loss (Y(NO)) in heat-tolerant accessions. Moreover, a significant inverse correlation between heat tolerance indexes (HTI) and Y(II) was observed. Therefore, maintaining a lower photochemical activity in heat-tolerant accessions could be a crucial strategy to improve their thermotolerance. This finding could be attributed to the structural difference in the reaction center, and for heat-tolerant accessions, it could simultaneously limit energy input into linear electron transport, and dissipate more energy through non-regulated non-photochemical energy loss processes.

Keywords Photosystem II · Thermotolerance · Tall fescue · OJIP transient · Slow Chl fluorescence kinetics · Light response curves

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Introduction

Abiotic stresses cause extensive losses to worldwide agricultural production (Mittler 2006). Particularly, heat stress disrupts a broad spectrum of cellular components and metabolism (Sung et al. 2003), resulting in cellular homeostasis disturbance which leads to severe growth and development retardation (Kotak et al. 2007). Tall fescue (*Festuca arundinacea Schreb*) is a major cool season forage and turf grass species grown in the temperate regions of the world, and susceptible to heat stress (Mian et al. 2008; Jiang and Huang 2001). Heat stress has become one of the major abiotic stresses limiting the growth and development of tall fescue. High temperature could lead to the photosynthesis inhibition, cell membrane damage,

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Genetic diversity and population structure of the medicinal orchid *Gastrodia elata* revealed by microsatellite analysis

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ABSTRACT

The wild resources of *Gastrodia elata* are currently threatened with extinction due to over-harvesting because of their high medicinal value. Genetic diversity plays a key role in the survival of endangered orchid species. In this study we investigated the genetic pattern in eight microsatellite loci within eight *G. elata* populations from central China. Compared with the other orchids, *G. elata* showed a low level of genetic variation within populations ($H_E = 0.356–0.622$). The main factors responsible for the genetic pattern were the plant's inbreeding system due to mating within clone patches, and the genetic bottlenecks and genetic drift caused by a long-history over-collecting. The significant heterozygote deficit was detected in all the populations. The *F* statistics calculated by different approaches consistently revealed a clear genetic differentiation among populations, contributing about 20% of the total gene diversity. The results are discussed in relation to both *in situ* and *ex situ* conservation efforts of the species. The populations with a high level of genetic diversity or with great genetic distinction were identified, which should be a high priority for conservation managers.

1. Introduction

Orchidaceae is one of the most diverse families of flowering plants, occurring on all vegetated continents and even some Antarctic islands. However, primarily as a result of mass collection and habitat loss, the family has a high proportion of threatened genera, with most containing threatened species (Swarts and Dixon, 2009). *Gastrodia* R. Br., a genus of Orchidaceae, is achlorophyllous orchid with approximate 20 species, mainly distributed in East Asia, Southeast Asia and Oceania (Chen et al., 1999). Unlike most other orchids which are famous for the high floricultural value, *Gastrodia* species have been noticed for their pharmaceutical value. With the acceleration of anthropogenic processes, wild resources of *Gastrodia* have

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RESEARCH ARTICLE

Genetic variation in cultivated populations of *Gastrodia elata*, a medicinal plant from central China analyzed by microsatellites

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Abstract *Gastrodia elata*, a member of Orchidaceae, is a popular herbal medicine in oriental countries, and has been cultivated in China since the 1970s. Genetic diversity in six cultivated populations of *G. aelata* from central China was estimated using simple sequence repeats (SSRs, or microsatellites). For eight nuclear microsatellites, a medium level of genetic diversities ($A = 3.92$, $H_E = 0.495$) was found in the populations, as compared with microsatellite variations of the other orchids. The genetic variation observed might be associated with the multiple origins and weak artificial selection in the cultivated *G. elata* populations. *F* statistics, calculated using different approaches, consistently revealed that the genetic differentiation among populations accounted for about 18 % of total genetic diversity ($F_{ST} = 0.186$, $F_{coal} = 0.179$). The results suggested that primitive cultivation practices might be an effective way for the maintenance and conservation of gene pools of medicinal plants. In order to alleviate the heterozygote deficit, controlling crossing should be carried out by competent research groups and the hybrid seedlings introduced in populations.

Keywords Cultivated populations · Domestication · *Gastrodia elata* · Genetic bottleneck · Genetic variation · Germplasm conservation

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Introduction

Plant domestication is the genetic modification of wild species to establish new plant varieties that are altered to meet human needs (Doebley et al. 2006). Studies on domestication enhance researchers' understanding on how evolution operates under artificial selection (Otero-Arnaiz et al. 2005). Cultivation process always produces genetic bottlenecks thus resulting in loss of genetic diversity due to the limited number of progenitors and subsequent artificial selection (Doebley 1989; Doebley et al. 2006). Reduced genetic variation might limit the potential for plants improvement over the long term and reduce fitness of

Unraveling the Mechanism Underlying the Glycosylation and Methylation of Anthocyanins in Peach[1][C][W]

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Modification of anthocyanin plays an important role in increasing its stability in plants. Here, six anthocyanins were identified in peach (*Prunus persica*), and their structural diversity is attributed to glycosylation and methylation. Interestingly, peach is quite similar to the wild species *Prunus ferganensis* but differs from both *Prunus davidiana* and *Prunus kansueasis* in terms of anthocyanin composition in flowers. This indicates that peach is probably domesticated from *P. ferganensis*. Subsequently, genes responsible for both methylation and glycosylation of anthocyanins were identified, and their spatiotemporal expression results in different patterns of anthocyanin accumulation in flowers, leaves, and fruits. Two tandem-duplicated genes encoding flavonoid 3-*O*-glycosyltransferase (F3GT) in peach, *PpUGT78A1* and *PpUGT78A2*, showed different activity toward anthocyanin, providing an example of divergent evolution of *F3GT* genes in plants. Two genes encoding anthocyanin *O*-methyltransferase (AOMT), *PpAOMT1* and *PpAOMT2*, are expressed in leaves and flowers, but only *PpAOMT2* is responsible for the *O*-methylation of anthocyanins at the 3′ position in peach. In addition, our study reveals a novel branch of *UGT78* genes in plants that lack the highly conserved intron 2 of the *UGT* gene family, with a great variation of the amino acid residue at position 22 of the plant secondary product glycosyltransferase box. Our results not only provide insights into the mechanisms underlying anthocyanin glycosylation and methylation in peach but will also aid in future attempts to manipulate flavonoid biosynthesis in peach as well as in other plants.

Anthocyanins are important secondary metabolites and serve to protect plants against pathogenic attack and UV radiation and provide flowers and fruits with pigmentation to attract pollinators and seed dispersers (Winkel-Shirley, 2001). Anthocyanins are usually stored as glycosylated forms in vacuoles. The basic structure of anthocyanins is composed of an anthocyanidin aglycone and one or more sugar moieties linked to hydroxyl groups 3, 5, 7, 3′, and 5′, with the 3 position on the C-ring being dominant. The most common sugar moieties are Glc, Gal, Xyl, Ara, and Fru. With different types and/or numbers of sugar moieties attached to various positions, the structural diversity of anthocyanins increases significantly. Besides glycosylation, modifications such as methylation and acylation also contribute to the structural diversity of anthocyanins. To date, there have been more than 550 anthocyanins identified in nature (Kong et al., 2003).

The formation of glycosides is catalyzed by UDP-Glc:flavonoid glycosyltransferase (UFGT). Both monoglycosides and diglycosides are common in plants. For example, 3RT, 3GGT, and F3GGT1, responsible for further glycosylation of anthocyanidin 3-*O*-glycosides, have been reported in petunia (*Petunia hybrida*; Kroon et al., 1994), Japanese morning glory (*Ipomoea nil*; Morita et al., 2005), and kiwifruit (*Actinidia chinensis*; Montefiori et al., 2011), respectively. Glycosylation plays at least two roles in the accumulation of anthocyanins. One is to stabilize anthocyanins by attaching sugar moieties to the unstable anthocyanidin aglycones. Deficiency in UF3GT activity results in a significant reduction of anthocyanin accumulation in maize (*Zea mays*; Fedoroff et al., 1984) and Arabidopsis (*Arabidopsis thaliana*; Tohge et al., 2005), as anthocyanidins are highly unstable and easily susceptible to degradation. Another is to serve as a signal for transport of the anthocyanins to vacuoles (Ono et al., 2006). Anthocyanins are synthesized on the cytoplasmic face of the endoplasmic reticulum by metabolons, which are likely to function as a multienzyme complex (Winkel, 2004). Once synthesized, anthocyanins need to be transported to vacuoles by transporters such as multidrug and toxin extrusion transporters (Zhao et al., 2011). Vacuole

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[C] Some figures in this article are displayed in color online but in black and white in the print edition.

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PLOS ONE

Effects of Fertilization and Clipping on Carbon, Nitrogen Storage, and Soil Microbial Activity in a Natural Grassland in Southern China

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Abstract

Grassland managements can affect carbon (C) and nitrogen (N) storage in grassland ecosystems with consequent feedbacks to climate change. We investigated the impacts of compound fertilization and clipping on grass biomass, plant and soil (0–20 cm depth) C, N storage, plant and soil C: N ratios, soil microbial activity and diversity, and C, N sequestration rates in grassland *in situ* in the National Dalaoling Forest Park of China beginning July, 2011. In July, 2012, the fertilization increased total biomass by 30.1%, plant C by 34.5%, plant N by 79.8%, soil C by 18.8% and soil N by 23.8% compared with the control, respectively. Whereas the clipping decreased total biomass, plant C and N, soil C and N by 24.9%, 30.3%, 39.3%, 18.5%, and 19.4%, respectively, when compared to the control. The plant C: N ratio was lower for the fertilization than for the control and the clipping treatments. The soil microbial activity and diversity indices were higher for the fertilization than for the control. The clipping generally exhibited a lower level of soil microbial activity and diversity compared to the control. The principal component analysis indicated that the soil microbial communities of the control, fertilization and clipping treatments formed three distinct groups. The plant C and N sequestration rates of the fertilization were significantly higher than the clipping treatment. Our results suggest that fertilization is an efficient management practice in improving the C and N storage of the grassland ecosystem via increasing the grass biomass and soil microbial activity and diversity.

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Introduction

The grasslands in China cover an area of 3.92 million km^2 and provide 9% to 16% of the total C in the world grasslands [1,2,3]. Concerns about global warming has increased an attention to understand the role of potential C and nitrogen (N) sink in grasslands in mitigating the emission of greenhouse gases (i.e. CO_2 and N_2O) [4–6]. The C and N sequestration in terrestrial ecosystems constitutes a major mitigation strategy against the global warming [7]. China's grasslands make an important contribution to the world C and N storage and may have significant effects on C and N cycles worldwide [2]. Natural grasslands of southern China cover an area of 79.58 million km^2, and probably have a high yield owning to good hydrothermal conditions [8], which can be an important C and N pool.

The processes of C and N sequestration can be greatly affected by grassland managements [9], and good managements are critical for grasslands to enhance C and N sequestration [10–12]. Compound fertilizers or organic amendments affected grasslands C and N storage via increasing plant biomass [10,13,14]. Dersch and Böhm [15] reported that N, phosphorus (P), and potassium (K) fertilizers combined with farmyard manure application enhanced C storage to about 5.6 Mg ha^{-1} after 21 years in Australia. The N fertilization and cover cropping can increase soil organic C and total N by increasing the amount of plant residues returned to the soil [11,16]. Similarly, the application of manure can increase soil organic C and total N levels [17,18]. Clipping was found to affect the grassland C and N storage via reducing plant biomass [9] and changing grass species [19]. Particularly, the potentially dominant plants (i.e. usually larger than their neighbors) often lose a higher proportion of their biomass than their neighbors after clipping [9].

Soil microorganisms exert a dominant influence on the net C and N balance of terrestrial ecosystems by controlling soil organic matter (SOM) decomposition and plant nutrient availability [20,21]. The grassland SOM mainly derived from roots, senescent leaves and stems of the vegetations [22]. The processes and functions of breakdown of the plants residues in soil are greatly impacted by soil microorganisms [23]. Agricultural managements can affect soil microorganisms' condition and ultimately affect the C and N cycling in ecosystems [24,25]. Microbial populations were significantly increased in the soils amended with green manure throughout two-year experiment [26]. Soil microbial diversity and/or activity may be a sensitive indicator of ecosystem change, as it can be quickly affected by disturbances [27,28].

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Antioxidant Responses and Gene Expression in Bermudagrass under Cold Stress

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Additional index words. *Cynodon dactylon*, low temperature, physiology, membrane stability, reactive oxygen species

Abstract. **Cold stress is a key factor limiting resource use in bermudagrass (*Cynodon dactylon*). Under cold stress, bermudagrass growth is severely inhibited and the leaves undergo chlorosis. Therefore, rigorous investigation on the physiological and molecular mechanisms of cold stress in this turf species is urgent. The objective of this study was to investigate the physiological and molecular alteration in wild bermudagrass under cold stress, particularly the changes of transpiration rate, soluble sugar content, enzyme activities, and expression of antioxidant genes. Wild bermudagrass (*C. dactylon*) was planted in plastic pots (each 10 cm tall and 8 cm in diameter) filled with matrix (brown coal soil:sand 1:1) and treated with 4 °C in a growth chamber. The results displayed a dramatic decline in the growth and transpiration rates of the wild bermudagrass under 4 °C temperature. Simultaneously, cold severely destabilized the cell membrane as indicated by increased malondialdehyde content and electrolyte leakage value. Superoxide dismutase and peroxidase activities were higher in the cold regime than the control. The expression of antioxidant genes including *MnSOD, Cu/ZnSOD, POD*, and *APX* was vividly up-regulated after cold stress. In summary, our results contributed to the understanding of the role of the antioxidant system in bermudagrass' response to cold.**

Bermudagrass is widely used in the turf systems and is equally relevant in animal husbandry because of its high protein content and relatively low cost. Bermudagrass is a typical warm-season grass, which grows best under air temperature ranging from 29.4 to 37.8 °C and soil temperature ranging from 23.9 to 35 °C. The minimum air temperature required for growth is 12.8 °C (Zhou, 1996). Therefore, below optimum temperature is a significant factor that may limit resource use in bermudagrass. Low temperature can influence growth, development, and yield of botanical species (Zhu et al., 2007). Hughes and Dunn (1996) reported that when exposed to low but not freezing temperature, plants can obtain chilling and freezing tolerance (cold acclimation) (Hughes and Dunn, 1996). Some studies have reported that cold stress led to biochemical and physical changes in plants, particularly causing freezing injury, which was accounted for by the damage on the plasma membrane. Other studies found that low temperature damaged cell membrane systems and lipid peroxidation by decreasing the fluidity of cell membranes of most plants (Levitt, 1980).

Malondialdehyde (MDA) and electrolyte leakage (EL) served as indicators of lipid peroxidation. Södergren (2000) reported that MDA was higher under low-temperature conditions (Södergren, 2000). In *Forsythia* species treated at 4 °C, MDA contents increased by ≈66.7% (Yan et al., 2010). Similarly, MDA levels were higher in wheat (*Triticum aestivum*) seedlings and strawberry (*Fragaria ananassa*) leaves subjected to 4 and 0 °C, respectively (Hou et al., 2010; Luo et al., 2011). Electrical conductivity is widely applied to detect membrane injury caused by various biotic and abiotic stresses in plants (Whitlow et al., 1992). EL declined in bermudagrass cultivars after 8 °C day temperature and 4 °C night temperature cold acclimation (Zhang et al., 2006). EL of leaves significantly increased by 55% and 26.3% in naked oats (*Avena nuda*) treated by –10 and 1 °C, respectively (Liu et al., 2013), indicating that low temperature could affect cell membrane penetrability.

Sufficient evidence suggests that extremely low temperature can induce oxidative stress through the generation of reactive

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ORIGINAL PAPER

Myb14, a direct activator of *STS*, is associated with resveratrol content variation in berry skin in two grape cultivars

Linchuan Fang · Yanlin Hou · Lijun Wang · Haiping Xin · Nian Wang · Shaohua Li

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Abstract

Key message **High and low resveratrol (Res) contents in two cultivars are correlated with the expression abundance of *Myb14*, which could directly activate transcriptional expression of stilbene synthase gene (*STS*).**

Abstract Resveratrol (3,5,4′-trihydroxystilbene) is one of the natural polyphenols produced by secondary metabolism in some plants. Stilbene synthase (STS) is the key enzyme for the final step of precursor formation of resveratrol (Res) in grapevines. In this study, we found that Res contents in ripe berry skin were completely different in two grape cultivars, namely, 'Z168' (*Vitis monticola* × *Vitis riparia*) with high-Res and 'Jingzaojing' (*Vitis vinifera*) with low-Res. Moreover, the level of expression of *STS* gene was higher in the ripe berry skin of 'Z168' than in that of 'Jingzaojing'. To further investigate the underlying mechanisms, we conducted a co-expression analysis through transcriptomic data. We confirmed that *Myb14*, an *R2R3 Myb* transcription factor, is the direct regulator of *STS by binding to Box-L5 motif*. Moreover, the expression pattern of *Myb14* is associated with the variation of Res content. To test this prediction, we conducted a number of experiments in vivo and in vitro. The expression patterns of *Myb14* and *STS* in grapevine leaves were identical under a series of stimulus. *Myb14* showed higher expression in the ripe berry skin of 'Z168' than in that of 'Jingzaojing'. Yeast one-hybrid assay indicated that grapevine *Myb14* could interact with the promoter of *STS* in vitro, and the

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A New Species of *Eutrema* (Brassicaceae) from Central China

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ABSTRACT. A new species of the genus *Eutrema* R. Br. (Brassicaceae) is described and illustrated from Hubei Province in China. This species is similar to *E. yungshunensis* (W. T. Wang) Al-Shehbaz & Warwick in its leafy stems and ovaries with more than 10 ovules, but the new species differs in having glaucous stems, larger leaves abaxially purplish, larger sepals and petals, and fewer seeds per fruit.

Key words: Brassicaceae, China, *Eutrema*, IUCN Red List, *Neomartinella*.

Eutrema R. Br. (Brassicaceae) consists of 26 species mainly distributed in Asia (Al-Shehbaz & Warwick, 2005). During extensive investigation of the flora in Zhuxi County, Hubei, China, an interesting species of the genus new to science was discovered and is reported here. The new species, *E. zhuxiense* Q. L. Gan & X. W. Li, is similar to *E. yungshunensis* (W. T. Wang) Al-Shehbaz & Warwick in its leafy stems and ovaries with more than 10 ovules. The new species is distinguished from *E. yungshunensis* by having glaucous stems (vs. glabrate but not glaucous), leaves that are abaxially purplish green and larger, 3–16 × 3–16 cm (vs. 2–6 × 1–5 cm, with no purple tint), sepals obovate to spatulate, 2.8–3 × ca. 2.5 mm and larger (vs. ovate, 1.5–2 × 1–1.2 mm), petals obovate and larger, 6–7 × 4–5.5 mm (vs. obcordate, 4–5 × 2–2.5 mm), and generally fewer seeds per fruit that are longer (six to 20 seeds in fruit 5–12 mm vs. 20 to 40 seeds in fruit 1.5–3.5 mm). In addition, *E. yungshunensis* is distributed at lower elevations (500–600 m) and is not sympatric, known only from adjacent Hunan Province to the south.

Eutrema zhuxiense Q. L. Gan & X. W. Li, sp. nov. TYPE: China. Hubei: Zhuxi Co., Taoyuan, Liushu-ping, Wangjiashan, 1100 m, 28 Mar. 2007, *Q. L. Gan 1890* (holotype, HIB). Figure 1.

Haec species *Eutremati yungshunensi* (W. T. Wang) Al-Shehbaz & Warwick similis, sed ab eo caulibus glaucis, foliis majoribus abaxialiter purpureis, sepalis et petalis majoribus, seminibus paucioribus et fructibus longioribus differt.

Perennial herbs, 10–55 cm tall; rhizomes fleshy; stems erect, simple, or 2- to 4-branched in distal portion, leafy, few to several from base, glabrous, glaucous. Basal leaves 5 to 16, rosulate; petiole 5–19 cm, glabrous, purple or green; leaf blades ovate or broadly ovate, 3–16 × 3–16 cm, glabrous on both sides, green adaxially, purplish green abaxially, papery, base cordate, margin repand or coarsely dentate, with apiculate callosities, apex obtuse and slightly emarginate; middle cauline leaves 2 or 3, sometimes more, similar to basal ones but smaller, coarsely dentate on leaf margin. Inflorescence a raceme, with 10 to 30 flowers, glabrous, ebracteate; pedicels filiform, 1–3 cm, ascending when fruiting. Flowers with sepals ovate to spatulate, 2.8–3 × ca. 2.5 mm, white or abaxially green in middle portion with the margin being white, easily caduous; petals white, obovate, 6–7 × 4–5.5 mm; filaments white, 2–2.2 mm; anthers ovate, 1.5 mm; ovary oblong, style absent, stigma rounded and flat; ovules 6 to 20 per ovary. Fruit oblong or linear, 5–12 × 1.5–3 mm, terete or slightly compressed, not torulose; valves without evident midvein; seeds rhombic, 1 mm long.

Distribution and habitat. *Eutrema zhuxiense* is endemic to Zhuxi County in Hubei Province in central China. The new taxon grows in damp places or among rocks in montane forests. It has been found from altitudes of 700–1500 m.

IUCN Red List category. *Eutrema zhuxiense* is known from only three populations in Zhuxi County. The three populations have ca. 500 individuals in total. Further study is needed to assess its distribution. At this time, the new species is assessed as Data Deficient (DD) based on IUCN criteria (IUCN, 2001).

Phenology. The authors observed that *Eutrema zhuxiense* flowers from February to March, with fruits seen in April to May.

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Stellaria zhuxiensis (Caryophyllaceae), a new species from Hubei, China

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Gan, Q. L. & Li, X. L. 2014: *Stellaria zhuxiensis* (Caryophyllaceae), a new species from Hubei, China. — *Ann. Bot. Fennici* 51: 22–24.

Stellaria zhuxiensis Q.L. Gan & X.W. Li *sp. nova* (Caryophyllaceae) is described and illustrated. The plants are covered with stellate hairs on stems, inflorescences, leaves and sepals. The hairs and the lax, cymose inflorescence are similar to those of *S. vestita* and *S. infracta*. *Stellaria zhuxiensis* can be distinguished from *S. vestita* by its broader leaves and longer pedicels, longer petals and longer capsules. *Stellaria infracta* is readily distinguished from *S. zhuxiensis* by having lanceolate or linear-lanceolate leaves while the leaves of *S. zhuxiensis* are broadly ovate or ovate-cordate.

Stellaria (Caryophyllaceae) is a genus consisting of about 190 species mainly in temperate and cold–temperate regions and there are 64 species in China (Lu *et al*. 2001). Some taxa such as *S. dichotoma var. lanceolata*, *S. chinensis*, *S. vestita* var. *vestita*, *S. media* var. *media*, *S. yunnanensis*, and *S. neglecta*, are commonly used in traditional Chinese medicine. During our botanical expeditions to the mountains of Zhuxi County, Hubei Province, China, we discovered two populations of a peculiar species of *Stellaria*. The leaves of this species are very similar to those of *Pseudostellaria davidii*, but its petals are 2-cleft nearly to base and it has no root tubers, belying a placement in *Pseudostellaria*. The stellate hairs and lax cymose inflorescence of the plants resemble those of *S. vestita*, but the flowers are much larger than in the coexisting *S. vestita*. After consulting national and local floras (Wu *et al*. 1996, Fu 2001, Lu *et al*. 2001) and herbarium specimens, the morphological differences between our collected species and the already described species led us to conclude that we had a species new to science at hand.

Stellaria zhuxiensis Q.L. Gan & X.W. Li, *sp. nova* (Fig. 1).

Type: China. Hubei, Zhuxi County, Jiangjiayan Town, Longyang Village, in the grassland by the mountain road, altitude 800 m, 109°31´55.57´´E, 32°17´03.06´´N, 23 May 2013 *X.W. Li 550* (holotype HIB!). — Paratypes: China. Hubei, Zhuxi County, Longba Town, Laoyinshan Village, in the grassland in the mountain, altitude 820 m, 109°33´37.28´´E, 32°26´01.54´´N, 11 May 2013 *Q.L. Gan 3450* (HIB!); same region, Jiangjiayan Town, Longyang Village, in the grassland by the mountain road, 2 July 2013, *Q.L. Gan 3458* (HIB!).

Etymology: The specific epithet refers to the Zhuxi County where the new species was discovered.

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ORIGINAL PAPER

Hetero-diploid pollen grains that represent self-compatibility are incompatible with non-self receptors in tetraploid Chinese cherry (*Prunus pseudocerasus* Lindl)

Chao Gu · Qing-Zhong Liu · M. Awais Khan · Jun Wu · Shao-Ling Zhang

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Abstract Chinese cherry (*Prunus pseudocerasus*) has many natural tetraploid species within *Prunus*. The pollen grains of tetraploid Chinese cherry are hetero-diploid, the two *S*-haplotypes in the pollen are a combination of two of the four possible *S*-haplotypes. The abnormal segregation ratios of pollen-*S* indicate that a few hetero-diploid pollen grains could inactivate self-stylar S-RNase inside the pollen tube and grow better into the self-ovaries than to the others. In this study, three Chinese cherry cultivars, "Daiba" ($S_1S_2S_5S_8$), "Taishanganying" ($S_1S_2S_4S_6$), and "Laiyangduanzhi" ($S_1S_2S_8S_x$), were used to investigate the inheritance of hetero-diploid pollen-*S* alleles in non-self receptors. Genetic analysis showed that the distribution of *S*-haplotypes is unequally expressed in self- and cross-pollinated progenies. The S_2-haplotype, which is found with lowest frequency in all progeny plants, is a likely lethal mutation. Moreover, the number of individuals with two different *S*-haplotypes was also unequal in the two cross-pollinated progenies. Notably, the number of individuals with S_1S_5 and S_1S_8 genotypes was larger than other genotypes in the cross-pollinated progeny of "Laiyangduanzhi"×"Daiba", and the number of individuals with S_1S_4, S_1S_6, and S_4S_6 genotypes was larger than S_2-haplotypes in the cross-pollinated progeny of "Laiyangduanzhi"×"Taishanganying". These results indicate that only a few genotypes of hetero-diploid pollen grains have the capability to grow into the ovaries of "Laiyangduanzhi". Interestingly, the pollen grains with S_5S_8 genotypes, which is self-compatible, was incompatible with the styles of "Laiyangduanzhi", while the pollen grains with S_1S_8, S_1S_6 and S_4S_6 genotypes, which are self-incompatible, were compatible with the styles of "Laiyangduanzhi".

Keywords *Prunus pseudocerasus* · Self-incompatibility · Genetic · Hetero-diploid pollen · *S*-genotypes

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Introduction

Self-incompatibility (SI) is a genetic mechanism in hermaphroditic plants that prevents self-fertilization by enabling the pistil to reject pollen from genetically related individuals. *Prunus* species exhibit typical gametophytic self-incompatibility (GSI). This incompatibility is controlled by a single multi-allelic locus, called the *S*-locus, which contains at least two polymorphic genes (stylar-*S* and pollen-*S*). *S-RNase* gene is the pistil determinant (stylar-*S*) that is specifically expressed in the pistil and had been identified in the Rosaceae (Sassa et al. 1996; Tao et al. 1997), Solanaceae (Anderson et al. 1986; McClure et al. 1989), and Plantaginaceae (Xue et al. 1996). *S*-locus *F-box* [*SLF*] or *S*-haplotype specific *F-box* [*SFB*] is a good candidate gene for the pollen determinant

Regulation of Anthocyanin Biosynthesis in Tissues of a Teinturier Grape Cultivar under Sunlight Exclusion

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Abstract: Yan-73 (*Vitis vinifera*) is a teinturier grape cultivar, which accumulates anthocyanins in skin, pulp, pedicels, and rachis. The effects of sunlight on anthocyanin biosynthesis and regulation in various tissues of this teinturier cultivar were investigated. Light transmission measurements showed that covering with opaque boxes substantially reduced light intensity around clusters; ≤0.25% of incident light reached the berry skin and <0.05% reached the pulp. The pulp naturally experiences increasing sunlight exclusion by skin during ripening. Sunlight exclusion reduced and delayed anthocyanin biosynthesis in skin and pulp during berry development, while both tissues nevertheless accumulated enough anthocyanins to turn dark red. No strong reduction of anthocyanin concentration was observed in pedicels or rachis. Sunlight exclusion decreased transcript abundance of *VvUFGT, VvMybA1, VvMybA2*, and *VvMyc1* in both skin and pulp and also decreased transcript abundance of *VvMycA1* in pulp. Sunlight exclusion differently influenced the anthocyanin components: it decreased the relative proportion of 3′,4′,5′-hydroxylated anthocyanins and increased that of 3′,4′-hydroxylated anthocyanins in berry skin and pulp, which corresponded to the change in the ratio of *VvF3′H* to *VvF3′5′H*. These results allow for insight on anthocyanin biosynthesis in various grape tissues in absence of sunlight.

Key words: teinturier, anthocyanin, sunlight exclusion, tissues, development

Anthocyanins are a class of flavonoids responsible for the color of grape berries. Berry coloration is an important factor for market acceptance of table-grape cultivars and red wines. For most grape cultivars, berry skin is the main or only tissue to accumulate anthocyanins. However, teinturier grape cultivars (also called dyers) synthesize anthocyanins in both skin and pulp and are commonly used for blending to give an intense color to red wine (Ageorges et al. 2006, Jeong et al. 2006b). Much is known about the genetic relationships between teinturier varieties (Santiago et al. 2008) and about anthocyanin content (Castillo-Muñoz et al. 2009), seasonal accumulation profile (Guan et al. 2012), and biosynthesis-related gene expression (Castellarin et al. 2011) in teinturier skin and pulp. Some teinturier grape cultivars may also accumulate anthocyanins in other tissues such as pedicels, rachis, leaf lamina and veins, petioles, living bark at the base of shoots, and seeds (Falginella et al. 2012, Guan et al. 2012, Jeong et al. 2006b).

Teinturier cultivars generally have a much higher anthocyanin concentration per unit juice volume or fresh mass than non-teinturier cultivars (Ageorges et al. 2006, Balík and Kumšta 2008), and among nine cultivars originating from south Moravia, the teinturier variety Neronet contained the highest concentration (2.15 to 4.49 g/kg fresh grapes) (Balík and Kumšta 2008). There are also notable differences in anthocyanin quantity and composition between berry skin and pulp of teinturier cultivars (Ageorges et al. 2006, Castillo-Muñoz et al. 2009, Falginella et al. 2012). We have previously shown that malvidin derivatives predominate in berry skin and peonidin derivatives predominate in the pulp but that similar concentrations of malvidin and peonidin derivatives were found in pedicels, rachis, leaf lamina and veins, petioles, and living bark of Yan-73 (Guan et al. 2012).

Anthocyanin biosynthesis is widely studied in grape berry skin. It is primarily regulated at the transcription level of structural and regulatory genes. Among structural genes, the expression level of UDP-glucose:flavonoid 3-*O*-glucosyltransferase (*UFGT*) is closely related to anthocyanin biosynthesis (Zheng et al. 2013), and the expression of flavonoid 3′,5′-hydroxylase (*F3′5′H*) and flavonoid 3′-hydroxylase (*F3′H*) affects anthocyanin composition in berry skin (Castellarin et al. 2006, Jeong et al. 2006a). A prevalence of *F3′5′H* over *F3′H* would lead to greater production of delphinidin, the precursor of petunidin and malvidin, and would yield less cyanidin, the

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PLANT & CELL PHYSIOLOGY

In-Depth Proteomic Analysis of Rice Embryo Reveals its Important Roles in Seed Germination

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Regular Paper

Seed germination is a complex physiological process that allows the seed embryo to grow and develop into a photosynthetic organism. The two major constituents of rice seed include the embryo and endosperm, with embryo being of much significance despite its small size. In this study, we conducted a systematic proteomic analysis of the embryos dissected from rice seed at different stages of germination through a combination of gel-based and gel-free strategies. In total, 343 differentially expressed proteins were identified. Among them, 191 were decreased and 152 were increased in terms of expression levels. All these proteins could be sorted into 11 functional groups based on MapMan analysis. Some starch biosynthesis-related enzymes such as starch branching enzyme, granule-bound starch synthase 1 and starch synthase increased during the early stage of germination and then decreased at the late stage, which was similar to the expressional patterns of glycolysis-related enzymes. However, tricarboxylic acid cycle-related enzymes only increased at the later stage. It was also found that sucrose might be an important intermediate for the biosynthesis of starch in embryos. Furthermore, gel-based proteomic analysis of the dissected endosperm showed that the biological processes in the endosperm were heavily regulated by the embryo. This study could provide some new insights into the distinct roles of the embryo and endosperm in rice seed germination.

