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

2012 年报

Annual Report

中国科学院武汉植物园

Wuhan Botanical Garden,Chinese Academy of Sciences

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目　　录

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

实验室代码：2009DP173234

依托单位：中国科学院武汉植物园

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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	草坪草狗牙根对干旱、水淹胁迫的反应机制；植物激素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；草坪草及生态修复用草新品种选育与应用；草坪草逆境分子生理和代谢调控机制
3	植物功能代谢组学	郭明全	基于功能代谢组学的药用植物资源品种筛选、功能活性成分分布和调控规律；药用植物功能化合物的药理活性；基于药用植物功能活性成分的可持续性利用
4	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改良与新种质的创制
5	植物保育遗传学	黄宏文	猕猴桃遗传资源的收集与评价；植物的濒危机制和保育原理研究；猕猴桃特异资源发掘及育种改良
6	系统与进化植物学	李建强	重要关键植物类群的分类学、系统发育和适应性进化
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	种群遗传学	王　艇	萝卜雄性不育育性恢复分子机理及基因挖掘；叶绿体进化基因组学；隐花色素基因家族的适应性进化
10	比较功能基因组学	王　瑛	特种药用植物资源（淫羊藿、枸杞、甘草、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
11	农业环境与保护	王　勇	三峡水库消落区植被恢复及植物水淹适应机理；华中特色农业种质发掘与创制；生态农业园的规划设计；三峡库区特有珍稀植物保护
12	资源植物繁殖生物学	杨平仿	植物有性生殖过程中花粉与雌蕊的识别机制；植物种子萌发的分子调控机制；莲经济性状的评价及其形成的遗传机理
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 114 项，总经费 10475 万元，当年实到经费 2158 万元（见附录一），其中 2012 年新增课题 35 项， 新增科研经费 2506 万元。在研课题包含：

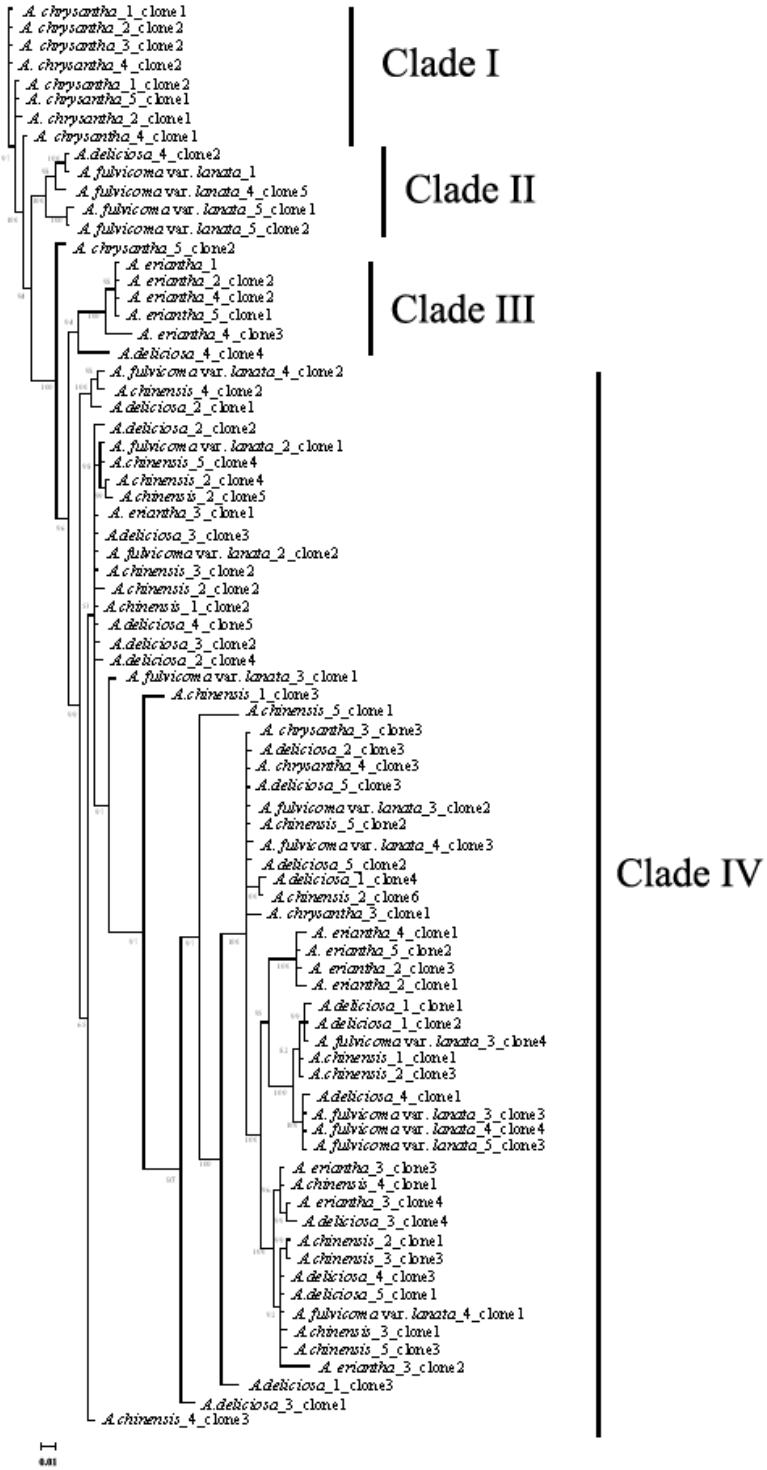
973 项目	2 项
863 项目	1 项
国家重大专项	5 项
行业重大专项	4 项
国家基金重大项目	1 项
国家基金重点项目	2 项
国家自然科学基金面上和青年基金项目	24 项
国际合作项目	4 项
院重大项目	6 项
省部委项目	11 项
横向合作及其它项目	54 项

2012 年度获国家自然科学基金资助项目（2013 年开始执行）12 项，其中面上项目 7 项，青年科学基金项目 5 项，资助经费总额 620 万元。

（一）特色农业资源植物保育原理

1. 基于 EPIC 标记的猕猴桃属植物同域分布物种的基因渐渗

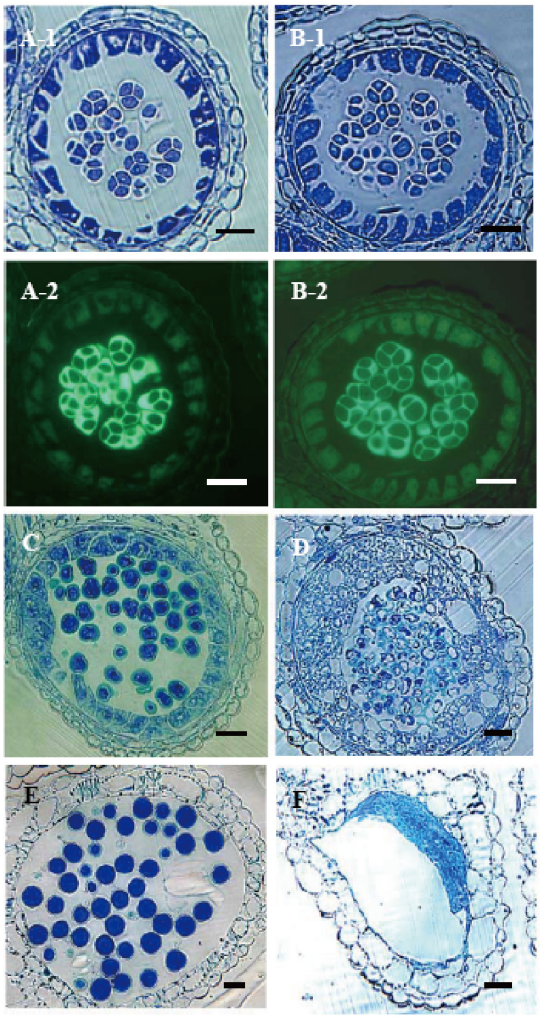
EPIC 标记是一种基于基因内含子的分子标记。采用生物信息学方法以葡萄基因组作为参考序列，与中华和美味猕猴桃的非冗余 EST 序列比较，成功开发 21 对 EPIC 引物。采用其中的 3 对 EPIC 引物和 1 对叶绿体基因组引物（trnL-trnF）对同域分布的 5 种猕猴桃属植物的杂交渐渗情况进行了检测。系统发育分析结果表明 3 对核标记基因树的拓扑结构相似，但与叶绿体基因树的拓扑结构相比存在差异，这说明猕猴桃属植物可能存在杂交渐渗事件和网状进化格局。此外还发现同域分布的中华猕猴桃与美味猕猴桃、黄毛猕猴桃与毛花猕猴桃、金花猕猴桃和毛花猕猴桃与中华-美味猕猴桃间存在一定程度的基因流。研究对于理解同域分布物种有基因流存在下的物种形成模式以及物种的适应性进化具有重要的理论意义。



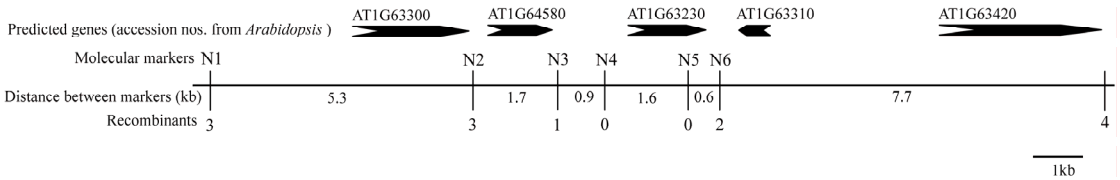
基于 EPIC1 的猕猴桃属植物的系统发育树

2. 植物细胞质雄性不育育性恢复机理研究

利用育性分离大群体，采用同源搜索 BAC 文库和图位克隆策略，阐明了杂合位点控制育性恢复的分子特征，为植物杂种优势利用提供了潜在价值基因。



雄性不育花药组织学分析

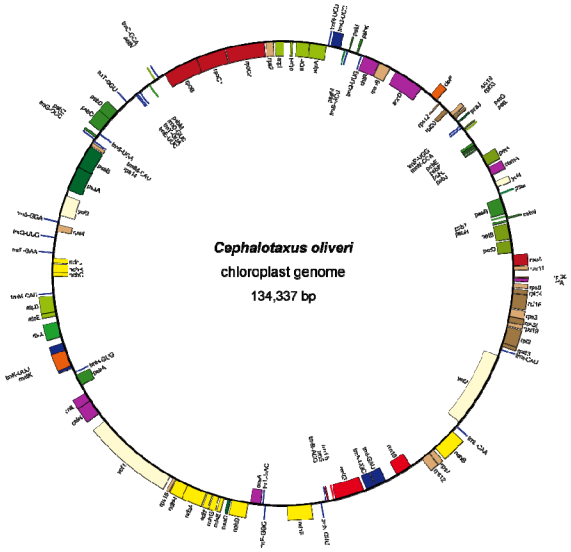


杂合位点遗传物理图谱

3. 篦子三尖杉的叶绿体进化基因组学研究

裸子植物篦子三尖杉属三尖杉科（Cephalotaxaceae）三尖杉属（*Cephalotaxus*），为我国特有珍稀濒危植物。通过测定篦子三尖杉的叶绿体基因组全序列，进行了裸子植物范围内的进化基因组学分析。在篦子三尖杉和台湾三尖杉叶绿体全序列水平，考察重复序列、插入缺失（Indel）和碱基替换三者之间的相互关系，发现三者之间存在显著相关性，且重复序列和其他两者的相关度最高，暗示重复序列可能对插入/缺失和碱基替换的发生起关键作用；在重建松杉类叶绿体基因组的基因排列顺序形成过程时，发现由于叶绿体 DNA 存在异构现象，所以目前还无法确定松科和非松科植物是否丢失了相同的反向重复区；解释了重复序列在篦子三尖杉等的 *accD* 基因扩增中的重要作用。上述研究结果为进

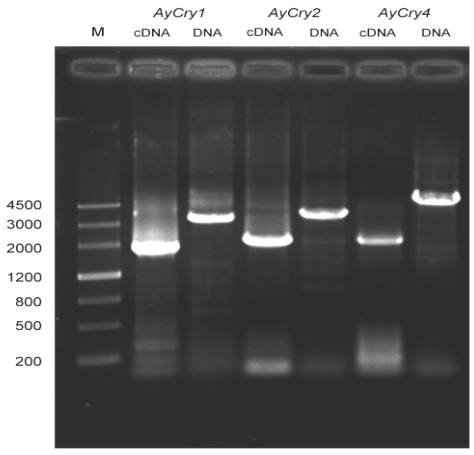
一步开展篦子三尖杉这一重要植物资源在全球气候变化背景下的分子适应性及保育研究奠定基础。



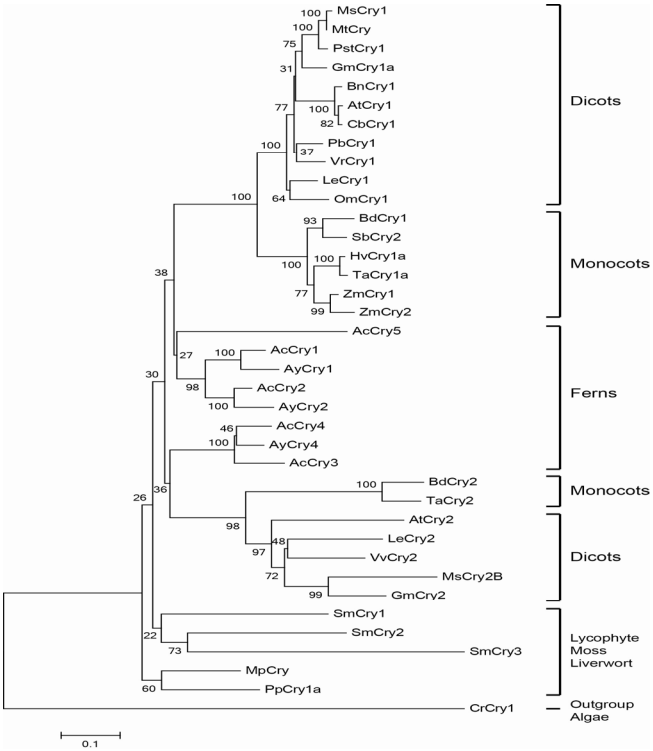
篦子三尖杉叶绿体基因组图谱

4. 蕨类植物隐花色素基因家族的适应性进化

通过设计简并引物进行 PCR 扩增和基因组步移技术，获得了云南铁角蕨和长叶铁角蕨隐花色素基因家族 3 个成员的全长序列；根据已知序列设计引物扩增铁角蕨科其它植物的 PHR 结构域，目前已经获得包括巢蕨、华中铁角蕨等在内的 10 种植物隐花色素基因 PHR 结构域的序列。上述研究结果将为下一步进行蕨类植物隐花色素基因家族的适应性进化研究奠定基础。



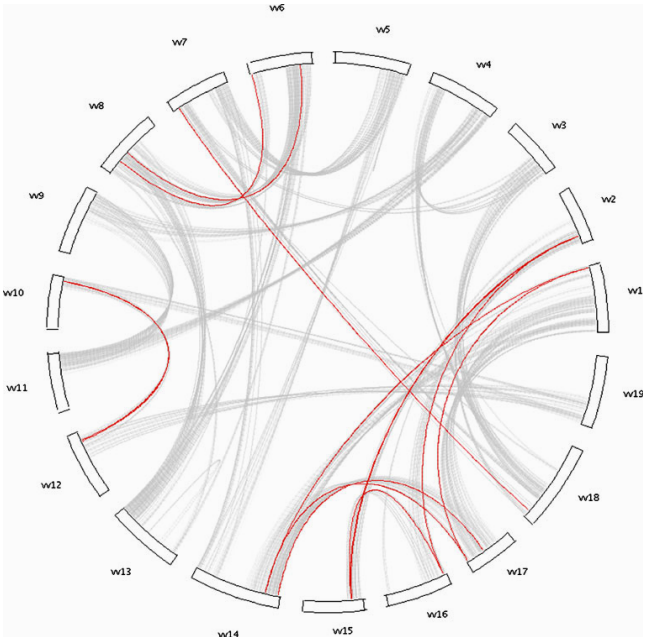
云南铁角蕨隐花色素基因家族 3 个成员的 RT-PCR 结果图



植物隐花色素基因 PHR 结构域的系统发育树

5. 葡萄基因组加倍和 NAC 基因家族产生方式解析

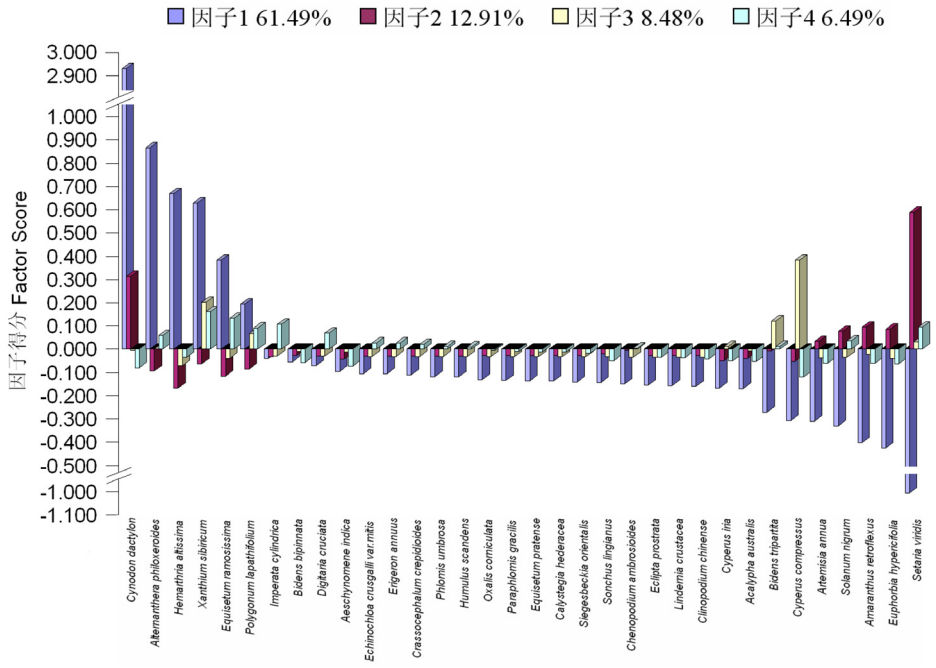
利用国际上公布的葡萄参考基因组序列，对葡萄基因组在进化过程中的加倍事件进行了深入解析。研究发现，葡萄基因组内不同加倍方式产生新基因的进化选择模式显著不同，对产生大基因家族的贡献也存在明显差异。此外，通过比较几个不同物种的关键性状基因数目发现，控制葡萄果实风味的一类转录因子具有显著的冗余性。基于这些进化规律，研究人员对葡萄 NAC 基因家族成员的产生、进化时间、基因结构和组织表达特异性进行了深入分析，并发现数个极具抗逆生物工程育种应用价值的 NAC 基因。