Keywords: Embryo • Endosperm • Proteomics • Rice • Seed germination.

Introduction

Rice is one of the most important cereals, which feeds more than half of the world population. Successful germination of rice seed is essential for its cultivation and production. Germination of orthodox seed is a triphasic process (Bewley 1997), during which the seed transits from a physiological quiescent stage to an active status (Han et al. 2013). Usually, large amounts of reserves and related enzymes are synthesized and stored in the seed during maturation. Upon imbibition, the degradation of the reserves is triggered (Muntz et al. 2001, Tan-Wilson and Wilson 2012). Degradation of storage reserves not only supplies the necessary ATPs and precursors, but also mediates the transition between different kinds of compounds (Leonova et al. 2010). In some plants, the degradation of reserves or other compounds is also a prerequisite for radicle extrusion. Inhibition of the degradation process can delay or even prevent germination in some plant species. For example, inhibition of pectin degradation could arrest radicle extruding teata, and hence prevent germination in Arabidopsis (Kanai et al. 2010). Although inhibition of lipid degradation did not prevent germination in Arabidopsis, it could delay this process (Dave et al. 2011).

Seed germination is regulated by environmental factors, such as temperature (Chiu et al. 2012) and light (Neff 2012), and phytohormones including gibberellic acid (GA) (Ogawa et al. 2003), abscisic acid (ABA) (Kim et al. 2013) and others (Holdsworth et al. 2008, Tan et al. 2013). GA and ABA have antagonistic roles during germination. GA promotes seed germination, while ABA inhibits seed germination (Finkelstein et al. 2008). This antagonistic behavior between GA and ABA is realized in barley aleurone cells by ABA-induced protein kinase, PKABA1, which mediates the ABA suppression of *α-amylase* expression (Gomez-Cadenas et al. 2001). The environmental factors affect germination through regulation of biosynthesis and catabolism of GA and ABA (Holdsworth et al. 2008). Recently, studies have proved that histone methylation is important for Arabidopsis seed germination (Cho et al. 2012). Reactive oxygen species (ROS) and nitric oxygen (NO), which act as signaling molecules, also participate in regulation of seed germination (Liu et al. 2010).

Rice seed is composed of embryo and endosperm. During germination, degradation of starches, which occurs in the endosperm, is regulated by the embryo when GA biosynthesis is commenced. The GAs are then transported to the aleurone layer (Fincher 1989) where they trigger expression of *α-amylase* by inducing the expression of a positive transcriptional factor (Gubler et al. 1995). As a result, α-amylase is secreted from the aleurone layer into the endosperm to catalyze the hydrolyzing reaction of stored starch (Bush et al. 1986, Kumagai et al. 1990). Recently, two crucial transcription factors, *MYBS1* and *MYBGA* were proved to be induced by GA and to form a stable bipartite MYB–DNA complex to activate the *α-amylase* gene expression in rice and barley (*Hordeum vulgare*) (Hong et al. 2012). In addition, G protein signaling and Ca^{2+} signaling are also involved in activation of *α-amylase* during rice seed

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Gel-Based Comparative Phosphoproteomic Analysis on Rice Embryo During Germination

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Regular Paper

Seed germination is a well regulated process, which incorporates many events including signal transduction, mobilization of reserves, reactive oxygen species scavenging and cell division. Although many transcriptomic and proteomic studies have been conducted on this process, regulation of protein modification has not been studied. To better understand the mechanism, a gel-based comparative phosphoproteomic study was performed on rice embryo during the germination process. In total, 168 protein spots exhibited significantly changed Pro-Q staining intensity during germination. Using matrix-assisted laser deionization-time of flight/time of flight mass spectrometry (MALDI-TOF/TOF MS) analysis, 193 proteins were identified. By combining Pro-Q and Coomassie brilliant blue stain intensity analyses, 109 proteins were verified to be phosphorylation regulation proteins. Functional analyses indicated that phosphorylation of proteins involved in stress response and storage was gradually enhanced. Phosphorylation of signal transduction proteins was mainly activated during the early stage of germination, while stress response and storage protein phosphorylation were enhanced at the late stage. Enzyme assays proved that the phosphorylation of fructokinase, pyruvate kinase, malate dehydrogenase, GDP-mannose 3,5-epimerase1, ascorbate peroxidase and glutathione *S*-transferase could consistently enhance their activity. This study showed the dynamic changes of protein phosphorylation status in rice embryo during germination and provided new insight into understanding the mechanism underlying this process.

Keywords: Embryo • Germination • Phosphoproteomics • Rice.

Abbreviations: ABRE, ABA-responsive element-binding factor; AGPlar, glucose-1-phosphate adenylyltransferase; APX, ascorbate peroxidase; CBB, Coomassie brilliant blue; CDNB, 1-chloro-2,4-dinitrobenzene; CHCA, α-cyano-4-hydroxycinnamic acid; 2-DE, two-dimensional electrophoresis; DTT, dithiothreitol; FK, fructokinase, GME, GDP-mannose 3,5-epimerase1; GST, glutathione *S*- transferase; HSP, heat shock protein; IEF, isoelectric focusing; MALDI-TOF/TOF MS, matrix-assisted laser deionization-time of flight/time of flight mass spectrometry; MDH, malate dehydrogenase; PEP, phosphoenolpryuvate; PGAM, phosphoglycerate mutase; PK, pyruvate kinase; PLC, inositol phospholipid-specific phospholipase C; PMSF, phenylmethylsulphonyl fluoride; PPDK, pyruvate orthophosphate dikinase; PRXIIC, peroxiredoxin-2C; PTM, post-translational modification; ROS, reactive oxygen species; sHSP, small heat shock protein; SnRK2, SNF1-related protein kinase 2 protein; TCA, tricarboxylic acid; TFA, trifluoroacetic acid; TPI, triosephosphate isomerase; UAM1, UDP-arabinopyranose mutase 1; UDP-Araf, UDP-L-arabinofuranose; UDP-Arap, UDP-L-arabinopyranose; UGDH, UDP-glucose 6-dehydrogenase; UGP2, UDP-glucose pyrophosphorylase.

Introduction

Seed germination is a crucial process for establishing the next generation and for the initiation of the new life cycle in plants. Optimal seed germination is a prerequisite for successful seedling establishment and plant growth. Understanding the mechanisms involved in the complex process of seed germination is therefore not only of academic interest, but is also relevant to solving the problems of the world food supply. By definition, seed germination starts with imbibition of mature dry seeds and terminates with the protrusion of radicles (Bewley 1997). During seed germination, quiescent dry seeds are transformed into a metabolically active status. Dramatic metabolic changes happen during this process, including providing energy, mobilization of reserves, signaling transduction, transcription activation, protein synthesis, reactive oxygen species (ROS) scavenging, and so on (Botha et al. 1992, Rajjou et al. 2012).

Based on the water uptake, germination can be divided into three phases: a rapid uptake of water (phase I); a plateau phase of water uptake (phase II); and post-germinative growth (phase

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Quantitative Proteomics Reveals the Role of Protein Phosphorylation in Rice Embryos during Early Stages of Germination

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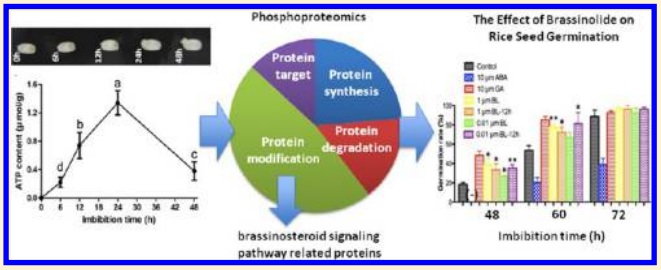
Supporting Information

ABSTRACT: Seed germination begins with water uptake and ends with radicle emergence. A gel-free phosphoproteomic technique was used to investigate the role of protein phosphorylation events in the early stages of rice seed germination. Both seed weight and ATP content increased gradually during the first 24 h following imbibition. Proteomic analysis indicated that carbohydrate metabolism- and protein synthesis/degradation-related proteins were predominantly increased and displayed temporal patterns of expression. Analyses of cluster and protein−protein interactions indicated that the regulation of sucrose synthases and alpha-amylases was the central event controlling germination. Phosphoproteomic analysis identified several proteins involved in protein modification and transcriptional regulation that exhibited significantly temporal changes in phosphorylation levels during germination. Cluster analysis indicated that 12 protein modification-related proteins had a peak abundance of phosphoproteins at 12 h after imbibition. These results suggest that the first 12 h following imbibition is a potentially important signal transduction phase for the initiation of rice seed germination. Three core components involved in brassinosteroid signal transduction displayed significant increases in phosphoprotein abundance during the early stages of germination. Brassinolide treatment increased the rice seed germination rate but not the rate of embryonic axis elongation. These findings suggest that brassinosteroid signal transduction likely triggers seed germination.



KEYWORDS: *phosphorylation, proteomics, rice, seed germination*

INTRODUCTION

Seed germination, which begins with water imbibition and ends with radicle extrusion,[1] is an essential step in the initiation of a new life cycle in plants. Completion of this step requires the coordination of several signal transduction and biochemical processes, including those involved in responding to environmental factors and mobilizing seed reserves, finally leading to elongation of the embryonic axis.[2] The seed germination process is predominantly controlled by phytohormones, including gibberellins (GAs) and abscisic acid (ABA), which play antagonistic roles as positive and negative regulators, respectively, during germination.[3,4]

The seed germination process is largely programmed during seed maturation.[2] In mature *Arabidopsis* seeds, numerous stored mRNAs are specifically involved in initiating germination.[5] The translation of stored mRNA during maize germination is mediated by the initiation factor eIFiso4E.[6] The importance of stored mRNA for the germination process was demonstrated in *Arabidopsis*, as the double mutant *i4gl/i4g2* exhibited a low germination rate and poor seed viability.[7] In addition, it was demonstrated that treatment with the translation inhibitor cycloheximide completely blocked seed germination of both *Arabidopsis* and rice and that treatment with the transcription inhibitor α-amanitin strongly affected the germination rate but did not prevent radicle protrusion.[8,9] These findings indicate that stored transcripts are more vital for germination than de novo synthesized transcripts. Supporting this conclusion, newly synthesized proteins radiolabeled with ^{35}S-methionine strongly increased after 8−24 h in *Arabidopsis* compared with those synthesized from 0 to 8 h during germination, indicating that the translation of stored mRNA occurred during the early stages of germination and was followed later by protein de novo synthesis.[10]

Reversible modification of proteins by phosphorylation is critical for the regulation of signal transduction in plants and other organisms. Many protein kinases and phosphatases participate in ABA signaling to regulate seed germination.[11] For

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Article

The Mitochondrion-Located Protein OsB12D1 Enhances Flooding Tolerance during Seed Germination and Early Seedling Growth in Rice

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Abstract: B12D belongs to a function unknown subgroup of the *Balem* (Barley aleurone and embryo) proteins. In our previous work on rice seed germination, we identified a B12D-like protein encoded by LOC_Os7g41350 (named *OsB12D1*). OsB12D1 pertains to an ancient protein family with an amino acid sequence highly conserved from moss to angiosperms. Among the six *OsB12D*s, *OsB12D1* is one of the major transcripts and is primarily expressed in germinating seed and root. Bioinformatics analyses indicated that *OsB12D1* is an anoxic or submergence resistance-related gene. RT-PCR results showed *OsB12D1* is induced remarkably in the coleoptiles or roots by flooding during seed germination and early seedling growth. The *OsB12D1*-overexpressed rice seeds could protrude radicles in 8 cm deep water, further exhibiting significant flooding tolerance compared to the wild type. Moreover, this tolerance was not affected by the gibberellin biosynthesis inhibitor paclobutrazol. OsB12D1 was identified in the mitochondrion by subcellular localization analysis and possibly enhances electron transport through mediating Fe and oxygen availability under flooded conditions. This work indicated that *OsB12D1* is a promising gene that can help to enhance rice seedling establishment in farming practices, especially for direct seeding.

Keywords: OsB12D1; flooding tolerance; mitochondrion; rice

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PLOS ONE

Comparative Analysis of Genome-Wide Chromosomal Histone Modification Patterns in Maize Cultivars and Their Wild Relatives

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Abstract

Recent advances demonstrate that epigenome changes can also cause phenotypic diversity and can be heritable across generations, indicating that they may play an important role in evolutionary processes. In this study, we analyzed the chromosomal distribution of several histone modifications in five elite maize cultivars (B73, Mo17, Chang7-2, Zheng58, ZD958) and their two wild relatives (*Zea mays* L. ssp. *parviglumis* and *Zea nicaraguensis*) using a three-dimensional (3D) epigenome karyotyping approach by combining immunostaining and 3D reconstruction with deconvolution techniques. The distribution of these histone modifications along chromosomes demonstrated that the histone modification patterns are conserved at the chromosomal level and have not changed significantly following domestication. The comparison of histone modification patterns between metaphase chromosomes and interphase nuclei showed that some of the histone modifications were retained as the cell progressed from interphase into metaphase, although remodelling existed. This study will increase comprehension of the function of epigenetic modifications in the structure and evolution of the maize genome.

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Introduction

Chromatin in eukaryotes is composed of DNA and its associated core histones and is subject to various post-translational modifications in the amino-terminal tails of histones and methylation in the cytosine residues of DNA [1]. Some modifications such as histone acetylation and H3K4 methylation are enriched in euchromatin, while H3K9 methylation, H3K27 methylation, and DNA methylation are generally thought to be the marks of condensed heterochromatin [2,3]. These epigenetic modifications have an effect on gene expression and phenotype and can be heritable across generations, indicating that they also play a role in evolution [4–6]. Although histones and their modifications are conserved, extensive studies have revealed that there are some differences in the distributions and functional meanings of histone modifications between the genomes of animals and plants as well as between different plant species [2]. In mouse nuclei, H3K9 trimethylation and H4K20 trimethylation preferentially mark constitutive heterochromatin [7], whereas in *Arabidopsis thaliana*, they are not typical marks for heterochromatin [2]. H3K9me1, a heterochromatin-specific mark in angiosperms has been found to be enriched in euchromatic domains in gymnosperm species [8]. However, very little is known about how the histone modification patterns change during plant evolution from wild species to cultivated species.

Genome-wide analysis of the epigenome can be revealed at the molecular level by the chromatin immunoprecipitation-sequencing (ChIP-seq) technique [9]. However, highly repetitive DNAs hinder sequencing-based analysis of the plant genome and ChIP-seq is not suitable for non-sequenced genomes [10]. There are some changes in epigenetic states from interphase to metaphase, but ChIP-seq cannot distinguish cells in different phases of the cell cycle. Immunocytological analysis with antibodies specific to histone modification and DNA methylation is a powerful technique for identifying individual chromosomes and analysis of the entire epigenome at the chromosomal level [3]. The distribution of histone modifications can be traced along metaphase chromosomes of animals and plants by this technique [2,3]. Many plants such as *A. thaliana* [11], *Zea mays* [12], *Vicia faba* [13] and *Secale cereale* [14] have been investigated so far with regard to the chromosomal distribution of histone modifications. To date, few systematic comparative analyses of epigenome between wild species and cultivated species have been performed at the cellular level. Exploring the variations in epigenetic patterns during plant evolution is very important for biological research and agriculture.

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SYSTEMATIC POSITIONS OF *MEDICAGO EDGEWORTHII* AND *M. ARCHIDUCIS-NICOLAI* (LEGUMINOSAE) INFERRED FROM PLASTID *TRN*K/*MAT*K, NUCLEAR *GA30X1* AND ITS SEQUENCES

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Abstract

This paper characterizes the systematic positions of *Medicago edgeworthii* and *M. archiducis-nicolai*. The combined data set of chloroplast *trn*K/*mat*K, nuclear *GA3ox1* and ITS sequences provided a substantial amount of informative characters. The methods of Maximum parsimony, Bayesian inference, and Maximum likelihood were employed. The results showed that *M. edgeworthii* formed a monophyletic group with *M. biflora* and *M. brachycarpa*, both of which are members of section *Lunatae*; *M. archiducis-nicolai* is closely related to *M. platycarpa* and *M. ruthenica*. Our study supports the previous view that *M. edgeworthii* belongs to section *Lunatae*, and *M. archiducis-nicolai* belongs to section *Platycarpae*. In addition, the study suggests that *M. lupulina* is a member of a clade having *M. tenoreana* and *M. minima*, which indicates that *M. lupulina* and *M. secundiflora* should probably not be placed in the same section.

Introduction

Medicago L. (Leguminosae) is distributed from the Mediterranean to central Asia and consists of about 87 species including some important forage species such as *M. sativa* L., *M. scutellata* (L.) Mill. and *M. lupulina* L. (Small, 2011). Systematists had been progressively revising the genus *Medicago* and clarifying the systematic position of its species (Urban, 1873; Lesins & Lesins, 1979; Small, 1987a, b; Small & Jomphe, 1989a, b). Bena (2001) concluded that 23 *Trigonella* species previously known as medicagoids were better placed in *Medicago* rather than assigned to a new genus by using nrDNA ITS and ETS sequences. In addition, the phylogenetic researches of Steele *et al*., (2010) using 73 *Medicago* species and plastid *trn*K/*mat*K and nuclear *GA3ox1* supported certain currently recognized taxonomic groups, e.g., section *Medicago* (with *M. sativa*) and section *Buceras*. But some strongly supported clades, related to *M. lupulina, M. murex*, *M. polymorpha*, and *M. truncatula*, contradict the current classification. Small (2011) divided the genus into 14 sections based on both morphological and nucleotide sequences from molecular data, e.g. plastid gene (*trn*K/*mat*K), mitochondrial region (*rpS14-cob*), nuclear genes (*GA3ox1*, *CNGC* 5, *β-cop*, ITS and ETS).

The systematic position of *M. edgeworthii* is controversial (Small & Jomphe, 1989b; Maureira-Butler *et al*., 2008; Small, 2011). Moreover, *M. archiducis-nicolai*, a valuable forage species was not included in previous studies. In the present study, we focus on the systematic positions of *M. edgeworthii* and *M. archiducis-nicolai* inferred from the combined sequences of plastid *trn*K/*mat*K and nuclear *GA3ox1* and ITS, based on Bena (2001) and Steele *et al*., (2010).

This paper firstly explores the systematic positions of *M. edgeworthii* and *M. archiducis-nicolai*, using the combined dataset of chloroplast *trn*K/*mat*K, nuclear *GA3ox1* and ITS sequences. In addition, this is the first study that uses molecular data to examine the systematic position of *M. archiducis-nicolai*.

Materials and Methods

Plant samples: The species used for the present study are listed in Table 1. They were all collected in the field in China. Healthy, clean leaves were fast-dried using silica gel. The voucher specimens have been deposited at the Herbarium of Wuhan Botanical Garden, Chinese Academy of Science (HIB).

DNA sequencing and alignment: Total genomic DNA was isolated using the modified CTAB method (Doyle & Doyle, 1987). The polymerase chain reaction (PCR) was used for double stranded DNA amplification. Each 25 μL reaction contained 0.25 μL of Ex Taq (2.5 u/μL), 2.5 μL of 10× Ex Taq buffer (Mg^{2+} concentration of 25 mM), 2.0 μL of dNTP mix (at 2.5 mM concentration for each dNTP), 1 μL of each, forward and reverse primers at 5 μmol/μL. The following molecular markers primers were used: plastid *trn*K/*mat*K (Hu *et al*., 2000; Steele and Wojciechowski, 2003; Wojciechowski *et al*., 2004; Bruneau *et al*., 2008), nuclear *GA3ox1* (Steele *et al*., 1999) and ITS (Bena, 2001) sequences. For PCR amplifications, predenaturation was first conducted at 94°C for 5min, followed by 30 cycles of (1) denaturation at 94°C for 30 s, (2) annealing at 50°C-58°C for 30 s, and (3) extension at 72°C for 1 min. At the end of the cycles, a final extension was used at 72°C for 10 min. The PCR products were purified and sequenced by Sangon Biotech (Shanghai) Co., Ltd.

Clustal X (Thompson *et al*., 1997) was used to produce an aligned matrix, which was corrected manually using the BioEdit program (Hall, 1999). All gaps were treated as missing characters. Finally, the sequences of *trn*K/*mat*K, *GA30x1* and ITS were combined for phylogenetic analyses.

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An RNA sequencing transcriptome analysis of the high-temperature stressed tall fescue reveals novel insights into plant thermotolerance

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RESEARCH PAPER

Leaf functional trait variation associated with salt tolerance in perennial ryegrass

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Keywords

Functional trait; *Lolium perenne*; mathematical model; relative growth rate; salt tolerance.

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ABSTRACT

Salinity is one of major environmental stresses that dramatically threaten plant growth, and variations in genetic structure and functional traits have important effects on the salt tolerance of perennial ryegrass (*Lolium perenne* L.). The objectives of this study were to: (i) assess the inter-clonal variation of functional traits of accessions among geographic groups or between wild and commercial groups in response to salt stress; (ii) develop a mathematical model to effectively assess salt tolerance of perennial ryegrass accessions originating from different geographic populations; and (iii) determine the relation between spatial genetic structure and salt tolerance in perennial ryegrass. Wide variations were found among the accessions for seven functional traits. One regression model ($F = 0.49 \times F_1 + 0.303 \times F_2 + 0.207 \times F_3$) was established to ascertain salt tolerance of each accession. The highest variation of the traits and salt tolerance were obtained for accessions from the European group. Wild accessions exhibited more variation in functional traits and salt tolerance than commercial cultivars. Both molecular marker techniques and functional traits were used to conduct phylogenetic analysis, and the majority of accessions from the same or adjacent regions were clustered into the same group or subgroup. The perennial ryegrass accessions with similar salt tolerance had a close phylogenetic background. The patterns in functional trait variations associated with salt tolerance might allow acceleration of the process for improving salt stress resistance in perennial ryegrass.

INTRODUCTION

Salinity is a major abiotic stress threatening plant growth and yield (Shabala & Cuin 2008). At least 20% of the world's cultivated land and nearly half of all irrigated lands are salt-affected to some extent (Rhoades & Loveday 1990). Developing genetically salt-resistant plants is considered a promising approach for alleviating the threats from soil salinity (Rhoades & Loveday 1990; Zhu 2001). The high variation in genetic structure and traits in plants provides potential genetic resources to select salt-tolerant germplasm.

Plant functional traits have been defined as any morphological, physiological or phenological features that directly or indirectly influence plant fitness *via* their effects on growth, survival and reproduction (Violle *et al.* 2007). Saline environments induce adaptive changes in morphological, physiological and biochemical traits of plants, including changes in ion absorption (Hajibagheri *et al.* 1987), plant hormones (Munns 1993), enzyme activity (Greenway & Osmond 1972), photosynthesis (Meloni *et al.* 2003), growth (Cooper 1982) and osmotic adjustment (Ghoulam *et al.* 2002). For perennial plant species, different functional traits have been studied with regard to adaptive responses to salt stress. For example, a low salinity treatment increased the relative growth rate (RGR) of shoots. In contrast, high salinity treatment substantially inhibited growth during a 30-day treatment period in *Atriplex griffithii* var. *stocksii* (Khan *et al.* 2000). Previous research suggested that plant growth rate could be used as an index for evaluating salt stress tolerance (Ball 1988; Neves-Piestun & Bernstein 2001; Li *et al.* 2010). Since photosynthesis, cell division and cell expansion are all inhibited at high salt concentrations, there will be a reduction in plant growth rate. Zhu (2001) reported that plants slow growth and use cellular organelles and other resources (e.g. mitochondria, energy, building blocks) to cope with environmental stress, which is an adaptive strategy for survival in stressful environments. Chlorophyll content also decreases with increasing sodium chloride (NaCl) concentration in both salt-tolerant and salt-sensitive *Eucalyptus camaldulensis* (Woodward & Bennett 2005) and *Lolium perenne* L. (Hu *et al.* 2011).

Salt tolerance in plants is complex and involves changes of many functional traits (Khan *et al.* 2000). Development of a mathematical model based on these functional traits would be a useful strategy to identify salt tolerance in plants. Some studies have analysed toxicity after abiotic stress by constructing mathematical models. Trapp *et al.* (2008) developed a non-linear model to determine NaCl uptake and toxicity in willow. Pishchik *et al.* (2002) simulated the beneficial effects of rhizobacteria in growth promotion of barley under cadmium stress using non-linear ordinary differential equations. However,

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MICROSATELLITE LETTERS

A set of microsatellite markers developed for *Dacrydium pectinatum* (Podocarpaceae), a vulnerable conifer in China

Lu Huang · Qi Deng · Ning Li · Yingjuan Su · Ting Wang

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Abstract *Dacrydium pectinatum* de Laub. (Podocarpaceae) is the sole species of *Dacrydium* in China. Excessive logging for more than 30 years has resulted in its vulnerable status in China. Fifteen microsatellites were developed for *D. pectinatum*, 12 of which displayed polymorphisms. The number of alleles ranged from one to seven, the expected and observed heterozygosities varied from 0.000 to 0.819 and from 0.000 to 1.000, respectively. Significant linkage disequilibrium and deviations from the Hardy–Weinberg equilibrium were found. These microsatellite markers give a comprehensive understanding of the forces driving population evolution and local adaptation of *D. pectinatum*, which may also contribute to its management and conservation.

Keywords *Dacrydium pectinatum* · Microsatellite marker · FIASCO · Population genetics

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Dacrydium pectinatum de Laub. (Podocarpaceae) is the sole species of *Dacrydium* in China, restrictedly distributed in mountain regions of central Hainan Island and historically widely used in constructing buildings and ships (Fu et al. 1999). Its present vulnerable status is due to more than 30 years of excessive logging. In 1992, *D. pectinatum* was entered on the Red List of Endangered Plants in China.

The island populations on Hainan Island of *D. pectinatum* provide an ideal natural experiment to study its adaptation. ISSR-based investigations have revealed its high level of genetic variation, low degree of differentiation, and the presence of severe bottlenecks (Su et al. 2010). However, its phylogeography and founder effects, especially its adaption to island environments, remain unexplored (Zheng 1991). Codominant SSR is a powerful tool for surveying local adaptation processes of trees in forests, including *D. pectinatum* (Lefort et al. 1999). Here, we developed and characterized 12 polymorphic microsatellite loci for *D. pectinatum.*

Genomic DNA was extracted from the leaves of two individuals from the Diaoluoshan population using a modified CTAB method with −20 °C propanone pretreatment to eliminate polysaccharides. A microsatellite enrichment library was constructed with an $(AC)_{15}$ probe using the FIASCO protocol (Zane et al. 2002). From 178 sequenced positive clones, sixty sequences containing SSRs and sufficient flanking regions were used to design primers using PRIMER PREMIER 5.0. Each primer pair

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Sodium selenite regulates phenolics accumulation and tuber development of purple potatoes

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ABSTRACT

It has been reported that selenium could increase nutritional components as carbohydrates, organic selenium, proteins and total amino acids of potatoes. However, there have been few reports on selenium-enriched purple potatoes as yet. In this study, the effects of selenium on phenolics and tuber development of purple potatoes were investigated. The main phenolic components in purple potatoes were separated and identified as chlorogenic acid, caffeic acid, malvidin-5-glu-3-dirhamnose-glucose, caffeic acid-acetylrhamnose ester and caffeic acid-prenylrhamnose ester. The total phenolics including malvidin-5-glu-3-dirhamnose-glucose could be directly quantified at 325 nm, with chlorogenic acid as a standard. The main phenolic components were significantly increased by selenium. Total tuber weight was significantly increased by selenium ($R^2 = 0.964$, $P = 0.002$). In conclusion, selenium significantly increased phenolic components and promoted tuber formation of purple potatoes; in this sense, selenium-enriched purple potato should be a better functional food.

1. Introduction

Phenolics are the main nutritional components in potatoes. Purple potatoes are the main sources for phenolics and anthocyanins (Jansen and Flamme, 2006). Anthocyanins contained in purple potatoes were found to show functions in antioxidation (Steed and Truong, 2008; Han et al., 2006a,b, 2007; Reyes et al., 2005; Brown et al., 2003; Lachman and Hamouz, 2005; Brown et al., 2007), antitumor (Yoshimoto and Okuno, 2001; Kazuya and Hiroshige, 2006) and liver protection (Han et al., 2006a). Anthocyanins could be easily absorbed by human body (*Harada* et al., 2004). So, compared to "white potatoes", purple potatoes were more valuable in health protection. Nowadays, purple potatoes were widely used as functional food in Europe, North America and Southeast Asia.

Selenium, a trace element, as a constituent of selenoproteins and some important enzymes as GSH-Px and some dehydrogenases, were reported to have physiological functions as antioxidation (Wong et al., 2010), anticancer (Tara et al., 2010), immunity stimulation (Ramoutar and Brumaghim, 2010) and inhibiting HIV (Silva et al., 2010). The bio-activity of selenium depends on its chemical form. Organic selenium-containing compounds such as selenomethionine and methylselenocysteine were better tolerated and exhibit anticarcinogenic activity (Yu et al., 2007). Many plants showed abilities in absorbing and transforming inorganic selenium into organic selenium (Sors et al., 2005). So selenium tolerant plants were widely used for selenium supplementation. In fact, selenium-enriched food was used as either selenium supplementation or functional food, since selenium could enhance accumulation of some active compounds (Dong et al., 2012, 2013). There have been some reports on selenium increasing carbohydrates, organic selenium, total amino acids, antioxidation activity and photosynthesis of potatoes (Turakainen et al., 2004; Cyrus et al., 1991; Seppänen et al., 2003; Cuderman et al., 2008; Germ et al., 2007). However, no reports about selenium-enriched purple potato have been seen as yet. In this study, cultivation of selenium-enriched purple potatoes

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Article

GA_3 and Other Signal Regulators (MeJA and IAA) Improve Xanthumin Biosynthesis in Different Manners in *Xanthium strumarium* L.

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Abstract: Xanthanolides from *Xanthium strumarium* L. exhibit various pharmacological activities and these compounds are mainly produced in the glandular trichomes of aerial plant parts. The regulation of xanthanolide biosynthesis has never been reported in the literature. In this study, the effects of phytohormonal stimulation on xanthumin (a xanthanolide compound) biosynthesis, glandular trichomes and germacrene A synthase (GAS) gene expression in *X. strumarium* L. young leaves were investigated. The exogenous applications of methyl jasmonate (MeJA), indole-3-acetic acid (IAA), and gibberrellin A_3 (GA_3) at appropriate concentrations were all found to improve xanthumin biosynthesis, but in different ways. It was suggested that a higher gland density stimulated by MeJA (400 μM) or IAA (200 μM) treatment caused at least in part an improvement in xanthumin production, whereas GA_3 (10 μM) led to an improvement by up-regulating xanthumin biosynthetic genes within gland cells, not by forming more glandular trichomes. Compared to the plants before the flowering stage, plants that had initiated flowering showed enhanced xanthumin biosynthesis, but no higher gland density, an effect was similar to that caused by exogenous GA_3 treatment.

Keywords: *Xanthium strumarium* L.; glandular trichomes; xanthanolide; phytohormones

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ORIGINAL PAPER

Genetic diversity in kiwifruit polyploid complexes: insights into cultivar evaluation, conservation, and utilization

Dawei Li · Yifei Liu · Xinwei Li · Jingyun Rao · Xiaohong Yao · Caihong Zhong

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Abstract Understanding the extent and partitioning of crop genetic diversity is necessary for conserving and utilizing their genetic potentials for breeding. In the present study, fluorescence-labeled amplified fragment length polymorphism markers were used to characterize the genetic diversity and relationships of 79 cultivars and also of 122 F1 hybrids which resulted from six kiwifruit interploid crosses. A high level of mean genetic diversity was detected (Hj > 0.22) for all cultivars investigated, without significant differences among diploids (2*x*), tetraploids (4*x*), and hexaploids (6*x*). This suggested that no significant genetic erosion occurred in these cultivars, which were directly selected from natural resources or created from crosses. The Unweighted Pair Group Method with Arithmetic Mean analysis of the genetic dissimilarity between cultivars showed three main groups mostly based on their three ploidy levels. Among these, the red-fleshed cultivars which were originally derived from 'Hongyang' clustered into one subgroup of group I, suggesting their unique genetic background despite they were marked as different cultivars used in the current kiwifruit industry. By analyzing the genetic variation of hybrids with variable ploidy levels, our genetic analyses further revealed that interploid crosses can increase the genetic diversity of F1 offsprings, especially from the parental combinations of 6*x*–2*x* and 6*x*–4*x*, in which both parents showed significant differences in morphology and genetic backgrounds. Based on these findings, strategies were proposed for the conservation and utilization of the current kiwifruit genetic resources for future breeding programs.