葡萄基因组加倍和 NAC 基因家族产生方式解析

6. 三峡水库消落区不同海拔间植物多样性和植物群落结构差异研究

从 α 多样性上来看，上部和中部消落区物种丰富度和均匀度不存在显著差异，但下部消落区丰富度指数明显低于中上部。下部消落区植物种间相遇几率较大，植物种间相互依存性较强。从 β 多样性上来看，由上部到中部再到下部，随着海拔下降，水库消落区植物物种的替代性均质地减少；不同地区间 β 多样性差异不显著，但不同海拔间存在显著差异。水库消落区植物群落结构稳定性中部＜上部＜下部，上部消落区水淹胁迫较小，植物物种多为竞争种（C-对策种），竞争力较强的杂草偏向形成优势群落；下部消落区水淹胁迫最强，植物物种多为耐胁迫种（S-对策种），能忍受高强度水淹环境的物种形成了植物群落；中部消落区，处于物种定居和水淹胁迫的双重压力下，竞争种和耐胁迫种间竞争明显，更偏向于形成共优群落，其群落稳定性较差。在目前情况下，消落区下部的植物群落组成比较单一，但是随着水库蓄水高程稳定在 175 m，预计消落区上中部群落组成也会逐渐趋于单一化。

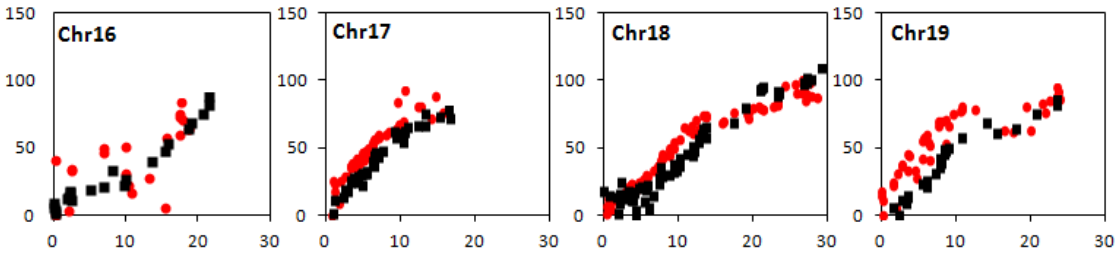


三峡水库消落区下部植物群落结构因子得分图

（二）特色农业资源植物优质和抗性性状的生物学基础

1. 构建葡萄高密度遗传图谱

首先构建了葡萄种间杂交 F1 群体，利用 RAD-seq 分子标记技术，构建了一张目前文献报道的最高密度遗传图谱。该图谱含有完整的序列信息 SNP 分子标记约 4400 个，全部 19 个连锁群总遗传距离约 2000 厘摩。该遗传图谱将用于葡萄关键农艺性状 QTL 定位，并开发与优异性状紧密连锁的分子标记，应于用葡萄分子标记辅助育种。



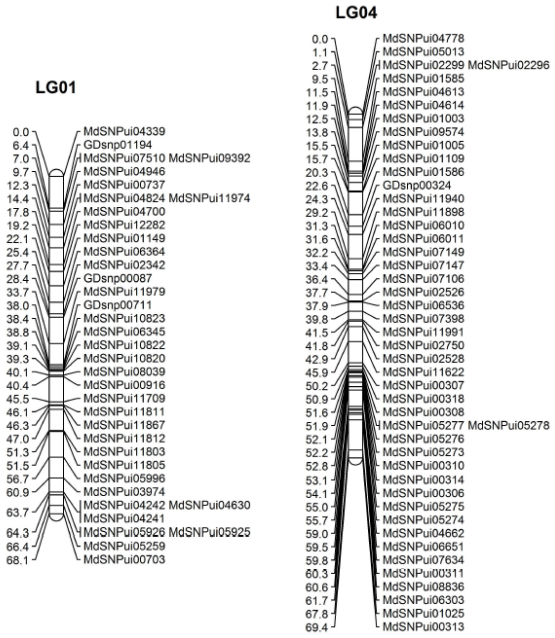
葡萄部分染色体物理图谱和遗传图谱共线性分析

2. 苹果果实糖酸代谢调控机制

围绕苹果糖、酸品质性状，首先利用红玉/金冠 F_1 分离群体构建了苹果基因组连锁遗传图谱，测定了分离群体果实品质性状，包括苹果酸、果糖、葡萄糖、蔗糖等组分含量，在第 8 号染色体检测到 1 个与苹果酸含量相关的 QTL，该 QTL

可以解释表型变异的 13%，在第 5 号连锁群发现了两个分别控制果糖和蔗糖含量的 QTLs，贡献率 13-14%。

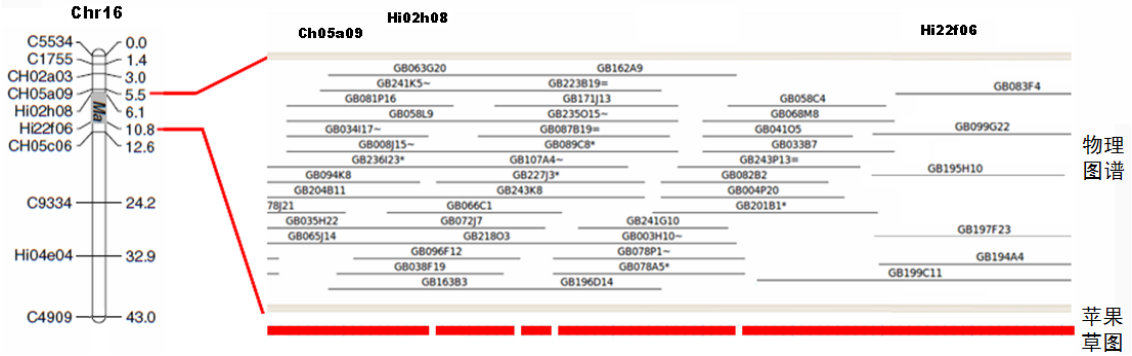
其次，分析了来自 14 个苹果品种的 27.2 万个 ESTs 序列，发掘了 37807 个 SNPs 位点，其中，12299 个 SNPs 两侧序列长度大于 60 bp，用于合成了包含 1536 个 SNPs 标记的 Illumina GoldenGate 芯片。利用该 SNP 芯片对‘望山红’×‘鸡冠’F_1 分离群体进行基因型检测，构建了基于 SNP 标记的遗传图谱，同时，完成了该群体的糖、酸组分与含量的测定，果实品质性状的 QTL 定位研究也正在进行中。



基于 SNP 标记的苹果遗传连锁群

最后，构建了覆盖第 16 号染色体上控制苹果酸含量 *Ma* 基因位点的 BAC 重叠群，该 contig 物理距离总长~ 428 kb，位于 Hi0h08 和 Hi22f6 两个 SSR 分子标记之间，两标记之间的遗传距离为 4.7 cM，*Ma* 基因位于一个“hot”染色体区段，在“hot”区段开发了 15 个新的 SSR 标记，结合 389 份苹果种质资源采用区间关联作图分析方法获得了与 *Ma* 基因精密连锁标记。

上述研究不仅对于认识苹果果实糖酸代谢调控机制具有重要的理论意义，而且也为苹果果实口感品质性状的遗传改良提供了分子工具。



覆盖苹果 Ma 基因区段的 BAC 物理图谱

3. 黑麦草耐盐机制研究

以耐盐黑麦草为研究对象，分别从生理、生化和分子角度深入探讨了黑麦草耐盐的机制。发现盐胁迫能降低草坪质量、相对蒸腾速率和叶绿素含量，提高脂质过氧化水平，并使黑麦草地上和地下部分积累更多的 Na^+，而降低 K^+/Na^+比，以及 Mg^{2+}和 P 的含量。在耐盐黑麦草中，CAT 酶活性在盐处理后显著高于对照；而在盐敏感基因型黑麦草中，CAT 酶活性一直低于对照，且其 MDA 含量、EL、H_2O_2 受盐胁迫上升幅度更大。此外，在耐盐黑麦草中，抗氧化酶基因的表达水平相对较高。表明黑麦草的耐盐性可能与抗氧化酶基因的组成型/诱导型表达有关。利用 SSH 技术构建了黑麦草盐胁迫差减杂交 cDNA 文库，发掘了一批可能与黑麦草耐盐有关的特异基因，这些基因根据所编码的蛋白功能可分为 11 个类别。克隆得到了 11 个关键基因的全长 cDNA 序列，发现其中部分基因（如 *PrP5CS* 等）可能与黑麦草耐盐性具有较强的联系。上述研究结果为从不同层面深入理解黑麦草的耐盐机制以及高效发掘黑麦草优异耐盐基因奠定了基础，并为植物转基因抗逆育种提供了基因材料。

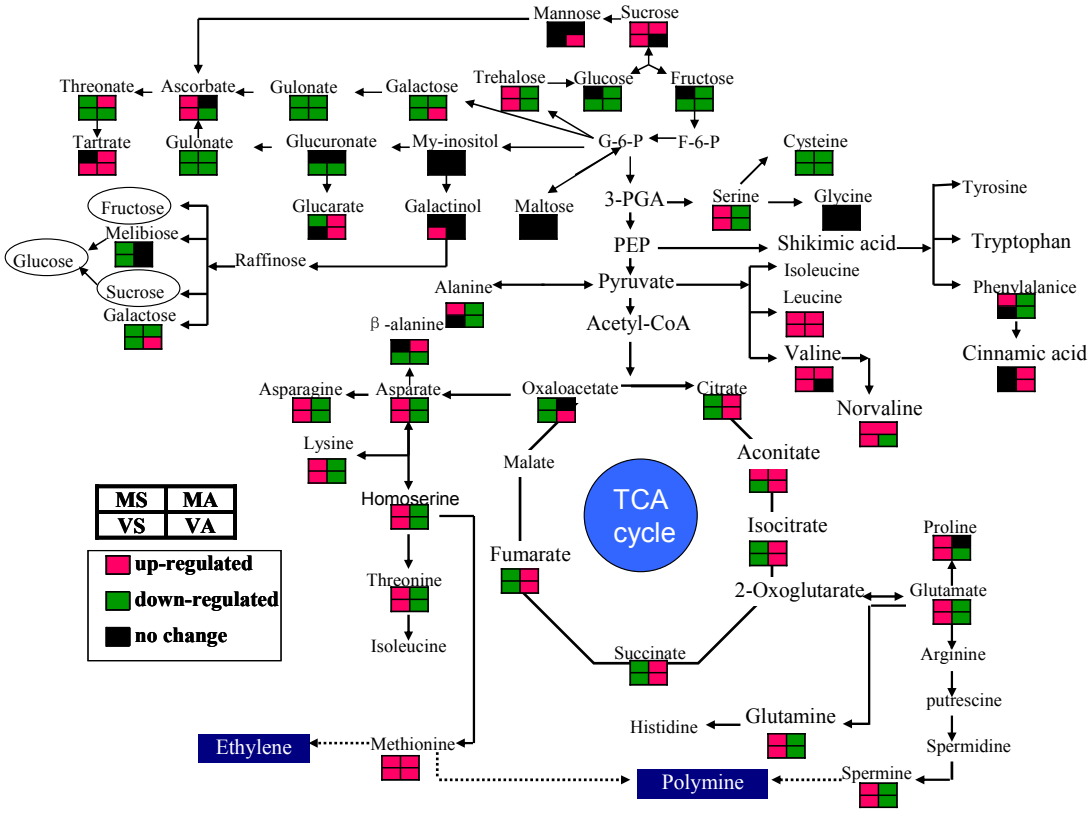
Journal of the American Society for Horticultural Science（2012, 137(1)）杂志

采用重点实验室草坪种质资源学学科组集体照为封面

4. 早熟禾耐盐碱的机制研究

对早熟禾耐盐碱的代谢机制进行研究发现，碱性盐比中性盐对早熟禾的伤害更大，两种不同的盐胁迫适应性机制在代谢途径上具有明显的差异；在碱性盐胁迫下，有机酸（糖酸和 TCA 中间产物）含量升高，而在中性盐胁迫下，以 TCA 循环中间产物为碳骨架的氨基酸（天冬氨酸家族和谷氨酸家族）的含量升高。在碱性盐胁迫下，植株内以 pH 调控为主要目的的有机酸合成代谢加强，而在中性

盐胁迫下，体内以合成渗透调节物质为主要目的的氨基酸合成途径加强。草地早熟禾对碱性盐和中性盐胁迫具有不同的代谢响应机制的阐明，对培育抗盐新品种具有重要的价值。



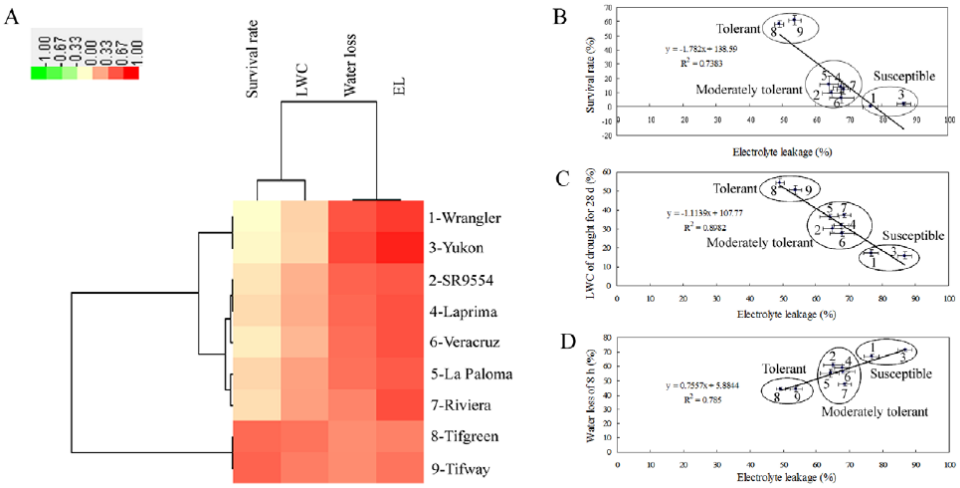
盐碱胁迫下的早熟禾代谢调控网络

MS: 早熟禾品种 1 盐处理；MA: 早熟禾品种 1 碱处理

VS: 早熟禾品种 2 盐处理；VA: 早熟禾品种 2 碱处理

5. 狗牙根抗旱机理研究

评价了 9 个商业化的狗牙根品种对干旱以及水淹胁迫的抗性，鉴定出了抗性较强的‘Tifgreen’和抗性较弱的‘Yukon’狗牙根材料。发现了狗牙根抗旱性强弱与狗牙根体内活性氧代谢渗透调节物质的积累相关。抗性强的‘Tifgreen’材料抗氧化酶活性强、活性氧含量积累量低、渗透调节物质含量高、膜氧化损伤小，从而在干旱条件下成活率高。上述研究为揭示草坪草狗牙根的抗旱机理和抗旱品种选育提供了理论依据。



9 种不同的商业化狗牙根材料对干旱胁迫的抗性差异

A：狗牙根材料抗性差异的聚类分析图；B：狗牙根材料干旱处理以后的存活率；C：狗牙根材料干旱处理以后的叶片水分含量；D：狗牙根材料的失水率。

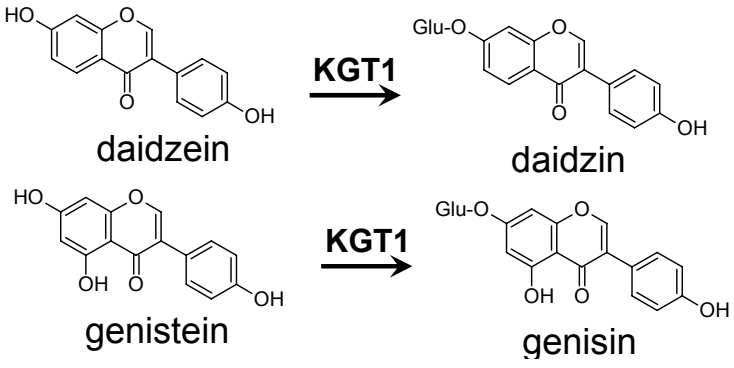
6. 不同逆境胁迫间的信号传导通路交叉

通过转录组学的分析，发现不同逆境胁迫之间存在信号传导通路上的交叉。在对盐胁迫和干旱胁迫的研究中，我们发现低浓度的盐胁迫处理，可以提高包括草坪狗牙根以及模式植物在内的多种植物对干旱胁迫的抗性。低浓度的盐胁迫处理以后，诱导植物大量逆境相关基因表达水平的上调（包括 DREB，COR，LTI 等基因），同时在生理以及生化水平发生相应的变化（包括活性氧代谢以及渗透调节物质的变化）。这些研究结果为在生产上利用轻微的逆境胁迫来提高植物的抗性提供理论依据以及实际操作的可能性。

（三）特色农业资源植物的种质创新和可持续利用

1. 野葛

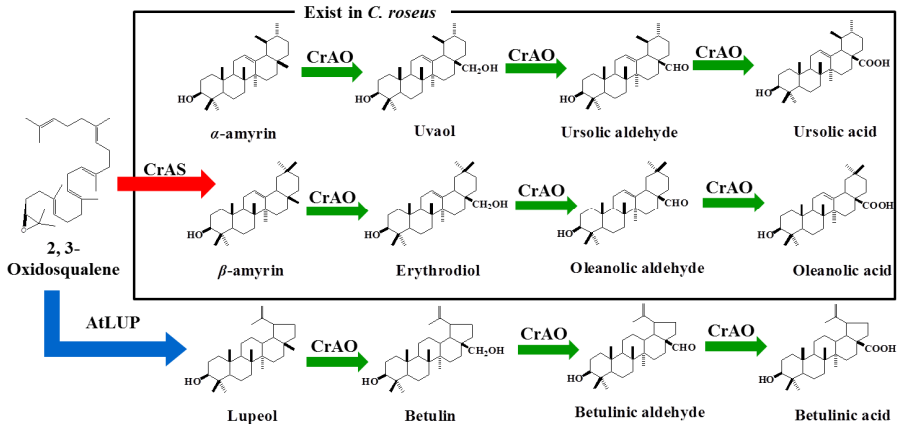
成功地从野葛的根中分离到异黄酮特异的糖基转移酶基因 ***KGT1***，该基因主要参与野葛体内异黄酮化合物大豆苷与染料木苷的生物合成。



分离的 KGT1 基因参与野葛大豆苷与染料木苷的生物合成

2. 长春花

结合长春花 EST 文库的分析与 RACE-PCR 技术的应用，从药用植物长春花中分离了参与三萜物质合成的环化酶基因（***CrAS***）与 P450 基因（***CrAO***），尤其重要的是所分离的 P450 基因可用于抗癌化合物“白桦脂酸”的合成，上述研究为白桦脂酸的生物合成提供了必需的基因资源。

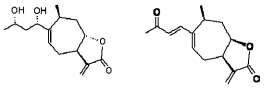
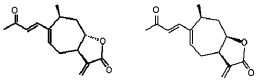
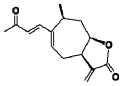
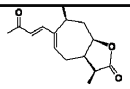


分离的 CrAS 与 CrAO 基因参与五环三萜类物质的生物合成

3. 苍耳

采用 LC-MS 分析结合核磁共振技术，对中国 10 多个省份的苍耳植物资源进行了化学品质特征分析，共发现 4 种化学生态型，对每一种化学型中的主要化合物进行了分离纯化，并利用核磁共振技术对其结构进行了鉴定，为进一步进行苍耳分子生物学研究提供了指导作用。