Keywords Kiwifruit cultivars · Genetic diversity · Polyploidy · Interploid cross · Conservation

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Introduction

Crop genetic diversity is the raw material for breeding new crop varieties in response to the needs of diverse agricultural systems (Brussaard et al. 2010). Domestication or plant breeding per se can be harmful for maintaining crop diversity (Esquinas-Alcázar 2005). However, reduction in genetic diversity during crop breeding is variable, mostly depending on the biological nature of plant and also the differences in domestication activities (Zhao et al. 2014). Assessing the level and pattern of the genetic variation for crop cultivars or the conserved genetic resources is thus crucial, in particular, for determining and constructing the core or mini-core collections (Zhang et al. 2011), assisting the selection of parental combinations to create hybrids with superior agronomic characters (Glaszmann et al. 2010), and developing conservation strategies to impede genetic erosion during domestication and breeding programs (Gepts 2006; Frankham 2010).

The genus *Actinidia* Lindl., well known as kiwifruit, contains 54 species which are generally dioecious, deciduous, and scrambling vines (Li et al. 2007). Currently, kiwifruit cultivars were mainly developed based on *Actinidia chinensis* var. *chinensis* and *A. chinensis* var. *deliciosa* (Li et al. 2007) in different breeding programs launched in China, New Zealand, and Italy during the last two decades (Ferguson and Huang 2007). Most cultivars are direct selections from the wild or seedling populations of the two varieties with expected flavor,

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Expression profiles of *Pr5CS1* and *Pr5CS2* genes and proline accumulation under salinity stress in perennial ryegrass (*Lolium perenne* L.)

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With 5 figures and 1 table

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Abstract

Proline is an important osmoprotectant in plant in response to osmotic stresses. Delta 1-pyrroline-5-carboxylate synthetase (*P5CS*) is a key enzyme in proline biosynthesis. In this study, two *P5CS* genes (*PrP5CS1* and *PrP5CS2*) were isolated for the first time from perennial ryegrass. Expression analysis revealed that the transcript of *PrP5CS1* in leaves was significantly up-regulated in two ryegrass cultivars exposed to 255 mM NaCl. The up-regulated level of *PrP5CS1* was higher in salt-tolerant 'Overdrive' than in sensitive 'Pizzazz'. *PrP5CS2* was significantly induced in 'Overdrive' but suppressed in 'Pizzazz' by NaCl treatment. In stems, however, there was no significant transcript change for both genes under salt treatment. The proline accumulation was significantly induced in both cultivars after salt treatment, and it was higher in 'Overdrive' than in 'Pizzazz' after 2 days of salt treatment. The results suggested that both genes are salt inducible and may be associated with salt-stress tolerance in perennial ryegrass.

Key words: gene cloning — gene expression — perennial ryegrass — *P5CS* — proline accumulation — salt stress

Soil salinization is a serious problem that constrains plant growth and distribution. The saline soil accounts for about 1 billion ha all over the world (Cheong and Yun 2007). In China, the area of saline soil accounts for almost 10% of the total area in the world. Salinity stress affects plants primarily through both the creation of osmotic stress and the direct action of excess Na^+ and Cl^- ions (Hasegawa et al. 2000, Munns 2005).

Proline accumulation was considered to be correlated with salt tolerance in plants (Delauney and Verma 1993), because it can function as an osmoprotectant (Christian 1955) and plays a role in stabilizing subcellular structure and scavenging free radicals (Smirnoff and Cumbes 1989). In response to osmotic stress caused by high salinity or water deficit, many plants could accumulate proline (Yancey et al. 1982, Kuznetsov and Shevyakova 1997). In higher plants, proline can be synthesized via two pathways: glutamate or ornithine. The glutamate pathway was the predominant pathway, especially under stress conditions (Zhang et al. 1995). In this pathway, proline is synthesized from glutamic acid via the intermediate γ-glutamic semialdehyde (GSA), and delta 1-pyrroline-5-carboxylate synthetase (P5CS) is the key enzyme that catalyses the first two reactions (Hu et al. 1992). Moreover, the γ-glutamyl kinase activity of P5CS is the rate-limiting step in this pathway and can be feedback inhibited by proline (Zhang et al. 1995). Previous studies demonstrated that the induction of *P5CS* gene preceded proline accumulation in *Arabidopsis thaliana*, suggesting that *P5CS* played a key role in proline biosynthesis under osmotic stress (Savoure′ et al. 1995, Yoshiba et al. 1995).

P5CS genes have been isolated and identified from many plant species (Yoshiba et al. 1995, Igarashi et al. 1997, Su et al. 2011). Moreover, in some species, two different but closely related *P5CS* genes were identified. For example, two independent *P5CS* genes were identified in *Arabidopsis thaliana* (Strizhov et al. 1997, Yoshiba et al. 1999). The *P5CS1* gene was expressed in almost all organs and could be rapidly induced by various stresses (Strizhov et al. 1997, Yoshiba et al. 1999), while *P5CS2* was expressed mainly in dividing cells (Ginzberg et al. 1998). In *Brassica napus* and rice (*Oryza sativa*), the transcript of both *P5CS* genes could be up-regulated by various stresses (Hur et al. 2004). In tomato (*Solanum lycopersicum* L.), the transcriptional profiles of two *P5CS* homologous genes (*tomPRO1* and *tomPRO2*) were also different. The *tomPRO2* was induced significantly, whereas the *tomPRO1* transcript was undetectable under NaCl stress (Fujita et al. 1998). Recently, two *P5CS* genes were also cloned from bioenergy sorghum (*Sorghum bicolor* (Linn.) Moench), and their expression profiles under abiotic stresses were examined (Su et al. 2011).

Previous studies have also demonstrated the correlation between the induction of *P5CS* gene and the accumulation of proline in *Arabidopsis thaliana*, rice and sorghum (Yoshiba et al. 1995, Igarashi et al. 1997, Su et al. 2011). It was found that there was a cause-and-effect relationship between the increasing of *P5CS* transcript and the accumulation of proline. In addition, overexpression of *Vigna aconitifolia P5CS* gene (*VaP5CS*) in transgenic tobacco resulted in a significant accumulation of proline under drought stress, when compared with the non-transgenic parents (Kishor et al. 1995). Furthermore, the increased proline concentration was often accompanied by the improvement of salt tolerance in transgenic plants (Zhu et al. 1998, Han and Hwang 2003).

Perennial ryegrass (*Lolium perenne* L), a major cool-season forage and turfgrass species, is widely used in temperate regions worldwide (Kubik et al. 2001). Salt stress is a major limiting factor in perennial ryegrass culture and management. To cope with this obstacle, development of salt-tolerant ryegrass cultivars is an economical and effective approach. As conventional breeding is laborious and time consuming, molecular breeding via genetic modification has become an alternative approach in developing stress-tolerant plants. In previous study, we have obtained cDNA fragments of two distinct *P5CS* genes from a salt-tolerant perennial ryegrass cultivar using SSH technique, and

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ORIGINAL PAPER

Molecular cloning and characterization of an isoflavone 7-*O*-glucosyltransferase from *Pueraria lobata*

Jia Li · Zhaobo Li · Changfu Li · Junbo Gou · Yansheng Zhang

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Abstract

Key message **A novel isoflavone 7-*O*-glucosyltransferase PlUGT1 was isolated from *Pueraria lobata*. PlUGT1 could convert daidzein to daidzin, genistein to genistin as well as formononetin to ononin.**

Abstract *Pueraria lobata* roots are traditionally consumed as a rich source of isoflavone glycosides that have various human health benefits. However, to date, the genes encoding isoflavone UDP-glycosyltransferases (UGTs) have only been isolated from the roots of soybean seedlings (GmIF7GT), soybean seeds (UGT73F2) and *Glycyrrhiza echinata* cell suspension cultures (GeIF7GT). To investigate the isoflavone metabolism in *P. lobata*, 40 types of partial UGT cDNAs were isolated from *P. lobata*, and seven full-length UGT candidates with preferential expression in roots were identified. Functional assays in yeast (*Saccharomyces cerevisiae*) revealed that one of these UGT candidates, designated PlUGT1 (official UGT designation UGT88E12), efficiently glycosylated isoflavone aglycones at the 7-hydroxy group. Recombinant PlUGT1 purified from *Escherichia coli* cells was characterized and shown to be relatively specific for isoflavone aglycones, while flavonoid substrates were poorly accepted. The biochemical results suggested that PlUGT1 was an isoflavone 7-*O*-glucosyltransferase. The deduced amino acid sequence of PlUGT1 shared only 26 % identity with GeIF7GT, 27 % with UGT73F2 and 63 % with GmIF7GT. The *PlUGT1* gene was highly expressed in *P. lobata* roots relative to other organs and strongly induced by methyl jasmonate signal in *P. lobata* cell suspension culture. The transcript abundance of *PlUGT1* was correlated with the accumulation pattern of isoflavone glycosides such as daidzin in *P. lobata* plants or in cell suspension culture. The biochemical properties and gene expression profile supported the idea that PlUGT1 could play a role in isoflavone glycosylation in *P. lobata*.

Keywords Glucosyltransferase · Isoflavone · Methyl jasmonate · *Pueraria lobata*

Abbreviations

cDNAs	Complementary DNAs
HID	Trihydroxyisoflavanone dehydratase
HPLC	High-performance liquid chromatography
IFS	Isoflavone synthase
IPTG	Isopropyl-*D*-thiogalactopyranoside

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J. Li and Z. Li contributed equally to this work.

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Increase of betulinic acid production in *Saccharomyces cerevisiae* by balancing fatty acids and betulinic acid forming pathways

Jing Li · Yansheng Zhang

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Abstract Betulinic acid is a plant-based triterpenoid that has been recognized for its antitumor and anti-HIV activities. The level of betulinic acid in its natural hosts is extremely low. In the present study, we constructed betulinic acid biosynthetic pathway in *Saccharomyces cerevisiae* by metabolic engineering. Given the betulinic acid forming pathways sharing the common substrate acetyl-CoA with fatty acid synthesis, the metabolic fluxes between the two pathways were varied by changing gene expressions, and their effects on betulinic acid production were investigated. We constructed nine *S. cerevisiae* strains representing nine combinations of the flux distributions between betulinic acid and fatty acid pathways. Our results demonstrated that it was possible to improve the betulinic acid production in *S. cerevisiae* while keeping a desirable growth phenotype by optimally balancing the carbon fluxes of the two pathways. Through modulating the expressions of the key genes on betulinic acid and fatty acid pathways, the difference in betulinic acid yield varied largely in the range of 0.01–1.92 mg L^{-1} OD^{-1}. The metabolic engineering approach used in this study could be extended for synthesizing other triterpenoids in *S. cerevisiae*.

Keywords Betulinic acid · Fatty acid · *Saccharomyces cerevisiae* · Balancing carbon flux

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Introduction

As a pentacyclic lupane type of triterpenoid, betulinic acid shows great pharmacological properties such as anticancer and anti-HIV activities (Yogeeswari and Sriram 2005). Because of its specific cytotoxicity against tumor cells, betulinic acid was considered to be a future promising anticancer compound (Zuco et al. 2002). Bevirimat, a betulinic acid derivative, has recently been successfully used in phase IIb clinical trials for the treatment of acquired immune deficiency syndrome (Smith et al. 2007). Despite its great potential for clinical applications, the insufficient supply of betulinic acid in its natural hosts is a major obstacle in commercializing this compound. Birch bark is the major plant source for extracting betulinic acid, but the minute amount of betulinic acid in their tissues has limited its production in a large scale for the market (Jäger et al. 2009). Betulin, a precursor of betulinic acid, has been successfully converted to betulinic acid, but some issues remain, including low yields, safety, pollutions, and high costs (Kim et al. 1997). Microbial biotransformation is another approach for converting betulin to betulinic acid, but the conversion efficiency is pretty low, and this approach is also limited by the supply of betulin (Liu et al. 2011).

Rapid developments in metabolic engineering and synthetic biology provide alternative approaches for the high production of natural products in microbial hosts (Ajikumar et al. 2010; Paddon et al. 2013; Ro et al. 2006). In many cases, synthetic biology efforts have successfully facilitated the high production of plant monoterpenes, sesquiterpenes, and diterpenes in microorganisms (Kirby and Keasling 2008; Withers and Keasling 2007); however, the metabolic engineering for the microbial synthesis of plant triterpenoids is very limited. To the best of our knowledge, the professional engineering efforts have only been made to produce the beta-amyrin which

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Characterization of two *VvICE1* genes isolated from 'Muscat Hamburg' grapevine and their effect on the tolerance to abiotic stresses

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ABSTRACT

To investigate the molecular nature of the cold responsive genes and elucidate its regulatory network, two putative bHLH transcription factors, named *VvICE1a* and *VvICE1b*, were isolated from table grape cultivar 'Muscat Hamburg' (*Vitis vinifera*). The expression of *VvICE1a* and *VvICE1b* were induced by a wide spectrum of abiotic stresses, including cold, exogenous abscisic acid (ABA), drought, salinity, and cold-drought conditions. The constitutive expression of *VvICE1a* and *VvICE1b* improved the tolerance to cold accompanying increased resistance to drought and salinity in transgenic arabidopsis. Moreover, in the *VvICE1a* and *VvICE1b* transgenic plants, the transcript levels of two stress-responsive genes *AtRD29A* and *AtCOR47* were enhanced under normal growth conditions.

1. Introduction

Cold stress adversely affects plant growth and development and limits crop distribution and productivity (Boyer, 1982). Many plant species in temperate regions can acquire freezing tolerance by a prior exposure to low (non-freezing) temperatures (Thomashow, 1999). In response to cold temperatures, plants reprogram the expression of genes involved in multiple cold signaling pathway in order to modify their metabolism (Chinnusamy et al., 2004).

The ICE1-CBF/DREB-COR (Inducer of CBF expression 1-C-repeat/DRE binding factor-cold-regulated gene) cold-response pathway is critical for configuring the primary cold signaling transduction and is better understood in arabidopsis (*Arabidopsis thaliana*) (Shinozaki and Yamaguchi-Shinozaki, 2000; Chinnusamy et al., 2003; Lee et al., 2005; Zhou et al., 2011). *COR* genes in arabidopsis encode proteins that help protect against cold stress (Steponkus et al., 1998). The promoter regions of 12% of *COR* genes contain copies of the C-repeat/dehydration-responsive *cis*-element (CRT/DRE) (Yamaguchi-Shinozaki and Shinozaki, 1994; Jaglo-Ottosen et al., 1998; Fowler and Thomashow, 2002). The central component in the pathway is a small family of AP2 DNA-domain-containing transcriptional activators, known as DREB/CBF, bind to the CRT/DRE *cis*-element and activate transcription (Stockinger et al., 1997; Jaglo-Ottosen et al., 1998; Medina et al., 1999). These *DREB/CBF* transcription factors are also induced by cold, and the three *CBF* genes *DREB1B/CBF1*, *DREB1A/CBF3* and *DREB1C/CBF2* in arabidopsis have been found to participate in the cold-response pathway (Gilmour et al., 2004; Novillo et al., 2004; Van Buskirk and Thomashow, 2006). Enhanced cold tolerance has been observed in transgenic arabidopsis, tobacco, tomato, rice, and apple after overexpression of *DREB/CBF* genes (Gilmour et al., 2000; Hsieh et al., 2002; Ito et al., 2006; Shukla et al., 2006; Wisniewski et al., 2011). Additionally, heterologous overexpression of a grape-derived *VvCBF4* gene also improved freezing survival and reduced freezing-induced electrolyte leakage in non-cold-acclimated vines (Tillett et al., 2012).

The bHLH transcription factors are ubiquitously involved in the response of higher plants to cold (*AtICE1*), drought (*AtMYB2*) and high salinity (*AtNIG1*) (Chinnusamy et al., 2003; Abe, 2003; Kim and Kim, 2006). *ICE1* encodes a MYC-like bHLH transcription activator that regulates the expression of *CBF3* and *COR* genes in response to cold stress (Chinnusamy et al., 2003; Lee et al., 2005). ICE1 protein binds specifically to the MYC recognition sequence (CANNTG) in the

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Molecular Cloning and Characterization of the *HOS1* Gene from 'Muscat Hamburg' Grapevine

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Additional index words. abiotic stress, *Vitis vinifera*, *VvHOS1*

Abstract. Cold stress is an important factor that limits grape (*Vitis* sp.) production around the world. The high expression of osmotically responsive genes 1 (*HOS1*) protein acts as a repressor of cold-responsive genes in plants. To increase understanding of mechanism regulating cold tolerance in grape, we isolated and characterized a novel *HOS1* gene, designated *VvHOS1* from 'Muscat Hamburg' grapevine (*Vitis vinifera*). Real-time polymerase chain reaction (PCR) analysis revealed that the expression of *VvHOS1* could be induced by the application of exogenous abscisic acid and various abiotic environmental conditions such as low temperature, drought, and salinity. Moreover, *VvHOS1* expression could also be induced by cold plus drought conditions (4 °C, 10% polyethylene glycol 6000). In addition, overexpression of *VvHOS1* in arabidopsis (*Arabidopsis thaliana*) decreased the plants' tolerance to cold, drought, and salt as well as negatively regulated the expression level of two stress-responsive genes, *AtRD29A* and *AtCOR47*. The results obtained in this study should help us to elucidate the function of *VvHOS1* and understand the cold-responsive pathway in grapevine.

Cold stress, including chilling (0 to 15 °C) and freezing (less than 0 °C), is one of the limiting environmental factors affecting plant growth (Levitt, 1980). To adapt to a cold environment, many plants have evolved a cold acclimation process when exposed to low non-freezing temperatures mediated by complex and elaborate signaling networks (Guy, 1990; Tang et al., 2006; Thomashow, 1999). This process is associated with the accumulation of compatible osmolytes and the stability of biomembranes (Orvar et al., 2000; Suzuki et al., 2000; Suzuki and Mittler, 2005). For the past several years, considerable attention has been devoted to the transcriptional activation of the positive cold-responsive genes such as C-repeat binding factor (*CBF*) and its downstream genes: responsive to dessication 29A (*RD29A*), cold-regulated 15A (*COR15A*), cold-regulated 47 (*COR47*), etc. A large number of genes that respond to cold acclimation have been identified in a number of plants (Fursova et al., 2009; Ganeshan et al., 2008; Knight et al., 2009; Lee and Thomashow, 2012; Medina et al., 2011; Provart et al., 2003; Thomashow, 2010; Xiao et al., 2006). Water-deficit stress such as drought and high salinity results in a marked reduction in crop productivity on as much as half of the irrigated land in the world. Signal transduction pathways triggered by various stresses, including drought and high salt content, share a number of signaling components that transduce the signal into downstream processes, which subsequently endow resistance to such stresses. In contrast, continuous activation of the plant's cold-responsive genes is metabolically expensive and may result in permanent damage to the cellular components of the plant itself; thus, the expression of the cold-responsive genes must be controlled by negative regulation to maintain the balance of gene expression in

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The molecular diversity analysis of *Auricularia auricula-judae* in China by nuclear ribosomal DNA intergenic spacer

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ABSTRACT

Background: For the crossbreeding of *Auricularia auricula-judae*, selecting the appropriated parents in hybridization is very important. However, the classification and diversity analysis of *A. auricula-judae* has been equivocal, due to the similarity of the fruiting body morphology and its susceptibility to environmental influences. For this purpose, the molecular diversity of 32 *A. auricula-judae* commercial cultivars in China was analyzed by using the nuclear ribosomal DNA intergenic spacer.
Results: The complete nuclear rDNA gene complex of *A. auricula-judae* isolate is 11,210 bp long, and contains the 18S, 5.8S, and 28S rRNA gene as well as the ITS and IGS regions. Based on the sequence data, four more effective primer combinations for the IGS region of *A. auricula-judae* were designed. Nucleotide sequence variation in the IGS among 32 *A. auricula-judae* commercial cultivars in China sorted into three strongly supported clades, which is correlated with geographical regions. Most strains originated from the same area were with a narrow genetic basis and could possibly be domesticated from the local wild-type strains.
Conclusion: The grouping information obtained in the present work provides significant information for further genetic improvement in *A. auricula-judae*, and suggested that the IGS region can be used as an excellent tool for identification of genetic variation.

1. Introduction

Auricularia auricula-judae (Bull.) Quel. which has a global distribution in tropical, sub-tropical and temperate region, has been first cultivated in China more than one thousand years ago. It is an important edible and medical mushroom, and the annual production is fourth in the world, following *Agaricus bisporus*, *Pleurotus ostreatus* and *Lentinula edodes* [1].

To protect the rights of mushroom breeders, it is very important to discriminate among main cultivars of *A. auricula-judae*. However, the classification and diversity analysis of edible mushroom has been equivocal, due to the similarity of the fruiting body morphology and its susceptibility to environmental influences. Thus, problems frequently arise if the analysis is based entirely on morphological characteristics.

Fortunately, molecular biology techniques provide a useful methodology for systematic analysis of genetic polymorphism. The spacer regions of ribosome DNA genes are useful for examination of close relationships between organisms, because of the divergence in their nucleotide sequences. The internal transcribed spacer (ITS) between the 18S and 25S rRNA genes is moderate and has been widely applied in phylogenetic studies of basidiomycetes [2]. The intergenic spacer (IGS) between the 25S and 18S rRNA genes is evolving fastest in the rDNA complex [3,4], and has been applied in the polymorphism analysis of edible fungi, such as *Agaricus bisporus* [5], *Laccaria bicolor* [6], *Hebeloma cylindrosporum* [7], *Lentinula edodes* [8], *Pleurotus eryngii* [9], *Tricholoma matsutake* [10], *Ferula sinkiangensis* [11], *Tuber borchii* [12] and *Rhodocollybia laulaha* [13]. However, sequence analysis of the IGS regions of *A. auricula-judae* has not been reported.

In this study, the complete rDNA repeat unit of *A. auricula-judae* was firstly sequenced and analyzed, particular emphasis was placed on the IGS region and the more effective primer combinations were designed. Based on the nucleotide sequence variation in the IGS, the genetic polymorphism of 32 *A. auricula-judae* commercial cultivars in China will be discussed.

2. Materials and methods

2.1. Mushroom strains

Thirty-two main cultivars of *A. auricula-judae* in China were used throughout this study. All cultivars were collected from local professional

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Morphological and Proteomic Analysis Reveal the Role of Pistil under Pollination in *Liriodendron chinense* (Hemsl.) Sarg.

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Abstract

Pollination is an important physiological process during which interaction between pollen and pistil occurs. This interaction could determine whether or not fertilization will occur and hence the ratio of plant seed setting. *Liriodendron chinense* (Hemsl.) Sarg. (*L. chinense*) exhibits a distinct phenomenon where seed setting ratio is not more than 10% in natural environment. To explore the origin of this phenomenon, we conducted a comparative morphological and proteomic analysis on *L. chinense* pistils upon pollination. The morphological analysis showed that pollen grows well *in vitro*, but much slower on pistil or nutrient medium containing pistil extract. Proteomic analysis showed that 493 proteins had changed the expression after pollination. Among them, 468 and 51 proteins were identified by isobaric tags for relative and absolute quantitation and two-dimensional gel electrophoresis respectively, and 26 proteins were common in the two methods. After proteins functional categorization, 66 differentially expressed proteins that are involved in reproduction process were found. Further analysis showed that among the reproductive process related proteins, protein disulfide-isomerase A6 and four embryo-defective proteins showed closer relations with the low seed setting phenomenon. The results indicated that the element from pistil might be the main reason leading to low seed setting in *L. chinense*, which will provide new insights in the mechanisms underlying *L. chinense* reproduction process.

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Introduction

Liriodendron is a genus in *Magnoliaceae* family. Plants in genus *Liriodendron* are distinctive and produce valuable hardwood with great ecological and economic values. They grow fast and the wood is light and soft, and therefore, are cultivated in many temperate mountains of America and China for wood production [1–5]. They are flowering plants with beautiful leaves and are used in urban landscaping as they also provide shading. In addition, *Liriodendron* is valued as source material for honey production, chemical extracts [6–8], and biofuels [9,10].

The genus *Liriodendron* survived from the last Ice Age and was distributed in temperate regions in the northern hemisphere over great geographical ranges [11,12]. Currently it comprises of only two morphologically similar species, *Liriodendron tulipifera* L. and *Liriodendron chinense* (Hemsl.) Sarg., derived from North American and East Asian respectively [11]. However, *Liriodendron chinense* (Hemsl.) Sarg. (*L. chinense*) has been deemed a rare and endangered species because it occurs in small, insular and sparse populations [12]. In 1992, *L. chinense* was listed in the Red List of Endangered Plants in China [13], and in 1998, it was classified as near-threatened species in IUCN Red List of Threatened Species by the International Union for Conservation of Nature and Natural Resources.

Low seed setting percentage is a marked trait in sexual reproduction of *L. chinense*. After years of studies, the setting percentage of *L. chinense* has been shown to be not more than 10% in natural conditions, and it is hard to find the seedling in natural environments [14]. In the last two decades, numerous researchers have conducted studies, such as examining the relative contribution of pollen fertility and transfer, availability of resources, flower, or seed predation and genetics, to determine why *L. chinense* only produces so few seeds [15–18]. Unfortunately, there has been no consistent conclusion. Pollination, which is a key event in reproductive processes of plants, especially in rare or endangered plant species like *L. chinense* that have low seed production, is probably one of the weak links in the reproductive cycle. Any barrier occurring between pollen and stigma interaction will lead to low seed production. However, few studies have focused on pollination in *L. chinense*. Zhou and Fan examined pollen quality, pollen germination and growth on stigma using fluorochroma method. The results indicated that *in vivo* the pollen grains can load on about 64% pistils of the gynoecium, but the rate of pollen tube passing the style is low, only 24% [19]. In addition to few pollen tubes passing the style, the pollen tubes may grow twined or in no direction, suggesting that only a smaller percent of the pollen tubes penetrates the micropyle and enter the ovule [20,21]. The results showed that interactions between pollen and stigma occur in

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Isolation and characterization of ten polymorphic microsatellite loci for a vulnerable species *Dacrycarpus imbricatus* (Podocarpaceae) in China

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1. Introduction

Dacrycarpus imbricatus (Blume) de Laub. (Podocarpaceae) is the only species of the genus *Dacrycarpus* naturally occurring in China (Fu et al., 1999). *D. imbricatus* is mainly distributed in southern China, including northeastern Guangxi, southern Yunnan, Guangdong, and Hainan Island (Fig. 1(a)) (Cheng and Fu, 1978). The plant is one of the most important forest trees on Hainan Island, including Bawangling, Jianfengling, Wuzhishan, and Diaoluoshan. *D. imbricatus* grows primarily in the mixed evergreen broad-leaved forests in the Chinese mainland (Tang, 1982). Compared with its island populations, *D. imbricatus* populations in the Chinese mainland are distributed in scattered stands (Chen, 1998; Chen et al., 2004). *D. imbricatus* is a dioecious conifer tree up to 40 m tall with red brown bark (Fu et al., 1999). The species presents two types of leaves: juvenile leaves 2-ranked and adult leaves needlelike or scalelike (de Laubenfels, 1969). The timber of *D. imbricatus* had been used in construction and for furniture (Cheng and Fu, 1978). The overexploitation, as well as the destruction of forests for farmland and to accommodate fast human population growth, has resulted in a decline of its population size over recent decades

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Molecular Cloning and Functional Characterization of Two Divergent 4-Coumarate : Coenzyme A Ligases from Kudzu (*Pueraria lobata*)

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As part of the efforts to understand isoflavonoid metabolism in *Pueraria lobata* at the molecular level, the cDNAs encoding two divergent 4-coumarate : coenzyme A ligases (4CLs, designated Pl4CL1 and Pl4CL2, respectively) were isolated from *P. lobata* roots. Sequence analysis revealed that Pl4CL1 had an N-terminal extension of twenty-one amino acid residues compared to Pl4CL2. Phylogenetic analysis showed that Pl4CL1 and Pl4CL2 fell into angiosperm Class II and Class I, respectively. Through *in vitro* biochemical assays, both Pl4CLs were found to have the capacity to utilize 4-coumarate and *trans*-cinnamate as substrates, while neither of them could convert sinapate. Pl4CL2 had a broader substrate specificity than Pl4CL1. The affinity of Pl4CL1 for 4-coumarate was 2.6-fold higher than that of Pl4CL2 (with the K_m values of 3.5 μM and 9.1 μM, respectively). Combining the dataset including gene expression profiles, metabolites measurements, and biochemical properties, our results indicated that Pl4CL1, just as other angiosperm Class II 4CLs, might play a role in isoflavone biosynthesis in *P. lobata*, while Pl4CL2 belongs to angiosperm Class I, and may function as a housekeeping enzyme concerning lignification.

Key words 4-coumarate : coenzyme A ligase (4CL); isoflavonoid; *Pueraria lobata*; puerarin

Human have used *Pueraria lobata* as a herbal drug for many years.[1,2] Its pharmacological effects are due to the presence of isoflavonoids, which include puerarin, daidzin, and other related metabolites. Puerarin exhibits diverse medicinal properties including hypotensive,[3] hypolipidemic,[4] hypoglycemic,[5] anti-oxidant,[6] anti-ischaemia,[7] vasodilation[8] and estrogen-like effects.[9] As an isoflavonoid, the biosynthesis of puerarin should derive from phenylpropanoid metabolism, in which the enzymes in the early steps such as phenylalanine ammonia lyase (PAL), cinnamate 4-hydroxylase (C4H), 4-coumarate : coenzyme A ligase (4CL), chalcone synthase (CHS), chalcone reductase (CHR), and chalcone isomerase (CHI) are conserved in many plant species. Although puerarin was proposed to exhibit diverse pharmacological activities, the genes for the enzymes involved in the pathway have limitedly been isolated and characterized from *P. lobata*. As part of understanding the isoflavanoid metabolism in *P. lobata* at the molecular level, we have undertaken standard amplifications of cDNAs on PAL, C4H, 4CL, CHS, CHR, CHI and isoflavone synthase (IFS) by degenerate primers from the *P. lobata* roots that are deemed as the source tissues mostly accumulating puerarin. Interestingly, the partial cDNAs corresponding to two divergent 4CLs, designated as Pl4CL1 and Pl4CL2, were amplified, and showed only 65% amino acid sequence identity between each other. In plants, 4CLs usually have many isoenzymes, *e.g.* structurally and functionally different 4CLs were discovered in *Arabidopsis thaliana*,[10] *Glycine max*,[11] *Lithospermum erythrorhizon* [12], *Lolium perenne*,[13] *Oryza sativa*,[14] *Phyllostachys edulis*,[15] *Populus tremuloides*,[16] *Panicum virgatum*,[17] and *Rubus idaeus*.[18] As the last enzyme of the general phenylpropanoid pathway, 4CLs directs carbon fluxes through different pathways into the biosynthesis of monolignols and other phenolic metabolites such as flavones (Fig. 1).

Methyl jasmonate (MeJA) has been suggested to be an important signal for inducing isoflavonoid biosynthesis in *P. lobata*.[19] Therefore, in our preliminary experiments, the gene expression of PAL, C4H, 4CL, CHS, CHI and IFS were examined in *P. lobata* suspension cultures in response to MeJA treatment. Interestingly, among the genes, *Pl4CL1* was shown to be strongest one to be up-regulated by MeJA treatment (unpublished data). For studying the regulatory mechanism on isoflavonoid biosynthesis in *P. lobata*, it will be important to experimentally elucidate the role of Pl4CLs. Here we reported on the full-length cDNA isolation and functional characterization of the two distinct 4CLs, Pl4CL1 and Pl4CL2, from *P. lobata*. Combined with gene expression profiles and *in vitro* biochemical characterizations, this study demonstrated that Pl4CL1 played a role in isoflavone production in *P. lobata* while Pl4CL2 most likely was involved in lignin biosynthesis. The connection of the 4CLs primary sequence to their functions was discussed.

MATERIALS AND METHODS

Plant Materials and Chemicals Seeds of *P. lobata* in this study were collected from Langxi county, Anhui province, China. Seed explants were scarified by grinding with quartz sand in a mortar for 3 min at most, avoiding damage to the embryos. After treatment with 20 mg/L kinetin (KT) for 24 h at room temperature, seeds were surface sterilized by being immersed in 75% (v/v) ethanol for 10–15 s, followed by three washes in sterile distilled water and 10 min immersion in 10% hydrogen peroxide. Afterwards, another three washes were necessary. Axenic seeds were then placed on the agarized (0.8%) Murashige and Skoog (MS) basal medium[20] at 25°C in darkness. The medium was autoclaved at 121°C for 20 min. After germination, the seedlings were incubated under sterile conditions in the growth chamber at 25±2°C with a 14 h photoperiod. After 30 d, seedlings were divided into two groups. One was for callus induction, the other was harvested

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Physicochemical effects on sulfite transformation in a lipid-rich *Chlorella* sp. strain*

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Abstract SO_2 is very rapidly hydrated to sulfurous acid in water solution at pH value above 6.0, whereby sulfite is yielded from the disassociation of protons. We aimed to improve the sulfite transformation efficiency and provide a basis for the direct utilization of SO_2 from flue gas by a microalgal suspension. *Chlorella* sp. XQ-20044 was cultured in a medium with 20 mmol/L sodium sulfite under different physicochemical conditions. Under light conditions, sulfite concentration in the algal suspension reduced linearly over time, and was completely converted into sulfate within 8 h. The highest sulfite transformation rate (3.25 mmol/(L·h)) was obtained under the following conditions: 35°C, light intensity of 300 μmol/(m^2·s), $NaHCO_3$ concentration of 6 g/L, initial cell density (OD_{540}) of 0.8 and pH of 9–10. There was a positive correlation between sulfite transformation rate and the growth of *Chlorella*, with the conditions favorable to algal growth giving better sulfite transformation. Although oxygen in the air plays a role in the transformation of SO_3^{2-} to SO_4^{2-}, the transformation is mainly dependent on the metabolic activity of algal cells. *Chlorella* sp. XQ-20044 is capable of tolerating high sulfite concentration, and can utilize sulfite as the sole sulfur source for maintaining healthy growth. We found that sulfite ≤20 mmol/L had no obvious effect on the total lipid content and fatty acid profiles of the algae. Thus, the results suggest it is feasible to use flue gas for the mass production of feedstock for biodiesel using *Chlorella* sp. XQ-20044, without preliminary removal of SO_2, assuming there is adequate control of the pH.

Keyword: *Chlorella*; sulfite transformation; sulfur dioxide; flue gas

1 INTRODUCTION

Global climate change, which has resulted from extensive CO_2 emissions into the atmosphere from human activities, has become an increasingly important environmental issue. Among CO_2 mitigation strategies, biological sequestration using photosynthetic microalgae has received considerable attention. Such microalgae have a high CO_2 fixation ability (Yoo et al., 2010) and are able to capture and utilize CO_2 to create substantial biomass that can be converted into biofuels, chemicals and health foods. These abilities make them the only source of renewable biodiesel that is capable of meeting the global demand for transport fuel (Chisti, 2007). Moreover, these microalgae can yield three to five times more biomass per land area than land-based plants (Zeiler, 1995). Several studies have examined CO_2 removal from flue gas using microalgae (Brown, 1996; Olaizola, 2003).

Flue gas contains not only large amounts of CO_2 but also 10^{-4}–3×10^{-4} SO_2 and NO_X, which can both cause harmful acid rain when emitted into the atmosphere. A major problem in the microalgal fixation of CO_2 from flue gas is the toxic oxides of

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Phylogenetic analyses provide the first insights into the evolution of OVATE family proteins in land plants

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• *Background and Aims* The *OVATE* gene encodes a nuclear-localized regulatory protein belonging to a distinct family of plant-specific proteins known as the OVATE family proteins (OFPs). *OVATE* was first identified as a key regulator of fruit shape in tomato, with nonsense mutants displaying pear-shaped fruits. However, the role of OFPs in plant development has been poorly characterized.
• *Methods* Public databases were searched and a total of 265 putative OVATE protein sequences were identified from 13 sequenced plant genomes that represent the major evolutionary lineages of land plants. A phylogenetic analysis was conducted based on the alignment of the conserved OVATE domain from these 13 selected plant genomes. The expression patterns of tomato *SlOFP* genes were analysed via quantitative real-time PCR. The pattern of *OVATE* gene duplication resulting in the expansion of the gene family was determined in arabidopsis, rice and tomato.
• *Key Results* Genes for OFPs were found to be present in all the sampled land plant genomes, including the early-diverged lineages, mosses and lycophytes. Phylogenetic analysis based on the amino acid sequences of the conserved OVATE domain defined 11 sub-groups of OFPs in angiosperms. Different evolutionary mechanisms are proposed for *OVATE* family evolution, namely conserved evolution and divergent expansion. Characterization of the *AtOFP* family in arabidopsis, the *OsOFP* family in rice and the *SlOFP* family in tomato provided further details regarding the evolutionary framework and revealed a major contribution of tandem and segmental duplications towards expansion of the *OVATE* gene family.
• *Conclusions* This first genome-wide survey on OFPs provides new insights into the evolution of the OVATE protein family and establishes a solid base for future functional genomics studies on this important but poorly characterized regulatory protein family in plants.

Key words: OVATE family proteins, OFP, land plants, angiosperm, phylogenetic analyses, *Arabidopsis thaliana*, *Oryza sativa*, *Solanum lycopersicum*, segmental duplication, tandem duplication.

INTRODUCTION

The *OVATE* gene was first identified as an important regulator of fruit shape in tomato, in which a naturally occurring premature stop codon in *OVATE* results in pear-shaped fruit with longitudinal elongation and neck constriction (Liu *et al*., 2002). This revealed a previously uncharacterized class of regulatory genes in plant development, which encode proteins with a conserved 70 amino acid C-terminal domain. This domain was designated as the OVATE domain, also known as DUF623 (Domain of Unknown Function 623), and proteins containing this domain were designated OVATE family proteins (OFPs), which are found exclusively in plants (Hackbusch *et al*., 2005; Wang *et al*., 2007, 2011).

To date, OFPs have been primarily characterized in arabidopsis (AtOFPs) and demonstrated to regulate plant growth and development (Hackbusch *et al*., 2005; Pagnussat *et al*., 2007; Wang *et al*., 2007, 2010; Li *et al*., 2011). AtOFPs were shown to have close functional interactions with three amino acid loop extension (TALE) homeodomain proteins, and AtOFP1 and AtOFP5 regulate the sub-cellular localization of TALE homeoproteins (Hackbusch *et al*., 2005). The plant TALE proteins are a conserved superclass of homeodomain proteins characterized by an extension of three amino acids between helices 1 and 2 of the homeodomain (Bertolino *et al*., 1995), and comprise two sub-classes called the KNOTTED-like homeobox (KNOX) and BEL1-like homeodomain (BELL) proteins (Hay and Tsiantis, 2009, 2010; Hamant and Pautot, 2010). Furthermore, it has been well documented that interactions between KNOX and BELL proteins result in heterodimers regulating plant development in a connected and complex network (Bellaoui *et al*., 2001; Smith *et al*., 2002; Smith and Hake, 2003; Chen *et al*., 2004; Hackbusch *et al*., 2005; Cole *et al*., 2006). AtOFP1 has been reported to function as an active transcriptional repressor of *AtGA20ox1* in the gibberellin (GA) biosynthesis pathway, suppressing cell elongation (Wang *et al*., 2007). A recent study also indicated that AtOFP1 interacts with AtKu70, a protein involved in DNA repair through the non-homologous

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Differential proteomic analysis of grapevine leaves by iTRAQ reveals responses to heat stress and subsequent recovery

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Abstract

Background: High temperature is a major environmental factor limiting grape yield and affecting berry quality. Thermotolerance includes the direct response to heat stress and the ability to recover from heat stress. To better understand the mechanism of the thermotolerance of *Vitis*, we combined a physiological analysis with iTRAQ-based proteomics of *Vitis vinifera* cv Cabernet Sauvignon, subjected to 43°C for 6 h, and then followed by recovery at 25/18°C.

Results: High temperature increased the concentrations of TBARS and inhibited electronic transport in photosynthesis apparatus, indicating that grape leaves were damaged by heat stress. However, these physiological changes rapidly returned to control levels during the subsequent recovery phase from heat stress. One hundred and seventy-four proteins were differentially expressed under heat stress and/or during the recovery phase, in comparison to unstressed controls, respectively. Stress and recovery conditions shared 42 proteins, while 113 and 103 proteins were respectively identified under heat stress and recovery conditions alone. Based on MapMan ontology, functional categories for these dysregulated proteins included mainly photosynthesis (about 20%), proteins (13%), and stress (8%). The subcellular localization using TargetP showed most proteins were located in the chloroplasts (34%), secretory pathways (8%) and mitochondrion (3%).

Conclusion: On the basis of these findings, we proposed that some proteins related to electron transport chain of photosynthesis, antioxidant enzymes, HSPs and other stress response proteins, and glycolysis may play key roles in enhancing grapevine adaptation to and recovery capacity from heat stress. These results provide a better understanding of the proteins involved in, and mechanisms of thermotolerance in grapevines.

Keywords: Cabernet sauvignon, Heat stress, iTRAQ, Photosynthesis, Proteomics, Recovery

Background

Temperature is one of the pivotal factors influencing plant growth and development. Both yield and quality are reduced when the temperature is above or below optimal levels [1]. The IPCC (Intergovernmental Panel on Climate Change) forecasts that the extreme annual daily maximum temperature (i.e., return value) will likely increase by about 1-3°C by mid-twenty-first century and by about 2-5°C by the late twenty-first centry (http://www.ipcc.ch), and direct grape yield losses in the range of 2.5-16% for every 1°C increase in seasonal temperatures have been observed [2]. Therefore, a better understanding of the mechanisms involved in thermotolerance would be greatly significant and would lay the theoretical foundation for formulating the strategies of adaptation to high temperatures.

Direct injuries associated with high temperatures include protein denaturation, aggregation, and increased fluidity of membrane lipids. Indirect or slower heat injuries include inactivation of enzymes in chloroplasts and mitochondria, inhibition of protein synthesis, protein

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Comparative physiological analysis of lotus (*Nelumbo nucifera*) cultivars in response to salt stress and cloning of *NnCIPK* genes

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ABSTRACT

Saline or salt water in the ocean accounts for 96.5% of total fresh water resource in the planet. Salinity is a global social and economic problem which severely inhibited plant growth and development. Utilization of marginal salt affected land and/or water resource becomes increasingly important because of the explosion of world population and climate change. In this study, salt stress resistance of fifteen *N. nucifera* cultivars was firstly evaluated. The results showed that Welcoming Guests was the most resistant cultivar, while Hunan Lotus was the most sensitive one. Resistant cultivar Welcoming Guests accumulated significant higher amount MDA and proline than Hunan Lotus prior to salt stress treatment, indicating Welcoming Guests was pre-conditioned to salt stress. Salt sensitive lotus cultivar exhibited relative lower antioxidant enzyme activities and higher reactive oxygen species accumulation than resistant one after salt treatment. Since calcineurin B-like protein interaction protein kinase (CIPK)/SALT OVERLY SENSITIVE2 gene family played essential roles during plant salt stress response, three *NnCIPK* genes were successfully cloned in this study. Phylogenetic analysis showed that these genes were high homologous to Arabidopsis and grape *CIPK* genes. Expression level analysis indicated that *NnCIPK6* was highly induced by NaCl treatment in resistant cultivar, while expression levels of *NnCIPK14s* showed fluctuation in susceptible cultivar after salt treatment. These results partially characterized mechanisms of lotus salt stress resistance and provided useful information for utilization of lotus cultivars in salt water.

1. Introduction

During the course of their life cycle, plants encounter numerous harsh environmental conditions and have developed various resistance strategies to cope with biotic and abiotic stresses. Salinity is a globally social and economic problem. Approximately 75% of the Earth's surface is surrounded by water and the Earth is therefore referred to as the "blue planet". However, the vast bulk of the water on Earth is regarded as saline or salt water in the ocean which accounts for 96.5% of total water resource in the planet with an average salinity of 35‰ (Shiklomanov, 1993a,b). This salt solution has affected, and continues to affect, the land on which plants are, or might be, grown. The area of salt affected land is already more than 900×10^6 ha, comprising nearly 7% of the world's total land area and one-third of irrigated land, which is sufficient to pose a threat to agriculture (Flowers and Yeo, 1995; Shabala and Cuin, 2008). Salt stress involves a combination of dehydration or osmotic-related stress effects, and damage due to excess sodium ions (Hasegawa et al., 2000). Osmotic stress reduces the ability of plants to take up water and minerals. It not only reduces the growth rate in proportion to the salinity level, but also the tiller numbers in plants (Husain et al., 2003; Munns et al., 2006). Ion toxicity inhibits a variety of processes such as K^+ sorption, vital enzyme reactions, protein synthesis and photosynthesis (Hall and Flowers, 1973; Murguia et al., 1995). Secondary effects include the production of reactive oxygen species (ROS). As important signaling molecules, ROS including H_2O_2 and $O_2^{\bullet -}$ was induced immediately after stress treatment (Shi et al., 2013a,b; Wang et al., 2013a). The over-production of H_2O_2 can lead to oxidative damages by oxidizing proteins, damaging nucleic acids and causing lipid peroxidation.

Abbreviations: CAT, catalase; CIPK, calcineurin B-like (CBL) protein interaction protein kinase; H_2O_2, hydrogen peroxide; MDA, malondialdehyde; $O_2^{\bullet -}$, superoxide radical; POD, peroxidase; ROS, reactive oxygen species; SOD, superoxide dismutase; SOS, salt overly sensitive.

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Long-term water balance and sustainable production of *Miscanthus* energy crops in the Loess Plateau of China

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ABSTRACT

Sustainable production of second-generation energy crops on marginal land holds a great potential for renewable energy development. Because a vast area of marginal land is located in the arid and semiarid regions of the world, water shortage is the most serious environmental limitation. In this study, we developed a water balance model to address the question of whether *Miscanthus* energy crops can be sustainably produced in the Loess Plateau of China, a region of more than 60 million hectares particularly abundant in semiarid marginal land. The simulation of 20-year soil water content in bare soil, the winter wheat field, and the *Miscanthus* field across the Loess Plateau suggested that the long-term production of *Miscanthus* would not cause water depletion in deep soil. This finding addressed a serious concern that growing high-biomass plants in the Loess Plateau might lead to deep-soil water depletion, which was suggested to be the cause of previous failure of afforestation. Planting *Miscanthus* was effective in reducing surface runoff and consequently preventing water and soil loss in this heavily eroded region. The model and analyses illustrated where in the Loess Plateau this perennial energy crop could be produced with stable and sufficient yield.

1. Introduction

Bioenergy contributes to the global sustainability by providing a source of renewable energy and by mitigating climate change. Second-generation energy crops that are developed to grow on marginal land have the great potential to contribute to regional sustainability [1–3]. The Loess Plateau of China is such a region facing serious threat of environmental and economic problems. Long-term human disturbance, especially the overuse of the land for food crop and feedstock production, has turned the Loess Plateau into one of the most seriously eroded

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Virus-induced gene silencing in two novel functional plants, *Lycium barbarum* L. and *Lycium ruthenicum* Murr.

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ABSTRACT

Two species of Goji, *Lycium barbarum* L. and *Lycium ruthenicum* Murr., are novel functional vegetables and functional fruits widely used in China and other Asian countries. Both species possess complex secondary metabolic pathways and show high tolerance and adaptability to saline-alkali stress, making them novel targets for functional genetic analysis of the biochemical pathways involved. Although stable transgenic Goji lines have been produced, the process is very labor-intensive and time-consuming. Virus-induced gene silencing (VIGS) presents an effective and rapid alternative for creating targeted gene knock-outs to study gene function in plants. In this study, the first application of VIGS in *Lycium* species is presented, using the *Tobacco rattle virus* (TRV) vectors. A number of vector delivery methods were trialed, including leaf syringe-infiltration, agrodrench, seedling vacuum-infiltration and sprout vacuum-infiltration (SVI). Vacuum-infiltration was the most effective method and was used to successfully silence two reporter genes, *phytoene desaturase* (*PDS*) and *Mg-chelatase H subunit* (*Chl H*), concomitant with photobleaching and yellow leaf phenotypes, respectively. The proven application of VIGS to these *Lycium* sp. will expedite the functional characterization of novel genes involved in the biosynthesis of functional components both in leaves and fruits, as well as the abiotic stress tolerances.

1. Introduction

"Goji" refers to the Chinese species of genus *Lycium* (family Solanaceae). *Lycium barbarum* L. (generally called Goji berry or Wolfberry) and *Lycium ruthenicum* Murr. have been used as functional fruits and functional vegetables, as well as traditional Chinese herbs (Potterat, 2010). *L. barbarum*, in particular, is a crop of high economic importance in Northwest China, with the fruits (*Gouqizi* in Chinese) having high contents of *L. barbarum* polysaccharides (LBP), flavonoids and carotenoids that can improve eyesight, liver and kidney function (Wang et al., 2010; Yao et al., 2011). Young leaves of *L. barbarum* have also been used as functional vegetables with high contents of microelement, alkaloid, carotenoids and flavonoids (Potterat, 2010; Zhang and Yang, 2010). *L. ruthenicum* is another traditional herb used for treating heart disease, abnormal menstruation and menopause. Functional compounds in *L. ruthenicum* fruit have been recently investigated and include various pigments, essential oils and polysaccharides (Altintas et al., 2006; Li et al., 2006; Peng et al., 2012; Zheng et al., 2011). In addition to their nutritive values, *L. barbarum* and *L. ruthenicum* have high saline-alkali resistance, and can be functional specialty crops with a high economic value on harsh marginal lands (Wei et al., 2006; Zhang and Zhang, 2004).

Despite the importance of Goji in traditional Chinese herbs, its rapidly increasing popularity in the global novelty and functional foods market (Potterat, 2010), and its tolerance of abiotic stress, little information is currently available on the genetic pathways involved in the biosynthesis of secondary metabolites and

Abbreviations: VIGS, virus-induced gene silencing; TRV, *Tobacco rattle virus*; PDS, phytoene desaturase; Chl H, Mg-chelatase H subunit; SVI, sprout vacuum-infiltration; TCM, traditional Chinese medicine; LBP, *L. barbarum* polysaccharide; EST, expressed sequence tag; PTGS, post-transcriptional gene silencing; RISC, RNA-induced silencing complex; GOI, gene of interest.

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RESEARCH ARTICLE Open Access

Comparative analysis of carotenoid accumulation in two goji (*Lycium barbarum* L. and *L. ruthenicum* Murr.) fruits

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Abstract

Background: The traditional Chinese medicinal plants *Lycium barbarum* L. and *L. ruthenicum* Murr. are valued for the abundance of bioactive carotenoids and anthocyanins in their fruits, respectively. However, the cellular and molecular mechanisms contributing to their species-specific bioactive profiles remain poorly understood.

Results: In this study, the red fruit (RF) of *L. barbarum* was found to accumulate high levels of carotenoids (primarily zeaxanthin), while they were undetectable in the black fruit (BF) of *L. ruthenicum*. Cytological and gene transcriptional analyses revealed that the chromoplast differentiation that occurs in the chloroplast during fruit ripening only occurs in RF, indicating that the lack of chromoplast biogenesis in BF leads to no sink for carotenoid storage and the failure to synthesize carotenoids. Similar enzyme activities of phytoene synthase 1 (PSY1), chromoplast-specific lycopene β-cyclase (CYC-B) and β-carotene hydroxylase 2 (CRTR-B2) were observed in both *L. ruthenicum* and *L. barbarum*, suggesting that the undetectable carotenoid levels in BF were not due to the inactivation of carotenoid biosynthetic enzymes. The transcript levels of the carotenoid biosynthetic genes, particularly *PSY1*, *phytoene desaturase* (*PDS*), *ζ-carotene desaturase* (*ZDS*), *CYC-B* and *CRTR-B2*, were greatly increased during RF ripening, indicating increased zeaxanthin biosynthesis. Additionally, *carotenoid cleavage dioxygenase* 4 (*CCD4*) was expressed at much higher levels in BF than in RF, suggesting continuous carotenoid degradation in BF.

Conclusions: The failure of the chromoplast development in BF causes low carotenoid biosynthesis levels and continuous carotenoid degradation, which ultimately leads to undetectable carotenoid levels in ripe BF. In contrast, the successful chromoplast biogenesis in RF furnishes the sink necessary for carotenoid storage. Based on this observation, the abundant zeaxanthin accumulation in RF is primarily determined via both the large carotenoid biosynthesis levels and the lack of carotenoid degradation, which are regulated at the transcriptional level.

Keywords: Carotenoids, Chromoplast, Fruit development, Gene expression, *Lycium barbarum*, *L. ruthenicum*

Background

Carotenoids are isoprenoids that are synthesized by all photosynthetic organisms as well as some non-photosynthetic bacteria and fungi. In plants, chloroplastic carotenoids are constituents of light-harvesting complexes and the photosynthetic reaction center, where they also play important roles in protecting tissues against photo-oxidative damage [1,2]. When accumulated in the chromoplasts of flowers and fruits, carotenoids act as visual attractants for pollinating insects and seed-dispersing animals [3,4]. Furthermore, carotenoids are the precursors of important apocarotenoids, such as volatile flavor/aroma terpenes, and the growth regulators abscisic acid (ABA) and strigolactone [5-7]. Recently, oxidized products from plant carotenoids have been implicated as signals induced by environmental stressors [8]. In addition to these biological functions, carotenoids serve as major micronutrients in the human diet [9,10]. In particular, β-carotene, α-carotene and β-cryptoxanthin are precursors for vitamin

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REVIEW

Chemical proteomic strategies for the discovery and development of anticancer drugs

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Cancer is one of the leading causes of death globally. Drug discovery and development against cancer is thus among the most pursuing goals nowadays. Although the majority of anticancer drugs targeted on proteins, the identification and validation of drug targets and their regulated pathways remain a bottleneck in the drug R&D processes. Fortunately, chemical proteomic strategies based on the perfect combination of various targeted affinity chromatography and high-throughput MS analysis have emerged as a powerful tool for the large-scale identification of proteome-wide drug–protein interactions, and demonstrated great promise in elucidating complex underlying mechanisms of drug action against cancers. In this context, an updated overview of the chemical proteomic strategies, such as activity-based protein profiling (ABPP), compound-centric chemical proteomics (CCCP), and other targeted affinity chromatographic approaches for modern anticancer drug discovery and development will be provided. Some most recent successful applications in this area will be highlighted. Future perspectives on this subject will also be discussed with a particular emphasis on small molecule natural products and their derivatives.

Keywords:
Activity-based protein profiling / Affinity chromatography / Anticancer / Biomedicine / Chemical proteomics / MS

1 Introduction

Due to the rapid population growth and aging, as well as lifestyle and environmental influences, cancer has become one of the leading causes of deaths worldwide. Cancer brought about 7.6 million deaths (around 13% of all deaths) in 2008, and this number was projected to continue rising, with an estimated 13.1 million deaths in 2030 based on the GLOBOCAN 2008 statistics [1]. Since cytotoxic drugs (e.g., Taxol) and molecular targeted anticancer therapies (e.g., Gleevec) often caused deleterious side effects or drug-resistance and coupled with high cost, the effective treatment for most cancer remains an extremely difficult task [2–4]. Therefore, there is an urgent need to develop novel anticancer drugs with higher effectiveness and lower toxicity.

It is widely accepted that the majority of anticancer drugs target on proteins [5], however, the identification and validation of drug targets and their regulated pathways remain a bottleneck in the drug R&D processes. On one hand, the medical community and pharmaceutical companies are facing a dire need for the discovery of new drug targets. Although currently developed drugs are based on no more than 500 molecular targets [6], it was estimated that there are actually about 3000–10 000 druggable targets for the treatment of a wide spectrum of human diseases [7, 8]. In fact, there are

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Abbreviations: ABPP, activity-based protein profiling; **ABP**, activity-based probe; **APL**, acute promyelocytic leukemia; **CCCP**, compound-centric chemical proteomics; **CK2**, casein kinase 2; **CML**, chronic myeloid leukemia; **DARTS**, drug affinity responsive target stability; **FP**, fluorophosphonate; **HAA**, hardwickiic acid; **MoA**, modes/mechanisms of action; **MudPIT**, multidimensional protein identification technology; **PML**, promyelocytic leukemia protein; **PML-RARα**, promyelocytic leukemia-retinoic acid receptor α; **SHs**, serine hydrolases; **TEV**, tobacco etch virus; **TOP**, tandem orthogonal proteolysis

Colour Online: See the article online to view Figs. 1–3 in colour.

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Guanacastane-type diterpenoids from *Coprinus plicatilis*

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ABSTRACT

Four new guanacastane-type diterpenoids (**1**–**4**), named plicatilisins E-H, together with the known compound, plicatilisin D (**5**), were isloated from two fermentation extracts (i.e., solid state fermentation on PDA medium & static fermentation of malt extract broth culture medium) of the fungal strain *Coprinus plicatilis* 82. Their structures were elucidated on the basis of extensive spectroscopic analysis, including 1D and 2D NMR as well as FT-ICR-MS, UV, and IR, and comparison with literature data. All the compounds showed insignificant cytotoxicities against the cancer cell line HCT116.

1. Introduction

Fungi belonging to the genus *Coprinus* are recognized as prolific producers of bioactive terpenoids (Schüffler and Anke, 2009). Since 1970s, a number of sesquiterpenoids with anti-bacterial activities such as the lagopodins A-B (Bottom and Siehr, 1975; Bu'Lock and Darbyshire, 1976), coprinol (Johansson et al., 2001), coprinolone (Starratt et al., 1989), illudins (Del Val et al., 2003; Reina et al., 2004), coprinastatins (Pettit et al., 2010) and so on, were isolated from the *Coprinus* species. In the course of our search for potential diverse secondary metabolites from the macro-fungi sources (Li and Shen, 2010; Zheng and Shen, 2009; Zheng et al., 2008), a series of new di- and tri-terpenoids were identified from *Coprinus* sp. (strain *C. radians M65*, *C. plicatilis 82*, and *C. cinereus 120*) which collected from Fujian and Yunnan province in China (Liu et al., 2012a,b; Ou et al., 2012). Notably, of these terpenoids, the guanacastane-type diterpene lactone—plicatilisin A displayed strong cytotoxicities toward a series of tumor cell lines (Liu et al., 2012a). In order to explore more guanacastane-type diterpenoids with anti-tumor activities from the strain *C. plicatilis 82*, we decided to change its culture medium and the fermentation method (i.e., solid state fermentation on PDA medium & static fermentation of malt extract broth culture medium in this experiment). Based on the "OSMAC (One Strain-Many Compounds)" theory, a single strain can produce many novel compounds if culture conditions slightly changed (Bode et al., 2002). Interestingly, the further investigation of secondary metabolites from this strain led to the discovery of four new guanacastane-type diterpenoids, named plicatilisins E-H, (**1**–**4**) (Fig. 1), and one known compound—plicatilisin D (**5**). Here we report the isolation, structure elucidation and anti-tumor activity evaluation of these new natural products.

2. Results and discussion

Compound **1** was obtained as a yellow oil, and the molecular formula was determined to be $C_{20}H_{28}O_4$ via the FT-ICR-MS data (*m/z* 355.1888 for $[M+Na]^+$, *calcd*. 355.1885). The IR absorptions at 3388, 1743, 1710 and 1653 cm^{-1} indicated the presence of OH and C=O groups, respectively. Comparison of the ^1H and ^{13}C NMR data suggested that **1** is structurally similar to radianspene C (Ou et al., 2012), except for the presence of one more double bond between C-6 (δ_C 125.6) and C-7 (δ_C 158.2) (Table 1). These were demonstrated by the clear ^1H–^1H COSY correlations between H-6 (δ_H 6.15) and H-7 (δ_H 6.88). In HMBC experiment on compound **1**, H-6 signal gave cross peaks with C-4 (δ_C 133.7) and C-8 (δ_C 41.4), the H-7 with C-3 (δ_C 159.0) and C-5 (δ_C 186.0), and also Me-16 (δ_H 1.32) showed correlation with C-7 (δ_C 158.2). Besides, the coupling constant between H-6 and H-7 (J = 10.1 Hz) indicated that the double bond should be *cis*-configured. Therefore, the cyclohexa-2,5-dienone moiety was established and the upfield shift of C-5 (at δ_C 199.8 in Radianspene C) could be thereby explainable. Then the structure of **1** was determined, and named as plicatilisin E.

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ORIGINAL PAPER

Inactivation of a Gene Encoding Carotenoid Cleavage Dioxygenase (CCD4) Leads to Carotenoid-Based Yellow Coloration of Fruit Flesh and Leaf Midvein in Peach

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Abstract Yellow fruit flesh color, resulting from the accumulation of carotenoids, is one of the most important commercial traits of peach. Yellow flesh is controlled by a single locus (*Y*), with white flesh dominant over yellow flesh. In this study, the *Y* locus was narrowed to a 2.6-cM interval flanked by two markers, SSRy and W2691. SSRy, which is located on the first exon of a gene encoding carotenoid cleavage dioxygenase (CCD4), was cosegregated with the *Y* locus in two peach F_1 populations. RNA-Seq and qRT-PCR analysis revealed transcript level of *CCD4* was consistent with carotenoid degradation in peach fruits. All these results suggest that *CCD4* is responsible for white and yellow coloration of peach fruit flesh. In fruits of white-fleshed peach, carotenoids are synthesized but subsequently degraded into colorless compounds, leading to the formation of white color. CCD4 is likely to utilize β-carotene as the substrate in peach. Interestingly, *CCD4* also controls white and yellow coloration of leaf midveins of peach. Moreover, *LCYE* was highly expressed in peach leaves, whereas its transcript was not detectable in fruits. This suggests the difference of carotenoid biosynthesis between peach fruits and leaves. Our study not only shows for the first time the pleiotropic effects of *CCD4* gene in peach but also provides a morphological marker for easy selection of new peach cultivars with desirable white or yellow flesh colors.

Keywords Peach · Carotenoid · Leaf vein coloration · Yellow fruit flesh

Introduction

Carotenoids are tetraterpenoid organic pigments that are naturally synthesized in chloroplasts and chromoplasts of plants. In chloroplasts, carotenoids play indispensable roles in photosynthesis, whereas in chromoplasts, they are recognized as secondary metabolites (Hirschberg 2001). Carotenoids are essential to both plants and humans. First, carotenoids provide flowers and fruits with distinct colors, ranging from yellow to orange or red, to attract insects and animals for pollination as well as seed dispersal. Second, carotenoids have multiple benefits to human health. For example, carotenoids act as antioxidants by oxidizing the superoxide radical anion, and may thus reduce the risk of certain cancers (Fraser and Bramley 2004). Some carotenoids can also serve as precursors to vitamin A, which is required for healthy skin and mucus membranes, and for night vision. Hence, carotenoid biosynthesis is becoming a hot topic worldwide in recent years.

The carotenoid metabolism pathway has been well established in higher plants (Isaacson 2002; Park et al. 2002; Schwartz et al. 2003; Li et al. 2007; Maass et al. 2009; Chen et al. 2010). Briefly, the biosynthesis pathway of carotenoids begins with the condensation of geranylgeranyl diphosphate, leading to the formation of phytoene (Fig. 1), and this reaction is catalyzed by the enzyme phytoene synthase (PSY). Phytoene

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Carbon sequestration by *Miscanthus* energy crops plantations in a broad range semi-arid marginal land in China

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ABSTRACT

Carbon sequestration is an essential ecosystem service that second-generation energy crops can provide. To evaluate the ability of carbon sequestration of *Miscanthus* energy crops in the Loess Plateau of China, the yield and soil organic carbon (SOC) changes were measured for three *Miscanthus* species in the experimental field in Qingyang of the Gansu Province (QG). With the highest yield of the three species, *Miscanthus lutarioriparius* contributed to the largest increase of SOC, 0.57 t ha^{-1} yr^{-1}, comparing to the field left unplanted. Through modeling *M. lutarioriparius* yield across the Loess Plateau, an average increase of SOC was estimated at 0.46 t ha^{-1} yr^{-1} for the entire region. Based on the measurements of SOC mineralization under various temperatures and moistures for soil samples taken from QG, a model was developed for estimating SOC mineralization rates across the Loess Plateau and resulted in an average of 1.11 t ha^{-1} yr^{-1}. Combining the estimates from these models, the average of net carbon sequestration was calculated at a rate of 9.13 t ha^{-1} yr^{-1} in the Loess Plateau. These results suggested that the domestication and production of *M. lutarioriparius* hold a great potential for carbon sequestration and soil restoration in this heavily eroded region.