红色星号表示采集省份

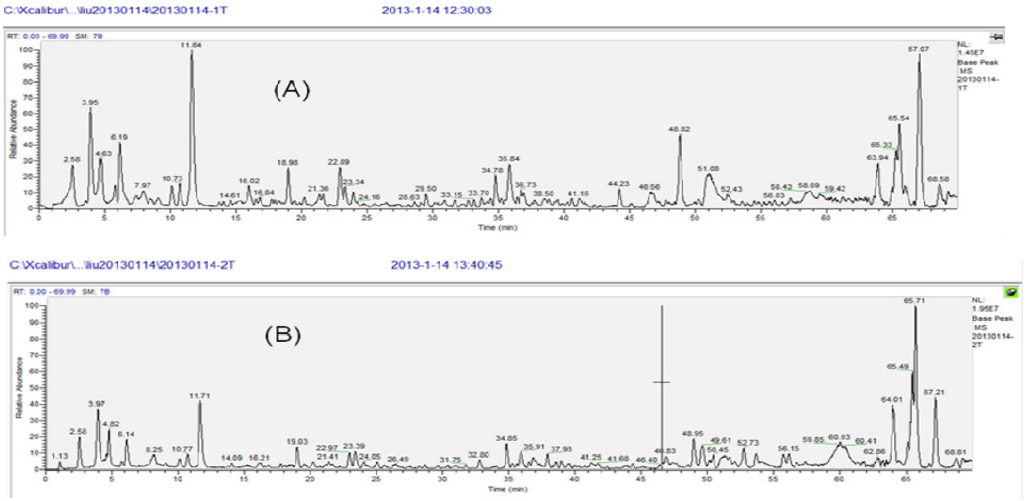
采集地点	主要倍半萜化合物结构
湖北-咸宁; 湖北-武汉; 湖北-房县;安徽-郎溪; 湖南-怀化; 四川-随宁; 四川-青城; 江西; 浙江	
甘肃; 河南-三门峡	
河南-南阳	
贵州遵义	

发现 4 种化学型苍耳植物

4. 马齿苋

在马齿苋科药食两用植物的综合开发利用研究方面，对栽培马齿苋和荷兰马齿苋进行了比较代谢组学研究，利用 GC-MS 分析检测到了 30 多种挥发性成分，根据保留时间和与标准质谱库的比对，鉴定出至少 20 种挥发性成分，发现其中至少近 10 种品种特异的化合物；同时，结合植物化学系统提取与 LC-MS 分析从两种马齿苋中检测到约 60 种可能的极性成分，发现其中可能含有多种品种特异

的功能化合物，上述研究不仅对马齿苋相关产品的质量控制与深度开发利用具有重要的应用价值，而且为基于马齿苋品种特异化合物的品种鉴定与品种创新奠定了良好的基础。



两种马齿苋的总提取物的比较代谢组学研究图谱

(A) 栽培马齿苋总提取物的 LC-MS 图谱；(B) 荷兰马齿苋总提取物的 LC-MS 图谱。

5. 莲

采用高压液相色谱-二极管阵列检测-电喷雾质谱（HPLC-PAD-ESI-MS）技术，分析了 108 个品种的莲花瓣的花青素、黄酮及黄酮醇的成分及含量，并对这些色素与颜色的相关性进行了分析，这 108 个莲品种包括 32 个红色品种、46 个粉红色品种、6 个黄色品种、14 个白色品种及 10 个红/白杂色品种。

共检测到 5 种花青素、14 种黄酮和黄酮醇，后者根据苷元又可以分为四大类：Myr（杨梅黄酮衍生物）、Qc（槲皮素衍生物）、Iso（异鼠李素衍生物）和 Kae（山奈酚衍生物）。发现了白色的莲花瓣中不含花青素，两种黄色品种及两种红/白品种含有少量的 Mv（二甲花翠素衍生物）和 Dp（飞燕草色素衍生物）两种花青素，红色和粉红色品种含有较高的花青素，前者含量高于后者，其中 Mv 和 Dp 含量最高，约占花青素总含量的 70%。所有品种中都含有黄酮及黄酮醇，其中粉红色含量最高，其次是红色和红/白杂色，而黄色和白色品种中含有较少的黄酮及黄酮醇。红色，粉红色，白色及红/白杂色这四种花色中，Kae>Iso>Qc>Myr，而黄色品种中，Iso>Qc > Kae>Myr。同时对这些色素成分及含量与花瓣颜色进行了主成分分析（PCA），根据 PC1 和 PC2 我们将大部分的莲品种分为 3 组（A,B 和 C），A 组是花青素含量较高的，含大部分的红色品种及几种粉红色品种；B 组含有较高的黄酮及黄酮醇，分布在这区域的有大部分的黄色品种，少量的粉红色、红/白杂色及白色品种，而 C 组是含两种色素都较少的，包括大部分的白色品种、及粉红色品种。从以上结果可以得出，红色与花青素相关性很高，花青素含量越高，红色越深，而黄色可能与黄酮及黄酮醇含量有关，尤其与 Iso 含量有很高的相关性。

(A)　　(B)

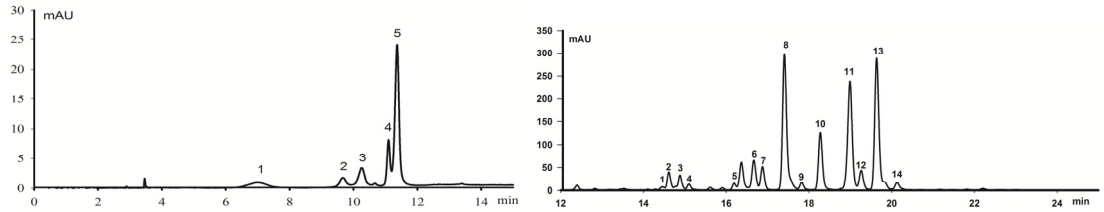


图 1.（A）和（B）是采用 HPLC 分别在 520nm 和 350nm 检测到的花青素、黄酮和黄酮醇的色谱图，分离得到 5 种花青素和 14 种黄酮和黄酮醇

莲花瓣中检测到的花青素的液相色谱和质谱数据

Peak No[a]	RT[b] (min)	NI-ms	MS^2	Identity[c]
1	6.068	463 [M− H]−	300 [A−2 H]−	Dp-3-glu
2	9.673	447 [M−H]−	284 [A−2 H]−	Cy-3-glu
3	10.255	477 [M−H]−	314 [A−2 H]−	Pt-3-glu
4	11.097	461 [M−H]−	298 [A−2 H]−	Pn-3-glu
5	11.365	491 [M−H]−	328 [A−2 H]−	Mv-3-glu

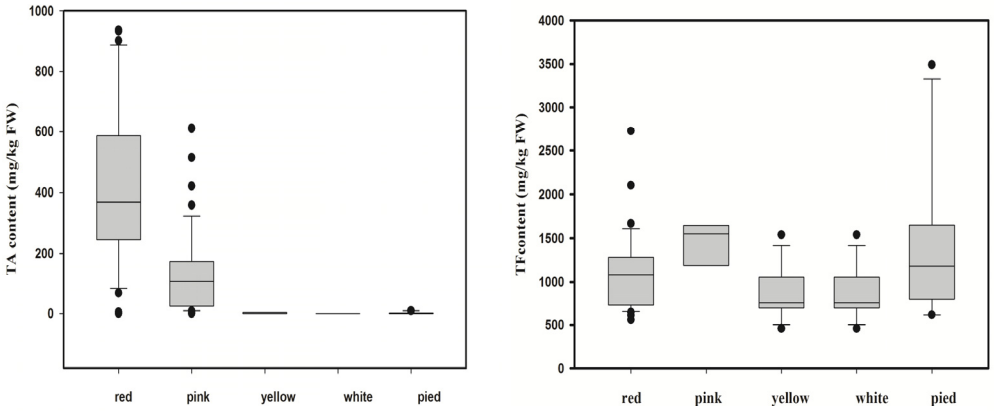
[a] 图 1（A）中的峰编号; [b] RT，保留时间

[c] Dp-3-glu=飞燕草色素-3-葡糖苷； Cy-3-glu=矢车菊素-3-葡糖苷； Pt-3-glu=矮牵牛花色素-3-葡糖苷； Pn-3-glu=芍药色素-3-葡糖苷； Mv-3-glu=二甲花翠素-3-葡糖苷

莲花瓣中检测到的黄酮和黄酮醇的液相色谱和质谱数据

Peak No[a]	RT[b] (min)	NI-ms	MS^2	Identity
1	14.455	479 [M− H]−	316 [A−2H]−	Myricetin 3-o-galatoside (Myr-3-Gal)
2	14.614	479 [M− H]−	316 [A−2H]−	Myricetin 3-o-glucoside (Myr-3-Glu)
3	14.884	595 [M−H]−	300 [A−2H]−	Quercetin 3-o-arabinopyanosyl-(1→2)-galactop-yranoside (Qc-3-Ara-Gal)
4	15.099	493 [M−H]−	317 [A−H]−	Myricetin 3-0-glucuronide (Myr-3-Gln)
5	16.192	609 [M−H]−	301[A−H]−	Quercetin 3-o-rhamopyranosyl-((1→6)-galactopyranoside (rutin)
6	16.669	463 [M−H]−	300 [A−2H]−	Quercetin 3-o-galacoside (hyperoside)
7	16.878	463 [M−H]−	300 [A−2H]−	Quercetin 3-o-glucoside (isoquercitrin)
8	17.400	477 [M−H]−	301 [A−H]−	Quercetin 3-o-glucuronide (Qc-3-Gln)
9	17.825	623 [M−H]−	315 [A−H]−	Isorhamnetin 3-o-rutinoside (Iso-3-Rut)
10	18.276	447 [M−H]−	284[A−2H]−	Kaempferol 3-o-galactoside (Kae-3-Gal)
11	18.993	447 [M−H]−	284[A−2H]−	Kaempferol 3-o-glucoside (astragalin)
12	19.264	477 [M−H]−	314[A−2H]−	Isorhamnetin 3-o-glucoside (Iso-3-Glu)
13	19.637	461 [M−H]−	285 [A−H]−	Kaempferol 3-o-glucuronide (Kae-3-Gln)
14	20.130	491 [M−H]−	491[M− H]− 315 [A−H]−	Isorhamnetin 3-o-glucuronide (Iso-3-Gln)

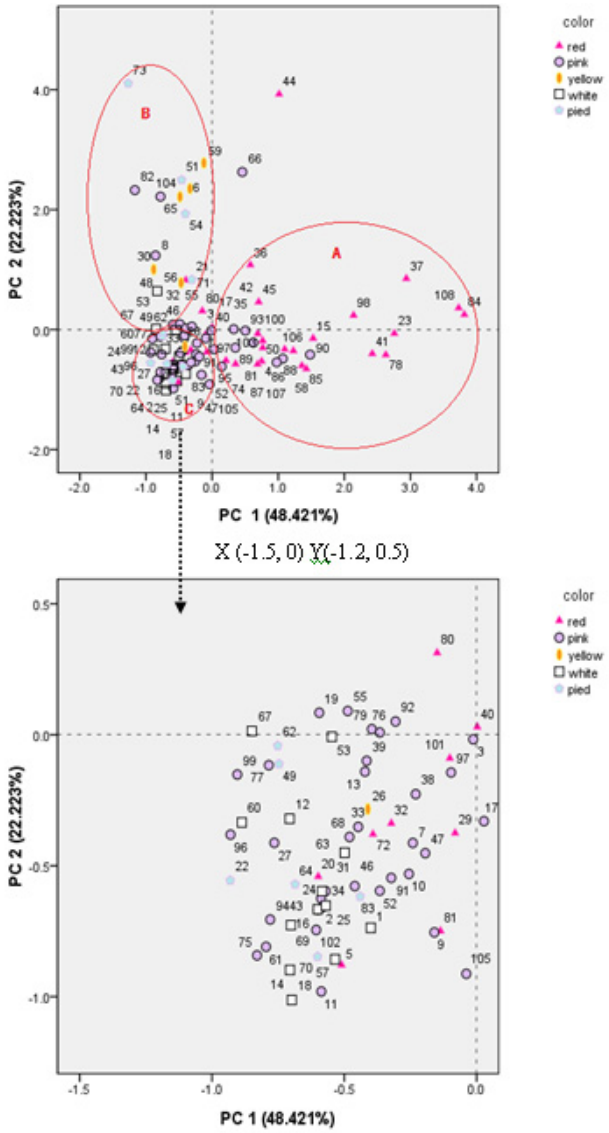
[a] 图 1（B）中的峰编号； [b] RT，保留时间



（A）　　（B）

(A) 和 (B) 是五种花色的莲花瓣中花青素总含量 (TA), 以及黄酮和黄酮醇总含量 (TF)的范围及分布情况

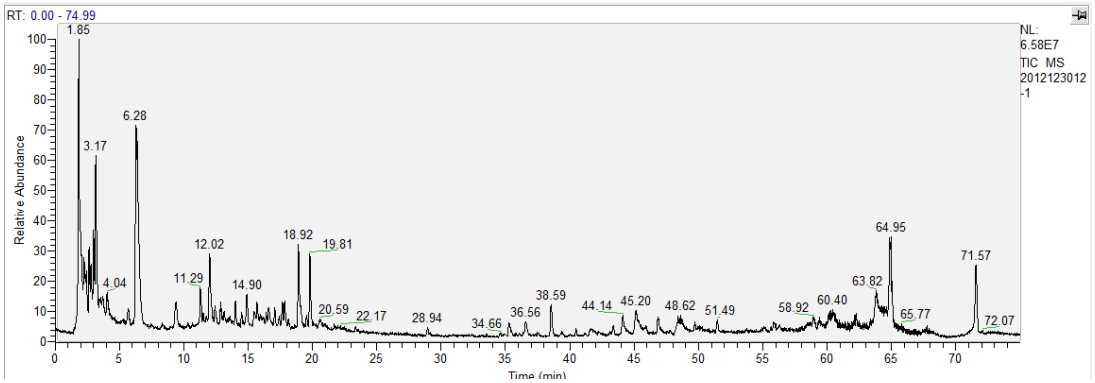
框内的横线代表中值



色素成分及含量与花瓣颜色的主成分分析

数字代表 108 个莲品种的编号；不同的形状代表不同的颜色

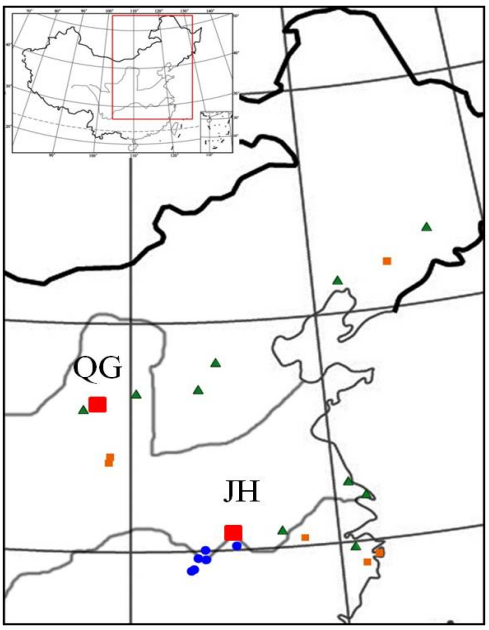
在莲子功能化合物的代谢组学研究方面，继续对不同品种莲子的功能化合物进行了系统深入的研究，利用气质联用(GC-MS)分析检测到了约 90 种挥发性成分，初步鉴定出其中的 14 种化合物，包括脂肪酸，胆甾醇和谷甾醇等；利用植物化学系统提取并结合液质联用(LC-MS)分析从莲子中检测到约 180 种可能次生代谢产物，初步分析发现其中可能含有多种以前未见文献报到的新成分，这些阶段性成果的取得不仅为莲资源的进一步综合开发利用提供了重要的基础数据，同时也将为莲品种鉴定和莲种质创新提供新的依据。



莲子乙醇提取物的 LC-MS 指纹图谱分析

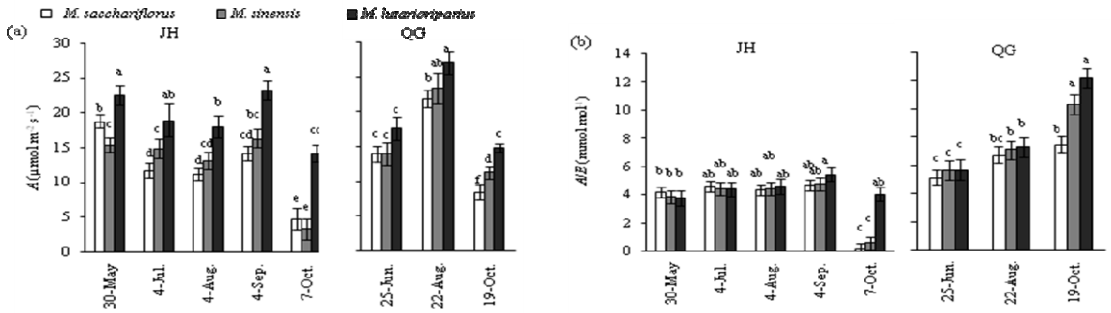
6. 芒草

将来自于中国自然分布地区的 93 个种群（15 个基因型/种群）的芒（*Miscanthus sinensis*）、荻（*M. sacchariflorus*）和南荻（*M. lutarioriparius*）分别种植在甘肃和武汉，通过对这些芒属植物整个生长季的形态变异性和生态适应性的评价，每个样地分别筛选出 22 个能代表芒草多样适应性的种群（4 个基因型/种群），在两地进行光合作用、CO_2 响应曲线和光响应曲线的测定，发现南荻在整个生长季都保持较高的光合速率，并且在 15℃的低温时仍保持较高的光合效率；甘肃南荻水分利用效率高于武汉，尤其在生长季末，其水分利用效率约是武汉的 2~3 倍。在 7 月之前，南荻的最大电子传递效率(J_{max})，PEP 羧化效率(V_{pmax})，Rubisco 羧化效率（V_{cmax}）均未表现出优势，但是在之后的生长季，这些与 C 固定相关的关键酶均表现出高于其它两种的优势。这些研究结果表明南荻的生物量大的主要原因是其光合速率高，关键酶活性强，光化学反应效率高；而水分利用效率高是南荻能够在甘肃长势良好的关键因素之一。本研究从生理方面解释了芒草，尤其是南荻生态适应性强和高产的原因。另一方面也确立了芒草可以在黄土高原地区推广种植的可能性。



在两个样地测定光合所选物种的采样点

绿色三角△：荻；橙色方块□：芒；蓝色圆圈○：南荻。红色方块□表示三个种植地。QG，甘肃庆阳；JH，湖北江夏。

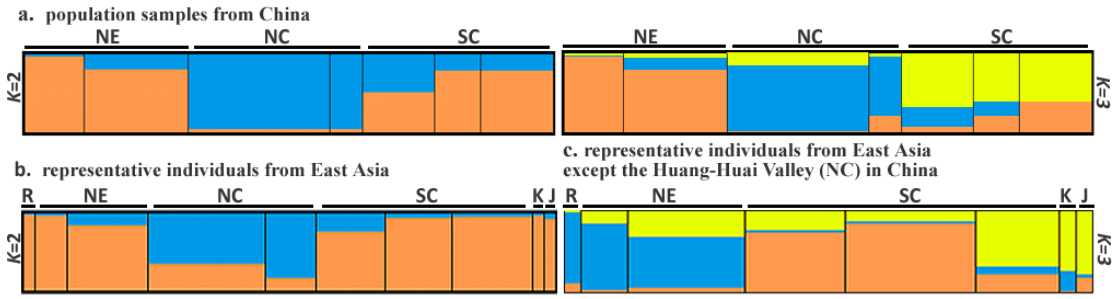


2011 年整个生长季各个物种的(a)光合速率和(b)水分利用效率

从 2010 年 5 月底到 10 月中旬，分别每月和每两月在武汉和甘肃各测一次光合作用

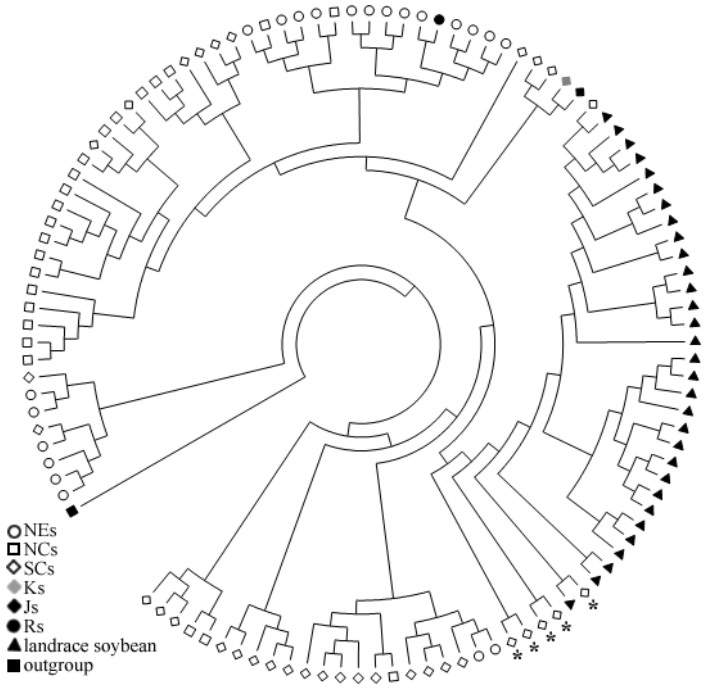
7. 大豆

在大豆的起源和进化方面，通过分析野大豆和栽培大豆的遗传多样性格局，提出了野大豆可能从中国的东南部经过远古冰川时期的大陆架扩散到我国东北部的假说，发现栽培大豆的单起源现象，提出了中国栽培大豆可能单一起源于中国南部地区的假说。



野大豆的遗传结构分析

a: 野大豆中国分布范围内的 40 个野生群体的 20 个 SSR 的分析；b: 基于 56 个 SSR 的东亚收集 231 份野大豆的遗传结构分析；c: 除黄淮海流域外的个体的 56 个 SSR 的遗传结构分析。NE: 东北野大豆；NC: 黄淮海流域野大豆； SC: 中国南部野大豆； R: 前苏联远东地区的野大豆；K: 韩国的野大豆；J: 日本的野大豆。

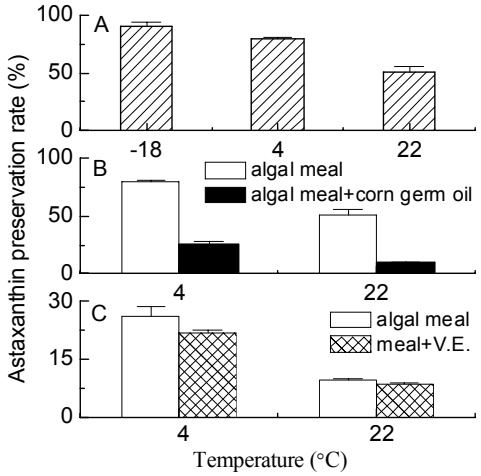


基于 56 个 SSR 标记构建的野大豆和栽培大豆的 Neighbor-Joining 系统树

表明了栽培大豆的单一起源，并且日本和韩国的野大豆与我们南方地区的野大豆具有很近的亲缘关系。

8. 微藻

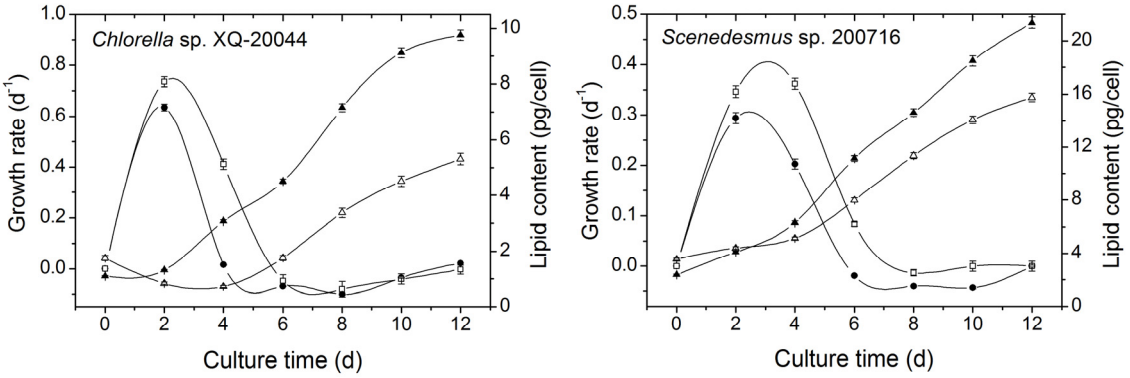
监测红球藻虾青素酯在长期保存（96 周）过程中组份和含量的变化，研究温度、氧、抗氧化剂和玉米油对红球藻天然虾青素酯稳定性的影响。研究结果表明，高温和氧气显著降低虾青素的稳定性，抗氧化剂（维生素 E，抗坏血酸）和玉米油不仅不具有保护虾青素酯免除氧化损伤的作用，反而促进了虾青素的降解。掌握了虾青素酯保存率与保存条件、保存周期的关系。以虾青素保存率 80%为目标，真空、黑暗条件下，温度不高于 4 ℃, 保存周期可达 96 周；室温条件下，保存周期只有 30 周。发现了虾青素单酯的相对含量下降，虾青素双酯的相对含量上升，虾青素双酯/单酯比值可以反映虾青素是否保存完好，如果这一比值高于 0.2，表明保存过程中虾青素酯的降解率已超过 20%。建立了经济、高效保存红球藻破壁藻粉和虾青素酯产品的工艺方法：不添加任何抗氧化剂，真空包装，黑暗、低温（≤4 ℃）保存。这一保存方法已在云南绿 A 生物工程公司红球藻规模化生产中应用。



温度、玉米油和抗氧化剂对虾青素稳定性影响

（A）温度的影响；（B）玉米油的影响；（C）抗氧化剂的影响

利用环形培养池模拟系统培养 2 株绿藻（*Scenedesmus* sp. 200716 and *Chlorella* sp. XQ-20044），在严格控制光照强度、光暗周期、温度、培养液 pH 值和藻液流速的条件下，研究了细胞密度、干重及总脂含量的动态变化。培养 12 天，小球藻总脂含量达到 55%，栅藻总脂含量达到 32%。发现 2 株绿藻在分批培养条件下，产油具有明显的二步法特征：第一阶段, 细胞快速生长繁殖，细胞密度大幅度增长，这一阶段，细胞不积累或积累少量油脂；第二阶段, 细胞停止繁殖，油脂含量开始大幅度上升。绿藻产油二步法特征的发现，对于优化培养条件，提高产油效率具有重要指导作用。



小球藻（*Chlorella*）和栅藻(*Scenedesmus*)分批培养过程中生长速率、总脂含量变化

四、科研产出

本年度实验室共发表科研论文 75 篇（见附录二），其中 SCI 论文 62 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 50 篇（含 Top 30% 21 篇，Top 10% 6 篇）；主编或参与编写的论著 1 部；通过国家级和省级审定的新品种各 1 个；授权专利 7 项，申请专利 2 项。

五、人员信息

1. 队伍建设

重点实验室现有固定人员 56 人（见附录三），包括研究员 16 人（其中 9 人入选中国科学院“百人计划”），副研究员 16 人，助理研究员 21 人，获博士学位的人数占固定人员总数的 87.5%。

人才引进：

中科院“百人计划”入选者：郭明全

青年博士： 胡涛、黄文俊、李黎、李书涛、施海涛、王中杰

2. 研究生培养情况

现有博士生导师 12 人，硕士生导师 21 人，在读研究生 91 人（见附录四），其中博士 37 人（含 1 名外籍留学生），硕士 54 人。本年度毕业研究生 26 人，其中博士 12 人，硕士 14 人，目前在站博士后 2 人，研究生培养取得的成绩：

3 名研究生获昌华奖学金；

3 名研究生获国家奖学金；

15 名研究生获院“优秀学生干部”、“三好学生”或“优秀毕业生”荣誉称号；

2 名研究生获武汉教育基地“优秀毕业生”荣誉称号。

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 16 人次赴国外参加国际会议或开展合作交流。邀请 8 人次前来实验室进行学术交流。邀请国内外专家来室讲学 9 人次，其中来自国外单位的专家 7 人次，来自国内单位专家 2 人次（见附录五）。

1. 非洲生物多样性合作研究进展顺利

受院国际合作局、生物局等支持，武汉植物园非洲生物多样性研究中心对非工作取得了新的进展。1 月 5 日-20 日，武汉植物园组织北京植物研究所、昆明植物研究所、上海辰山植物园等单位的 6 名科研人员，对肯尼亚山（非洲第二高峰）地区进行了高山植物调查。4 月 5 日-18 日，武汉植物园 3 名科研人员赴肯

尼亚维多利亚湖、纳库鲁湖等地区开展热带地区雨季湖泊水生植物调查，采集了大量沉水、挺水和浮水植物标本。8 月 20 日-9 月 20 日，武汉植物园再次联合北京植物研究所、北京动物研究所、昆明植物研究所、昆明动物研究所、武汉病毒研究所、上海辰山植物园、仙湖植物园等国内 8 家单位的 12 名科研人员及肯尼亚乔莫·肯尼亚塔农业与技术大学 6 名外籍科研人员，组成植物动物微生物联合考察队，赴肯尼亚蒙巴萨海滨带、埃贡山（海拔 4000 米）地区及马赛马拉草原地区进行生物多样性综合调查，采集了大量红树林植物、高山草甸植物及稀树草原植物标本，同时也获得了大量的昆虫和微生物病毒标本，极大地丰富和补充了科学院各所标本馆非洲地区的标本馆藏，同时也对非方科研人员进行了技术支持。

2. 组织召开国际草坪学研究与发展策略论坛

6 月 30-7 月 1 日，由中国科学院武汉植物园发起并主办的国际草坪学研究与发展策略论坛在武汉成功召开。

本次论坛由国际知名草坪逆境生理学专家、长江学者讲座教授、美国罗格斯大学教授黄炳茹博士担任主席，武汉植物园傅金民研究员担任秘书长，包括国际知名的草坪育种学家 Dr. William Meyer、草坪育种和遗传学家 Dr. Paul Raymer、美国 USGA 高尔夫草坪专家 Dr. Michael P. Kenna 在内的 70 多名国内外从事草坪学研究的知名专家和学者参加了本次论坛。

本次论坛特邀国内外知名草坪学专家作学术报道 21 个，内容涉及草坪遗传育种、草坪逆境分子生理、草坪生物技术等草坪学研究的各个方面的前沿性进展与成果。报告后的集中讨论时段，与会专家对我国的草坪科研现状进行了深入的剖析，并针对我国草坪学的发展战略提出许多建设性意见。本次论坛的成功召开，将极大地促进我国草坪界学者之间的联系与合作，并加强我国与国际草坪学研究人员的交流与合作。

3. 组织召开第四届国际农业蛋白质组学前沿论坛

11 月 9 日-11 日，由亚洲大洋洲农业蛋白质组组织（AOAPO）主办，中国科学院武汉植物园承办的第四届国际农业蛋白质组学前沿论坛在武汉市隆重召开。来自中国、美国、德国、日本等 9 个国家的 150 多位代表参加了本次论坛。

会议期间，与会代表通过 25 个专题学术报告和墙报展示，对目前国际上农业蛋白质组学研究的关键问题、最新进展以及蛋白质组学技术在农业中的应用等科学问题进行了深入交流和讨论。通过研讨，专家们一致提出蛋白质组学研究技术及数据格式标准的建立、蛋白质组数据库的建立和共享、亚细胞结构蛋白质组、动态修饰蛋白质组的研究将是今后研究的重点；同时，还提出蛋白质组学研究应

该更多地和细胞生物学、分子遗传学等研究技术相结合，着重探讨生命现象的内在机理和解决科学问题，特别是农业科学中的关键问题。

4. 开放课题

目前实验室在研的开放课题共 11 项，每项课题支持 3 万元。

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实时荧光定量 PCR 检测系统、水果品质无损检测仪、遗传分析仪、多功能细胞分析系统、微波消解仪、蛋白质等电聚焦仪及大型垂直电泳槽、高效液相色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 71 台（套），设备总值 1900 余万元（见附录六）。

八、2012 年度大事记

3 月 5 日，郭明全顺利通过中科院“百人计划”初评答辩。

4 月，助理研究员何冬丽获“2012 年度中国科学院王宽诚教育基金会访问学者项目——优秀女科学家专项”资助。

4 月 27 日，重点实验室举行 2012 年首场学术交流会，相关学科组博士二年级研究生作了 7 场学术报告，陈莎获优秀报告奖。

5 月 11 日，武汉植物园举行药用植物项目 2012 年度研讨会，项目组成员围绕“药用植物黄酮类化合物的代谢调控、生物合成和开发利用”主题进行了阶段性进展报告，专家对子课题的研究工作提出了建议和意见，并对该项目的总体进展及后续研究重点进行了讨论。

5 月 27 日，重点实验室召开第一届学术委员会第三次会议，依据 2011 年度工作报告，委员们就重点实验室科学问题凝练、科研平台建设、人才队伍建设和国际合作交流等方面提出了建议。

6 月 30 日-7 月 1 日，由武汉植物园发起并主办的国际草坪学研究与发展策略论坛在武汉召开，70 余名国内外从事草坪学研究的知名专家和学者参加了本次论坛。

7 月 6 日，武汉植物园与广水市东晨农业技术有限公司共同合作的“特种功能蔬菜广水产业化示范基地”正式落户湖北省广水市。

9 月 17 日，重点实验室举行 2012 年第二场学术交流会，相关学科组硕士研究生作了 12 场学术报告，60 余名科研人员和研究生参加了会议。

10 月 26 日，重点实验室青年科研人员学术交流活动在湖北黄陂举行。

11 月 8 日，吕世友顺利通过中科院“百人计划”初评答辩。

11 月 9 日-11 日，由亚洲大洋洲农业蛋白质组组织（AOAPO）主办，武汉植物园承办的第四届国际农业蛋白质组学前沿论坛在武汉召开，来自中国、美国、德国、日本等 9 个国家的 150 余位代表参加了本次论坛。

11 月 13 日，内蒙古农牧业科学院生物技术研究中心主任刘红葵一行 5 人考察重点实验室。

12 月 7 日，重点实验室举行 2012 年第三场学术交流会，4 位学科组长和 2 位青年科研人员作了学术报告，科研人员和研究生共 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	意大利	新品种商业授权开发	1000	87.3	2005-8-1	2028-12-1	黄宏文
2	加拿大国际植物营养研究所	棉花钾效率基因型差异的分子遗传机理研究	18	0	2009-5-1	2013-12-31	李作洲
3	美国国家科学基金会和盖茨基金会促进农业发展的基础研究计划(NSF-Gates)	Inactivating rust resistance suppressors to unlock multiple defense responses in wheat	40	0	2010-5-31	2013-5-31	傅金民
4	日本 “丸善制药株式会社	甘草资源调查和优良品种培育	140	80	2010-12-1	2014-12-31	陈建军
合计	--	--	1198	167.3	--	--	--

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	非粮柴油能源植物与相关微生物资源的调查、收集与保存	42	7	2008-12-1	2013-12-30	傅金民
2	国家重大专项	中国产油微藻调查	105	21	2012-5-1	2017-4-30	李夜光
3	国家重大专项	青藏高原特殊生境下野生植物种质资源的调查与保存	60	0	2008-1-1	2013-12-31	李建强
4	国家重大专项	优质加工品质转基因小麦新品种培育	125	0	2010-5-1	2012-12-31	傅金民
5	国家重大专项	抗病、优质转基因小麦新种质遗传鉴定	38	0	2011-11-1	2012-12-31	傅金民
6	973	小麦高产优质品种设计和选育的应用基础研究	50	0	2009-1-1	2013-12-30	傅金民
7	973	重要园艺作物果实	123	59	2011-1-1	2015-12-31	李绍华

		品质形成机理与调控					
8	863	多年生黑麦草高效育种技术研究和抗逆新品种创制	56	56	2012-1-1	2015-12-31	傅金民
9	国家基金重大	芒草的驯化性状评价和群体遗传学分析	30	15	2012-1-1	2015-12-31	李建强
10	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	2014-12-31	傅金民
11	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	120	2012-1-1	2016-12-31	李绍华
12	国家自然基金	中国淫羊藿属的分类学研究	19	0	2010-1-1	2012-12-31	张燕君
13	国家自然基金	缬草属植物雌花两性花同株的适应意义研究	19	0	2011-1-1	2013-12-31	卢　洋
14	国家自然基金	中国野生花苜蓿由大格局到精密尺度格局的居群遗传变异模式和生态适应性进化初探	19	0	2011-1-1	2013-12-31	闫　娟
15	国家自然基金	铁角蕨属不同生态型植物隐花色素基因家族的适应性进化研究	22	0	2011-1-1	2013-12-31	周　媛
16	国家自然基金	基于表达谱的山葡萄抗寒调控研究	18	0	2011-1-1	2013-12-31	辛海平
17	国家自然基金	淫羊藿 A-E 类 MADS-box 基因与花型演化的关系	21	0	2011-1-1	2013-12-31	李志能
18	国家自然基金	孑遗植物栾椤的适应性种群分化研究	30	0	2010-1-1	2012-12-31	王　艇
19	国家自然基金	基于 SSR 遗传图谱的苹果糖酸品质性状的基因定位	21	0	2010-1-1	2012-12-31	韩月彭
20	国家自然基金	葡萄果实发育过程中香气物质形成关键时期的研究	38	0	2010-1-1	2012-12-31	李绍华
21	国家自然基金	欧亚北美间断高山特征成分山莓草属的扩散和分化研究	34	0	2011-1-1	2013-12-31	王恒昌