Abbreviations: SOC, soil organic carbon; GHG, greenhouse gas; GGP, Grain for Green Project; NCS, net carbon sequestration; WFPS, water filled pore space; TC, total carbon; IC, inorganic carbon; GLMM, generalized linear mixed model; ANOVA, analysis of variance.

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ORIGINAL PAPER

Identification of Quantitative Trait Loci (QTLs) for Fruit Quality Traits in Apple

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Abstract A segregating mapping population of "Co-op 17" × "Co-op 16" was used to identify quantitative trait loci (QTLs) associated with various fruit quality traits in apple. Phenotypic data were collected over 2 years for fruit circumference (in centimeter), diameter at midpoint (in centimeter), length (in centimeter), weight (in gram), total soluble solids (in degree Brix), and total titratable acids (in percent) for the segregating population. The phenotypic data along with a previously constructed genetic map, based on simple sequence repeat markers derived from expressed sequence tag and bacterial artificial chromosome end sequence databases, were used in marker–trait association analysis. Interval mapping identified two QTLs linked to fruit size components on linkage groups 03 and 05 with limit of detection scores of 3.27–4.06 and 3.29–4.02 along with phenotypic variation accounting for 15.4–46.4 and 18.3–21.9 %, respectively.

Keywords *Malus* × *domestica* Borkh · Microsatellite markers · Simple sequence repeats (SSRs) · Quantitative trait loci (QTLs) · Fruit quality traits

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Introduction

Screening of seedlings for desirable traits at an early stage of development is important for the efficiency of apple breeding programs. However, due to high levels of heterozygosity present in apple, it is difficult to predict phenotypes of resulting progeny from controlled crosses. Most traits important to apple growers and consumers, including disease resistance and fruit quality, are complex and quantitatively controlled (Kenis et al. 2008). Fruit quality traits, e.g., size, sweetness, and crispiness, are important indicators for marketability of apples; however, breeding and/or selecting for these traits is rather difficult and time-consuming. Thus, identifying quantitative trait loci (QTLs) for fruit quality traits as well as markers linked to these traits is critical for designing marker-assisted breeding (MAB) strategies (Longhi et al. 2012).

Molecular markers have been used extensively in apple breeding and genetic studies. Several linkage maps have been constructed for specific apple crosses to enhance efficiency of apple breeding programs (Liebhard et al. 2003b; Kenis and Keulemans 2005; Silfverberg-Dilworth et al. 2006; Han et al. 2011). Liebhard et al. (2003b) used molecular markers to construct a linkage map consisting of 17 linkage groups from the cross of "Fiesta" × "Discovery" for the purpose of aligning apple linkage maps. Linkage maps can be useful in identifying QTLs for specific crosses and can be used for studying various traits including disease resistance, fruit quality, and physiological characteristics, among others. Kenis et al. (2008) identified QTLs for fruit quality traits using the linkage map of Liebhard et al. (2003b). Use of the same linkage map for studying different traits within the same population demonstrated the inherent value of such linkage maps and the markers aligned along these linkage groups.

Recently, an integrated physical and genetic map for apple has been constructed using simple sequence repeat markers derived from expressed sequence tag and bacterial artificial

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Improvement of plant abiotic stress tolerance through modulation of the polyamine pathway

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Minireview

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Abstract Polyamines (mainly putrescine (Put), spermidine (Spd), and spermine (Spm)) have been widely found in a range of physiological processes and in almost all diverse environmental stresses. In various plant species, abiotic stresses modulated the accumulation of polyamines and related gene expression. Studies using loss-of-function mutants and transgenic overexpression plants modulating polyamine metabolic pathways confirmed protective roles of polyamines during plant abiotic stress responses, and indicated the possibility to improve plant tolerance through genetic manipulation of the polyamine pathway. Additionally, putative mechanisms of polyamines involved in plant abiotic stress tolerance were thoroughly discussed and crosstalks among polyamine, abscisic acid, and nitric oxide in plant responses to abiotic stress were emphasized. Special attention was paid to the interaction between polyamine and reactive oxygen species, ion channels, amino acid and carbon metabolism, and other adaptive responses. Further studies are needed to elucidate the polyamine signaling pathway, especially polyamine-regulated downstream targets and the connections between polyamines and other stress responsive molecules.

Keywords: Abiotic stress; crosstalk; polyamine; polyamine metabolic pathway; transgenic plants
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INTRODUCTION

Polyamines, including putrescine (Put), spermidine (Spd), and spermine (Spm), are low molecular weight natural compounds with aliphatic nitrogen structure, and exist in almost all organisms from bacteria to animals and plants (Hussain et al. 2011). For plant growth and development, polyamines are widely implicated in cell division and differentiation, root elongation, floral development, fruit ripening, leaf senescence, programmed cell death, DNA synthesis, gene transcription, protein translation, and chromatin organization (Su et al. 2006; Alcázar et al. 2011; Feng et al. 2011; Wimalasekera et al. 2011a; Zhang et al. 2011; Alet et al. 2012; Tavladoraki et al. 2012). Additionally, polyamines also play important roles in almost all diverse environmental stresses, including salt, drought, low and high temperature, wounding, ozone, flooding, heavy metals (Cu, Cr, Fe, and Ni), acid, and oxidative stresses (Liu et al. 2005; Cheng et al. 2009; Wimalasekera et al. 2011a; Tavladoraki et al. 2012). On one hand, polyamine biosynthetic and metabolic pathways, as well as polyamine levels, are modulated by multiple abiotic stresses. Polyamines share common substrates with nitric oxide (NO), ethylene, and proline as well as N metabolism, so finding the link between polyamines and abiotic stress is complicated (Shi et al. 2013a, 2013b). On the other hand, exogenous application of polyamines and transgenic plants with overproduced polyamines through modulating polyamine metabolic pathways shows protective roles of polyamines under abiotic stress conditions, while reduced *in vivo* polyamine levels result in decreased stress tolerance (Alcázar et al. 2011; Alet et al. 2012). Therefore, polyamines are potentially ideal targets for future genetic engineering technology to improve abiotic stress tolerance in plants.

In this review, changes of polyamine biosynthetic and metabolic pathways in response to abiotic stress in different plant species were discussed. Improvement of abiotic stress tolerance in various plant species through modulation of polyamine metabolic pathways and exogenous polyamine application were also included. Finally, the potential mechanisms of polyamines involved in plant responses to abiotic stress were further summarized.

POLYAMINE BIOSYNTHETIC AND METABOLIC PATHWAYS IN PLANTS

The polyamine biosynthetic and metabolic pathways in plants have been thoroughly dissected (Wimalasekera et al. 2011a;

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Molecular, Biological, Physiological and Clinical Aspects of Melatonin

The cysteine2/histidine2-type transcription factor *ZINC FINGER OF ARABIDOPSIS THALIANA 6*-activated *C-REPEAT-BINDING FACTOR* pathway is essential for melatonin-mediated freezing stress resistance in *Arabidopsis*

Abstract: Melatonin (*N*-acetyl-5-methoxytryptamine) is not only a widely known animal hormone, but also an important regulator in plant development and multiple abiotic stress responses. Recently, it has been revealed that melatonin alleviated cold stress through mediating several cold-related genes, including *C-REPEAT-BINDING FACTORs* (*CBFs*)/*Drought Response Element Binding factors* (*DREBs*), *COR15a*, and three transcription factors (*CAMTA1*, *ZINC FINGER OF ARABIDOPSIS THALIANA 10* (*ZAT10*), and *ZAT12*). In this study, we quantified the endogenous melatonin level in *Arabidopsis* plant leaves and found the endogenous melatonin levels were significantly induced by cold stress (4°C) treatment. In addition, we found one cysteine2/histidine2-type zinc finger transcription factor, ZAT6, was involved in melatonin-mediated freezing stress response in *Arabidopsis*. Interestingly, exogenous melatonin enhanced freezing stress resistance was largely alleviated in *AtZAT6* knockdown plants, but was enhanced in *AtZAT6* overexpressing plants. Moreover, the expression levels of *AtZAT6* and *AtCBFs* were commonly upregulated by cold stress (4°C) and exogenous melatonin treatments, and modulation of *AtZAT6* expression significantly affected the induction *AtCBFs* transcripts by cold stress (4°C) and exogenous melatonin treatments. Taken together, AtZAT6-activated *CBF* pathway might be essential for melatonin-mediated freezing stress response in *Arabidopsis*.

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Key words: *Arabidopsis*, cold stress, *C-REPEAT-BINDING FACTOR*, melatonin, *ZINC FINGER OF ARABIDOPSIS THALIANA 6*, zinc finger protein

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Introduction

Cold stress, including chilling stress (<20°C) and freezing stress (<0°C), is a serious environmental stress that adversely limits plant growth and impacts the world's agriculture [1–7]. To date, there are at least three efficient approaches for improvement of plant cold stress resistance. One approach is screening cold tolerant varieties from multiple plant varieties because of naturally occurring genetic variation [8]; the second approach is exogenous application of chemicals such as abscisic acid, cytokinin, ethylene, jasmonate, calcium, hydrogen sulfide (H_2S), etc [9–12]; and the third approach is genetic engineering via modulating some core genes' expression such as *C-REPEAT-BINDING FACTORs* (*CBFs*)/*Drought Response Element Binding factors* (*DREBs*) [1–7, 11, 12].

In *Arabidopsis*, *CBFs*/*DREBs* pathway is widely known to play essential roles in cold stress response [1–7]. Briefly, inducer of CBF expression (ICE1/2), encoding basic helix-loop-helix (bHLH) transcription factors, directly binds to CANNTG box in the promoter of CBF/DREBs which interact with C-repeat/dehydration responsive element (DRE) of downstream cold-regulated (CORs) and regulate cold stress response [1–7]. Moreover, ICE1 is negatively regulated by the ubiquitin E3 ligase high expression of osmotically responsive genes 1 (HOS1) and is positively regulated by the small ubiquitin-related modifier (SUMO) E3 ligase SIZ1 (SAP and Miz) at the post-translational level [1–7].

Melatonin (*N*-acetyl-5-methoxytryptamine) was initially identified as an important animal hormone that is involved in circadian rhythms, mood, sleep, retina physiology, temperature homeostasis, antioxidative activity, sexual behavior, and immunologic enhancement [13–15]. In last two decades, melatonin has been commonly examined in leaves, roots, stems, fruits, and seeds of various plant species including popular beverage (beer, coffee, tea, and wine), crops (rice, wheat, barley, grape, corn, tobacco, and oats), as well as *Arabidopsis* [16–28]. In addition, the modulation of melatonin in plants has been evidenced in flowering, circadian rhythms, photosynthesis [21], root system architecture [29,30], senescence [31–34], and in response to stress treatments such as cold stress [35–40], heat stress [41], drought stress osmotic stress [42], drought stress [43], salt stress [44], and pathogen infection [45].

To date, at least seven reports indicated the in vivo role of melatonin in alleviated cold stress in plants [35–40]. Recently, Bajwa et al. [40] found that melatonin enhanced cold tolerance in *Arabidopsis* and upregulated the expression of cold-inducible transcriptional activators including

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Research article

Modulation of auxin content in Arabidopsis confers improved drought stress resistance

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ABSTRACT

Auxin is a well-known plant phytohormone that is involved in multiple plant growth processes and stress responses. In this study, auxin response was significantly modulated under drought stress condition. The *iaaM-OX* transgenic lines with higher endogenous indole-3-acetic acid (IAA) level and IAA pre-treated wild type (WT) plants exhibited enhanced drought stress resistance, while the *yuc1yuc2yuc6* triple mutants with lower endogenous IAA level showed decreased stress resistance in comparison to non-treated WT plants. Additionally, endogenous and exogenous auxin positively modulated the expression levels of multiple abiotic stress-related genes (*RAB18*, *RD22*, *RD29A*, *RD29B*, *DREB2A*, and *DREB2B*), and positively affected reactive oxygen species (ROS) metabolism and underlying antioxidant enzyme activities. Moreover, auxin significantly modulated some carbon metabolites including amino acids, organic acids, sugars, sugar alcohols and aromatic amines. Notably, endogenous and exogenous auxin positively modulated root architecture especially the lateral root number. Taken together, this study demonstrated that auxin might participate in the positive regulation of drought stress resistance, through regulation of root architecture, ABA-responsive genes expression, ROS metabolism, and metabolic homeostasis, at least partially.

1. Introduction

Drought is one of the major environmental stresses that drastically limit crop growth and production worldwide (Harb et al., 2010; Shi et al., 2012). To respond to drought stress, plants have evolved complex biochemical and physiological strategies to allow them adapt to sudden environmental changes (Cutler et al., 2010; Harb et al., 2010; Hirayama and Shinozaki, 2010; Krasensky and Jonak, 2012; Qin et al., 2011; Shi et al., 2013a, b). Most of these adaptation responses are regulated by several secondary messengers including some plant hormones (Cutler et al., 2010; Du et al., 2013a,b; Harb et al., 2010; Hirayama and Shinozaki, 2010; Qin et al., 2011). For example, plant endogenous abscisic acid (ABA) is largely activated in response to drought stress, and the induced ABA can trigger stomatal closure and modulate the expression of downstream genes and physiological changes (Cutler et al., 2010; Harb et al., 2010; Hirayama and Shinozaki, 2010; Qin et al., 2011; Seki et al., 2007).

As a well-known plant phytohormone, auxin is an important regulator of several diverse processes including seed dormancy, tropistic growth, root patterning, cell differentiation, flower organ development (Zhao, 2010; Zhao et al., 2001). In recent years, accumulating evidence suggests the potential link between auxin response and abiotic and biotic stresses, especially the crosstalks between auxin and other plant hormones such as salicylic acid (SA) and ABA (Kazan and Manners, 2009; Wang et al., 2007). For biotic stress response, increasing evidence indicates that auxin and SA act individually or via antagonistic crosstalk, suggesting the fine balance between them may be critical for plant–pathogen interaction

Abbreviations: ABA, abscisic acid; BSA, bovine serum albumin; CAT, catalase; DW, dry weight; EL, electrolyte leakage; FW, fresh weight; GC-TOF-MS, gas chromatography time-of-flight mass spectrometry; GR, glutathione reductase; GSH, reduced glutathione; GSSG, oxidized glutathione; H_2O_2, hydrogen peroxide; IAA, indole-3-acetic acid; MS, Murashige and Skoog; MU, 4-methylumbelliferone; MUG, 4-methylumbelliferyl-β-glucuronide; $O_2^{\bullet-}$, superoxide radical; POD, peroxidase; ROS, reactive oxygen species; SA, salicylic acid; SOD, superoxide dismutase; WT, wild type.

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The Cysteine2/Histidine2-Type Transcription Factor *ZINC FINGER OF ARABIDOPSIS THALIANA6* Modulates Biotic and Abiotic Stress Responses by Activating Salicylic Acid-Related Genes and *C-REPEAT-BINDING FACTOR* Genes in Arabidopsis[1][C][W]

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The cysteine2/histidine2-type zinc finger proteins are a large family of transcription regulators, and some of them play essential roles in plant responses to biotic and abiotic stress. In this study, we found that expression of C_2H_2-type *ZINC FINGER OF ARABIDOPSIS THALIANA6* (*AtZAT6*) was transcriptionally induced by salt, dehydration, cold stress treatments, and pathogen infection, and AtZAT6 was predominantly located in the nucleus. *AtZAT6*-overexpressing plants exhibited improved resistance to pathogen infection, salt, drought, and freezing stresses, while *AtZAT6* knockdown plants showed decreased stress resistance. AtZAT6 positively modulates expression levels of stress-related genes by directly binding to the TACAAT motifs in the promoter region of pathogen-related genes (*ENHANCED DISEASE SUSCEPTIBILITY1*, *PHYTOALEXIN DEFICIENT4*, *PATHOGENESIS-RELATED GENE1* [*PR1*], *PR2*, and *PR5*) and abiotic stress-responsive genes (*C-REPEAT-BINDING FACTOR1* [*CBF1*], *CBF2*, and *CBF3*). Moreover, overexpression of *AtZAT6* exhibited pleiotrophic phenotypes with curly leaves and small-sized plant at vegetative stage and reduced size of floral organs and siliques at the reproductive stage. Modulation of *AtZAT6* also positively regulates the accumulation of salicylic acid and reactive oxygen species (hydrogen peroxide and superoxide radical). Taken together, our findings indicated that *AtZAT6* plays important roles in plant development and positively modulates biotic and abiotic stress resistance by activating the expression levels of salicylic acid-related genes and *CBF* genes.

In nature, plants live in complex environmental conditions in which various abiotic stresses and multiple microbial pathogens with different infection strategies and lifestyles influence plant growth and development (Bent and Mackey, 2007; Shi et al., 2013a, 2013b). As sessile organisms, plants cannot avoid unfavorable circumstances by adjusting their location. Therefore, they have evolved complex strategies to perceive stress signals and further translate the perception into effective plant responses (Gimenez-Ibanez and Solano, 2013; Shi et al., 2014a, 2014b).

In recent years, much attention has been paid to the roles of the hormones in biotic and abiotic stress responses and especially hormone interactions under stress conditions (Pieterse et al., 2012; Yang et al., 2012a; Liu et al., 2013c). Several plant hormone receptors are located in the nucleus, such as jasmonate and auxin, while the signaling perception of salicylic acid (SA), ethylene, and abscisic acid initiates in the cytoplasm and then translocates to the nucleus. Plant transcription factors (TFs) serve as important mediators during hormone cross talks under biotic and abiotic stress conditions (Kazan and Manners, 2009; Kieffer et al., 2010; Liu et al., 2013c). Currently, various TFs have been shown to be involved in biotic and abiotic stress responses via activating stress-responsive gene expression, such as C-repeat-binding factors (CBFs)/dehydration-responsive element-binding proteins (DREBs), WRKYs, ethylene-responsive element-binding factors, MYBs, MYCs, basic domain-Leu zipper families, and zinc finger proteins (ZFPs; Qiu et al., 2008; Yu et al., 2008; Bi et al., 2010; Li et al., 2010a, 2010b; Seo and Park, 2010; Zhang et al., 2010a, 2010b; Zhu et al., 2010; Cheng et al., 2011).

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[C] Some figures in this article are displayed in color online but in black and white in the print edition.

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Research article

Comparative proteomic responses of two bermudagrass (*Cynodon dactylon* (L). Pers.) varieties contrasting in drought stress resistance

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ABSTRACT

Drought (water-deficit) stress is a serious environmental problem in plant growth and cultivation. As one of widely cultivated warm-season turfgrass, bermudagrass (*Cynodon dactylon* (L). Pers.) exhibits drastic natural variation in the drought stress resistance in leaves and stems of different varieties. In this study, proteomic analysis was performed to identify drought-responsive proteins in both leaves and stems of two bermudagrass varieties contrasting in drought stress resistance, including drought sensitive variety (Yukon) and drought tolerant variety (Tifgreen). Through comparative proteomic analysis, 39 proteins with significantly changed abundance were identified, including 3 commonly increased and 2 decreased proteins by drought stress in leaves and stems of Yukon and Tifgreen varieties, 2 differentially regulated proteins in leaves and stems of two varieties after drought treatment, 23 proteins increased by drought stress in Yukon variety and constitutively expressed in Tifgreen variety, and other 3 differentially expressed proteins under control and drought stress conditions. Among them, proteins involved in photosynthesis (PS), glycolysis, N-metabolism, tricarboxylicacid (TCA) and redox pathways were largely enriched, which might be contributed to the natural variation of drought resistance between Yukon and Tifgreen varieties. These studies provide new insights to understand the molecular mechanism underlying bermudagrass response to drought stress.

1. Introduction

Drought (water-deficit) stress is one of the major environmental stresses that seriously limits plant distribution, growth and yield worldwide (Shi et al., 2012, 2013a, b; 2014; Zhao et al., 2011). With the changes of environment and global climate, drought stress not only seriously affects plant growth and development, but also breaks the ecological balance of the ecosystem (Shi et al., 2012, 2013a, b; 2014; Zhao et al., 2011).

Bermudagrass (*Cynodon dactylon* (L). Pers.) is one of wildly cultivated warm-season turfgrass on lawns, parks and sport fields, with drastic natural variation in the drought stress resistance among different varieties (Shi et al., 2012; Zhao et al., 2011). Recently, we identified three groups of bermudagrass that differed in drought resistance, including drought sensitive variety (Yukon), moderately tolerant variety (SR9554) and drought tolerant variety (Tifgreen) (Shi et al., 2012). Comparative physiological analysis among these varieties suggested that changes of water status, osmolyte accumulation and antioxidant defense system during drought stress might contribute to the natural variation of bermudagrass drought resistance (Shi et al., 2012). However, the molecular mechanisms underlying bermudagrass response to drought stress remain largely unknown. Recent studies in plant proteome have made it powerful and effective approach to identify proteins

Abbreviations: 2-DE, two-dimensional electrophoresis; Asc, ascorbate; BSA, bovine serum albumin; CBB, coomassie brilliant bluered; CHAPS, 3-[(3-cholamidopropyl)-dimethylammonio]-1-propane sulfonate; DHA, dehydroascorbate; DHAR, dehydroascorbate reductase; EL, electrolyte leakage; GAPC, cytosolic glyceraldehyde 3-phosphate dehydrogenase; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; GS, glutamine synthetase; H_2O_2, hydrogen peroxide; HSP, heat shock protein; IEF, isoelectric focus; IPG, immobilized pH gradient; MALDI-TOF–MS, matrix-assisted laser desorption/ionization time of flight mass spectrometry; MDH, malate dehydrogenase; NF, normalized frequency; OEC, oxygen-evolving complex; PEP, phosphoenolpyruvate; PEPC, phosphoenolpyruvate carboxylase; PLD, phospholipase D; PRK, Phosphoribulokinase; PS, photosynthesis; ROS, reactive oxygen species; RuBisCO, ribulose-1,5-bisphosphate carboxylase oxygenase; RuBP, ribulose-1,5-bisphosphate; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; SOD, superoxide dismutase; TCA, tricarboxylicacid; TFA, trifluoroacetic acid.

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Research article

Nitric oxide-activated hydrogen sulfide is essential for cadmium stress response in bermudagrass (*Cynodon dactylon* (L). Pers.)

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ABSTRACT

Nitric oxide (NO) and hydrogen sulfide (H_2S) are important gaseous molecules, serving as important secondary messengers in plant response to various biotic and abiotic stresses. However, the interaction between NO and H_2S in plant stress response was largely unclear. In this study, endogenous NO and H_2S were evidently induced by cadmium stress treatment in bermudagrass, and exogenous applications of NO donor (sodium nitroprusside, SNP) or H_2S donor (sodium hydrosulfide, NaHS) conferred improved cadmium stress tolerance. Additionally, SNP and NaHS treatments alleviated cadmium stress-triggered plant growth inhibition, cell damage and reactive oxygen species (ROS) burst, partly via modulating enzymatic and non-enzymatic antioxidants. Moreover, SNP and NaHS treatments also induced the productions of both NO and H_2S in the presence of Cd. Interestingly, combined treatments with inhibitors and scavengers of NO and H_2S under cadmium stress condition showed that NO signal could be blocked by both NO and H_2S inhibitors and scavengers, while H_2S signal was specifically blocked by H_2S inhibitors and scavengers, indicating that NO-activated H_2S was essential for cadmium stress response. Taken together, we assigned the protective roles of endogenous and exogenous NO and H_2S in bermudagrass response to cadmium stress, and speculated that NO-activated H_2S might be essential for cadmium stress response in bermudagrass.

1. Introduction

Cadmium (Cd), which is primarily generated by industrial activities and mining and highly soluble in water, can be quickly taken up by plant roots in soil, and enter into animals and humans-related food chains (Arasimowicz-Jelonek et al., 2012a; Arasimowicz-Jelonek et al., 2012b; Gallego et al., 2012; Uraguchi and Fujiwara, 2013). Cd is a non-redox heavy metal which is difficult to degradation, therefore Cd is one of the major environmental pollutants, resulting in serious toxic problems to all living organisms including human and various plant species (Arasimowicz-Jelonek et al., 2012a; Arasimowicz-Jelonek et al., 2012b; Gallego et al., 2012; Uraguchi and Fujiwara, 2013; Agami and Mohamed, 2013; Liu et al., 2013). To alleviate Cd-caused health problems in humans, exploring the Cd-induced toxicity in plants is very important, including antioxidative response, Cd transport and uptake, lipid peroxation, photosynthesis, the metabolism of nitrogen (N) and sulfur (S) (Gill et al., 2013).

As two important gaseous molecules, both nitric oxide (NO) and hydrogen sulfide (H_2S) were shown to play essential roles in various plant developmental processes and stress responses (Gupta et al., 2011; Shi et al., 2012; Shi et al., 2013d; Simontacchi et al., 2013). For plant developmental processes, both NO and H_2S are important signal messengers in seed germination and root organogenesis, while NO also functions in root elongation and formation, photomorphogenesis, floral repression, plant senescence, programmed cell death (PCD) and stomatal movement (Gupta et al., 2011; Shi et al., 2012). Additionally, endogenous NO could be rapidly and largely induced by most hormonal and stress treatments, which might be essential for stress sensing, signal transduction, and activation of adaptive stress response (Gill et al., 2013; Gupta et al., 2011;

Abbreviations: CAT, catalase; Cd, cadmium; c-PTIO, 2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxy-3-oxide; EL, electrolyte leakage; FW, fresh weight; GR, glutathione reductase; GSH, reduced glutathione; GSNO, nitrosoglutathione; GSSG, oxidized glutathione; H_2O_2, hydrogen peroxide; H_2S, hydrogen sulfide; HA, hydroxylamine; HT, hypotaurine; LCD, L-cysteine desulfhydrase; L-NAME, L-N^G-nitro arginine methylester; MDA, malondialdehyde; MS, Murashige and Skoog; N, nitrogen; NaHS, sodium hydrosulphide; Na_2S, sodium sulfide; NO, nitric oxide; NOS, nitric oxide synthase; $O_2^{\bullet-}$, superoxide radical; PCD, programmed cell death; POD, peroxidase; PP, potassium pyruvate; ROS, reactive oxygen species; S, sulfur; SNAP, S-nitroso-N-acetyl-D-penicillamine; SNP, sodium nitroprusside; SOD, superoxide dismutase; TBA, thiobarbituric acid.

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New Phytologist

AtHAP5A modulates freezing stress resistance in Arabidopsis through binding to CCAAT motif of *AtXTH21*

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Key words: Arabidopsis, CCAAT motif, chromatin immunoprecipitation (ChIP), freezing stress, HAP5A, XTH21.

Summary

- Several eukaryotic Heme-associated proteins (HAPs) have been reported to bind specifically to DNA fragments containing CCAAT-box; however, the physiological functions and direct targets of these HAP proteins in plants remain unclear.
- In this study, we showed that AtHAP5A as a transcription factor interacted with CCAAT motif *in vivo*, and *AtXTH21*, one direct target of AtHAP5A, was involved in freezing stress resistance. The *AtHAP5A* overexpressing plants were more tolerant, whereas the loss-of-function mutant of *AtHAP5A* was more sensitive to freezing stress than wild-type plants. Chromatin immunoprecipitation (ChIP) assay demonstrated that AtHAP5A could bind to five fragments that contained CCAAT motifs in the *AtXTH21* promoter.
- Similarly, the *AtXTH21* overexpressing plants exhibited improved freezing resistance, while *xth21* knockdown mutants displayed decreased freezing resistance. Notably, the modulated freezing resistance of *AtHAP5A* overexpressing plants and knockout mutant could be reversed by the *xth21* mutant and *AtXTH21* overexpressing plants, respectively, indicating that *AtHAP5A* might act upstream of *AtXTH21* in freezing stress. Additionally, modulation of *AtHAP5A* and *AtXTH21* expression had the same effects on abscisic acid (ABA) sensitivity and reactive oxygen species (ROS) metabolism.
- Taken together, these results demonstrated that AtHAP5A modulates freezing stress resistance in Arabidopsis through binding to the CCAAT motif of *AtXTH21*.

Introduction

Low temperature (cold stress), including chilling (< 20°C) and freezing (< 0°C), is a serious environmental stress that disrupts cellular homeostasis and limits plant growth (Doherty *et al.*, 2009; Guo *et al.*, 2013). To respond to seasonal variations in temperature, immotile plants have developed complex biochemical and physiological processes (Doherty *et al.*, 2009; Qin *et al.*, 2011; Guo *et al.*, 2013). A number of plant hormones and genes, and several transcription factors in particular, play pivotal roles in plant cold stress response (Xiong *et al.*, 2002; Vogel *et al.*, 2005; Yamaguchi-Shinozaki & Shinozaki, 2005; Dong *et al.*, 2006; Jeon *et al.*, 2010; Hu *et al.*, 2013). Plant transcription factors including basic domain-leucine zipper (bZIP) families, MYBs, MYCs and zinc finger proteins, not only serve as important mediators during hormone crosstalks under stress conditions, but also function in the early stress signaling transduction via directly activating or inhibiting the expression of several stress-responsive genes (Qin *et al.*, 2011; Shi *et al.*, 2012b).

In Arabidopsis, a widely known model involved in plant cold stress response is the C-repeat (CRT)/Dehydration responsive element (DRE) BINDING FACTORs (CBF/DREBs)-mediated pathway (Mäntylä *et al.*, 1995; Thomashow, 1999, 2010; Agarwal *et al.*, 2006; Dong *et al.*, 2006). Briefly, INDUCER OF CBF EXPRESSION 1/2 (ICE1/2), encoding basic helix-loop-helix (bHLH) transcription factors, directly bind to CANNTG box in the promoter of *CBF/DREBs* which interact with CRT/DRE of downstream Cold-Regulated (COR) genes and regulate cold stress response (Gilmour *et al.*, 1998; Jaglo-Ottosen *et al.*, 1998; Chinnusamy *et al.*, 2003; Zarka *et al.*, 2003; Cook *et al.*, 2004; Zhang *et al.*, 2004; Canella *et al.*, 2010). Recently, abscisic acid (ABA), cytokinin, ethylene and jasmonate (JA) were found to be involved in plant cold resistance through the *CBFs*-mediated pathway (Jeon *et al.*, 2010; Shi *et al.*, 2012b; Hu *et al.*, 2013). More recently, Guo *et al.* (2013) found that Arabidopsis *Lipid transfer protein 3* (*AtLTP3*) might act as a target of AtMYB96 to be involved in plant resistance to cold stress independent of the *CBF* pathway. Because plant cold stress response is a complex signaling pathway, many unknown mechanisms need to be further dissected, especially the *in vivo* roles of several transcription factors.

The CCAAT motif is a very common cis-element in the promoters of many eukaryotic genes (McNabb *et al.*, 1995; Edwards *et al.*, 1998; Kato, 2005; Yazawa & Kamada, 2007). Plant HAP (for histone- or haem-associated protein) complex has been widely confirmed to be required for CCAAT binding in yeast and

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RESEARCH PAPER

Constitutive production of nitric oxide leads to enhanced drought stress resistance and extensive transcriptional reprogramming in *Arabidopsis*

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Abstract

Nitric oxide (NO) is involved in plant responses to many environmental stresses. Transgenic *Arabidopsis* lines that constitutively express rat neuronal NO synthase (nNOS) were described recently. In this study, it is reported that the nNOS transgenic *Arabidopsis* plants displayed high levels of osmolytes and increased antioxidant enzyme activities. Transcriptomic analysis identified 601 or 510 genes that were differentially expressed as a consequence of drought stress or nNOS transformation, respectively. Pathway and gene ontology (GO) term enrichment analyses revealed that genes involved in photosynthesis, redox, stress, and phytohormone and secondary metabolism were greatly affected by the nNOS transgene. Several *CBF* genes and members of zinc finger gene families, which are known to regulate transcription in the stress response, were changed by the nNOS transgene. Genes regulated by both the nNOS transgene and abscisic acid (ABA) treatments were compared and identified, including those for two ABA receptors (*AtPYL4* and *AtPYL5*). Moreover, overexpression of *AtPYL4* and *AtPYL5* enhanced drought resistance, antioxidant enzyme activity, and osmolyte levels. These observations increase our understanding of the role of NO in drought stress response in *Arabidopsis*.