22	国家自然基金	列当科植物叶绿体基因组进化及其与寄生性的关系	32	0	2011-1-1	2013-12-31	李建强
23	国家自然基金	中国高羊茅种质资源耐热生理鉴定及分子遗传基础研究	33	0	2011-1-1	2013-12-31	傅金民
24	国家自然基金	蓖子三尖杉种群遗传分化中的气候和环境效应研究	32	0	2011-1-1	2013-12-31	王　艇
25	国家自然基金	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	49.5	2012-1-1	2015-12-31	章焰生
26	国家自然基金	猕猴桃属种复合体的综合分类学研究	55	49.5	2012-1-1	2015-12-31	李新伟
27	国家自然基金	基于高密度遗传图谱的葡萄果实白藜芦醇含量 QTL 定位	60	54	2012-1-1	2015-12-31	汪　念
28	国家自然基金	温度影响红肉猕猴桃呈色的色素降解机制研究	60	54	2012-1-1	2015-12-31	王彦昌
29	国家自然基金	水稻柱头与花粉识别机理的蛋白质组学研究	28	21.1	2012-1-1	2014-12-31	王　坤
30	国家自然基金	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	15.4	2012-1-1	2014-12-31	汪志伟
31	国家自然基金	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	16.1	2012-1-1	2014-12-31	何冬丽
32	国家自然基金	猕猴桃维生素 C 遗传机理及 Vc 代谢关键基因发掘	25	17.5	2012-1-1	2014-12-31	李大卫
33	国家自然基金	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	32	26.5	2012-1-1	2014-12-31	周　莹
34	国家自然基金	狗牙根适应盐胁迫的要/冠异速生长特性及机理研究	23	16.1	2012-1-1	2014-12-31	胡龙兴
35	国家自然基金	NtGNL1 调控根极性	20	17.7	2011-1-1	2013-12-31	王　鲁

		生长的分子和细胞机制					
36	行业性重大专项	重要果树基因资源发掘与创新的关键技术合作研发	100	16	2011-1-1	2015-12-31	韩月彭
37	行业性重大专项	黄河上中游次生盐碱地农业高效利用技术模式研究与示范	20	5	2009-1-1	2013-12-31	傅金民
38	行业性重大专项	东北野生猕猴桃保护、开发和利用研究	68	13.2	2009-1-1	2013-12-31	王彦昌
39	行业性重大专项	国家猕猴桃种质资源圃	270	19.8	2010-10-1	2012-10-31	黄宏文
40	院重大项目	猕猴桃和葡萄绿色生态产业化生产技术集成研究与示范	50	0	2009-6-1	2012-12-30	钟彩虹
41	院重大项目	软枣猕猴桃新品种主试验区示范	5	0	2011-1-1	2012-12-31	钟彩虹
42	院重大项目	武汉植物园优秀青年科技专项	80	20	2011-1-1	2013-12-31	姚小洪
43	院重大项目	新一代能源作物芒草的驯化生物学	40	20	2012-1-1	2014-12-31	闫　娟
44	院重大项目	考古遗存典型农作物-野生植物鉴定	140	27	2011-1-1	2015-12-31	韩月彭
45	院重大项目	生态系统固碳现状、速率、机制和潜力	120	36	2011-1-1	2015-12-31	傅金民
46	部委项目	河南省高产高效现代农业示范工程二期	40	40	2012-6-1	2012-12-31	钟彩虹
47	部委项目	外籍特聘研究员计划	9.9	9.9	2012-5-1	2012-12-30	韩月彭
48	部委项目	外籍特聘研究员计划	30.5	30.5	2012-7-1	2013-12-30	杨平仿
49	部委项目	湖南重金属超富集草坪草的选育与示范	40	2.8	2011-1-1	2013-12-31	李惠英
50	部委项目	壳斗科和猕猴桃科 DNA 条形码研究	6.75	0	2009-1-1	2012-8-30	李新伟
51	部委项目	能源植物油桐种质资源油脂提取及评价	2	2	2012-1-1	2012-12-30	潘　越
52	部委项目	植物雄性不育研究	40	10	2012-1-1	2014-12-31	汪志伟
53	部委项目	植物药用化合物合成途径功能基因的	260	75	2011-1-1	2013-12-31	章焰生

		挖掘					
54	部委项目	植物繁殖过程中种子形成与萌发的分子机理	260	80	2011-1-1	2013-12-31	杨平仿
55	部委项目	果树果实重要品质改善的遗传机制与分子改良	200	0	2011-1-1	2013-12-31	韩月彭
56	部委项目	植物资源保护与可持续利用创新团队	50	0	2009-1-1	2013-12-31	李绍华
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注：1. 第 29 项的总经费和实到经费中已包含基金委支持蛋白质组学国际会议的 5 万元。

2. 第 48 项总经费和实到经费中已包含中科院支持蛋白质组学国际会议的 4 万元。

3. 横向合作及其它项目

序号	项目名称	类别	总经费	本年实到经费	开题时间	结题时间	负责人
1	药用植物化学与功能代谢组学	自主部署	70	0	2012-8-1	2015-8-30	郭明全
2	药用植物黄酮类化合物的代谢调控、生物合成和开发利用	自主部署	140	20.5	2011-1-1	2013-12-31	王　瑛
3	莲种质创新与可持续利用	自主部署	135	34.2	2011-1-1	2013-12-31	杨平仿 韩月彭
4	川东-鄂西植物多样性形成及维持机制	自主部署	120	33.7	2011-9-1	2014-8-31	李建强
5	果树分子育种	研究所自选	100	0	2008-11-1	2014-12-30	韩月彭
6	草坪种质资源收集、评价与种质创新	研究所自选	77	0	2009-1-1	2012-12-30	傅金民
7	植物分典编纂 PP	研究所自选	20	10	2010-9-1	2013-12-31	李建强
8	省重点实验室开放课题 2010	研究所自选	10	0	2010-6-1	2012-5-31	王　艇
9	资源植物繁殖生物学	研究所自选	77	0	2010-1-1	2013-12-31	杨平仿
10	天然药物生物合成学	研究所自选	77	0	2010-1-1	2013-12-31	章焰生
11	草坪草-狗牙根对水分胁迫的应答机制	研究所自选	77	0	2011-11-1	2014-12-31	产祝龙
12	MYB 及其结构域对菊花花青素合成影响的研究	地方政府委托	6	0	2010-1-1	2012-12-31	李志能
13	三峡库区秭归县生态农业园建设规划	地方政府委托	30	0	2009-7-1	2020-12-30	王　勇
14	中国外来入侵植物志	地方政府委托	12	1.2	2010-6-1	2013-12-31	陈　丽
15	猕猴桃特色资源收集与新品种选育研究	地方政府委托	60	0	2011-11-1	2014-12-31	王彦昌

16	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	地方政府委托	30	0	2012-1-1	2014-12-31	杨　帆
17	秭归县三峡水库消落区治理一期规划设计	地方政府委托	22.14	32.5	2012-3-1	2014-12-31	杨　帆
18	莲藕的叶绿体基因组学研究	地方政府委托	3	0	2009-1-1	2012-12-30	王　艇
19	萝卜源 OguraCMS 新恢复基因 Rfo2 的克隆及功能解析	地方政府委托	4	0	2011-1-1	2012-12-31	汪志伟
20	葡萄果实白藜芦醇合成调控因子筛选及其功能验证	地方政府委托	4	0	2011-1-1	2012-12-31	汪　念
21	都江堰猕猴桃产业链技术集成研究与示范 2	地方政府委托	20	9	2011-10-1	2013-12-31	钟彩虹
22	兴山县低效生态公益林改造一期项目	地方政府委托	20	4	2012-8-2	2015-12-31	王　勇
23	中国喜马拉雅地区蒿属(Artemisia)分类修订	其他国家费	10	6	2012-6-1	2014-5-31	李晓东
24	葛根素生物合成途径中关键糖基转移酶基因的分离	其他国家费	3	3	2012-8-1	2013-8-30	章焰生
25	苹果 EST-SSR 标记在其它蔷薇科果树中可转移性研究	其他国家费	3	3	2012-8-1	2013-8-30	韩月彭
26	全国中草药汇编第三版修订	其他国家费	10	0	2008-12-1	2013-12-30	王　瑛
27	猕猴桃种质资源收集、编目、更新与利用	其他国家费	18	18	2012-1-1	2012-12-31	钟彩虹
28	三峡库区三种特有植物丰都车前、宜昌黄杨、鄂西鼠李的保护工程	其他国家费	125	40	2008-4-1	2012-12-30	王　勇
29	猕猴桃新品种研究	其他委托	260	100	2005-7-1	2012-12-31	黄宏文
30	宁夏耐盐碱草筛选	其他委托	12.5	11.1	2011-11-1	2013-12-31	傅金民
31	螺旋藻技术优化	企业委托	300	0	2004-12-28	2019-12-28	李夜光
32	红球藻中试及规模化养殖	企业委托	58	0	2006-11-15	2016-11-14	李夜光
33	微藻生物柴油成套技术开发	企业委托	420	123.3	2010-1-1	2013-12-31	李夜光
34	黄鹤楼（红坪）百草园适栽植物栽培与示范	企业委托	105	17	2008-1-1	2014-12-30	王　庆
35	泰州重要中药材选育及高通量筛选研发	企业委托	30	0	2008-11-13	2012-12-30	王　庆
36	合作建设成都猕猴桃	企业委托	120	53.3	2009-8-20	2014-8-20	钟彩虹

	资源基因库						
37	武汉花山生态新城启动区植被调查	企业委托	5.8	0	2009-12-18	2012-12-31	李晓东
38	宜昌年产3亿株现代化种苗工厂项目	企业委托	70	20	2010-9-1	2012-12-31	王　勇
39	万州区生态农业园建设实施规划	企业委托	20	0	2010-8-1	2013-8-1	王　勇
40	特种功能蔬菜推广种植	企业委托	35	10.6	2010-10-1	2013-9-30	王　瑛
41	三峡库区设施农业园及生态农业园设计	企业委托	810	0	2010-9-1	2015-12-31	王　勇
42	猕猴桃新品种中试及开发	企业委托	60	45.4	2011-1-1	2016-12-31	钟彩虹
43	三峡库区巴东县溪丘湾生态茶叶示范园建设	企业委托	27	0	2011-1-1	2013-12-31	王　勇
44	中药材及植物新品种示范推广研究	企业委托	40	2.2	2011-7-28	2015-5-30	王　庆
45	猕猴桃科研开发-华夏联诚果业	企业委托	490	50	2011-11-10	2021-11-10	钟彩虹
46	特种功能蔬菜的产业化	企业委托	35	33.3	2011-12-1	2012-12-31	王　瑛
47	红肉猕猴桃新品种东红	企业委托	950	150.3	2012-2-24	2032-2-24	钟彩虹
48	抗癌化合物阿可拉定异戊稀侧链的生物合成	企业委托	10	10	2012-1-1	2012-12-31	章焰生
49	兴山生态农业园可研	企业委托	55.5	8	2012-1-1	2014-12-31	王　杰
50	三峡库区后续工程生态农业一期实施规划	企业委托	100	20	2012-3-1	2014-12-31	王　勇
51	微藻生物能源示范工程-红球藻培养	企业委托	50	30	2012-5-1	2013-5-30	李夜光
52	功能蔬菜示范及推广种植	企业委托	30	10	2012-10-1	2015-10-30	王　庆
53	猕猴桃新品种及配套栽培技术	企业委托	128	38.4	2012-11-27	2015-11-27	钟彩虹
54	功能蔬菜和神农金菊的种植	企业委托	50	20	2012-4-26	2014-12-31	王　庆
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4. 2012 年国家自然科学基金获批项目

序号	类别	项目名称	批准经费	开始时间	结题时间	负责人
1	面上项目	箭叶淫羊藿叶片发育过程中花青素苷和黄酮醇苷代谢分支的分子调控	82	2013-1-1	2016-12-31	王　瑛

2	面上项目	药用植物苍耳腺体细胞特异性表达倍半萜合酶基因功能分析	15	2013-1-1	2013-12-31	章焰生
3	面上项目	孑遗植物鹅掌楸分布范围限制的进化机制研究	75	2013-1-1	2016-12-31	姚小洪
4	面上项目	美洲黑杨对冬季水淹及后期恢复的性别特异性响应	83	2013-1-1	2016-12-31	杨　帆
5	面上项目	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	2013-1-1	2016-12-31	杨平仿
6	面上项目	野生狗牙根抗寒关键基因发掘和功能解析	80	2013-1-1	2016-12-31	傅金民
7	面上项目	微藻产油、固碳、脱硫、除硝一体化模式研究	80	2013-1-1	2016-12-31	李夜光
8	青年科学基金	一氧化氮介导植物对干旱胁迫的反应机理研究	25	2013-1-1	2015-12-31	施海涛
9	青年科学基金	药用植物箭叶淫羊藿类黄酮异戊烯转移酶基因的克隆与功能研究	23	2013-1-1	2015-12-31	黄文俊
10	青年科学基金	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	2013-1-1	2015-12-31	吕海燕
11	青年科学基金	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	2013-1-1	2015-12-31	谷　超
12	青年科学基金	高羊茅铅富集关键基因的发掘与功能解析	25	2013-1-1	2015-12-31	李惠英
合计	--	--	620	--	--	--

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) Chan ZL. Expression profiling of ABA pathway transcripts indicates crosstalk between abiotic and biotic stress responses in Arabidopsis. **Genomics** 2012, 100: 110-115 (SCI, IF:3.019)
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12) Guan L, Li JH, Fan PG, Chen S, Fang JB, Li SH, Wu BH. Anthocyanin accumulation in various organs of a teinturier cultivar (*Vitis vinifera* L.) during the growing season. **American Journal of Enology and Viticulture** 2012, 63(2): 177-184 (SCI, IF:1.826)

13) Guo J, Liu YF, Wang YS, Chen JJ, Li YH, Huang HW, Qiu LJ, Wang Y. Population structure of the wild soybean (*Glycine soja*) in China: implications from microsatellite analyses. **Annals of Botany** 2012, 110, 777-785 (SCI, IF:4.03)

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2. 专著

黄宏文，钟彩虹，姜正旺，李新伟，姚小洪，李大卫，王圣梅，李作洲，龚俊杰，刘义飞，石涛，张蕾，贾硕威，陈彬. 猕猴桃属分类资源驯化栽培. 科学出版社. 2013

3. 新品种

良种名称	树种	第一完成人	发证机关	时间	良种证编号
中科绿川 1 号	枸杞	王瑛	国家林业局林木品种审定委员会	2011.12.30	（2011）第 22 号
‘斑叶’吐烟花	多年生草本	王庆	湖北省林木品种审定委员会	2012.6.8	鄂 2011 第 015 号

4. 专利

授权专利 7 项

1）一种从紫草中分离出萘醌类有效成分的方法（专利号：ZL 201110164992.8，袁晓、袁萍）

2）猕猴桃天然香料提取方法（专利号：ZL 200910062371.1，徐丽云、黄宏文、钟彩虹、王圣梅）

3）一种葛根的快速繁殖方法（专利号：ZL 201110029788.5，李长福、章焰生）

4）从大黄根茎中提取高纯大黄酚的方法（专利号：ZL200910272509.0，袁 晓、袁萍）

5）一种高纯度大黄素的分离方法（专利号：ZL200910272508.6，袁 晓、袁萍）

6）油桐幼叶基因组 DNA 提取方法　（专利号：ZL 201110453531.2，彭俊华、张玲玲）

7）油桐成熟叶片和老叶片基因组 DNA 提取方法（专利号：ZL201110454394.4，彭俊华、张玲玲）

申请专利 2 项

1）一种神农药酒及其制备方法（申请号：201210056944.1，王庆、王淑慧 辛春梅）

2）一种经济快速盐渍土壤草坪种植的方法（申请号：201210378866.7，傅金民、胡涛）

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
彭俊华	研究员	孟山都公司	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
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	专业	最后学位	职　务	职称	性质
1	产祝龙	男	1975.9	植物学	博士		研究员	研究
2	傅金民	男	1961.12	园艺学	博士	重点实验室副主任	研究员	研究
3	郭明全	男	1975.10	化学	博士		研究员	研究
4	韩月彭	男	1968.11	作物遗传育种	博士		研究员	研究
5	黄宏文	男	1957.1	园艺学	博士		研究员	研究
6	李建强	男	1954.11	植物学	博士		研究员	研究
7	李绍华	男	1957.9	园艺园林	博士	重点实验室主任	研究员	研究
8	李夜光	男	1962.5	植物学	硕士		研究员	研究

9	王恒昌	男	1967.3	植物学	博士		研究员	研究
10	王　庆	女	1955.10	药学	学士		研究员	研究
11	王　艇	男	1969.3	生物化学	博士		研究员	研究
12	王　瑛	女	1973.10	植物遗传学	博士		研究员	研究
13	王　勇	男	1968.10	植物学	博士		研究员	研究
14	吴金清	男	1963.6	植物学	硕士		研究员	研究
15	杨平仿	男	1975.7	植物蛋白质组学	博士		研究员	研究
16	章焰生	男	1972.12	植物学	博士		研究员	研究
17	胡龙兴	男	1982.7	园艺学	博士		副研究员	研究
18	姜正旺	男	1965.6	果树学	学士		副研究员	研究
19	李惠英	女	1977.3	植物学	博士		副研究员	研究
20	李晓东	男	1966.11	植物学	博士		副研究员	研究
21	李新伟	男	1974.10	植物学	博士		副研究员	研究
22	李作洲	男	1967.5	植物学	博士		副研究员	研究
23	汪　念	男	1982.1	发育生物学	博士		副研究员	研究
24	汪志伟	男	1978.12	种群遗传学	博士		副研究员	研究
25	王彦昌	男	1973..9	农学	博士		副研究员	研究
26	辛海平	男	1980.2	发育生物学	博士		副研究员	研究
27	闫　娟	女	1982.10	植物学	博士		副研究员	研究
28	杨　帆	男	1979.6	植物学	博士		副研究员	研究
29	姚小洪	男	1975.11	植物学	博士		副研究员	研究
30	袁　晓	男	1962.7	园艺学	硕士		副研究员	研究
31	张燕君	女	1980.9	植物学	博士		副研究员	研究
32	钟彩虹	女	1968.2	植物学	博士		副研究员	研究
33	陈方方	女	1982.2	作物生物技术	博士		助理研究员	研究
34	陈建军	男	1979.12	植物学	博士		助理研究员	研究
35	陈　丽	女	1982.10	植物学	博士		助理研究员	研究
36	陈　良	男	1981.2	植物分子遗传学	博士		助理研究员	研究
37	高　磊	男	1981.5	植物学	博士		助理研究员	研究
38	谷　超	男	1985.8	发育生物学	博士		助理研究员	研究
39	何冬丽	女	1977.11	藻类遗传与生物技术	博士		助理研究员	研究
40	胡　涛	男	1981.10	植物学	博士		助理研究员	研究
41	黄文俊	男	1981.5	植物学	博士		助理研究员	研究

42	黎　佳	女	1982.2	微生物学	博士		助理研究员	研究
43	李大卫	男	1983.5	植物学	博士		助理研究员	研究
44	李　黎	女	1985.12	微生物学	博士		助理研究员	研究
45	李书涛	男	1984.1	生物化学与分子生物学	博士		助理研究员	研究
46	李志能	男	1980.2	园林植物与观赏园艺	博士		助理研究员	研究
47	卢　洋	男	1981.7	植物学	博士		助理研究员	研究
48	施海涛	男	1984.12	发育生物学	博士		助理研究员	研究
49	王　坤	男	1981.12	遗传学	博士		助理研究员	研究
50	王　鲁	男	1976.12	发育生物学	博士		助理研究员	研究
51	王艳平	女	1983.01	发育生物学	博士		助理研究员	研究
52	王中杰	男	1984.8	水生生物学	博士		助理研究员	研究
53	周　莹	女	1981.6	分子生物学	博士		助理研究员	研究
54	耿亚洪	女	1962.6	经济管理学	本科		高级实验师	技术
55	李长福	女	1971.12	昆虫学	硕士		工程师	技术
56	梁　琼	女	1975.5	植物学	博士		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

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	韩月彭	湖北省遗传学会	理事	2009-
2	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-
		中国植物学会	理事	2008-2013
		中国植物学会植物园分会	副理事长	2008-2013
		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
3	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-
4	李夜光	中国海洋湖沼学会	理事	2008-2012
5	王　庆	中国植物学会第 14 届药用植物和植物药专业委员会	委员	2009-2013
6	王　艇	中国植物学会植物分类与系统进化专业委员会	委员	2008-2013
		中国花卉协会蕨类植物分会第四届理事会	理事	2010-2014
7	王　瑛	中国植物学会药用植物和植物药专业委员会	副主任	2009-
		湖北省植物学会	理事	2007-
		湖北省细胞生物学会	理事	2009-
8	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-
9	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-
10	钟彩虹	中国园艺学会猕猴桃分会	理事	2010
		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	郭明全	Current Analytical Chemistry	客座编辑	2011-2012
		The Scientific World Journal	编委	2011-
		Asian Journal of Chemistry	编委	2008-
2	傅金民	Ecotoxicology	编委	2010-
3	韩月彭	Plant Molecular Biology Reporter	副编辑	2008-
		Canadian Journal of Plant Science	编辑	2010-
4	李建强	Journal of Systematics and Evolution	副主编	2009-
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-
		《生物多样性》	编委	2006-2013

附录四 人才培养

1. 2012 年毕业研究生学位和论文情况

序号	姓名	性别	学位	所学专业	导师姓名	论文题目
1	黄文俊	男	博士	植物学	王　瑛	箭叶淫羊藿类黄酮代谢途径相关基因的克隆与功能分析
2	宋　驰	男	博士	植物学	王　瑛	基于植物基因组共线性及全基因组复制的物种特异和共性基因发掘
3	向巧彦	女	博士	植物学	李绍华	遮光对菊花品种‘丽金’花色素苷合成相关基因表达的影响
4	李峰奇	男	博士	植物学	彭俊华	小麦种质资源对麦长管蚜抗虫性和耐虫性的关联分析
5	梁　燕	女	博士	植物学	彭俊华	野生二粒小麦锈病抗性基因发掘、遗传变异和遗传转化研究
6	朱婷婷	女	博士	植物学	彭俊华	植物中NAC基因家族的进化与野生二粒小麦NAM-B1 基因等位变异的研究
7	梁　琼	女	博士	植物学	黄宏文	箭叶淫羊藿特异居群形态、主要化学成分及遗传多样性研究
8	钟彩虹	女	博士	植物学	黄宏文	中华猕猴桃（Actinidia chinensis Planch）倍性遗传及多倍体杂交育种研究
9	石　涛	男	博士	植物学	黄宏文	猕猴桃科与近缘科古多倍化与基因家族进化
10	张紫刚	男	博士	植物学	李建强	中国千金藤属的分类学和花形态发生研究
11	胡　涛	男	博士	植物学	傅金民	多年生黑麦草种质耐盐鉴定及耐性机理研究
12	张　琼	女	博士	植物学	韩月彭	苹果遗传图谱构建及糖酸品质性状的 QTLs 定位
13	王　博	男	硕士	植物学	彭俊华	能源植物油桐和中国芒种质资源的初步评价
14	戴李菁	女	硕士	植物学	彭俊华	高粱 SSR 分子标记对能源植物中国芒的可转移性研究
15	黄泽辉	男	硕士	植物学	傅金民	源于中国不同生境的狗牙根耐盐生理及遗传多样性研究
16	江丽丽	女	硕士	植物学	李夜光	淡水产油微藻的分子鉴定
17	罗曼曼	女	硕士	植物学	李晓东	中国特有珍稀濒危植物崖白菜属的遗传多样性分析
18	饶静云	女	硕士	植物学	黄宏文	中华猕猴桃不同倍性间杂交后代倍性分离和遗传变异分析
19	申健勇	男	硕士	植物学	吴金清	嘉陵江流域河岸带维管植物区系与植被研究
20	魏国燕	女	硕士	植物学	王瑛	同园栽培淫羊藿主要活性成分比较及基因组 DNA 甲基化研究
21	颜　菱	女	硕士	植物学	黄宏文	猕猴桃倍性混合居群基因组遗传及表观遗传变异分析
22	袁　珊	女	硕士	植物学	王恒昌	东亚特有珍稀濒危植物连香树的居群遗传学

						研究
23	祝　铭	女	硕士	植物学	李建强	同质园芒、荻、南荻的花粉基因流研究
24	阮咏梅	女	硕士	生态学	叶其刚	黄梅秤锤树孤立居群的空间遗传结构和基因流
25	张萍萍	女	硕士	植物学	傅金民	草地早熟禾对碱胁迫的生理响应
26	朱奉霞	女	硕士	植物学	杨　波	蕙兰内生细菌可分泌吲哚乙酸特性及在兰科组培中的共培养研究

2. 在读博士后

陈英明（指导教师：彭俊华）

陈柯（指导教师：傅金民）

3. 2012 年在读博士研究生

年级	姓　名	导师姓名	专业	年级	姓　名	导师姓名	专业
2008	肖　贡	王　瑛	植物学	2011	任　景	傅金民	植物学
2008	易　轩	王　艇	植物学	2011	杜志敏	傅金民	植物学
2009	李吉涛	李绍华	植物学	2011	孙小艳	傅金民	植物学
2009	李文彬	黄宏文	植物学	2011	孙延霞	李建强	植物学
2009	刘　迪	王　瑛	植物学	2011	李　明	杨平仿	植物学
2009	王淑慧	王　瑛	植物学	2011	韩　超	杨平仿	植物学
2009	梁　芳	李夜光	植物学	2011	李　晶	章焰生	植物学
2010	陈　莎	李绍华	植物学	2012	孙小明	李绍华	植物学
2010	胡　蝶	李建强	植物学	2012	马娟娟	韩月彭	植物学
2010	胡伟明	王　瑛	植物学	2012	杜　奎	李夜光	植物学
2010	杨爱红	黄宏文	植物学	2012	刘永亮	王　瑛	植物学
2010	张玲玲	傅金民 彭俊华	植物学	2012	A.B.M. Khaldun	王　瑛	植物学
2010	程　钧	韩月彭	植物学	2012	李　佳	王　艇	植物学
2010	王　博	王　艇	植物学	2012	邓　娇	杨平仿	植物学
2010	温小斌	李夜光	植物学	2012	谢　燕	傅金民	植物学
2010	李兆波	章焰生	植物学	2012	叶甜甜	产祝龙	植物学
2011	方林川	李绍华	植物学	2012	苟君波	章焰生	植物学
2011	杨路路	王　瑛	植物学	2012	朱洺志	郭明全	生态学
2011	周　晖	韩月彭	植物学				

4. 2012 年在读硕士研究生

年级	姓　名	导师姓名	专业	年级	姓　名	导师姓名	专业
2009	许　可	王　艇	植物学	2011	王 欣	杨平仿	生物工程
2010	潘　越	傅金民 彭俊华	植物学	2011	沈 佳	章焰生	生物工程
2010	余江艳	傅金民 彭俊华	植物学	2011	王传德	汪志伟	生物工程
2010	刘　洁	李建强	植物学	2011	王莉娜	李绍华	园林植物与观赏园艺
2010	廖思红	王　瑛	植物学	2011	沈笑飞	王　瑛	园林植物与观赏园艺
2010	刘　磊	黄宏文	植物学	2011	赵状军	傅金民	园林植物与观赏园艺
2010	尹小建	杨平仿	植物学	2011	魏国超	韩月彭	园林植物与观赏园艺
2010	张　丹	李夜光	植物学	2011	王应丽	王　瑛	园林植物与观赏园艺
2010	朱晓艳	李夜光	植物学	2011	刘瑞杰	产祝龙	园林植物与观赏园艺
2010	李　倩	章焰生	植物学	2012	董　霞	郭明全	植物学
2010	孙志强	章焰生	植物学	2012	范荣艳	章焰生	植物学
2010	项　悦	李绍华	园林植物与观赏园艺	2012	符子阳	杨平仿	植物学
2010	郭慧娟	傅金民	园林植物与观赏园艺	2012	陶　珂	王　艇	植物学
2010	马百全	韩月彭	园林植物与观赏园艺	2012	薛定磊	王　瑛	植物学
2010	祝　为	李绍华	生物工程	2012	闫明科	姚小洪	植物学
2010	罗宏基	傅金民	生物工程	2012	杨　力	产祝龙	植物学
2010	韩艳妮	韩月彭	生物工程	2012	李菲菲	李建强	植物学
2010	刘司浪	吴金清	生物工程	2012	柴风梅	李绍华	园林植物与观赏园艺
2011	刘春燕	黄宏文	植物学	2012	韩春宇	王　勇	园林植物与观赏园艺
2011	范吉标	傅金民	植物学	2012	郑红玉	韩月彭	园林植物与观赏园艺
2011	姜 斌	王 艇	植物学	2012	成章敏	产祝龙	生物工程
2011	张 虎	李夜光	植物学	2012	蒋晓明	郭明全	生物工程
2011	冯 涛	李建强	植物学	2012	陶焕平	李夜光	生物工程
2011	张 慧	杨平仿	植物学	2012	周　晨	章焰生	生物工程
2011	李缘君	章焰生	植物学	2012	傅金磊	杨平仿	生物工程
2011	刘淑倩	傅金民	生物工程	2012	马持衡	吕世友	生物工程
2011	赵 双	韩月彭	生物工程	2012	李虹侠	吕世友	生物工程

5. 2012 年研究生获奖一览表

序号	获奖名称	获奖人员	指导教师
1	昌华奖学金特别奖	陈　莎	李绍华
2	昌华奖学金优秀奖	胡　涛	傅金民
3	昌华奖学金优秀奖	罗宏基	傅金民
4	国家奖学金	陈　莎	李绍华
5	国家奖学金	罗宏基	傅金民
6	国家奖学金	王传德	汪志伟
7	院“优秀学生干部”	韩　超	杨平仿
8	院“优秀学生干部”	温小斌	李夜光
9	院“三好学生”	杨爱红	黄宏文
10	院“三好学生”	谢　燕	傅金民
11	院“三好学生”	饶静云	黄宏文
12	院“三好学生”	罗宏基	傅金民
13	院“三好学生”	杨路路	王　瑛
14	院“三好学生”	张玲玲	傅金民、彭俊华
15	院“三好学生”	王　博	王　艇
16	院“三好学生”	张　琼	韩月彭
17	院“三好学生”	陈　莎	李绍华
18	院“三好学生”	孙延霞	李建强
19	院“三好学生”	李　倩	章焰生
20	院“三好学生”	刘司浪	吴金清
21	院“优秀毕业生”	胡　涛	傅金民
22	武汉教育基地“优秀毕业生”	朱婷婷	彭俊华
23	武汉教育基地“优秀毕业生”	魏国燕	王　瑛

附录五 合作与交流

1. 举办的国际国内学术会议

序号	会议名称	会议类别	主办单位	会议主席	会议日期	参加人数
1	国际草坪学研究与发展策略论坛	国际会议	中国科学院武汉植物园	美国罗格斯大学教授黄炳茹博士	6 月 30 日-7 月 1 日	70 余人
2	第四届国际农业蛋白质组学前沿论坛	国际会议	亚洲大洋洲农业蛋白质组组织	日本筑波大学 Setsuko Komatsu 教授、中国科学院武汉植物园李绍华研究员	11 月 9 日-11 日	150 余人

2. 出访项目

2011 年 8 月 4 日-2012 年 8 月 1 日，李新伟副研究员赴美国 The University of Texas at Austin 进修，在国际著名的表观遗传学家 Jeffrrey Z. Chen 教授的指导下，开展拟南芥 Mir172 对其靶基因表达的调控研究。

2011 年 11 月 18 日-2013 年 2 月 24 日，汪志伟副研究员赴英国 John Innes Centre，在英国皇家科学院院士、德国科学院院士及美国科学院外籍院士 Caroline Dean 教授指导下从事植物开花时间分子表观遗传调控合作研究。

1 月 8 日-2013 年 1 月 8 日，在教育部国家留学基金委的资助下，王彦昌副研究员应新西兰 The University of Waikato Dr. Michael Clearwater 邀请，前往该校从事猕猴桃溃疡病合作研究。

1 月 27 日-2 月 27 日，傅金民研究员赴美国开展草坪逆境生理和新品种选育的合作研究。

3 月 19 日-23 日，王庆研究员赴美国开展有关植物紫背天葵和土人参功能作用机理的合作研究。

3 月 25 日-29 日，李绍华研究员和韩月彭研究员赴新西兰，参加“第二届果树生物技术国际交流会”。

3 月 30 日-4 月 5 日，李绍华研究员赴肯尼亚，进行中肯共建“乔莫-肯尼亚塔农业与科技大学植物园”考察。

4 月 16 日-7 月 13 日，应加拿大研究理事会植物生物技术研究所邀请，章焰生研究员赴加拿大，开展青蒿素生物合成酶分子理性改造的合作研究。

6 月 17 日-27 日，李绍华研究员赴法国参加 2012 葡萄研究国际会议及创建中法葡萄研究联合实验室研讨。

6 月 18 日-22 日，王瑛研究员赴乌干达参加“21 世纪全球木薯伙伴科学会议”，并作了题为“Comparative Genomics of major Euphorbiaceae species”的报告。

8 月 15 日-21 日，王瑛研究员赴美国，开展药用植物可持续开发利用合作研究。

8 月 26 日-30 日，王瑛研究员赴瑞士参加“第九次茄科基因组学国际会议”，并作了题为“番茄和马铃薯基因组信息在枸杞研究中的应用”的报告。

10 月 4 日-10 日，郭明全研究员赴美国参加第 28 届 ASILOMAR 会议（质谱在食品安全与质量控制

方面的应用），并作了题为“Studies on plant flavonoid and their non-covalent complexes”的报告。

10 月 24 日-27 日，王瑛研究员赴韩国，参加韩国药用植物协会 2012 年年会，并作了题为“药用植物淫羊藿的转录组学研究”的报告。

11 月 11 日-15 日，王庆研究员赴澳大利亚，参加系统和网络生物学与中医药学学术大会。

3. 来访活动

4 月 14 日-16 日，应杨平仿研究员的邀请，国际著名的植物线粒体和种子蛋白质组学专家、丹麦奥胡斯大学 Ian Max Møller 教授于来重点实验室，就植物种子的蛋白质组学研究开展学术交流。

5 月 21 日-28 日，应韩月彭研究员的邀请，美国克莱姆森大学 Ksenija Gasic 博士来重点实验室，就果树育种及遗传学研究开展学术交流。

6 月 25 日，应李建强研究员的邀请，美国北卡罗来纳大学孟少武博士来重点实验室，就植物生物信息学和基因组学研究开展学术交流。

8 月 7 日-10 日，应韩月彭研究员的邀请，新西兰植物和食品研究所 Andrew Allen 副研究员来重点实验室，就果实着色研究开展学术交流。

8 月 4 日-16 日，应杨平仿研究员的邀请，国际著名植物蛋白质组学专家、日本国立作物研究所、筑波大学 Setsuko Komatsu 教授来重点实验室，就植物蛋白质组学研究开展合作交流。

9 月 10 日-11 月 10 日， 应韩月彭研究员的邀请，中科院特聘研究员美国伊利诺伊大学 Korban S Schuyler 教授来重点实验室，就苹果基因组学与分子育种开展合作交流。

10 月 6 日-11 月 29 日，应杨平仿研究员的邀请，国际著名植物蛋白质组学专家、日本国立作物研究所、筑波大学 Setsuko Komatsu 教授来重点实验室，就植物蛋白质组学研究开展合作交流。

12 月 25 日-26 日，应产祝龙研究员的邀请，美国科学院院士、中组部顶尖“千人计划”入选者朱健康教授来重点实验室，就植物抗逆分子生物学研究开展学术交流。

4. 学术报告

序号	时间	报告人	报告人单位	报 告 题 目
1	4 月 11 日	杨耀东	中国热带农业科学院椰子研究所	生长素转运载体蛋白的功能分析及线虫诱导的巨形细胞形成的相关性研究
2	4 月 16 日	Ian Max Møller	丹麦奥胡斯大学	植物种子的蛋白质组学研究
3	5 月 11 日	吴昌银	华中农业大学	水稻突变体库的创建及育性基因的功能分析
4	5 月 21 日	Ksenija Gasic	美国克莱姆森大学	Peach breeding program at clemson university
5	6 月 25 日	孟少武	美国北卡罗来纳大学	Gene ontology annotation of the rice blast fungus, *Maganaporthe grisea*

6	8 月 6 日	Setsuko Komatsu	日本筑波大学	Concept and Technique of Proteomics: Application of proteomics to functional analysis of soybean
7	8 月 7 日	Setsuko Komatsu	日本筑波大学	Application of Proteomics to Investigate Stress-induced Proteins
8	9 月 4 日	Korban S Schuyler	美国伊利诺伊大学	Genomic analysis of the flavonoid biosynthesis pathway
9	12 月 25 日	朱健康	美国普渡大学	Osmotic Stress Sensing and Signaling in Plants

5. 在研开放课题

序号	课题名称	起止时间	总经费（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

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	6.4	3
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	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	1
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	1
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

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Expression profiling of ABA pathway transcripts indicates crosstalk between abiotic and biotic stress responses in Arabidopsis

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ABSTRACT

Recent breakthrough on identification and characterization of PYR/PYLs as ABA receptors enables us to better understand the perception, signaling and transportation of ABA in plant. Based on publicly available microarray data, transcriptional levels of ABA signaling pathway core components were compared after stress and phytohormone treatments, including these involved in ABA metabolism, signal transduction, and catabolism. The results showed that both abiotic and biotic stress treatments increased the expression levels of ABA key metabolism and catabolism transcripts. The expression levels of PYR/PYLs were down-regulated and these of PP2Cs and ABFs were uniformly up-regulated after exogenous ABA application and under stress conditions. The results indicated that the increased ratio of PP2Cs:PYR/PYLs might be required for activation of the downstream ABA signal pathway under both abiotic and biotic stress conditions. We concluded that abiotic and biotic stress responses shared ABA signal pathway in Arabidopsis.