Key words: Abscisic acid, drought stress, *in vivo*, neuronal nitric oxide synthase, nitric oxide, physiological, PYL, transcriptomic.

Introduction

As a gaseous diatomic radical, nitric oxide (NO) is an essential endogenous signalling molecule involved in multiple physiological processes in plants, including growth, development, and response to environmental stresses (Shi *et al.*, 2012*b*, *c*). Interestingly, NO is rapidly induced by multiple hormonal and environmental stimuli, including abscisic acid (ABA) (Guo *et al.*, 2003), hydrogen peroxide (H_2O_2) (Bright *et al.*, 2006), polyamines (Tun *et al.*, 2006; Shi and Chan, 2013, 2014; Shi *et al.*, 2013*a*; Wang *et al.*, 2011), auxin (Kolbert *et al.*, 2007), salicylic acid (SA) (Zottini *et al.*, 2007), brassinosteroids (BRs) (Cui *et al.*, 2011), drought (Fan and Liu, 2012), salt (Zhao *et al.*, 2007; Corpas *et al.*, 2009), cold (Zhao *et al.*, 2009), and heat (Bouchard and Yamasaki, 2008). NO can also act as a secondary messenger in environmental stress signal transduction (Gupta *et al.*, 2011; Gill *et al.*, 2013).

Understanding the complex effects of NO in plants requires a detailed analysis of the physiological and molecular changes. In recent years, transcriptional analyses of

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Comparative proteomic and metabolomic analyses reveal mechanisms of improved cold stress tolerance in bermudagrass (*Cynodon dactylon* (L.) Pers.) by exogenous calcium

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Abstract As an important second messenger, calcium is involved in plant cold stress response, including chilling (<20 °C) and freezing (<0 °C). In this study, exogenous application of calcium chloride ($CaCl_2$) improved both chilling and freezing stress tolerances, while ethylene glycol-bis-(β-aminoethyl) ether-*N*,*N*,*N*-tetraacetic acid (EGTA) reversed $CaCl_2$ effects in bermudagrass (*Cynodon dactylon* (L.) Pers.). Pysiological analyses showed that $CaCl_2$ treatment alleviated the reactive oxygen species (ROS) burst and cell damage triggered by chilling stress, via activating antioxidant enzymes, non-enzymatic glutathione antioxidant pool, while EGTA treatment had the opposite effects. Additionally, comparative proteomic analysis identified 51 differentially expressed proteins that were enriched in redox, tricarboxylicacid cycle, glycolysis, photosynthesis, oxidative pentose phosphate pathway, and amino acid metabolisms. Consistently, 42 metabolites including amino acids, organic acids, sugars, and sugar alcohols were regulated by $CaCl_2$ treatment under control and cold stress conditions, further confirming the common modulation of $CaCl_2$ treatment in carbon metabolites and amino acid metabolism. Taken together, this study reported first evidence of the essential and protective roles of endogenous and exogenous calcium in bermudagrass response to cold stress, partially via activation of the antioxidants and modulation of several differentially expressed proteins and metabolic homeostasis in the process of cold acclimation.

Keywords: Antioxidant; bermudagrass; calcium; chilling; freezing; metabolite; proteomic
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Research Article

Free Access

INTRODUCTION

As a warm-season turfgrass, bermudagrass (*Cynodon dactylon* (L.) Pers.) is cultivated in warm climates all over the world for lawns, parks, and sport fields especially in golf courses (Shi et al. 2012c, 2013a, 2013b). Bermudagrass is tolerant to drought, salt and heat stresses, but does not grow well under chilling and freezing stress conditions (Anderson et al. 2002; Munshaw et al. 2011). Thus, improving chilling and freezing tolerances is very important for grass engineering, especially for warm-season turfgrass (Zhang et al. 2011a, 2011b; Shi and Chan 2014).

To date, screening cold tolerant varieties is an efficient approach for improvement of cold tolerance in bermudagrass (Anderson et al. 2002; Zhang et al. 2006). Another approach is exogenous application of chemicals such as abscisic acid (ABA), cytokinin, and ethephon to modulate cold tolerance in bermudagrass (Zhang et al. 2006, 2011a, 2011b; Munshaw et al. 2011). Although previous research identified several physiological and metabolic changes by cold stress in bermudagrass, including protein synthesis, chitinase and dehydrin expression, amino acid metabolism, etc. (Gatschet et al. 1994, 1996; Anderson et al. 2002; Zhang et al. 2006, 2011a, 2011b; Munshaw et al. 2011), the early signal transduction of cold stress responses in bermudagrass are largely unknown.

Low temperature (cold stress), which includes chilling (<20 °C) and freezing (<0 °C) temperatures, seriously disrupts cellular homeostasis, leads to cell damage and drastically impairs plant development and growth (Yang et al. 2006; Tester and Langridge 2010; Varshney et al. 2011; Shi et al. 2012b). As sessile organisms, plants have evolved complex mechanisms involving multiple biochemical and physiological processes to tolerate chilling and freezing stresses, such as the Inducer of Crepeat Binding Factor/Dre Binding Factor (CBF/DREB) Expression (ICE)-CBF/DREB pathway in *Arabidopsis* (Mäntylä et al. 1995; Jeon et al. 2010; Shi et al. 2012b; Hu et al. 2013). Additionally, exogenous applications of ABA, hydrogen sulfide (H_2S), cytokinin, and jasmonate (JA) modulate plant chilling and freezing tolerances (Jeon et al. 2010; Shi et al. 2012b; Hu et al. 2013). However, all these mechanisms of chilling and freezing stress responses were extensively dissected in model plants like *Arabidopsis*. The detailed changes in turfgrass especially

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PLOS ONE

Two Pear Glutathione *S*-Transferases Genes Are Regulated during Fruit Development and Involved in Response to Salicylic Acid, Auxin, and Glucose Signaling

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Abstract

Two genes encoding putative glutathione *S*-transferase proteins were isolated from pear (*Pyrus pyrifolia*) and designated *PpGST1* and *PpGST2*. The deduced PpGST1 and PpGST2 proteins contain conserved Glutathione S-transferase N-terminal domain (GST_N) and Glutathione S-transferase, C-terminal domain (GST_C). Using PCR amplification technique, the genomic clones corresponding to *PpGST1* and *PpGST2* were isolated and shown to contain two introns and a singal intron respectively with typical GT/AG boundaries defining the splice junctions. Phylogenetic analysis clearly demonstrated that PpGST1 belonged to Phi class of GST superfamilies and had high homology with apple MdGST, while PpGST2 was classified into the Tau class of GST superfamilies. The expression of *PpGST1* and *PpGST2* genes was developmentally regulated in fruit. Further study demonstrated that *PpGST1* and *PpGST2* expression was remarkably induced by glucose, salicylic acid (SA) and indole-3-aceticacid (IAA) treatments in pear fruit, and in diseased fruit. These data suggested that *PpGST1* and *PpGST2* might be involved in response to sugar, SA, and IAA signaling during fruit development of pear.

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Introduction

Glutathione *S*-transferases (GSTs; EC 2.5.1.18) are a superfamily of multifunctional enzymes that catalyze the nucleophilic conjugation of reduced tripeptide glutathione (GSH; g-Glu-Cys-Gly) into a variety of hydrophobic and electrophilic compounds to direct them to specific sites both intra- and extracellularly. GSTs protect tissues against oxidative stress or from toxic products produced during xenobiotic metabolism [1–3]. Additionally, plant GSTs are involved in development [2,4].

Plant GSTs have been mainly divided into eight classes: phi, tau, lambda, theta, zeta, EF1Bg, dehydroascorbate reductase (DHAR), and tetrachlorohydroquinone dehalogenase (TCHQD) [5–8]. Among these, phi, tau, DHAR, and lambda GSTs are specific to plants. Recently, two new GST classes, hemerythrin and iota, were identified in *Physcomitrella patens* that is a nonvascular representative of early land plants [9]. Phi and tau GSTs are the most abundant in plant and are involved mainly in xenobiotic metabolism [3,10]. However, evidence to substantiate plant development has been limited.

Plant *GST* genes form a large gene family. *GSTs* have been identified in some plants, such as tomato [11], Arabidopsis (*Arabidopsis thaliana*) [12], poplar [6], rice (*Oryza sativa*) [8], and barley [13]. The barley *SIGST* gene might play an important role during leaf senescence [13]. Moreover, plant *GST*s can be induced by a wide variety of phytohormones, including salicylic acid (SA), auxin, ethylene, methyl jasmonate, and abscisic acid (ABA) [14–17]. That all these hormones regulate many aspects of plant development implies that plant *GSTs* may play crucial roles in plant development as well. The study aims to elucidate the regulation of the pear *GST* genes during fruit ripening and senescence, under glucose, SA and auxin treatment, and disease resistance, which would provide valuable information for fruit senescence, disease resistance and sugar signaling studies in pear.

Materials and Methods

Collection of Plant Materials

Pear (*Pyrus pyrifolia* Nakai. cv. Whangkeumbae) fruit were harvested at 30, 60, 90, 120, 130, 140, 150 d after full bloom from the experimental farm of horticulture plants of Agricultural University of Hebei, China. The fruit, harvested at 150 d after full bloom that is natural harvest date, were placed for 10, 20, and 30 days at room temperature for the collection of 10, 20, and 30 d after harvest fruit respectively. The diseased fruit and the controls were chosen from the above 10 d after harvest pear fruit. The mesocarp of the pears was collected for further study. The other tissues (such as shoots, young leaves, petals, and anthers) were derived from the same pear trees of the local orchard. These samples were frozen immediately in liquid nitrogen, and then stored at –80°C for RNA isolation.

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Functional and evolutionary analysis of the *AP1/SEP/AGL6* superclade of MADS-box genes in the basal eudicot *Epimedium sagittatum*

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• *Background and Aims* MADS-box transcriptional regulators play important roles during plant development. Based on phylogenetic reconstruction, the *AP1/SEP/AGL6* superclade of floral MADS-box genes underwent one or two duplication events in the common ancestor of the core eudicots. However, the functional evolution of the *AP1/SEP/AGL6* superclade in basal eudicots remains uncharacterized. *Epimedium sagittatum* is a basal eudicot species valued for its medicinal properties and showing unique floral morphology. In this study, structural and functional variation of *FUL*-like (*AP1* subfamily), *SEP*-like and *AGL6*-like genes in this species was investigated to further our understanding of flower evolution in angiosperms. Detailed investigations into the microsynteny and evolutionary history of the floral A and E class MADS-box genes in eudicots were undertaken and used to trace their genomic rearrangements.
• *Methods* One *AP1*-like gene, two *SEP*-like genes and one *AGL6*-like gene were cloned from *E. sagittatum*. Their expression patterns were examined using quantitative RT-PCR in different vegetative and reproductive organs at two developmental stages. Yeast two-hybrid assays were carried out among *AP1/SEP/AGL6* superclade, *AP3/PI* and *AGAMOUS* subfamily members for elucidation of dimerization patterns. In addition, possible formation of a ternary complex involving B class proteins with the A class protein EsFUL-like, the E class SEP-like protein EsAGL2-1 or the AGL6-class protein EsAGL6 were detected using yeast three-hybrid assays. Transgenic *Arabidopsis* or tobacco plants expressing *EsFUL*-like, *EsAGL2-1* and *EsAGL6*-like under the cauliflower mosaic virus (CaMV) 35S promoter were generated and analysed. Genomic studies of *AP1* syntenic regions in arabidopsis, columbine, strawberry, papaya, peach, grapevine and tomato were conducted for microsyntenic analyses.
• *Key Results* Sequence and phylogenetic analyses showed that *EsFUL*-like is a member of the *AP1* (A class) subfamily, *EsAGL2-1* and *EsAGL2-2* belong to the *SEP*-like (E class) subfamily, and *EsAGL6*-like belongs to the *AGL6* (AGL6 class) subfamily. Quantitative RT-PCR analyses revealed that the transcripts of the four genes are absent, or minimal, in vegetative tissues and are most highly expressed in floral organs. Yeast two-hybrid results revealed that of the eight MADS-box proteins tested, only EsAGL6-like, EsAGL2-1 and EsAGL2 were able to form strong homo- and heterodimers, with EsAGL6-like and EsAGL2-1 showing similar interaction patterns. Yeast three-hybrid analysis revealed that EsFUL1-like, EsAGL6-like and EsAGL2-1 (representing the three major lineages of the *Epimedium* AGL/SEP/ALG6 superclade) could act as bridging proteins in ternary complexes with both EsAP3-2 (B class) and EsPI (B class), which do not heterodimerize themselves. Syntenic analyses of sequenced basal eudicots, rosids and asterids showed that most *AP1*-like and *SEP*-like genes have been tightly associated as neighbours since the origin of basal eudicots. Ectopic expression of *EsFUL*-like in arabidopsis caused early flowering through endogenous high-level expression of *AP1* and formation of secondary flowers between the first and second whorls. Tobacco plants with ectopic expression of *EsAGL2-1* showed shortened pistils and styles, as well as axillary and extra petals in the initial flower.
• *Conclusions* This study provides a description of *EsFUL*-like, *EsAGL2-1*, *EsAGL2-2* and *EsAGL6*-like function divergence and conservation in comparison with a selection of model core eudicots. The study also highlights how organization in genomic segments containing A and E class genes in sequenced model species has resulted in similar topologies of *AP1* and *SEP*-like gene trees.

Key words: Basal eudicots, AP1/SEP/AGL6 superclade, MADS-box, microsynteny analysis, evo-devo, *Epimedium sagittatum*.

INTRODUCTION

MADS-box genes, including type I and type II, encode transcription factors that control diverse developmental processes in flowering plants (Alvarez-Buylla *et al.*, 2000; Masiero *et al.*, 2011). In the four-whorled flower of *Arabidopsis*, type II MADS box genes (MIKC type) work together to specify the identity of

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REVIEW

Evaluation of genotypic variation in heat tolerance of tall fescue by functional traits

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Abstract Tall fescue is an important cool season turfgrass. Summer high temperature negatively affects the performance of tall fescue in transitional and warm climate zones. To identify heat tolerant material, 120 tall fescue accessions from different regions of the world were collected and subjected to high temperature under the greenhouse and the growth chamber conditions. Average temperature was 43 °C in the greenhouse trial. Meanwhile, in the growth chamber trial there were 38/30 °C (day/night) and 25/16 °C for heat stress and control, respectively. Leaf water content, leaf dry weight, leaf fresh weight, growth rate (GR), turf quality (TQ), survival rate (SR), chlorophyll content (Chl), evapotranspiration rate (ET) and electrolyte leakage were determined. Significant effects of accessions, duration time and heat treatment on most characteristics were observed. Wild accessions exhibited higher variations in most of the studied traits than commercial cultivars. There were differences in GR and ET with greater variation coefficients than other traits between accessions, suggesting GR and ET could be effective indices for evaluating heat tolerance of tall fescue accessions. Three principal components in growth chamber trial and two principal components in greenhouse trial were extracted. Principal component analysis indicated that common PC1, correlated with TQ, SR and Chl, was named as "turf performance component", which explained 43.17 % and 39.24 % of genetic variations in the greenhouse trial and the growth chamber trial, respectively. PC2 that defined as "growth potential component" could explain 23.26 % of total variability in the greenhouse trial and 20.36 % in the growth chamber trial. PC3 was named as "leaf water potential component". Two regression models ($F = 0.65 \times F1 + 0.35 \times F2$) and ($F = 0.54 \times F1 + 0.28 \times F2 + 0.18 \times F3$) were formulated by factor analysis to evaluate heat tolerance of tall fescue accessions in greenhouse and growth chamber trials, respectively. Accessions from subtropical monsoon climate zone generally exhibited better heat tolerance. In contrast, accessions from East Asia showed more heat sensitive. Finally, five accessions including PI 598574, PI 608787, PI 559374, Pure Gold and PI 527504 were selected as heat tolerant accessions for future tall fescue breeding.

Keywords Tall fescue · Heat stress · Functional traits · Genetic variation · Evaluating indices

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Introduction

High temperature is a main abiotic stress limiting the use of cool season turfgrass in transitional and warm climatic regions where the temperature would reaches

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ORIGINAL ARTICLE

Chloroplast phylogeography of the East Asian Arcto-Tertiary relict *Tetracentron sinense* (Trochodendraceae)

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ABSTRACT

Aim A phylogeographical study of the widespread but phylogenetically isolated East Asian endemic tree species *Tetracentron sinense* (Trochodendraceae) was performed to evaluate whether and how Pleistocene and pre-Pleistocene climate changes helped to influence current phylogeographical patterns, and to describe the current patterns of genetic diversity and their implications for conservation.

Location Southwestern and central subtropical China.

Methods Sequences of four chloroplast spacer regions were obtained from 157 individuals of *T. sinense*. A haplotype network was constructed using TCS. Genetic diversity and differentiation, spatial analysis of molecular variance (SAMOVA) and analysis of molecular variance (AMOVA) were used to test for genetic structure. BEAST was used to estimate the divergence times between haplotypes. Historical demographic expansion was tested using pairwise mismatch distribution analysis.

Results Of the 21 recovered haplotypes, three were widely distributed, but most were restricted to particular regions. Populations with high haplotype diversity were located in western Hubei, southern Sichuan and southern Chongqing. The two earliest-diverging haplotypes were found in southwestern China. The haplotype distribution of *T. sinense* demonstrated significant phylogeographical structure ($N_{ST} > G_{ST}$; $P < 0.05$). The best partitioning of genetic diversity by SAMOVA ($K = 5$) produced groups that matched the main TCS-derived clades. Two independent range expansions within SAMOVA-derived groups 2 and 3 were dated to approximately 399 and 311 ka, respectively. The time to the most recent common ancestor of all haplotypes was 9.6 (95% highest posterior density: 27.0–2.2) Ma, but most of the haplotype diversity appeared during the Quaternary.

Main conclusions The extant distribution of *T. sinense* is likely to have been shaped by both pre-Quaternary and Pleistocene climate changes. Southwestern China may have served as an important refugium for *T. sinense* throughout the Neogene, while the species also occupied multiple refugia during the late Pleistocene glacial periods. Populations of *T. sinense* were resolved into five allopatric groups, between which there is apparently no seed movement.

Keywords
China, climate change, conservation, interglacial period, Neogene, Pleistocene, range expansion, refugia.

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PLOS ONE

Identification of Novel Knockout Targets for Improving Terpenoids Biosynthesis in *Saccharomyces cerevisiae*

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Abstract

Many terpenoids have important pharmacological activity and commercial value; however, application of these terpenoids is often limited by problems associated with the production of sufficient amounts of these molecules. The use of *Saccharomyces cerevisiae* (*S. cerevisiae*) for the production of heterologous terpenoids has achieved some success. The objective of this study was to identify *S. cerevisiae* knockout targets for improving the synthesis of heterologous terpeniods. On the basis of computational analysis of the *S. cerevisiae* metabolic network, we identified the knockout sites with the potential to promote terpenoid production and the corresponding single mutant was constructed by molecular manipulations. The growth rates of these strains were measured and the results indicated that the gene deletion had no adverse effects. Using the expression of amorphadiene biosynthesis as a testing model, the gene deletion was assessed for its effect on the production of exogenous terpenoids. The results showed that the dysfunction of most genes led to increased production of amorphadiene. The yield of amorphadiene produced by most single mutants was 8–10-fold greater compared to the wild type, indicating that the knockout sites can be engineered to promote the synthesis of exogenous terpenoids.

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Introduction

Terpenoids, which are candidate drugs and fragrances, are important secondary metabolites derived from the universal precursors isopentenyl diphosphate (IPP) and its isomer dimethylallyl diphosphate (DMAPP) [1,2]. The low concentrations present in host organisms and the complicated structure of many terpenoids limits their suitability for commercial applications [3]. Although plant cell cultures and transgenic plants have been made to improve isoprenoid biosynthesis [4,5], plant-based efforts are less likely to supply sufficient commercial-scale quantities of terpenoids. The development of biological synthesis presented a promising alternative for producing large quantities of plant terpenoids in microorganisms such as *Escherichia coli* and *Saccharomyces cerevisiae* [6,7]. Compared to bacteria, *S. cerevisiae* is more advantageous for synthesizing plant terpenoids owing to its ability to express plant cytochrome P450 enzymes [8,9]. Monoterpenes, sesquiterpenes and diterpenes have been engineered using yeast cells as a living factory [10–12]. Very recently, the antimalarial drug artemisinin intermediate artemisinic acid was produced in *S. cerevisiae* at a titer of 25 $g{\cdot}L^{-1}$, which encourages the use of industrial manufacture of plant terpenes in microbial systems [13]. A sufficient supply of endogenous IPP for the heterologous terpenoid pathway is a crucial issue for metabolic engineering of plant terpenes in yeast cells [7,14]. Traditional engineering strategies designed to increase carbon flux toward IPP biosynthesis have relied heavily on amplifying the transcripts of several genes in the upstream pathway to IPP, often resulting in an unbalanced carbon flux and accumulation of toxic intermediates that inhibit yeast growth [10,15]. For this reason, there are two important challenges to be met by traditional engineering strategies. First, the simple up or down regulation of genes on the isoprenoid pathway might cause suboptimal yeast viability [16]. Second, owing to complex interactions between intracellular fluxes, modulation of gene targets solely on the isoprenoid pathway might be less able to direct carbon fluxes into the production of desired molecules. To overcome these difficulties, a genome-scale metabolic model of *S. cerevisiae*, iMM904, has been developed and used for metabolic engineering in yeast cells [17,18]. The aim of this study was to discover novel gene knockout targets outside the isoprenoid pathway that could improve isoprenoid fluxes in *S. cerevisiae* while not inhibiting yeast growth. First, *in silico* strategies based on iMM904 were used to identify possible knockout targets. To test their suitability, experiments were designed to knock out these targets from an *S. cerevisiae* strain maintained in our laboratory, which was then engineered to

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Patterns of genetic variation in the Chinese endemic *Psilopeganum sinense* (Rutaceae) as revealed by nuclear microsatellites and chloroplast microsatellites

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ABSTRACT

Psilopeganum sinense is a perennial herb endemic to Three-Gorges Reservoir Area (TGRA) in China. Genetic diversity of this endangered species was assessed by using 11 nuclear microsatellites and three chloroplast microsatellite (cpSSR) markers. A total of 8 haplotypes were identified in a survey of 212 individuals sampled from nine populations encompassing most of the natural range of the species. A low level of genetic diversity was detected: $H_E = 0.301$ for SSR and $H_E = 0.28$ for cpSSR. Populations were highly differentiated from one another: an AMOVA analysis that showed that 56.3% and 68.2% genetic variation resided between populations based on SSR and cpSSR analysis, respectively, and F_{ST} and F_{STc} (0.467 for SSR and 0.644 for cpSSR, respectively) were high. Significant differences were found between estimates of haplotypic differentiation calculated by using unordered alleles ($G_{STc} = 0.857$) and ordered alleles ($N_{STc} = 0.728$), which indicated the existence of phylogeographical structure in *P. sinense*. The indirect ratio of pollen flow/seed flow derived from estimates of haplotypic and nuclear DNA differentiation indicated that gene flow via pollen is less efficient than via seed. Two distinct evolutionary lineages (evolutionary significant units, ESUs) were recognized for *P. sinense* on the basis of both the PCoA and NCA analysis. Sampling strategies for conserving this endangered plant were discussed.

1. Introduction

Psilopeganum sinense Hemsl. is a perennial plant endemic to China and the only member of its genus (*Psilopeganum* Hemsl.) in the family Rutaceae (Wu et al., 2003). This species is typically distributed sporadically in the brushwood of hillsides or under roadside trees at altitudes from 100 to 320 m along the Yangtze River from Yichang, Hubei to Wuling, Chongqing, centre-west China, a biodiversity hotspot in China (Wang et al., 1995). Due to its wide usage as a traditional medicinal herb and spiceberry, *P. sinense* has suffered severe over-collection by local residents. Furthermore, because the natural distribution of *P. sinense* is located in the core area of the Three Gorges Reservoir (TGR) along the Yangtze River, the natural habitat of *P. sinense* below 145 m above sea level has been inundated since 2003, and the inundated range has extended to 175 m above sea level in 2009 when the Three-Gorges Dam (TGD) was fully completed. The TGD has increased the isolation of remaining

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Transcriptome analysis of the exocarp of apple fruit identifies light-induced genes involved in red color pigmentation

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ABSTRACT

Although the mechanism of light regulation of color pigmentation of apple fruit is not fully understood, it has been shown that light can regulate expression of genes in the anthocyanin biosynthesis pathway by inducing transcription factors (TFs). Moreover, expression of genes encoding enzymes involved in this pathway may be coordinately regulated by multiple TFs. In this study, fruits on trees of apple cv. Red Delicious were covered with paper bags during early stages of fruit development and then removed prior to maturation to analyze the transcriptome in the exocarp of apple fruit. Comparisons of gene expression profiles of fruit covered with paper bags (dark-grown treatment) and those subjected to 14 h light treatment, following removal of paper bags, were investigated using an apple microarray of 40,000 sequences. Expression profiles were investigated over three time points, at one week intervals, during fruit development. Overall, 736 genes with expression values greater than two-fold were found to be modulated by light treatment. Light-induced products were classified into 19 categories with highest scores in primary metabolism (17%) and transcription (12%). Based on the *Arabidopsis* gene ontology annotation, 18 genes were identified as TFs. To further confirm expression patterns of flavonoid-related genes, these were subjected to quantitative RT-PCR (qRT-PCR) using fruit of red-skinned apple cv. Red Delicious and yellow-skinned apple cv. Golden Delicious. Of these, two genes showed higher levels of expression in 'Red Delicious' than in 'Golden Delicious', and were likely involved in the regulation of fruit red color pigmentation.

1. Introduction

Apple (*Malus* × *domestica* Borkh.) is an important fruit crop that is widely grown all over the world. With increasing apple consumption, apple cultivars with highly desirable fruit quality characters such as fruit color, taste, flavor, and texture, among others, are in high demand. Among these characters, fruit coloration is a major important determinant of fruit quality as consumers have been reported to perceive red skin coloration to be correlated with better taste and flavor (King and Cliff, 2002).

Red color of apple fruit is derived from accumulation of anthocyanin pigments. It has been well known that coloration is genetically regulated by structural genes involved in the anthocyanin biosynthesis pathway (Davies and Schwinn, 2003); moreover, these genes are transcriptionally controlled by various transcription factors (TFs), including members of MYB, bHLH, WD40, WRKY, bZIP, and MADS-box protein families (Davies and Schwinn, 2003; Kubo et al., 1999; Ramsay and Glover, 2005).

The anthocyanin biosynthesis pathway has been well characterized genetically and biochemically (Grotewold, 2006; Holton and Cornish, 1995; Martin et al., 2001; Lancaster, 1992). The regulation of anthocyanin biosynthesis is dependent on critical stages of apple fruit development, known to occur at two peaks. The first peak occurs early in fruit development in both red and non-red cultivars; however, this stage is not economically important (Lancaster, 1992). The second peak occurs at the mature fruit stage, and it is markedly influenced by environmental factors, including temperature and light. It is well documented that environmental conditions, such as light, impact color development of apple fruit by inducing regulatory genes to act upstream of the anthocyanin biosynthesis cascade (Davies and Schwinn, 2003). Several studies have demonstrated that genes in the anthocyanin biosynthetic pathway are upregulated following exposure

Abbreviations: TF, transcription factor; qRT-PCR, quantitative real time-polymerase chain reaction; DAP, days after pollination; w, week; EST, expressed sequence tag.

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RESEARCH ARTICLE

Analysis of phosphoproteome in rice pistil

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As the female reproductive part of a flower, the pistil consists of the ovary, style, and stigma, and is a critical organ for the process from pollen recognition to fertilization and seed formation. Previous studies on pollen–pistil interaction mainly focused on gene expression changes with comparative transcriptomics or proteomics method. However, studies on protein PTMs are still lacking. Here we report a phosphoproteomic study on mature pistil of rice. Using IMAC enrichment, hydrophilic interaction chromatography fraction and high-accuracy MS instrument (TripleTOF 5600), 2347 of high-confidence (Ascore $\geq$ 19, $p \leq 0.01$), phosphorylation sites corresponding to 1588 phosphoproteins were identified. Among them, 1369 phosphorylation sites within 654 phosphoproteins were newly identified; 41 serine phosphorylation motifs, which belong to three groups: proline-directed, basophilic, and acidic motifs were identified after analysis by motif-X. Two hundred and one genes whose phosphopeptides were identified here showed tissue-specific expression in pistil based on information mining of previous microarray data. All MS data have been deposited in the ProteomeXchange with identifier PXD000923 (http://proteomecentral.proteomexchange.org/dataset/PXD000923). This study will help us to understand pistil development and pollination on the posttranslational level.

Keywords:
IMAC / *Oryza sativa* L. / Phosphoproteome / Pistil / Plant proteomics / Pollen–pistil interaction

Additional supporting information may be found in the online version of this article at the publisher's web-site

1 Introduction

In flowering plants, pollination is a critical process that results in production of seeds. Generally, it consists of the following six steps: pollen capture and adhesion, pollen hydration, pollen germination, penetration of stigma, growth of pollen tube through the stigma and style, pollen tube entering into the ovule and discharge of the sperm cells [1–3]. The six-step process, which is also called pollen–pistil interaction, depends on the recognition and communication between pollen and pistil [1, 3–5].

The accumulating understanding of pollen–pistil interaction has shed light on the molecular mechanism underlying this process in recent years. A large number of genes involved in the process have been identified by forward or reverse genetic strategies. For example, studies on sporophytic self-incompatibility (SI) in *Brassica* have identified a series of genes or proteins and uncovered an interesting network that mediates pollen–stigma interaction, since the

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Abbreviations: **ARC1**, ARM-repeat containing 1; **FDR**, false discovery rate; **GSK**, glycogen synthase kinase; **HILIC**, hydrophilic interaction chromatography; **pS**, phosphoserine; **pT**, phosphothreonine; **pY**, phosphotyrosine; **SI**, self-incompatibility

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Colour Online: See the article online to view Figs. 1–3 and 5–8 in colour.

BMC Plant Biology

RESEARCH ARTICLE Open Access

Genome-wide identification of *WRKY* family genes and their response to cold stress in *Vitis vinifera*

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Abstract

Background: *WRKY* transcription factors are one of the largest families of transcriptional regulators in plants. *WRKY* genes are not only found to play significant roles in biotic and abiotic stress response, but also regulate growth and development. Grapevine (*Vitis vinifera*) production is largely limited by stressful climate conditions such as cold stress and the role of *WRKY* genes in the survival of grapevine under these conditions remains unknown.

Results: We identified a total of 59 *VvWRKYs* from the *V. vinifera* genome, belonging to four subgroups according to conserved WRKY domains and zinc-finger structure. The majority of *VvWRKYs* were expressed in more than one tissue among the 7 tissues examined which included young leaves, mature leaves, tendril, stem apex, root, young fruits and ripe fruits. Publicly available microarray data suggested that a subset of *VvWRKYs* was activated in response to diverse stresses. Quantitative real-time PCR (qRT-PCR) results demonstrated that the expression levels of 36 *VvWRKYs* are changed following cold exposure. Comparative analysis was performed on data from publicly available microarray experiments, previous global transcriptome analysis studies, and qRT-PCR. We identified 15 *VvWRKYs* in at least two of these databases which may relate to cold stress. Among them, the transcription of three genes can be induced by exogenous ABA application, suggesting that they can be involved in an ABA-dependent signaling pathway in response to cold stress.

Conclusions: We identified 59 *VvWRKYs* from the *V. vinifera* genome and 15 of them showed cold stress-induced expression patterns. These genes represented candidate genes for future functional analysis of *VvWRKYs* involved in the low temperature-related signal pathways in grape.

Keywords: WRKY transcription factor family, Grapevine, Biotic and abiotic stress, Cold stress

Background

Plants have a variety of defense mechanisms to protect themselves from adverse environmental effects. Families of transcription factors are involved in these processes by functioning to reorganize gene expression patterns. The WRKY family is among them and plays key roles in modulating genes expression during plant defense in response to pathogens [1,2]. The WRKY transcription factors were first identified in sweet potato (SPF1) as DNA binding proteins [3]. Two similar genes (*ABF1* and *ABF2*) were found in wheat during germination [4]. Subsequently, Rushton et al. [5] reported the identification and characterization of *WRKY1*, *WRKY2* and *WRKY3* from parsley (*Petroselinum crispum*) and proposed these genes belong to a gene family. This gene family was named WRKY due to a conserved region (WRKYGQK) that was identified in the N-terminal amino acid sequence of all the members [4,5]. Further studies showed that the conserved WRKY domain had other forms such as WRKYGKK and WRKYGEK [6], or the WRKY domain could be replaced by WKKY, WKRY, WSKY, WIKY, WRIC, WRMC, WRRY or WVKY [7,8].

According to variation in WRKY domain and a zinc finger motif in the C-terminus, WRKY proteins were divided into four groups [9,10]. WRKY proteins with two WRKY domains composed group I. Groups II and III were characterized by a single WRKY domain. Group II

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PLOS ONE

Nitrite Promotes the Growth and Decreases the Lignin Content of *indica* Rice Calli: A Comprehensive Transcriptome Analysis of Nitrite-Responsive Genes during *In Vitro* Culture of Rice

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Abstract

As both major macronutrients and signal molecules, nitrogen metabolites, such as nitrate and nitrite, play an important role in plant growth and development. In this study, the callus growth of *indica* rice cv. 9311 was significantly enhanced by nitrite, whereas the soluble protein content remained unchanged. The deep RNA sequencing technology (RNA-seq) showed that the transcriptional profiles of cv. 9311 calli were significantly changed after adding nitrite to the nitrate-free medium, and these nitrite-responsive genes were involved in a wide range of plant processes, particularly in the secondary metabolite pathways. Interestingly, most of the genes involved in phenylpropanoid-related pathways were coordinately down-regulated by nitrite, such as four cinnamoyl-CoA reductase, and these in turn resulted in the decrease of lignin content of *indica* calli. Furthermore, several candidate genes related to cell growth or stress responses were identified, such as genes coding for expansins, *SMALL AUXIN UP RNA* (*SAUR*) and HSP20s, and these suggested that nitrite could probably serve as a transcriptome signal to enhance the *indica* calli growth by regulation of various downstream genes expression. This study contributes to a better understanding of the function of nitrite during the process of plant tissue culture and could aid in the application of this technology to improved *indica* genetic transformation efficiency.

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Introduction

Rice (*Oryza sativa* L) is one of the most important staple foods, and also a model species for molecular biology and functional genome in gramineae crops. *Indica* varieties account for approximately 70% of the cultivated rice, however, the tissue culture system in this subspecies is mostly specific and *Agrobacterium tumefaciens*-mediated genetic transformation remains difficult [1,2]. Establishment of a robust and widely applicable culture system for *indica* rice can provide an useful platform for basic biology research, and will also be helpful to develop high-quality cultivars by genetic manipulation [3,4].

Nitrogen (N) is an essential macronutrient and plays a key role in crop growth and development [5,6]. As the main source of inorganic nitrogen, nitrate is first reduced to nitrite by nitrate reductase (NR), then to ammonium by nitrite reductase (NiR), and is ultimately incorporated into amino acids. Besides its role as a nutrient, nitrate and its downstream metabolites are known to act as signal molecules to regulate global gene expressions, thus affecting plant physiology and architecture [7–9]. Microarray studies showed that the transcriptional profiles had been significantly changed after adding nitrate to the nitrogen-starved *Arabidopsis*, and these nitrate-responsive genes were involved in a wide range of plant processes, such as the nitrate uptake and assimilation process, pentose phosphate pathway and secondary metabolism [10–12]. A NR-null mutant of *Arabidopsis* was constructed to indentify a catalogue of NR independent nitrate-responsive genes, which were directly-regulated by nitrate, not downstream metabolites, served as the signal [13]. On the other hand, nitrite, a transient intermediate in the nitrate assimilation, is thought to be toxic metabolite if it is allowed to accumulate in plants. Similar to nitrate, nitrite might also function as a potential signal that regulates various gene expressions [14,15]. Global transcriptional analysis showed that there was extensive overlap between the nitrate and nitrite-responsive genes, and almost all of the pathways and processes induced by nitrate responded equivalently to nitrite [16].

High-quality embryonic callus is required for the successful *Agrobacterium tumefaciens*-mediated transformation of rice [17]. It has been reported that nitrite is one of determining factors for the

Antisense-mediated *FLC* transcriptional repression requires the P-TEFb transcription elongation factor

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The functional significance of noncoding transcripts is currently a major question in biology. We have been studying the function of a set of antisense transcripts called *COOLAIR* that encompass the whole transcription unit of the *Arabidopsis* floral repressor *FLOWERING LOCUS C (FLC)*. Alternative polyadenylation of *COOLAIR* transcripts correlates with different *FLC* sense expression states. Suppressor mutagenesis aimed at understanding the importance of this sense–antisense transcriptional circuitry has identified a role for *Arabidopsis* cyclin-dependent kinase C (CDKC;2) in *FLC* repression. CDKC;2 functions in an *Arabidopsis* positive transcription elongation factor b (P-TEFb) complex and influences global RNA polymerase II (Pol II) Ser^2 phosphorylation levels. CDKC;2 activity directly promotes *COOLAIR* transcription but does not affect an *FLC* transgene missing the *COOLAIR* promoter. In the endogenous gene context, however, the reduction of *COOLAIR* transcription by *cdkc;2* disrupts a *COOLAIR*-mediated repression mechanism that increases *FLC* expression. This disruption then feeds back to indirectly increase *COOLAIR* expression. This tight interconnection between sense and antisense transcription, together with differential promoter sensitivity to P-TEFb, is central to quantitative regulation of this important floral repressor gene.

lncRNA | autonomous pathway | transcriptional regulation | chromatin silencing

We are investigating the role of specific antisense transcripts in gene regulation through our analysis of the regulation of expression of *Arabidopsis thaliana FLOWERING LOCUS C* (*FLC*), an important regulator of flowering time (1, 2). Multiple genetic pathways have been defined that regulate *FLC* expression; some function in parallel whereas others function antagonistically. FRIGIDA (FRI) up-regulates *FLC* expression, causing plants to overwinter vegetatively (3). FRI function is antagonized by vernalization, a process through which prolonged cold epigenetically silences *FLC* (4–7). Acting in parallel with vernalization to repress *FLC* expression is the autonomous pathway. The autonomous pathway was initially characterized through mutations specifically affecting flowering time but has subsequently been shown to regulate many other targets in the *A. thaliana* genome (8–10). The autonomous pathway is composed of a number of factors: RNA-binding proteins FCA (11), FPA (12), and FLK (13, 14), RNA 3′ processing factors FY (15–17), CstF64, and CstF77 (18), a histone 3 lysine 4 (H3K4) demethylase FLD (19, 20), a homolog of MSI1 (multicopy suppressor of *ira*1) FVE (21), and a homeodomain protein LUMINIDEPENDENS (LD) (22). These factors link alternative processing of a set of antisense transcripts produced at the *FLC* locus, collectively termed *COOLAIR*, with chromatin modifications in the *FLC* gene body (8). FCA, FY, and FPA promote proximal polyadenylation of *COOLAIR* transcripts (18), FLD-dependent H3K4me2 demethylation across the body of the gene, and low *FLC* transcription (23). Their loss results in distal polyadenylation in *COOLAIR*, increased H3K4 methylation across the gene, and high expression (24), but how *COOLAIR* processing is linked to *FLC* transcription is still not fully understood. *COOLAIR* is also regulated transcriptionally via an extensive R-loop that covers the *COOLAIR* promoter and first exon (25).

To gain a better understanding of the sense–antisense mechanism regulating *FLC*, we have undertaken suppressor mutagenesis and have identified a requirement for cyclin-dependent kinase C (CDKC;2). This protein is an *Arabidopsis* ortholog of a component of the positive transcription elongation factor b (P-TEFb) (26–29). P-TEFb regulates transcription elongation and integrates mRNA synthesis with histone modification, pre-mRNA processing, and mRNA export (30). *Arabidopsis* CDKC;2 has previously been shown to be important for flowering time control and plant virus infection (26) and to colocalize with spliceosome components in nuclear bodies (31). Here, we show that CDKC;2 functions globally in the *A. thaliana* genome to influence the phosphorylation status of RNA polymerase II (Pol II), as anticipated from a P-TEFb function. We also investigate CDKC;2 function on sense *FLC* and *COOLAIR* transcription through analysis of transgenic lines, where both are expressed independently of each other. This analysis established that *cdkc;2* specifically reduces transcription of *COOLAIR*, which indirectly up-regulates *FLC* expression through disruption of a *COOLAIR*-mediated repression mechanism. The feedback mechanisms that link sense and antisense transcription in the endogenous gene context (18) then indirectly increase *COOLAIR* expression. This sensitivity of the *COOLAIR* promoter to P-TEFb function suggests that the antisense transcription is the driver quantitatively regulating expression levels at *FLC*.

Significance

Noncoding transcripts are found in high complexity but low abundance in most genomes, but their functional relevance is unclear. We investigate the function of a set of antisense transcripts expressed from an important floral repressor gene in *Arabidopsis*. Different polyadenylated forms of the antisense transcripts correlate with high or low expression states of the floral repressor gene. We now identify a mutation in a conserved transcription elongation factor that specifically disrupts the antisense transcription. The direct reduction of antisense transcription releases a repression mechanism that indirectly increases expression of both the floral repressor gene and antisense expression. This study reveals tight interplay between sense and antisense transcription and a mechanism that could have a widespread role in quantitative gene regulation.

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Enhanced lipid production in *Chlorella pyrenoidosa* by continuous culture

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HIGHLIGHTS

- *Chlorella pyrenoidosa* XQ-20044 is able to accumulate lipids in growing cells.
- One step production of algal lipid was achieved in chemostat culture.
- Proper SNI was the key for simultaneous algal growth and lipid accumulation.
- Lipid productivity was significantly enhanced by continuous culture.

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ABSTRACT

Usually microalgae growth and lipid accumulation do not run in parallel throughout cultivation, which necessarily lowers overall lipid productivity. However, we show through batch and feed-batch studies of *Chlorella pyrenoidosa* XQ-20044 that by varying the nitrate concentration, conditions which produce fairly high lipid content could be achieved without sacrificing algal growth. Simultaneous microalgae growth and lipid production was achieved in continuous chemostat culture when the specific nitrate input rate was in the range of 0.78–4.56 mmol g^{-1} d^{-1}. Moreover, the maximum lipid productivity (144.93 mg L^{-1} d^{-1}) in the continuous culture was significantly higher than in batch culture (96.28 mg L^{-1} d^{-1}), thus indicating the feasibility and great advantage of one-step production of microalgal lipids.

1. Introduction

Aquatic microalgae have a high capacity for photosynthesis, and many are able to use excess photosynthetically-fixed carbon for synthesis of neutral lipids, which are also known as triacylglycerols (TAGs) (Chisti, 2007). Oleaginous microalgae are thus considered to be ideal raw materials for biodiesel production, especially if their growth is coupled to the direct bio-fixation of waste CO_2 (Kwak et al., 2006). Over the past 50 years the concept and feasibility of microalgal biodiesel have been discussed extensively (Wijffels and Barbosa, 2010), but this renewable energy source has yet to be exploited. Limiting factors include the lack of appropriate microalgal strains, less-than optimal lipid productivity and ineffective culturing techniques for lipid accumulation.

To date, several hundred oleaginous species have been isolated and characterized (Gouveia et al., 2009). A common thread in these studies is that cell growth and lipid accumulation do not happen at the same time during cultivation (Lourenco et al., 2002; Merzlyak et al., 2007), which results in lower overall lipid productivity. In order to overcome this, researchers have explored two-stage cultivation strategies to enhance microalgal lipid production. In such strategies, which have been used mainly with batch cultures, the microalgal cells first grow rapidly under growth-optimized conditions, and then are transferred to conditions where light irradiance (Zhang et al., 2009), nutrition (Su et al., 2011), culture pH (Han et al., 2013), as well as other factors (Das et al., 2011; Liu et al., 2008) are adjusted to promote lipid accumulation at the expense of cell growth.

However, detailed study of the stress response of oleaginous microalgae under nitrogen deficiency may provide a foundation for the production of biomass and lipids in one step. Recent data showed that while *Neochloris oleoabundans* UTEX #1185 accumulated lipid under relatively high nitrogen stress conditions (i.e., low nitrogen levels), its growth was not severely limited (Adams

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TWO-STAGE CHARACTERISTICS OF LIPID PRODUCTION IN BATCH CULTURE OF TWO GREEN MICROALGAE

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ABSTRACT

In this paper, batch culture of two Chlorophyte *Scenedesmus* sp. 200716 and *Chlorella* sp. XQ-20044 with different nitrogen concentrations were carried out in a circular pond mimic system to characterize its growth and lipid production. Both of the strains grew quickly while the total lipid content was relatively constant (about 20% of dry biomass) before stationary phase. However, lipid accumulation began as cell division ceased in the stationary phase. After 12 days of cultivation with 1mM and 3mM sodium nitrate, total lipid content reached 55.0% and 54.3% dry weight of *Chlorella* sp. XQ-20044, 32.1% and 29.7% dry weight of *Scenedesmus* sp. 200716, respectively. The data clearly demonstrated the unparallel occurrence of cell growth and lipid accumulation, which was stated here as two-stage characteristics of lipid production: The first, cell production stage, algal cells divide quickly to produce large amount of biomass, then followed by lipid production stage, cell division cease and lipid accumulation begin. Based on these results, the strategies for efficient lipid production by mass culture of green algae were further discussed.

KEYWORDS:
Batch; *Chlorella*; Lipid production; *Scenedesmus*; Two-stage

1. INTRODUCTION

Microalgae are considered to be ideal raw materials for biodiesel production for many years. To date, numerous studies have been conducted to investigate the feasibility of microalgae for biodiesel production [1, 2], the effects of various factors on lipid accumulation [3-5] as well as the selection and characterization of microalgae species suitable for lipid production [6-9].

It is likely a general trend in microalgae towards accumulation of lipids in response to nitrogen deficiency [10]. However, biomass yield of microalgae cultivation under such conditions was rather low due to nitrogen limitation, and overall lipid productivity would be reduced consequently. Recently, some efforts were made to enhance the lipid productivity of microalgae. Under fed-batch cultivation mode, cell concentration of *Nannochloris* sp. UTEX LB1999 reached almost the same as those of cells cultured under batch mode while the intracellular lipid content increased from 31.0 to 50.9% [11]. Su et al. [12] reported that final yield of lipids obtained from the two-stage cultivation of *Nannochloropsis oculata* was 2.82 times higher than that from one-stage batch cultivations. Later, Probir Das et al. [13] reported another type of "two-phase cultivation process" and concluded that addition of organic carbon sources to the cultures of *Nannochloropsis* sp. at the "second growth phase" of culture (the later days of a single batch culture) resulted in higher lipid productivity.

Although microalgal lipid content and productivity can be improved by modulation of culture conditions, thorough understanding of microalgal lipid production lies ahead before the establishment of optimal cultivation mode for enhanced lipid production. In this paper, batch culture with different nitrogen concentrations were carried out in a circular pond mimic system to characterize growth and lipid production of *Chlorella* and *Scenedesmus*. Possible cultivation mode for mass culture of green microalgae for lipid production was also discussed.

2. MATERIALS AND METHODS

2.1 Algae investigated

Two microalgae strains were used in this study, specifically *Scenedesmus* sp. 200716 and *Chlorella* sp. XQ-20044. Both strains were obtained from the algae culture collection in Wuhan Botanical Garden, CAS. The seed cultures were grown photoautotrophically in Bold's Basal Medium [14] in 1000 mL flasks on a horizontal shaker. The culture conditions were as follows: light intensity 50 μmol $m^{-2}s^{-1}$, light-dark cycle 14h:10h, temperature 25℃, shaking speed 100 rpm. The seed algae were cultured for 4 d before inoculation.

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PLOS ONE

Genome-Wide Transcriptional Profiles of the Berry Skin of Two Red Grape Cultivars (*Vitis vinifera*) in Which Anthocyanin Synthesis Is Sunlight-Dependent or -Independent

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Abstract

Global gene expression was analyzed in the berry skin of two red grape cultivars, which can ('Jingyan') or cannot ('Jingxiu') synthesize anthocyanins after sunlight exclusion from fruit set until maturity. Gene transcripts responding to sunlight exclusion in 'Jingyan' were less complex than in 'Jingxiu'; 528 genes were induced and 383 repressed in the former, whereas 2655 genes were induced and 205 suppressed in 'Jingxiu'. They were regulated either in the same or opposing manner in the two cultivars, or in only one cultivar. In addition to *VvUFGT* and *VvMYBA1*, some candidate genes (e.g. *AOMT, GST*, and *ANP*) were identified which are probably involved in the differential responses of 'Jingxiu' and 'Jingyan' to sunlight exclusion. In addition, 26 *MYB*, 14 *bHLH* and 23 *WD40* genes responded differently to sunlight exclusion in the two cultivars. Interestingly, all of the 189 genes classified as being relevant to ubiquitin-dependent protein degradation were down-regulated by sunlight exclusion in 'Jingxiu', but the majority (162) remained unchanged in 'Jingyan' berry skin. It would be of interest to determine the precise role of the ubiquitin pathway following sunlight exclusion, particularly the role of *COP9* signalosome, cullins, *RING-Box* 1, and *COP1*-interacting proteins. Only a few genes in the light signal system were found to be regulated by sunlight exclusion in either or both cultivars. This study provides a valuable overview of the transcriptome changes and gives insight into the genetic background that may be responsible for sunlight-dependent versus -independent anthocyanin biosynthesis in berry skin.

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Introduction

Anthocyanins, which are derived from the phenylpropanoid pathway, are a class of secondary metabolites that contribute to the red, blue, and purple coloring of a diverse range of flowers and the skin and flesh of fruit, as well as leaves, shoots, roots, and seeds [1]. Among other environmental factors, light is a critical stimulus regulating anthocyanin accumulation and the effect of light and shade on anthocyanin accumulation has been widely studied [1–4]. In general, anthocyanin accumulation is reduced under low light conditions and increased under high light in the fruit of many crops, including grapes [5–13], although too much radiation in the ultraviolet-B (UV-B) wavelength range can inhibit anthocyanin synthesis [14].

Anthocyanin production requires a number of genes, the most studied of which are the structural genes encoding the biosynthetic enzymes and the *R2R3 MYB* regulator family. In various tissues of *Arabidopsis*, *AtMYB11*, *AtMYB12* and *AtMYB111* together regulate the early anthocyanin biosynthetic genes chalcone synthase (*CHS*), chalcone isomerase (*CHI*) and flavanone 3-hydroxylase (*F3H*) in response to light [15–17]. In grapes, shade also suppresses and retards the accumulation of *CHS*, *CHI*, *F3H*, *DFR* (dihydroflavonol 4-reductase), *LDOX* (leucoanthocyanidin dioxygenase), *UFGT* (UDP-glucose: flavonoid 3-O-glucosyltransferase) and *VvMYBA1* mRNA [11]. *MYB* regulators often regulate these structural genes by activating their promoters. PcMYB can interact with light-regulatory unit 1 (*LRU1*), comprising an ACGT-containing element (ACE) and an *MYB* recognition element (MRE), which is necessary to mediate light-dependent activation of *CHS* in *Petroselinum crispum* [18]. PfMYBP1 was able to bind to the *DFR* gene promoter and its expression was induced by light in *Perilla frutescens* [19]. When fruit grown in the dark were exposed to sunlight, *MdMYB1* transcript levels increased over several days, correlating with

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Identification of cadmium-resistant fungi related to Cd transportation in bermudagrass [*Cynodon dactylon* (L.) Pers.]

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HIGHLIGHTS

- A new Cd-resistant strain was isolated and identified as *Aspergillus aculeatus*.
- *A. aculeatus* improved bermudagrass Cd tolerance.
- Bermuda treated with the fungi had greater Cd in root, but less Cd in shoot.
- The fungi caused less Cd translocation from root to shoot in bermudagrass.
- *A. aculeatus* might be used to reduce Cd concentration in seed of other crop.

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ABSTRACT

Phytoremediation utilizing plants and microbes has been increasingly adopted as a green technology for cleaning up heavy metal polluted soils. Cd polluted soil and native bermudagrass from Liuyang and Zhuzhou in Hunan province of China were collected to investigate microbial diversity and isolate Cd resistant fungi, and then to determine the effect of Cd resistant fungi on Cd tolerance and transportation of bermudagrass. The functional diversity of microorganisms was evaluated using the BIOLOG Eco method. Cd-resistant fungi strain was isolated and identified as *Aspergillus aculeatus* based on the ribosomal internal transcribed spacer region sequence analysis. Bermudagrass was exposed to control, Cd only, and Cd plus *A. aculeatus* (Cd + *A. aculeatus*) with growth matrix (sawdust/sand = 3/1 in volume). Results indicated that Cd + *A. aculeatus* treated bermudagrass exhibited a higher photosynthetic activity compared to Cd only treated plants. Inoculation of *A. aculeatus* resulted in a decrease in stem and leaf Cd concentrations, to a greater extent for Cd-sensitive than for Cd-tolerant genotype. However, inoculation of *A. aculeatus* increased root Cd concentration under Cd stress conditions, significantly elevated soil pH, and decreased soil water-soluble Cd concentration. These results suggested that *A. aculeatus* might be potentially applied to improve Cd tolerance and to reduce Cd transportation to shoot of bermudagrass.

1. Introduction

Cd, a highly health-threatening heavy metal pollutant, is a widespread trace element pollutant with a long biological half-life (Wagner, 1993). It has been investigated that over 1.4×10^6 ha of farmland in China has been contaminated with Cd (Gu et al., 2003). Cd in the soil is taken up by plants and translocated to edible parts to threaten animal and human health. Consequently, relatively high Cd concentrations may result in bone disease and kidney damage in mammals (van de Mortel et al., 2008). Therefore, remediation of Cd-contaminated soil is urgent for human health and environmental conservation (Abou-Shanab et al., 2006).

Phytoremediation (i.e. the utilization of plants to take up or immobilize hazardous substances from contaminated soils) has been widely applied due to its relatively low cost, minimum secondary pollution, effectiveness and safety for humans and the environment (Chandra Sekhar et al., 2005). Of all phytoremediation technologies, phytoextraction and phytostabilization are the most commonly recognized approaches for remediation of heavy metals contaminated soils. However, there is no evident suitable plant for removing metals in a reasonably short time for phytoextraction. Effective phytoextraction does not entirely depend on bioaccumulation factor, but also on plant biomass, however currently, hyperaccumulator plants barely meet all these requirements (Burd

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Classification of genetic variation for cadmium tolerance in Bermudagrass [*Cynodon dactylon* (L.) Pers.] using physiological traits and molecular markers

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Abstract Cadmium (Cd) is one of the most toxic pollutants that caused severe threats to animal and human health. Bermudagrass is a dominant species in Cd contaminated soils, which can prevent Cd flow and spread. The objectives of this study were to determine the genetic variations in major physiological traits related to Cd tolerance in six populations of Bermudagrass collected from China, and to examine the genetic diversity and relationships among these accessions that vary in Cd tolerance using molecular markers. Plants of 120 accessions (116 natural accessions and 4 commercial cultivars) were exposed to 0 (i.e. control) or 1.5 mM $CdSO_4 \cdot 8/3H_2O$ for 3 weeks in hydroponic culture. Turf quality, transpiration rate, chlorophyll content, leaf water content and growth rate showed wide phenotypic variation. The membership function method was used to comprehensively evaluate Cd-tolerance. According to the average subordinate function value, four accessions were classified as the most tolerant genotypes and four accessions as Cd-sensitive genotypes. The trend of Cd tolerance among the six studied populations was as follows: Hunan > South China > North China > Central China > West South China and Xinjiang population. Phylogenetic analysis revealed that the majority of accessions from the same or adjacent regions were clustered into the same groups or subgroups, and the accessions with similar cadmium tolerance displayed a close phylogenetic relationship. Screening genetically diverse germplasm by combining the physiological traits and molecular markers could prove useful in developing Cd-tolerant Bermudagrass for the remediation of mill tailings and heavy metal polluted soils.

Keywords Bermudagrass · Cadmium · Genetic variation · Physiological traits · Molecular markers

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Introduction

Heavy metals are ubiquitous and persistent environmental pollutants because of their impact on plants, animals and the health of human beings via the food chain. Cadmium (Cd) is one of the most notorious toxic pollutants and a large amount of this chemical enters the environment annually due to the smelting of metals, alloy preparation, mining operations, municipal wastes, phosphate fertilizers and electroplating (Davidson 2013). Cd is highly mobile and can easily enter the food chain, so its presence in the environment, even at low concentrations posed a severe threat to animal and human health (Wagner 1993). In fact, over 1.4×10^6 ha of farmland in eleven provinces of China are contaminated with Cd (Gu et al. 2003).

Phytoremediation has been widely applied due to its low cost, in situ rehabilitation and no secondary pollution as plants can take up Cd and transport it from roots to shoots (Padmaja et al. 1990; Daghan et al. 2010). Equal essential in the development of phytoremediation technology is the identification and collection of new, naturally occurring species that are resistant to and accumulate metal. A substantial number of Cd tolerant species or ecotypes have been identified around the world (Baker 1987). Species such as *Viola baoshanensis*, *Salsola kali*, *Sedum alfredii* and *Thlaspi praecox* have been reported on the physiological and molecular mechanisms of heavy metal uptake, detoxification and translocation (Brooks 1998; Liu et al.

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RESEARCH ARTICLE Open Access

Comparison of investigation methods of heat injury in grapevine (*Vitis*) and assessment to heat tolerance in different cultivars and species

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Abstract

Background: In the context of global climate change, heat stress is becoming an increasingly important constraint on grapevine growth and berry quality. There is a need to breed new grape cultivars with heat tolerance and to design effective physiological defenses against heat stress. The investigation of heat injury to plants or tissues under high temperature is an important step in achieving these goals. At present, evaluation methods for heat injury include the gas exchange parameters of photosynthesis, membrane thermostability, chlorophyll content etc.; however, these methods have obvious disadvantages, such as insensitivity, inconvenience and delayed information. An effective and convenient method for investigating the heat injury of grapevine must be developed.

Results: In this study, an investigation protocol for a critical temperature (47°C) and heat treatment time (40 min) was developed in detached grape leaves. Based on the results, we found that the OJIP test was superior to measuring electrolyte leakage or photosynthetic O_2 evolution for investigating the heat injury of three cultivars of grapevine. Heat tolerance of 47 grape species and cultivars was evaluated through investigating heat injury using the OJIP test. Moreover, the electron transport chain (donor side, acceptor side and reaction center) of PSII in photosynthesis was further investigated.

Conclusions: The OJIP test was a rapid, sensitive and convenient method for investigating heat injury in grapevine. An analysis of PSII function using this method indicated that the acceptor side was less sensitive to heat than was the donor side or the reaction center in grape leaves. Among the 47 taxa evaluated (cultivars, hybrids, and wild species), heat tolerance varied largely in each genotype group: most wild species and hybrids between *V. labrusca* and *V. vinifera* had relatively strong heat tolerance, but most cultivars from *V. vinifera* had relatively weak heat tolerance.

Background

Grapevine is the most economically important fruit crop in the world, with its berries both eaten fresh and used for making wine, jam, juice, jelly, raisins and vinegar. Viticultural production is famously sensitive to climate [1-3], and temperature and moisture regimes are among the primary elements of grape terroir [3,4]. In many production regions, the maximum midday air temperature may exceed 40°C, with some regions exceeding 45°C [5-7]. High temperatures influence the development of plants and inhibit leaf photosynthesis. Exposure to high temperatures during flowering significantly inhibits berry set [8]. After fruit set, high temperatures are generally not favourable to the development secondary metabolites such as phenolic compounds [9,10] and aromatic volatiles [7]. High temperatures stimulate sugar accumulation [8], resulting in the production of wines with higher alcohol concentrations. To cope with heat stress, it is necessary to breed new cultivars with strong heat tolerance and to design effective physiological defenses against heat stress. Consequently, developing an effective and convenient method for evaluating the heat stress is a key goal.

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RESEARCH PAPERS

Low Sink-Induced Stomatal Closure Alters Photosynthetic Rates of Source Leaves in Beans as Dependent on H_2O_2 and ABA Accumulation in Guard Cells[1]

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Abstract—Low sink demand provided by pod removal and stem girdling of beans (*Vicia faba*, cv. Daqingshan) (–Sink) induced a significantly lower net photosynthetic rate (P_n), stomatal conductance (g_s), internal CO_2 concentration (C_i), and transpiration rate (E) compared with pod and root sink retention (CK). This depression in P_n was due to stomatal limitation. Low sink demand of –Sink plants resulted in a higher leaf sucrose content, but a lower sucrose content in guard cells. Moreover, the significant accumulation of H_2O_2 and ABA were observed in both leaves and guard cells of –Sink plants. The most intensive electron dense deposit of cerium perhydroxides, produced by H_2O_2 reaction with cerium chloride, was present in the cell walls, especially the dorsal walls of guard cells. Immunogold electron-microscopy localization of ABA showed that ABA was distributed in ventral walls of guard cells and the intercellular space of mesophyll cells of –Sink leaves in contrast to CK plants. Application of exogenous sucrose to isolated bean leaves increased H_2O_2 and ABA contents. H_2O_2 and ABA in leaves was likely generated by two independently regulated pathways, each affected by the high sucrose concentration induced by low sink demand. Increased sucrose in leaves in response to low sink demand may have caused the increase of H_2O_2 and ABA, and their accumulation in mesophyll cells and guard cells was likely the primary cause for stomatal closure under low sink demand.

Keywords: Vicia faba, low sink demand, photosynthesis, stomatal closure, abscisic acid, hydrogen peroxide

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INTRODUCTION

A decline in leaf photosynthesis in response to low sink demand has been observed in many higher plants, such as peach [1], citrus [2], soybean [3], and coffee [4]. Many studies have focused primarily on carbohydrate accumulation in source leaves with evidence supporting the hypothesis of end-product inhibition of photosynthesis [5]. However, some studies failed to show a relationship between carbohydrate accumulation and decreased net photosynthetic rate (P_n) caused by low sink demand [6]. Moreover, studies with peach indicated that the accumulation of end products did not reduce the potential activity of related carbon-metabolic enzymes despite a considerable decrease in P_n in response to low sink demand [7]. The specific mechanism for the effect of low sink demand on photosynthesis is still unclear, although the feedback regulation hypothesis was proposed over 150 years ago.

Over the last 20 or more years, numerous studies have demonstrated a strong positive correlation between g_s and P_n under a variety of different source–sink relations [4, 8]. Our previous studies have shown that both a decreased g_s and increased leaf temperature (T_{leaf}) have a relationship to decreased P_n in response to low sink demand [8]. We suggested that decreased g_s might be considered as the trigger or promoter and increased T_{leaf} as the regulator of photosynthesis under

[1] This text was submitted by the authors in English.

[2] These authors contributed equally to this work.

Abbreviations: C_i—internal CO_2 concentration; CK—control; DPI—diphenyleneiodonium chloride; E—transpiration rate; EDC—1-(3-dimethyl aminopropyl)-3-ethyl carbodiimide; g_s—stomatal conductance; PAR—photosynthetically active radiation; PBS—phosphate-buffered saline; P_n—net photosynthetic rate; PVP—polyvinylpyrrolidone; Suc—sucrose; TCA—trichloroacetic acid; T_{leaf}—leaf temperature; Tun—sodium tungstate; AsA—ascorbic acid.