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1. Introduction

The plant hormone ABA regulates many key processes in plants and serves as an endogenous messenger in biotic and abiotic stress responses [1–3]. Abiotic stress such as drought and high salinity results in strong increases of ABA levels accompanied by a major change in gene expression [4–7]. To date, many ABA signaling components have been identified. ABA receptors have until recently remained either elusive or contested. A family of novel START domain proteins, known as PYR/PYLs (also known as RCARs), were identified as ABA receptors by separate research groups. The results showed that several PYR/PYLs interact with and inhibit clade A PP2Cs [8–11]. Seventy-six Arabidopsis genes were identified as PP2C-type phosphatase candidates [12,13] and six of the nine PP2Cs in clade A have been identified as negative regulators of ABA response [14–19]. In contrast, SnRK2s act as positive signaling components in ABA signaling [3,20–23]. The default state of the SnRK2 protein kinases is an autophosphorylated, active state, and that the SnRK2 kinases are kept inactive by the PP2Cs through physical interaction and dephosphorylation [10]. PYR/PYLs interact with and are able to inactivate the PP2Cs after binding ABA. The ABA-bound receptors also disrupt or decrease the interaction between the PP2Cs and the SnRK2s, thus preventing the PP2C-mediated dephosphorylation and thereby relieving inhibition of the SnRK2s [10]. Accumulation of phosphorylated SnRK2s leads to subsequent phosphorylation of the basic leucine zipper (bZIP) transcription factors called ABFs/AREBs [24,25]. The ABFs then bind to ABA-responsive promoter elements (ABRE) to induce the expression of ABA-responsive genes [26].

ABA response eventually leads to changes in gene expression. Exogenous ABA as well as conditions that increase endogenous ABA redirect the expression of part of Arabidopsis genome [4,5,27,28]. However, it is not clear how short-term and long-term stress and phytohormone treatments affect expression levels of ABA pathway core components. Recently, two groups found that ABA and abiotic stress conditions or treatments altered the relative levels of PYR/PYL/RCAR and PP2C family members and increased the PP2Cs:PYR/PYLs ratio [11,29]. These results indicated that higher PP2Cs:PYR/PYLs ratio would lead to a desensitization of the ABA response. However, these data were obtained from one specific time point and only three abiotic stress conditions were applied in one study. Furthermore, only several ABA signaling transcripts were included in these manuscripts. Considering at least 252 different receptor/PP2C/SnRK2 complexes (14 receptors × 6 PP2Cs × 3 SnRK2s) in Arabidopsis, it is important to understand how different stresses and ABA treatment change the expression of the whole ABA pathway transcripts and thus alter the sensitivity and plasticity of the response. In addition, the high dynamic range of ABA levels intrigues us to check dynamic changes of these transcripts under stress conditions.

In this study, we reported transcriptional profiling of Arabidopsis ABA pathway core components after abiotic stress, biotic stress and plant hormone treatments based on publicly available microarray

Abbreviations: ABA, abscisic acid; PP2C, protein phosphatase 2C; SnRK, sucrose nonfermenting (SNF)-related kinase; ABF, abscisic acid-responsive element binding factor; PYR/PYL, *PYRABACTIN RESISTANCE 1/PYR like*; RCAR, regulatory components of ABA receptor.

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Diversity of Genome Size and Ty1-*copia* in *Epimedium* Species Used for Traditional Chinese Medicines

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Additional index words. Epimedium, genome size, Ty1-*copia,* NJ phylogenetic tree, FISH

Abstract. Epimedium **species are traditional Chinese medicinal plants as well as potential groundcover and ornamental plants. In this study, genome size and genome structures of *Epimedium* species were investigated using flow cytometric and fluorescence in situ hybridization (FISH). The nuclear DNA content of *Epimedium* species ranged from 8.42 pg/2C (8230.7 Mbp) to 9.97 pg/2C (9752.8 Mbp). The pairwise nucleotide diversity (π) of the fragments of the genes for reverse transcriptase (*rt*) of Ty1-*copia* retrotransposon within a species of *rt* fragments ranged from 0.251 to 0.428 in 10 *Epimedium* species. Phylogenetic analysis of the sequences revealed four major clades with the largest subclade containing 72 sequences of relatively low nucleotide diversity. FISH indicated that Ty1-*copia* retrotransposons are distributed unevenly along the pachytene chromosomes of *E. wushanense* and *E. sagittatum*, mostly associated with the pericentromeric and terminal heterochromatin. The relatively low sequence heterogeneity of Ty1-*copia rt* sequences implies that the *Epimedium* genomes have experienced a few relatively large-scale proliferation events of *copia* elements, which could be one of the major forces resulting in the large genome size of *Epimedium* species.**

Epimedium L. ($2n = 2x = 12$), referred to as yin yang huo in Chinese, belongs to the basal eudicot plant family, berberidaceae. The genus of *Epimedium* is composed of more than 50 species (Stearn, 2002), most of which are widely distributed in China and commonly used as traditional Chinese medicinal herbs (Ying, 2002) and as ornamental plants (Stearn, 2002). In particular, *Epimedium* species are of great interest because of their pharmacological properties in the treatment of impotence, spermatorrhea, infertility, amenorrhea, and in improving menopause symptoms (Wu et al., 2003). In China, Herba Epimedii is usually comacerated in wine with other traditional medicines contributing to prevent disease and strengthen immunity (Ma et al., 2011). Four flavonoids, epimedin A, epimedin B, epimedin C, and icarrin, were believed as the major active components in *Epimedium* and were regarded as markers for quality control (Chen et al., 2007; Xie and Sun, 2006; Xie et al., 2010). Five species are officially recorded as medicinal plants in the Chinese Pharmacopoeia, including *E. brevicornum* Maxim, *E. sagittatum* (Sieb. et Zucc), *E. pubescens* Maxim, *E. wushanense* T. S. Ying, and *E. koreanum* Nakai (Chinese Pharmacopoeia Commission, 2005).

Repetitive DNA is the major components of plant genomes (Kubis et al., 1998), which is the primary determinant of genome size and structure and plays an important role in genome evolution. Plant genome size differs as a result of variable amounts of repetitive DNA (Bennett and Leitch, 2011; Flavell et al., 1974). Polyploidization, unequal recombination, and illegitimate recombination leading to plant genome expansion and contraction may be the major driving forces for plant genome size variation (Bennetzen, 2002). Moreover, retrotransposon insertions through a "copy and paste" mechanism also can increase host genome size rapidly (Hawkins et al., 2006; Piegu et al., 2006).

Known as the most abundant repetitive DNA, plant transposable elements (TEs) are classified as RNA-mediated TEs (Class 1) and DNA-mediated TEs (Class 2) according to their transposition intermediate (Feschotte et al., 2002). Class 1 TEs are divided into long terminal repeat retrotransposons (LTR) and non-LTR retrotransposons. LTR retrotransposons can be further classified as Ty1-*copia* and Ty3-*gypsy* elements based on the order of their coding domains. Ty1-*copia* group retroransposons have been shown to be present throughout almost all plant genomes with high copy numbers (Flavell et al., 1992a). Sequence analyses of polymerase chain reaction (PCR) fragments of reverse transcriptase conserved domains have revealed very high degrees of sequence heterogeneity in many plants (Flavell et al., 1992b; Kumar et al., 1997). Phylogenetic studies of these LTR retrotransposon families provide information of unknown genomic components and suggest causes of genome size variation.

Most recent studies on *Epimedium* have concentrated on its chemical composition (Jiang et al., 2009; Wu et al., 2011; Zhao et al., 2008), pharmacological properties (Wong et al., 2009), phylogenetic relationship (Sun et al., 2005), karyotype (Sheng et al., 2010; Zhang et al., 2008), and genetic diversity (Xu et al., 2007; Zhou et al., 2007). Despite its great potential value, genomic characteristics, including genome size and genome structure of *Epimedium,* have received little attention. Genomic analyses of *Epimedium* will provide basic information on genome duplication, speciation, and its complex metabolism. In this study, we aimed to characterize the *Epimedium* genome in terms of the nuclear DNA contents, the sequence diversity, and genomic distribution of Ty1-*copia* retrotransposons.

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RESEARCH PAPER

The *Brassica napus* Calcineurin B-Like 1/CBL-interacting protein kinase 6 (CBL1/CIPK6) component is involved in the plant response to abiotic stress and ABA signalling

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Abstract

A CBL-interacting protein kinase (CIPK) gene, *BnCIPK6,* was isolated in *Brassica napus*. Through yeast two-hybrid screening, 27 interaction partners (including BnCBL1) of BnCIPK6 were identified in *Brassica napus*. Interaction of BnCIPK6 and BnCBL1 was further confirmed by BiFC (bimolecular fluorescence complementation) in plant cells. Expressions of *BnCIPK6* and *BnCBL1* were significantly up-regulated by salt and osmotic stresses, phosphorous starvation, and abscisic acid (ABA). Furthermore, *BnCIPK6* promoter activity was intensively induced in cotyledons and roots under NaCl, mannitol, and ABA treatments. Transgenic *Arabidopsis* plants with over-expressing *BnCIPK6*, its activated form *BnCIPK6M*, and *BnCBL1* enhanced high salinity and low phosphate tolerance, suggesting that the functional interaction of BnCBL1 and BnCIPK6 may be important for the high salinity and phosphorous deficiency signalling pathways. In addition, activation of BnCIPK6 confers *Arabidopsis* plants hypersensitive to ABA. On the other hand, over-expression of *BnCIPK6* in *Arabidopsis cipk6* mutant completely rescued the low-phosphate-sensitive and ABA-insensitive phenotypes of this mutant, further suggesting that *BnCIPK6* is involved in the plant response to high-salinity, phosphorous deficiency, and ABA signalling.

Key words: Abiotic stress tolerance, abscisic acid (ABA), *Brassica napus*, BnCBL1, BnCIPK6, interaction, regulation of gene expression.

Introduction

As an essential second messenger, calcium regulates diverse cellular processes in plants. Several Ca^{2+}-sensor protein families, including calmodulin (CaM), the superfamily of calcium-dependent protein kinases (CDPK), and calcineurin B-like (CBL) proteins, have been characterized and implicated in a variety of physiological functions in plants (Albrecht *et al*., 2001; Kim *et al*., 2003; Pandey *et al*., 2004). Ca^{2+} sensors can be classified into sensor responders and sensor relays (Sanders *et al*., 2002). Upon binding of Ca^{2+}, sensor responders change their conformation and modulate their own enzymatic activity or function through intramolecular interactions. By contrast, sensor relays must interact with their target proteins (such as protein kinases) to regulate their activity after binding Ca^{2+}. CDPKs act as sensor responders (Sanders *et al*., 2002; Kim *et al*., 2003), while CaM and CBL proteins are sensor relays (Luan *et al*., 2002; Sanders *et al*., 2002). However, unlike CaMs targeting a large variety of target proteins, CBLs specifically interact with a family of protein kinases referred to as CBL-interacting protein kinases (CIPKs) or SnRK3s (Luan *et al*., 2002). 10 CBLs and 25 CIPKs in *Arabidopsis* and 10 CBLs and 30 CIPKs in rice were

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Simultaneous qualitative assessment and quantitative analysis of flavonoids in various tissues of lotus (*Nelumbo nucifera*) using high performance liquid chromatography coupled with triple quad mass spectrometry

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ABSTRACT

Flavonoid composition and concentration were investigated in 12 different tissues of 'Ti-1' lotus (*Nelumbo nucifera*) by high performance liquid chromatography equipped with photodiode array detection tandem electrospray ionization mass spectrometry (HPLC-DAD-ESI-MS^n). A total of 20 flavonoids belonging to six groups (myricetin, quercetin, kaempferol, isohamnetin, diosmetin derivatives) were separated and identified. Myricetin 3-*O*-galactoside, myricetin 3-*O*-glucuronide, isorhamnetin 3-*O*-glucuronide and free aglycone diometin (3′,5,7-trihydroxy-4′-methoxyflavone) were first reported in lotus. Flavonoid composition varied largely with tissue type, and diverse compounds (5–15) were found in leaf and flower stalks, flower pistils, seed coats and embryos. Flower tissues including flower petals, stamens, pistils, and, especially, reproductive tissue fruit coats had more flavonoid compounds (15–17) than leaves (12), while no flavonoids were detectable in seed kernels. The flavonoid content of seed embryos was high, 730.95 mg 100 g^{-1} DW (dry weight). As regards the other tissues, mature leaf pulp (771.79 mg 100 g^{-1} FW (fresh weight)) and young leaves (650.67 mg 100 g^{-1} FW) had higher total flavonoid amount than flower stamens (359.45 mg 100 g^{-1} FW) and flower petals (342.97 mg 100 g^{-1} FW), while leaf stalks, flower stalks and seed coats had much less total flavonoid (less than 40 mg 100 g^{-1} FW).
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1. Introduction

Lotus (*Nelumbo nucifera* Gaertn.), a traditional Chinese medicinal herb, is a flavonoid-rich plant. It has been cultivated for more than 2000 years in China and consumed around the world [1]. Almost all the tissues of lotus, including leaves, leaf stalks, flower stalks, flower petals, flower stamens, flower pistils, seeds and rhizomes, are used as vegetables or traditional Chinese medicinal herbs [2]. Lotus seed kernels and rhizomes are usually used as a healthful cooked food, and they are often considered as human health immunomodulators [3,4]. Moreover, leaves and embryos have been evaluated as important Chinese herbal drugs [5]. Petals and stamens containing natural pigment and flavonols are made into healthy tea and functional food additions, and they also have ornamental value [6–8].

Lotus leaves and embryos are rich in flavonoids and other secondary metabolites, and they have been extensively studied for their antioxidant, antibacterial, anti-HIV and anti-obesity functions [9–14]. Eight flavonoids in lotus stamens were isolated and identified by nuclear magnetic resonance spectroscopy (NMR), and their antioxidant properties were revealed by some free radical ion scavenging activity tests [15]. Recently, twelve flavonoids were identified in lotus petals, and five of these flavonoids were also found in lotus fruit coats by HPLC-MS^n [16,17]. The successful identification and quantification of flavonoids has greatly helped the study of antioxidant and other healthy-protective properties in lotus. In addition, leaves and stamens of lotus have been identified as enriched sources of natural flavonoids [12,15]. However, flavonoid composition and accumulation varies with tissue, which may be largely modulated by genetic regulation [18]. Tissue-dependent assessment may not only be essential for quality control of the medicinal herb, but also effective as traceable markers for genetic and metabolic research [18–20].

Spectrophotometry, thin-layer chromatography (TLC), and high-performance liquid chromatography (HPLC) are the methods

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Analysis of flavonoids from lotus (*Nelumbo nucifera*) leaves using high performance liquid chromatography/photodiode array detector tandem electrospray ionization mass spectrometry and an extraction method optimized by orthogonal design

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ABSTRACT

The extraction protocol of flavonoids from lotus (*Nelumbo nucifera*) leaves was optimized through an orthogonal design. The solvent was the most important factor comparing solvent, solvent:tissue ratio, extraction time, and temperature. The highest yield of flavonoids was achieved with 70% methanol–water and a solvent:tissue ratio of 30:1 at 4 °C for 36 h. The optimized analytical method for HPLC was a multi-step gradient elution using 0.5% formic acid (A) and CH_3CN containing 0.1% formic acid (B), at a flow rate of 0.6 mL/min. Using this optimized method, thirteen flavonoids were simultaneously separated and identified by high performance liquid chromatography coupled with photodiode array detection/electrospray ionization mass spectrometry (HPLC/DAD/ESI-MS^n). Five of the bioactive compounds are reported in lotus leaves for the first time. The flavonoid content of the leaves of three representative cultivars was assessed under the optimized extraction and HPLC analytical conditions, and the seed-producing cultivar 'Baijianlian' had the highest flavonoid content compared with rhizome-producing 'Zhimahuoulian' and wild floral cultivar 'Honglian'.

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1. Introduction

Lotus (*Nelumbo nucifera* GAERTN.), which is distributed widely throughout East Asia, Australia and North America, is an aquatic plant that has been cultivated for thousands of years and holds particular religious significance [1]. All of the tissues of *N. nucifera*, including the leaves, stamens, flowers, rhizomes, seeds and the embryo of seeds, are commonly used as traditional medicines as well as being common foods. They are known to contain bioactive components such as flavonoids and alkaloids in addition to nutritional ingredients like carbohydrates, proteins and fats [2–6].

Flavonoids have been isolated and characterized from various plants [7], and previous studies have shown that lotus leaves are rich in flavonoids [8,9]. The antioxidant [9,10], antibacterial [8], anti-HIV [11], antimalarial and antifungal [12], anti-obesity [13–15] and potential anti-atherogenic [16] activities of lotus leaves have been evaluated and reported in recent years. The biological activities of lotus leaves that have led to its use as a traditional medicine have been identified. However, the physiological impacts are strongly dependent on the composition of flavonoids and their contents [17].

Classical separation and identification methods used for flavonoids are high performance liquid chromatography (HPLC) coupled with tandem mass spectrometry (MS) and high-speed counter-current chromatography coupled with nuclear magnetic resonance spectroscopy (HSCCC)-NMR. The former is a fast and reliable method for flavonoid analysis, and it has been widely applied during recent years due to its low limit detection. Compositional analysis of flavonoids by HPLC depends on the development of successful separation protocols. This means that the use of different experimental conditions including the mobile solvent system, elution gradient, column temperature and elution flow rate can have a significant influence on the separation of often closely-related compounds. The mobile solvent system is a particularly important factor in flavonoid separation. Formic acid was added to the mobile phase to allow the separation of flavonoids from lotus leaves by Goo et al. [18] and Deng et al. [19], and five

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PLoS one

Diversification of Genes Encoding Granule-Bound Starch Synthase in Monocots and Dicots Is Marked by Multiple Genome-Wide Duplication Events

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Abstract

Starch is one of the major components of cereals, tubers, and fruits. Genes encoding granule-bound starch synthase (GBSS), which is responsible for amylose synthesis, have been extensively studied in cereals but little is known about them in fruits. Due to their low copy gene number, *GBSS* genes have been used to study plant phylogenetic and evolutionary relationships. In this study, *GBSS* genes have been isolated and characterized in three fruit trees, including apple, peach, and orange. Moreover, a comprehensive evolutionary study of *GBSS* genes has also been conducted between both monocots and eudicots. Results have revealed that genomic structures of *GBSS* genes in plants are conserved, suggesting they all have evolved from a common ancestor. In addition, the *GBSS* gene in an ancestral angiosperm must have undergone genome duplication ~251 million years ago (MYA) to generate two families, *GBSSI* and *GBSSII*. Both *GBSSI* and *GBSSII* are found in monocots; however, *GBSSI* is absent in eudicots. The ancestral *GBSSII* must have undergone further divergence when monocots and eudicots split ~165 MYA. This is consistent with expression profiles of *GBSS* genes, wherein these profiles are more similar to those of *GBSSII* in eudicots than to those of *GBSSI* genes in monocots. In dicots, *GBSSII* must have undergone further divergence when rosids and asterids split from each other ~126 MYA. Taken together, these findings suggest that it is *GBSSII* rather than *GBSSI* of monocots that have orthologous relationships with *GBSS* genes of eudicots. Moreover, diversification of *GBSS* genes is mainly associated with genome-wide duplication events throughout the evolutionary course of history of monocots and eudicots.