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PLOS ONE

Perspectives on Screening Winter-Flood-Tolerant Woody Species in the Riparian Protection Forests of the Three Gorges Reservoir

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Abstract

The establishment of riparian protection forests in the Three Gorges Reservoir (TGR) is an ideal measure to cope with the eco-environmental problems of the water-level fluctuation zone (WLFZ). Thus, the information for screening winter-flood-tolerant woody plant species is useful for the recovery and re-establishment of the riparian protection forests in the TGR WLFZ. Therefore, we discussed the possibilities of constructing and popularizing riparian protection forests in the TGR WLFZ from several aspects, including the woody plant species distribution in the WLFZ, the survival rate analyses of suitable candidate woody species under controlled flooding conditions, the survival rate investigation of some woody plant species planted in the TGR WLFZ, and the physiological responses of some woody plant species during the recovery stage after winter floods. The results of woody species investigation showed that most woody plant species that existed as annual seedlings in the TGR WLFZ are not suitable candidates for the riparian protection forests. However, arbor species (e.g., *Salix matsudana*, *Populus* ×*canadensis*, *Morus alba*, *Pterocarya stenoptera*, *Taxodium ascendens*, and *Metasequoia glyptostroboides*) and shrub species (e.g., *Salix variegata*, *Distylium chinensis*, *Lycium chinense*, *Myricaria laxiflora*, and *Rosa multiflora*) might be considered suitable candidates for the riparian protection forests in the TGR WLFZ by survival rate analyses under controlled winter flooding conditions, and survival rate investigations of woody plant species planted in the TGR WLFZ, respectively. Physiological analyses showed that *P.* ×*canadensis*, *M. alba*, *L. chinense*, and *S. variegata* could develop specific self-repairing mechanisms to stimulate biomass accumulation and carbohydrate synthesis via the increases in chlorophyll pigments and photosynthesis during recovery after winter floods. Our results suggested these woody plant species could endure the winter flooding stress and recover well, and be used as candidate for the construction of riparian protection forests in the TGR WLFZ.

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Introduction

The portion of the Yangtze River Basin between Chongqing City and Yichang City is known as the Three Gorges Reservoir (TGR) Area (TGRA). The Three Gorges Dam (TGD) was designed to control floods, generate electricity, improve navigation, and create tourism opportunities on the Yangtze River [1–3]. The dam was initiated in 1994 and its first impoundment was conducted in 2003 with a water level rising of 60 m above former riverbank of the Yangtze River. The second impoundment was impounded in October 2006 and the water level rose to 156 m. The third impoundment occurred in October 2008 and resulted in a sustained water level at above 170 m for five months. The water was raised to the ultimate planned level of about 175 m above sea level in 2010 [4–6]. To operate the TGR at full capacity, the water level of the TGR fluctuates between 145 and 175 m, i.e., 145 m in summer for flood control and emission sediment and 175 m in winter for energy generation. In October, the water level rises gradually to 175 m. By the following January, the water level starts to fall, finally dropping to 145 m in June [3,5,7–9]. Thus, the peak flows of the TGR occurred during November, December, January, February and March (winter), low flows in June, July, August and September (summer). Therefore, the affecting riparian vegetation factors such as flooding timing, duration, frequency, rate of change, and magnitude [10] in the TGR differed from the natural Yangtze River.

The new hydrological regime, including the reversal of flooding time and the prolonged flooding duration caused by the TGD, dramatically alters the conditions of riparian ecosystems and results in the formation of reservoir water-level fluctuation zone (WLFZ), i.e., the area between the high (175 m) and low (145 m) water levels in the TGR [3,5]. The WLFZ forms a transitional zone between terrestrial and aquatic ecosystems and serves as an important pathway for exchanging of the fluxes of matter, energy, and information between terrestrial and aquatic ecosystems [7,11]. In the WLFZ, plants suffered serial submergence stress with durations as long as 210 days at depths of up to 30 m. Few plant species could tolerate such submergence stress. Therefore, the

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Molecular and quantitative trait variation within and among small fragmented populations of the endangered plant species *Psilopeganum sinense*

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• *Background and Aims* Natural selection and genetic drift are important evolutionary forces in determining genetic and phenotypic differentiation in plant populations. The extent to which these two distinct evolutionary forces affect locally adaptive quantitative traits has been well studied in common plant and animal species. However, we know less about how quantitative traits respond to selection pressures and drift in endangered species that have small population sizes and fragmented distributions. To address this question, this study assessed the relative strengths of selection and genetic drift in shaping population differentiation of phenotypic traits in *Psilopeganum sinense*, a naturally rare and recently endangered plant species.
• *Methods* Population differentiation at five quantitative traits (Q_{ST}) obtained from a common garden experiment was compared with differentiation at putatively neutral microsatellite markers (F_{ST}) in seven populations of *P. sinense*. Q_{ST} estimates were derived using a Bayesian hierarchical variance component method.
• *Key Results* Trait-specific Q_{ST} values were equal to or lower than F_{ST}. Neutral genetic diversity was not correlated with quantitative genetic variation within the populations of *P. sinense*.
• *Conclusions* Despite the prevalent empirical evidence for $Q_{ST} > F_{ST}$, the results instead suggest a definitive role of stabilizing selection and drift leading to phenotypic differentiation among small populations. Three traits exhibited a significantly lower Q_{ST} relative to F_{ST}, suggesting that populations of *P. sinense* might have experienced stabilizing selection for the same optimal phenotypes despite large geographical distances between populations and habitat fragmentation. For the other two traits, Q_{ST} estimates were of the same magnitude as F_{ST}, indicating that divergence in these traits could have been achieved by genetic drift alone. The lack of correlation between molecular marker and quantitative genetic variation suggests that sophisticated considerations are required for the inference of conservation measures of *P. sinense* from neutral genetic markers.

Key words: *Psilopeganum sinense*, Chinese privet, stabilizing selection, genetic drift, quantitative traits, Q_{ST}, neutral microsatellite markers, F_{ST}, local adaptation, habitat fragmentation.

INTRODUCTION

Understanding how natural populations respond to selection, gene flow and genetic drift is important for conservation and evolutionary biology (Merilä and Crnokrak, 2001; Leinonen *et al.*, 2013), particularly in the light of increasing habitat fragmentation. Habitat fragmentation imposes negative effects on the persistence of populations and species (McGarigal and Cushman, 2002; Fahrig, 2003) by reducing gene flow, elevating random genetic drift and lessening the effectiveness of selection (e.g. Young *et al.*, 1996; Sork *et al.*, 1999; Aguilar *et al.*, 2008; Charlesworth, 2009), which likely restrain the evolutionary response of populations to future changes (Caro and Laurenson, 1994; Young *et al.*, 1996; Lande, 1998; Booy *et al.*, 2000). Despite the awareness of the negative fitness consequences of population fragmentation, we know little about the relative strength of different evolutionary forces in shaping genetic and phenotypic differentiation in small and isolated natural populations (Frankham, 1999; Rogell *et al.*, 2010).

One approach for evaluating the relative importance of natural selection and genetic drift in determining the levels of adaptive trait divergence is to compare population differentiation at neutrally evolving genetic markers, as measured by F_{ST} (Wright, 1951), with differentiation for quantitative genetic traits, as measured by Q_{ST} (e.g. Lande, 1992; Merilä and Crnokrak, 2001; McKay and Latta, 2002; Leinonen *et al.*, 2008; Lamy *et al.*, 2012). The difference between Q_{ST} and F_{ST} is compared against the null expectation of $Q_{ST} = F_{ST}$ for neutral additive traits differentiating via genetic drift. As F_{ST} represents neutral divergence that depends on gene flow–drift equilibrium, significant $Q_{ST} > F_{ST}$ comparisons suggest the presence of local adaptation, whereas $Q_{ST} < F_{ST}$ comparisons imply that stabilizing selection has prevented populations from diverging by drift (Leinonen *et al.*, 2008). Because of the logistic difficulty of performing reciprocal transplantation among multiple populations, comparing Q_{ST} and F_{ST} is a particularly useful approach for studying local adaptation.

Meta-analyses of empirical Q_{ST}–F_{ST} contrast studies have shown that Q_{ST} typically exceeds F_{ST} (Merilä and Crnokrak, 2001; Leinonen *et al.*, 2008), suggesting that quantitative genetic variation is often under the influence of divergent selection. However, it is not clear whether the general tendency of $Q_{ST} > F_{ST}$ applies to small, fragmented and usually genetically impoverished populations in which genetic drift is presumably

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Identification and Validation of Reference Genes for Quantitative Real-Time PCR Normalization and Its Applications in *Lycium*

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Abstract

Lycium barbarum and *L. ruthenicum* are extensively used as traditional Chinese medicinal plants. Next generation sequencing technology provides a powerful tool for analyzing transcriptomic profiles of gene expression in non-model species. Such gene expression can then be confirmed with quantitative real-time polymerase chain reaction (qRT-PCR). Therefore, use of systematically identified suitable reference genes is a prerequisite for obtaining reliable gene expression data. Here, we calculated the expression stability of 18 candidate reference genes across samples from different tissues and grown under salt stress using geNorm and NormFinder procedures. The geNorm-determined rank of reference genes was similar to those defined by NormFinder with some differences. Both procedures confirmed that the single most stable reference gene was *ACNTIN1* for *L. barbarum* fruits, *H2B1* for *L. barbarum* roots, and *EF1α* for *L. ruthenicum* fruits. *PGK3*, *H2B2*, and *PGK3* were identified as the best stable reference genes for salt-treated *L. ruthenicum* leaves, roots, and stems, respectively. *H2B1* and *GAPDH1+PGK1* for *L. ruthenicum* and *SAMDC2+H2B1* for *L. barbarum* were the best single and/or combined reference genes across all samples. Finally, expression of salt-responsive gene *NAC*, fruit ripening candidate gene *LrPG*, and anthocyanin genes were investigated to confirm the validity of the selected reference genes. Suitable reference genes identified in this study provide a foundation for accurately assessing gene expression and further better understanding of novel gene function to elucidate molecular mechanisms behind particular biological/physiological processes in *Lycium*.

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Introduction

Lycium belong to the Solanaceae family and include seven Chinese species, *L. chinense* Miller, *L. ruthenicum* Murray, *L. truncatum* Y. C. Wang, *L. barbarum L.*, *L. cylindricum* Kuang et A. M. Lu, *L. truncatum* Y. C. Wang, and *L. yunnanense* Kuang. Of those, *L. barbarum* and *L. ruthenicum* have been extensively used as medicinal and functional foods in China for more than 2000 years. Several Chinese medicinal monographs depict their functions in nourishing the liver and kidney, enhancing eyesight, enriching blood, invigorating sex, reducing rheumatism, curing heart disease and correcting abnormal menstruation. These health-promoting phytochemical compounds, including anthocyanins and carotenoids, accumulate in *Lycium* fruits [1,2]. At this time, anthocyanin biosynthesis is well known [3] and the anthocyanin regulatory model of BMW tricomplex, formed by **b**HLH, **M**YB, and **W**D40 transcription factors, has been established [4]. The BMW tricomplex is responsible for transcription of several anthocyanin structural genes, including *flavonoid 3′hydroxylase* (*F3′H*, EC: 1.14.13.21) and *flavonoid 3′5′hydroxylase* (*F3′5′H*, EC 1.14.13.88). All anthocyanin structural genes were recently isolated and characterized in *L. ruthenicum* [5]. In addition, petunidin derivatives account for 95% of the anthocyanins in *L. ruthenicum* fruits [1], suggesting that metabolic flux was largely introduced into the delphindin branch by F3′5′H enzymes while not into the cyanidin branch by the F3′H enzyme. In the anthocyanin pathway, F3′5′H enzymes compete with F3′H enzymes for the same substrate, dihydrokaempferol, and the anthocyanin pathway in *L. ruthenicum* fruit has been predicted (Fig. S1).

L. barbarum and *L. ruthenicum* are widely cultivated and distributed in Northwest China because they are drought-, alkaline-, and salt-resistant. These unique characteristics enable *Lycium* to prevent soil desertification and improve soil salinity/alkalinity, which is necessary for ecosystem protection and agricultural stability in remote areas of Northwest China. Recently, *SlNAC1* transcripts were reported to be increased in tomato (*Solanum lycopersicum*) roots under salt-stress [6]. Thus, *SlNAC1* was thought to be a salt stress-responsive gene marker. *Lycium NAC*, which is homologous to *SlNAC1*, is a candidate gene for investigating molecular mechanisms behind *Lycium* tolerance to salt stress.

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Comparative analysis of anthocyanin biosynthesis during fruit development in two *Lycium* species

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Dietary consumption of functional foods enriched in anthocyanins benefit for human health by protection against far-ranging human diseases. Delphinidin-derived anthocyanins (valuable as blue pigments and antioxidants) are accumulated specifically in the fruits of *Lycium ruthenicum* but not in the fruits of *Lycium barbarum*, suggesting the differences of anthocyanin biosynthesis between the two species. In this study, anthocyanin profiling confirmed that anthocyanins were increasingly accumulated during fruit ripening in *L. ruthenicum*, and sharply increased at full expanded mature fruit, while no anthocyanin were detected at any stage of *L. barbarum* fruit development. Several genes involved in anthocyanin biosynthesis were characterized in *L. ruthenicum* and *L. barbarum* fruits. Expression profiling of these genes during fruit development showed a significant positive correlation between transcript abundance and anthocyanin accumulation in *L. ruthenicum* fruit. Meanwhile, transcripts in *L. barbarum* fruit were either undetectable or were downregulated during fruit ripening, before increasing slightly in the final stages of maturation. In addition, the ratio of *LrF3′5H*/*LrF3′H* transcription showed a gradual increase before 6 days after breaker (DAB) and a sharp enhancement at 10 DAB. Our results suggest that the expression patterns of both regulatory and structural genes and the transcriptional ratio of branch-node structural genes *F3′5′H*/*F3′H* may determine the phenotypic difference in anthocyanin biosynthesis between *L. ruthenicum* and *L. barbarum* fruits.

Introduction

Flavonoids are a large set of polyphenolic metabolites that function in pigmentation, fertility and signaling in plants. The flavonoid pathway is derived from the general phenylpropanoid pathway and is well-characterized and highly conserved in plant species (Grotewold 2006). The initial three steps of the flavonoid pathway convert 4-coumaroyl-CoA into dihydrokaempferol by the successive action of three enzymes; chalcone synthase (CHS, EC: 2.3.1.74), chalcone isomerase (CHI, EC: 5.5.1.6) and flavanone 3-hydroxylase (F3H, EC: 1.14.11.9; Fig. 1).

Abbreviations – 4CL, 4-coumaroyl: CoA-ligase; ANS, anthocyanin synthase; AT, anthocyanin acyltransferase; C4H, cinnamate 4-hydroxylase; CHI, chalcone isomerase; CHS, chalcone synthase; DFR, dihydroflavonol 4-reductase; F3H, flavanone 3-hydroxylase; F3′H, flavonoid 3′ hydroxylase; F3′5′H, flavonoid 3′5′ hydroxylase; GST, glutathione *S*-transferase; ORF, open reading frame; PCR, polymerase chain reaction; qRT-PCR, quantitative reverse transcription-PCR; UF3GT, flavonoid 3-glucosyl transferase; UTR, untranslated regions.

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Sunlight exclusion from Muscat grape alters volatile profiles during berry development

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ABSTRACT

The effects of sunlight exclusion on the volatile profiles of grapes during different stages of berry development were investigated by placing clusters of grapes in special boxes. Terpenes and aldehydes were the main volatile compounds in the ripe 'Jingxiangyu' berries. Sunlight exclusion was found to change volatile profiles at any stage. Sunlight exclusion from berries significantly inhibited the synthesis and accumulation of terpenes, which contribute to the characteristic aroma of Muscat grapes. However, sunlight exclusion during berry formation and veraison promoted the accumulation of aldehydes, alcohols, and ketones during the ripening stage. These results may provide important information regarding the metabolism of volatile compounds in grapes.

1. Introduction

Aroma is caused by low-molecular-weight volatile compounds, which vaporise at room temperature to emit a pleasant odour (Dunlevy, Kalua, Keyzers, & Boss, 2009). Aroma compounds are naturally present in all fruits. They are commonly classified into families based on the chemical nature of the substrate, such as volatile fatty acids or esters, lactones, aldehydes, alcohols, ketones, terpenes, and some other groups such as carotenoid-derived aroma compounds (Dastager, 2009). Aroma contributes to the desirable taste of food, which attracts the consumer's preference (Li, Gan, Bueckert, & Warkentin, 2010). It also determines the unique varietal character and the quality of fruit.

Several studies have focused on the identification and quantification of volatile aroma compounds in different grape cultivars. Grape cultivars can be often distinguished by the presence of these characteristic aroma compounds. Esters are the most abundant of the aroma compounds that contribute to the strawberry-like odour in the *Vitis labrusca* grape and its hybrids with *Vitis vinifera* and *Vitisamurensis* (Yang et al., 2009). Terpenes, especially monoterpenes, are the main contributors to the characteristic floral aroma of Muscat grapes (*V. vinifera* L.) (Strauss, Wilson, Gooley, & Williams, 2010). Terpenes play important roles not only in table Muscat grapes but also in Muscat wines (Bordiga et al., 2013; Selli, Canbas, Cabaroglu, Erten, & Gunata, 2006). They undergo minimal changes in wines during the fermentation process and can be defined as grape-derived wine aromatic compositions. Approximately 70 monoterpenes have been identified in grapes and wine (Rapp, 1998). The free monoterpenes most often found in grapes and wines are citronellol, 3,6-dimethyl-1,5-octadien-1,7-diol, linalool, geraniol, nerol, and α-terpineol (Rapp, 1998).

Sunlight is one of the natural terroir factors. These factors influence the growth of grapevines and chemical composition and the content of berries. Grape berries exposed to sunlight during development generally have higher levels of sugar, anthocyanins, and phenolics, but lower malate levels and less titratable acidity than berries shaded by canopies (Kliewer, 1977; Reynolds, Pool, & Mattick, 1986). Recently, the increasing interest in the manner in which sunlight affects fruit volatiles has encouraged remarkable research involving sunlight exclusion (Liu & Liu, 2012; Watson, Wright, McBurney, Taylor, & Linforth, 2002). Nevertheless, there has been only a handful of studies in grapes. The concentration of volatile compounds such as terpenes and C13-norisoprenoids tends to be low in sun-deprived bunches (Bureau, Razungles, & Baumes, 2000; Skinkis, 2010). However, excessive sun exposure can also negatively affect the concentration of terpenes (Belancic

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Optimization of linkage mapping strategy and construction of a high-density American lotus linkage map

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Abstract

Background: Lotus is a diploid plant with agricultural, medicinal, and ecological significance. Genetic linkage maps are fundamental resources for genome and genetic study, and also provide molecular markers for breeding in agriculturally important species. Genotyping by sequencing revolutionized genetic mapping, the restriction-site associated DNA sequencing (RADseq) allowed rapid discovery of thousands of SNPs markers, and a crucial aspect of the sequence based mapping strategy is the reference sequences used for marker identification.

Results: We assessed the effectiveness of linkage mapping using three types of references for scoring markers: the unmasked genome, repeat masked genome, and gene models. Overall, the repeat masked genome produced the optimal genetic maps. A high-density genetic map of American lotus was constructed using an F_1 population derived from a cross between *Nelumbo nucifera* 'China Antique' and *N. lutea* 'AL1'. A total of 4,098 RADseq markers were used to construct the American lotus 'AL1' genetic map, and 147 markers were used to construct the Chinese lotus 'China Antique' genetic map. The American lotus map has 9 linkage groups, and spans 494.3 cM, with an average distance of 0.7 cM between adjacent markers. The American lotus map was used to anchor scaffold sequences in the *N. nucifera* 'China Antique' draft genome. 3,603 RADseq markers anchored 234 individual scaffold sequences into 9 megascaffolds spanning 67% of the 804 Mb draft genome.

Conclusions: Among the unmasked genome, repeat masked genome and gene models, the optimal reference sequences to call RADseq markers for map construction is repeat masked genome. This high density genetic map is a valuable resource for genomic research and crop improvement in lotus.

Keywords: Chinese lotus, Genome assembly, Genotyping by sequencing, Restriction associated sequencing, Megascaffold

Background

The Nelumbonaceae is a perennial, aquatic plant family, which comprises only one genus, *Nelumbo,* consisting of two species: Chinese lotus *N. nucifera* Gaertn (Asia, Australia, Russia) and American lotus *N. lutea* Pers (eastern and southern North America) [1]. Lotus is a diploid plant (2n = 2× = 16) with agricultural, medicinal, and ecological significance. Chinese lotus (*N. nucifera* Gaertn) is cultivated for its edible rhizomes, seeds, and leaves, which have been consumed as food for thousands of years in Asia. Nearly all parts of lotus have been used as herbal medicines to treat cancer, depression, diarrhea, heart problems, hypertension, insomnia, pyrexia, and obesity [2-4]. Lotus has been shown to be an effective phytoremediator, playing a critical role in removal of heavy metals from polluted water [5,6]. *N. nucifera* 'Chinese Antique' and *N. lutea* 'AL1' are geographically isolated and have distinct morphological traits [7]. American lotus has been used to transfer desirable ornamental flower traits to Chinese lotus germplasm.

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The *Epimedium wushanense* (Berberidaceae) species complex, with one new species from Sichuan, China

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Abstract

Epimedium wushanense (Berberidaceae) as treated in the Flora of China includes four species similar in leaflet shape: *E. wushanense*, *E. ilicifolium*, *E. jinchengshanense* (sp. nov.), and *E. pseudowushanense*. Its seven type specimens represent three of the four species. In the present paper, *E. wushanense* is identified according to morphological characters of its holotype. Except for four of the seven type specimens belonging to *E. wushanense* and *E. ilicifolium*, the remaining three specimens represent a new species, *E. jinchengshanense*. Furthermore, *E. wushanense* from Guangxi and Guizhou as treated in the Flora of China is recognized as *E. pseudowushanense*. *Epimedium wushanense*, *E. ilicifolium*, *E. jinchengshanense,* and *E. pseudowushanense* differ by their distributions and flowers. Based on the floral characters, *E. jinchengshanense* is grouped into ser. *Dolichocerae*, *E. wushanense* is adjusted from ser. *Dolichocerae* to ser. *Davidianae*, and *E. ilicifolium* is moved from ser. *Davidianae* to ser. *Dolichocerae*.

Key words: Berberidaceae, *Epimedium wushanense*, species complex, revision.

Introduction

Epimedium L. (1753: 117) is the largest herbaceous genus of the Berberidaceae and contains approximately 58 species distributed disjunctly and very unevenly in the Mediterranean region and eastern Asia (Stearn 2002; Ying *et al*. 2011). As the diversity center of *Epimedium*, China possesses about 48 species of the genus which are all endemic except *Epimedium koreanum* Nakai (1936: 63). In his revision of *Epimedium*, Stearn (2002) grouped all of China's endemic species into section *Diphyllon* (Kom.) Stearn (2002: 48), which was divided into four series based mainly on floral morphology, particularly petal characteristics.

Epimedium wushanense T.S. Ying (1975: 55), of sect. *Diphyllon*, was published based on seven collections from Sichuan (and Chongqing), China. One of the most diagnostic characters of this species is its lanceolate or narrowly lanceolate leaflet. Stearn (2002) proposed *E. wushanense* with long-spurred petals lacking lamina, classifying it into series *Dolichocerae* Stearn (1938: 509). In the Flora of China, Ying *et al*. (2011) listed its distribution as Chongqing, Guangxi, Guizhou, Hubei, and Sichuan; however, *E. wushanense* from Guangxi and Guizhou was recognized as an insufficiently known species, *Epimedium pseudowushanense* B.L. Guo (2007: 814). Based on our extensive studies on plants in herbaria, the field, and cultivation, we found that *E. wushanense* described in the Flora of China actually includes four distinct species, which are similar in leaflet shape, with a new species *Epimedium jinchengshanense* Y.J. Zhang & J.Q. Li. In the present paper, we revise the *E. wushanense* species complex and discuss the differences of these four species in their morphology and distribution.

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Taxonomic and phylogenetic analysis of *Epimedium* L. based on amplified fragment length polymorphisms

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ABSTRACT

Epimedium is well known for its ornamental and medicinal value. The genus consists of ca. 58 species disjunctly distributed in the Mediterranean region and eastern Asia, with the highest species diversity concentrated in central-southeastern China. In the present research, we collected 144 accessions from 58 *Epimedium* species and one accession representing the outgroup *Vancouveria hexandra*. Using Bayesian analysis, the phylogeny of *Epimedium* was reconstructed based on amplified fragment length polymorphism data. The dendrogram suggested that two subgenera and four sections of *Epimedium* were monophyletic. Chinese sect. *Diphyllon* was divided into five well-supported clades related to flower morphology except that five species were either isolated or formed a general polytomy. The result also well supported the recent morphological revision on *E. reticulatum*, *E. wushanense*, *E. ilicifolium*, *E. jinchengshanense*, *E. simplicifolium*, *E. chlorandrum*, *E. brachyrrhizum*, and *E. dewuense*, and provided significant implications for *E. sagittatum* complex. The present research is of great implication for facilitating the utilization of natural germplasm of *Epimedium*, especially for further development of new cultivars for ornamental and medicinal purposes.

1. Introduction

Epimedium species has been known as an exotic perennial garden plant in western countries and also used as a herbal medicine in Asian countries. Bearing attractive foliage and flowers, *Epimedium* has become a popular shading plant with great commercial prospects (Lubell and Brand, 2005; Ren et al., 2008; Avent, 2010). Meanwhile, modern pharmaceutical studies have verified its wide-reaching activity against sexual dysfunction, osteoporosis, cardiovascular diseases, menstrual irregularity, asthma, chronic nephritis, and immunoregulation (Ma et al., 2011; Gao et al., 2012; Li et al., 2012; Yin et al., 2012). The recent focus on the research and development of *Epimedium* has prompted the need for in-depth taxonomic and phylogenetic studies of the genus, especially for the highly diversified Chinese taxa (Shen et al., 2007; Guo et al., 2008; Govindaraghavan et al., 2012).

The genus *Epimedium* L. (Berberidaceae) contains approximately 58 species of herbs in the North Temperate Zone, distributing disjunctly and very unevenly in the Mediterranean region and eastern Asia (Stearn, 2002; Ying et al., 2011). Based on flower and leaf morphology, C-banding of chromosomes (Takahashi, 1989), and geographical distribution, the updated system of the genus comprised two subgenera, four sections, and four series (Stearn, 2002). Subgenus *Rhizophyllum* comprised *E. perralderianum* endemic to Algeria and *E. pinnatum* from Caucasia. Subgenus *Epimedium* consisted of four sections: (1) section *Epimedium* with *E. alpinum* in Alps and Balkan areas and *E. pubigerum* from Caucasia; (2) section *Polyphyllon*, comprising *E. elatum*, limited to the western Himalaya; (3) section *Macroceras* with six species distributed in Japan, Korea, northeastern China, and Far Eastern Russia; and (4) section *Diphyllon*, with about 47 known species in central-southeastern China, was further subdivided into four series based mainly on flower morphology, particularly on the petal characteristics (Fig. 1).

China is the diversity center of *Epimedium*, containing over 80% of species of the genus (Stearn, 2002; Ying, 2002; Ying et al., 2011). Chinese *Epimedium* has presented a number of taxonomic questions with about 30 species published in the past 30 years (Ying et al., 2011; Govindaraghavan et al., 2012). The poor quality of type specimen and inadequate investigation for some species might result in inaccurate morphological descriptions, controversial subgenera classification, and publication of synonymous new

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The Putative E3 Ubiquitin Ligase ECERIFERUM9 Regulates Abscisic Acid Biosynthesis and Response during Seed Germination and Postgermination Growth in Arabidopsis[1][W][OPEN]

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The *ECERIFERUM9* (*CER9*) gene encodes a putative E3 ubiquitin ligase that functions in cuticle biosynthesis and the maintenance of plant water status. Here, we found that CER9 is also involved in abscisic acid (ABA) signaling in seeds and young seedlings of Arabidopsis (*Arabidopsis thaliana*). The germinated embryos of the mutants exhibited enhanced sensitivity to ABA during the transition from reversible dormancy to determinate seedling growth. Expression of the *CER9* gene is closely related to ABA levels and displays a similar pattern to that of *ABSCISIC ACID-INSENSITIVE5* (*ABI5*), which encodes a positive regulator of ABA responses in seeds. *cer9* mutant seeds exhibited delayed germination that is independent of seed coat permeability. Quantitative proteomic analyses showed that *cer9* seeds had a protein profile similar to that of the wild type treated with ABA. Transcriptomics analyses revealed that genes involved in ABA biosynthesis or signaling pathways were differentially regulated in *cer9* seeds. Consistent with this, high levels of ABA were detected in dry seeds of *cer9*. Blocking ABA biosynthesis by fluridone treatment or by combining an ABA-deficient mutation with *cer9* attenuated the phenotypes of *cer9*. Whereas introduction of the *abi1-1*, *abi3-1*, or *abi4-103* mutation could completely eliminate the ABA hypersensitivity of *cer9*, introduction of *abi5* resulted only in partial suppression. These results indicate that CER9 is a novel negative regulator of ABA biosynthesis and the ABA signaling pathway during seed germination.

Seed germination is a critical stage in the life cycle of higher plants, during which the imbibed mature seed must shift rapidly from a state of quiescence to one of active metabolism. The transition from embryo dormancy to germination requires the activation of a series of developmental programs with the simultaneous mobilization of seed storage reserves and the loosening of the seed coat; these processes cumulate in radicle emergence, seedling establishment, and subsequent photoautotrophic growth (Bewley, 1997; Finkelstein et al., 2008; Holdsworth et al., 2008; Rajjou et al., 2012). The decision for a seed to germinate depends on certain intrinsic factors and many environmental factors, including water availability, temperature, light, and mineral availability. Among the internal factors, endogenous abscisic acid (ABA) and GA are two important regulators that also mediate interactions with these environmental factors in modulating seed germination. In fact, seed germination or dormancy largely depends on the ratio of these two hormones, which play antagonistic roles in seed germination. ABA inhibits seed germination, and mutants defective in ABA biosynthesis or signaling have enhanced germination efficiency and more easily break dormancy under osmotic stress. Conversely, GA promotes seed germination, and GA-deficient mutants show delayed seed germination (Finkelstein et al., 2008; Holdsworth et al., 2008; Rajjou et al., 2012).

Protein degradation through the ubiquitination pathway is an important mechanism in regulating hormone biosynthesis and signaling. To date, there are over 1,000 predicted E3 ubiquitin ligases in Arabidopsis

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RESEARCH PAPER

Comprehensive analysis of cystatin family genes suggests their putative functions in sexual reproduction, embryogenesis, and seed formation

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Abstract

Cystatins are tightly bound and reversible inhibitors of cysteine proteases in C1A and C13 peptidase families, which have been identified in several species and shown to function in vegetative development and response to biotic/abiotic stresses in plants. Recent work revealed their critical role in regulating programmed cell death during embryogenesis in tobacco and suggested their more comprehensive roles in the process of sexual plant reproduction, although little is known about cystatin family genes in the processes. Here, 10 cystatin family genes in *Nicotiana tabacum* were identified using an expressed sequence tag (EST)-based gene clone strategy. Analysis of their biochemical properties showed that nine of them have the potency to inhibit the activities of both commercial cathepsin L-like proteases and extracted cysteine proteases from seeds, but with different K_i values depending on the types of proteases and the developmental stages of the seed tested. This suggests that cystatin-dependent cathepsin L-like proteolytic pathways are probably important for early seed development. Comprehensive expression profile analysis revealed that cystatin family genes showed manifold variations in their transcription levels in different plant cell types, including the sperm, egg, and zygote, especially in the embryo and seed at different developmental stages. More interestingly, intracellular localization analysis of each cystatin revealed that most members of cystatin families are recognized as secretory proteins with signal peptides that direct them to the endoplasmic reticulum. These results suggest their widespread roles in cell fate determination and cell–cell communication in the process of sexual reproduction, especially in gamete and embryo development, as well as in seed formation.

Key words: Cathepsin L-like proteases, cystatin, embryogenesis, seed development, sexual reproduction, tobacco.

Introduction

Cystatins are tightly bound and reversible inhibitors of papain-like and legumain-like proteases, which have been identified in vertebrates, invertebrates, plants, and other organisms. Notably, cystatins in plants form an independent subfamily clustering in a branch distinct from other cystatin families on the phylogenetic tree (Margis *et al.*, 1998). Most cystatins in plant have a molecular mass in the 12–16 kDa range (Misaka *et al.*, 1996; Martinez *et al.*, 2005), and a

Abbreviations: ER, endoplasmic reticulum; ORF, open reading frame; PCD, programmed cell death; RACE, rapid amplification of cDNA ends; RT–PCR, reverse transcription–PCR; RT–qPCR, quantitative real-time reverse transcription–PCR.

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Transcriptome analysis and transient transformation suggest an ancient duplicated MYB transcription factor as a candidate gene for leaf red coloration in peach

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