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Introduction

Plant starch consists of a mixture of two different components, amylose (20–30%) and amylopectin (70–80%). Amylose is a linear polymer of glucose (Glc) residues joined together by α-1,4-glucosidic bonds, while amylopectin is a highly branched glucose polymer with α-1,6-glucosidic bonds linking linear chains. Amylose synthesis is relatively simple, and it is mainly catalyzed by granule-bound starch synthase (GBSS), which is encoded by the *waxy* or the *GBSS* gene. In contrast, the synthesis of amylopectin is rather complex and involves coordinated activities of different classes of enzymes, including soluble starch synthases (SSs), starch branching enzymes (SBEs), and starch debranching enzymes (DBEs) [1,2]. Of these enzymes, SBEs introduce α-1,6-glucosidic linkages into polyglucans, while DBEs hydrolyze α-1,6-glucosidic linkages and play an important role in determining starch structure and granule characteristics during starch biolsynthesis [2]. SSs catalyze the transfer of Glc from ADP-Glcose (ADP-Glu) to non-reducing ends of glucan chains via an α-1,4-glucosidic linkage.

Genes encoding GBSS have been well characterized in starch crops as amylose content has a significant impact on physico-chemical properties of starch [3]. GBSS differs from other SS isoforms due to its localization in granules and its unique functional role in starch synthesis. Not only it can transfer glucosyl residues from ADP-Glu to glucan substrates to produce relatively long-chain amylose molecules, but it also acts on side chains of amylopectin to form long chains of amylopectin [2]. The latter activity may have been the original function of GBSS, early in its evolutionary path. Moreover, amylose synthesis may be responsible for starch density, and ultimately improving the efficiency of carbon storage, thus justifying the conservation of GBSS in higher plants [2].

In cereals, GBSS consists of two isoforms, GBSSI (also known as waxy protein) and GBSSII. The *GBSSI* gene is exclusively expressed in storage tissues such as endosperms and embryos of

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Selenium Enrichment on *Cordyceps militaris* Link and Analysis on Its Main Active Components

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Abstract To investigate the effects of selenium on the main active components of *Cordyceps militaris* fruit bodies, selenium-enriched cultivation of *C. militaris* and the main active components of the fruit bodies were studied. Superoxide dismutase (SOD) activity and contents of cordycepin, cordycepic acid, and organic selenium of fruit bodies were sodium selenite concentration dependent; contents of adenosine and cordycep polysaccharides were significantly enhanced by adding sodium selenite in the substrates, but not proportional to sodium selenite concentrations. In the cultivation of wheat substrate added with 18.0 ppm sodium selenite, SOD activity and contents of cordycepin, cordycepic acid, adenosine, cordycep polysaccharides, and total amino acids were enhanced by 121/145%, 124/74%, 325/520%, 130/284%, 121/145%, and 157/554%, respectively, compared to NS (non-selenium-cultivated) fruit bodies and wild *Cordyceps sinensis*; organic selenium contents of fruit bodies reached 6.49 mg/100 g. So selenium-enriched cultivation may be a potential way to produce more valuable medicinal food as a substitute for wild *C. sinensis*.

Keywords *Cordyceps militaris* · Selenium · Active components · Trace minerals · Medicinal value

Practical applications *C. militaris* is widely used as a nutraceutical in functional foods or a phytopharmaceutical for treating some serious illnesses; selenium, a newly discovered anticancer element in recent years, can significantly enhance the anticancer activity of functional foods. However, most areas of the earth lack selenium. Selenium-enriching cultivation can significantly enhance contents of organic selenium and cordycepin in *C. militaris* fruit bodies. This study showed a potential method to produce selenium-enriched *C. militaris* fruit bodies with higher medicinal value than the commonly used wild *C. sinensis*, selenium supplementation of food and a primary source of cordycepin which had been reported to have great potential as an anticancer compound. The results also suggested a potential way for liquid fermentation to produce selenium-enriched *C. militaris* products, especially to enhance cordycepin production.

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Effects of Selenium and Light Wavelengths on Liquid Culture of *Cordyceps militaris* Link

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Abstract To investigate the effects of selenium and light wavelengths on the growth of liquid-cultured *Cordyceps militaris* and the main active components' accumulation, culture conditions as selenium selenite concentrations and light of different wavelengths were studied. The results are: adenosine accumulation proved to be significantly selenium dependent (R^2=0.9403) and cordycepin contents were determined to be not significantly selenium dependent (R^2=0.3845) but significantly enhanced by selenium except for 20 ppm; there were significant differences in cordycepin contents, adenosine contents, and mycelium growth caused by light wavelengths: cordycepin, blue light > pink light > daylight, darkness, red light; adenosine, red light > pink light, darkness, daylight, blue light; and mycelium growth, red light>pink light, darkness, daylight > blue light. In conclusion, light wavelength had a significant influence on production of mycelia, adenosine, and cordycepin, so lightening wavelength should be changed according to target products in the liquid culture of *C. militaris*.

Keywords *Cordyceps militaris* · Cordycepin · Adenosine · Light wavelength · Selenium · Mycelium growth

Introduction

Cordyceps sinensis Sacc. and *Cordyceps militaris* Link, a kind of caterpillar-shaped Chinese traditional mushrooms, named Dong Chong Xia Cao in Chinese herbs, are entomopathogenic fungi in the class of Ascomycetes. *C. militaris* has been extensively used as a folk medicine in East Asia as Korea, China, and Japan for revitalization of various systems of the

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Rooting in a Creeping Bentgrass Putting Green in Response to Spring and Summer Coring

Jinmin Fu and Peter H. Dernoeden*

ABSTRACT

Understanding root growth response of creeping bentgrass (*Agrostis stolonifera* L.) to coring will help golf course superintendents maintain high quality putting green turf. The objective of this field study was to examine the effects of coring on summer rooting in young creeping bentgrass grown on a sand-based root zone and maintained as a putting green. The study was initiated on 7-mo-old 'Providence' creeping bentgrass in 2006 and compared spring (SP) only coring, spring plus three summer (SU) corings (SP + SU) and a noncored control through 2007. The minirhizotron imagining technique was used to measure total root count (TRC) and total root length (TRL) from late spring to late summer. The percentage of the TRC in the surface 0- to 6-cm root zone depth averaged over measurement dates was 48 to 53% and 33 to 44% among all treatments in 2006 and 2007, respectively. Greater TRC were observed in 2006 with 28, 51, and 50% lower TRC's found in SP + SU, SP only, and noncored plots in 2007, respectively. Spring + SU coring generally reduced TRC and TRL at various root zone depths and dates during the first year of establishment. In 2007, greater TRC and TRL were observed throughout the 0- to 24-cm root zone in SP + SU cored compared to SP only and noncored plots. Thus, SP + SU coring in the second study year promoted creeping bentgrass root growth and/or longevity, but coring during the first summer of establishment reduced rooting.

Creeping bentgrass is the most widely used cool-season turfgrass on golf greens. Creeping bentgrass is aggressively stoloniferous and produces a well-defined surface organic layer, which hereafter will be referred to as the thatch-mat layer (McCarty et al., 2007). Excessive thatch-mat layers commonly are associated with negative effects on biological and soil properties as summarized by McCarty et al. (2005). Managing thatch-mat layers on putting greens is difficult and involves numerous cultural practices, including core cultivation (McCarty et al., 2007). In turfgrass management the term cultivation refers to working the soil and/or thatch-mat layer without destroying the turf (Turgeon, 2008). Coring (i.e., hollow or solid tines are used) is a cultivation technique (Beard, 1973) and the term coring will be used hereafter. Coring is used to manage thatch-mat layers and improve turf quality by promoting water infiltration, reducing soil surface wetness, and improving aeration and rooting (Beard, 1973; Fry and Huang, 2004).

Some research efforts involving coring have examined rooting by destructive sampling methods (Harper, 1953; Murphy et al., 1993; Wiecko et al., 1993). Reports on turf rooting in response to coring, however, have not been consistent. In a fairway coring study, Harper (1953) reported that a single spring coring did not affect bentgrass root mass compared to noncored bentgrass. In a Georgia study, Tifway bermudagrass [*Cynodon transvaalensis* Burtt-Davy × *C. dactylon* (L.) Pers] was cored to a 7.6-cm soil depth using hollow tines between late April and early August (Wiecko et al., 1993). Data from 1 yr of that study indicated that coring resulted in an increase in root length, but had no effect on root length in the second year (Wiecko et al., 1993). Murphy et al. (1993) sampled roots in a Penneagle creeping bentgrass green grown on a modified loamy sand. In compacted and noncompacted soil, coring reduced both total root weight and root density (Murphy et al., 1993). Furthermore, summer coring did not enhance surface root development of creeping bentgrass.

Root production, growth, longevity, and mortality are critical components contributing to plant adaptation to environmental stresses. Most turfgrass root studies were conducted using destructive soil sampling techniques, which typically quantify living and dead root biomass at a singular time of the growing season. Destructive sampling techniques are not able to detect root initiation or root death. The minirhizotron imaging technique, however, allows for nondestructive monitoring of root production and growth (Murphy et al., 1994; Liu and Huang 2002). The minirhizotron allows for the quantification of various living root parameters throughout a 0 to 24-cm deep root zone. Its greatest advantage is that it provides information on seasonal changes of the same roots, which eliminates confounding spatial variation and permits a high frequency of visual root observations (Murphy et al., 1994).

Coring generally is performed in the spring and autumn. Little information is available on summer coring effects on seasonal and vertical changes in a creeping bentgrass root system grown on a sand-based root zone. Previous studies were conducted on modified or native soil research putting greens. Most putting greens today are constructed with a high sand content to reduce

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Abbreviations: SP, spring only coring; SP + SU, spring plus summer coring; TRC, total root count; TRL, total root length.

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Characterization of the *S-RNase* genomic DNA allele sequence in *Prunus speciosa* and *P. pseudocerasus*

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ABSTRACT

In this study, eight *S-RNase* alleles were isolated from two *Prunus pseudocerasus* and two *Prunus speciosa* accessions by PCR amplification from genomic DNA. Analysis of the amino acid sequences revealed five novel and three published *S*-alleles. These *S-RNases* share typical structural features with *S-RNases* from other *Prunus* species exhibiting gametophytic self-incompatibility. The deduced amino acid identities ranged from 60.8 to 75.6% among four *S-RNase* alleles in *Prunus speciosa* and ranged from 73 to 81.4% among four *S-RNase* alleles in *Prunus pseudocerasus*. The size of the first introns ranged from 197 to 341 bp, and the size of second introns ranged from 81 to 1182 bp. Sequence analysis demonstrated that the deduced amino acid identities, by comparison with other *Prunus* species, were often higher than those of intraspecific identities. Moreover, exceptionally high identities were found between *Pspe-S_1* and *Pd-S_{28}*; between *Pspe-S_{31}* and *Pm-S_6*; among *Pspe-S_{51}*, *Pa-S_{29}* and *Pweb-S_7*; and among *Pps-S_{13}*, *Psim-S_4* and *Ps-S_b*, indicating that the *S-RNase* alleles evolved before *Prunus* species divergence. Interestingly, the similarities of the first and second introns were also high between the two *S-RNase* alleles, which range from 83.63 to 100% among the first introns and from 46.13 to 100% among the second introns. These information could not increase our knowledge on the *S*-alleles of *Prunus* species, but is available for molecular breeding of fruit trees, to avoid cross-incompatibility.

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1. Introduction

Self-incompatibility (SI) is a widespread mechanism in flowering plants that prevents self-fertilization and the deleterious effects of inbreeding (De Nettancourt, 2001). Gametophytic self-incompatibility (GSI) is one such system that is controlled by a single multi-allelic locus, termed the *S*-locus (De Nettancourt, 1977). The *S*-locus is thought to contain at least two linked genes: one for the pistil determinant, and the other for the pollen determinant (Kao and Tsukamoto, 2004). Pollen-tube growth is arrested in the style when the haploid pollen *S*-allele matches either of the two *S*-alleles of the diploid pistil (Roalson and McCubbin, 2003).

S-RNase alleles have been determined as the stylar component of GSI in Rosaceae (Bŏsković and Tobutt, 1996; Sassa et al., 1996) and have been identified in many *Prunus* species including almond (*P. dulcis*; Ushijima et al., 1998; Tamura et al., 2000; Ortega et al., 2006), apricot (*P. armeniaca*; Romero et al., 2004; Vilanova et al., 2006; Wu et al., 2009), Japanese plum (*P. salicina*; Sapir et al., 2004; Zhang et al., 2008), Japanese apricot (*P. mume*; Yaegaki et al., 2001; Heng et al., 2008) and sweet cherry (*P. avium*; Tao et al., 1999; Sonneveld et al., 2001; Wünsh and Hormaza, 2004). The *S-RNases* are expressed in the pistil but not in the leaves and pollen and specifically degrade incompatible pollen RNAs (McClure et al., 1990). Recently, *S-RNase* alleles with exceptionally high identity had been found between different Rosaceae species, for example, *PtenS_8-RNase* have 99% identity with *PaS_1-RNase*, and *PtenS_1-RNase* have 99% identity with *ParS_4-RNase* (Šurbanovski et al., 2007). *PpyS_8-RNase* have 96.9% identity and the same recognition specificity with *MsS_3-RNase* (Heng et al., 2011). The phenomenon maybe makes against for molecular breeding of fruit trees.

The flowering cherry (*Prunus speciosa*) is self-incompatible and grows on Japanese islands, its *S-RNase* alleles have been surveyed and the distribution of *S*-alleles have been characterized among the populations on the Izu Peninsula and Izu Islands of Japan (Kato et al., 2007). The flowers of Chinese cherry (*P. pseudocerasus*) are

Abbreviations: Pspe, *Prunus speciosa*; Pps, *Prunus pseudocerasus*; Par, *Prunus armeniaca*; Pa, *Prunus avium*; Pd, *Prunus dulcis*; Pm, *Prunus mume*; Ps, *Prunus salicina*; Pten, *Prunus tenella*; Pweb, *Prunus webbii*; Psim, *Prunus simonii*; Pspi, *Prunus spinosa*; Pdom, *Prunus domestica*; Pbre, *Pyrus×bretschneideri*; Pcom, *Pyrus communis*; Ppy, *Pyrus pyrifolia*; Md, *Malus×domestica*; Ms, *Malus spectabilis*.

☆ Plant species from which each sequence is derived are represented by their initials in 'Abbreviations' section.

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Anthocyanin Accumulation in Various Organs of a Teinturier Cultivar (*Vitis vinifera* L.) during the Growing Season

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Abstract: The anthocyanin composition and concentration of various organs of teinturier grape Yan-73 were studied throughout the growing season. Nineteen anthocyanins were identified by HPLC-MS as monoglucosides and their derivatives. Anthocyanin composition and concentration varied among grape organs and by developmental stage. Skin anthocyanins were mainly composed of malvidin derivatives, while peonidin derivatives were the most dominant anthocyanins in the pulp. Both malvidin and peonidin derivatives were predominant components in carpopodia (swollen pedicel at point of berry attachment), berry pedicels, leaf lamina, vein and petioles, and living bark at the base of the shoot. Anthocyanins were very low before veraison, and then increased sharply at veraison in berry skin and pulp. Anthocyanins in carpopodia and berry pedicels also increased sharply, although occurring later than in berry skin and pulp. Anthocyanins were high in young and senescing leaf lamina and low in expanding and mature lamina. Anthocyanins did not vary much in leaf vein and petiole tissue, or in bark, throughout the growing season.

Key words: teinturier grape, Yan-73, anthocyanin, organ specificity, accumulation

Anthocyanins of grape berries are synthesized and accumulated in berry skin of most grape cultivars, and therefore wine color essentially relies on the composition and concentration of anthocyanins in the skin. However, some grape germplasm have anthocyanins in both skin and pulp, and these are called teinturier cultivars. Teinturier cultivars have in general much higher anthocyanin concentration per unit juice volume or fresh mass than nonteinturier cultivars and are commonly used for blending to give a very dense color to red wine (Ageorges et al. 2006, Balik and Kumsta 2008, Kobayashi et al. 2005). Because the profile and concentration of anthocyanins in teinturier berries may have significant effects on the color parameters of the red wines produced from them, studies on their anthocyanin composition and concentration are valuable for winemaking and quality assessment.

Teinturier cultivars, such as Lacryma (Ageorges et al. 2006) and Neronet (Balik and Kumsta 2008) contain a higher level of anthocyanins in the skin than nonteinturier cultivars. Anthocyanin composition in the pulp of teinturier cultivars, such as Lacryma (Ageorges et al. 2006), Alicante Bouschet (Castillo-Munoz et al. 2009), and Yan-73 (He et al. 2010), was similar to that in the skin, with malvidin (Mv) dominating in the skin and peonidin (Pn) in the pulp. In addition to berry skin and pulp, teinturier cultivars accumulate anthocyanins in other organs such as leaves, tendrils, and shoots. Color determination of various organs of teinturier grape cultivars might result from tissue-specific expression of *VvmybA1*, a *Myb*-like transcriptional activator gene for anthocyanin synthesis (Jeong et al. 2006). Pigmented berry skin and pulp color might be related to the expression of structure genes of the anthocyanin synthesis pathway, such as genes encoding UDP-glucose—flavonoid 3-*O*-glucosyltransferase (*UFGT*), chalcone synthase (isogene *CHS3*), glutathione *S*-transferase (*GST*), and caffeoyl methyl transferase (*CaOMT*) (Ageorges et al. 2006)—based on the association between gene expression and color evaluation of teinturier cultivars.

However, there have been few studies on the composition and developmental changes of anthocyanins in various organs of teinturier cultivars. Therefore, it is unclear whether other organs share the same anthocyanin composition and developmental changes in profile as berry skin. The variety Yan-73 (*Vitis vinifera*, Muscat Hamburg × Alicante Bouschet) has been cultivated in China for over 50 years and has been commonly used for wine blending. This teinturier cultivar can accumulate anthocyanins in different organs, including leaves, stems, tendrils, flower bracts, and berry pulp as well as berry skin.

In this study, the composition and concentration of anthocyanins in various organs of Yan-73 during development were investigated through HPLC-MS. The purpose was to investigate possible developmental and organ-specific characteristics of anthocyanin accumulation during the growing

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Population structure of the wild soybean (*Glycine soja*) in China: implications from microsatellite analyses

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• *Background and Aims* Wild soybean (*Glycine soja*), a native species of East Asia, is the closest wild relative of the cultivated soybean (*G. max*) and supplies valuable genetic resources for cultivar breeding. Analyses of the genetic variation and population structure of wild soybean are fundamental for effective conservation studies and utilization of this valuable genetic resource.
• *Methods* In this study, 40 wild soybean populations from China were genotyped with 20 microsatellites to investigate the natural population structure and genetic diversity. These results were integrated with previous microsatellite analyses for 231 representative individuals from East Asia to investigate the genetic relationships of wild soybeans from China.
• *Key Results* Analysis of molecular variance (AMOVA) revealed that 43·92 % of the molecular variance occurred within populations, although relatively low genetic diversity was detected for natural wild soybean populations. Most of the populations exhibited significant effects of a genetic bottleneck. Principal co-ordinate analysis, construction of a Neighbor–Joining tree and Bayesian clustering indicated two main genotypic clusters of wild soybean from China. The wild soybean populations, which are distributed in north-east and south China, separated by the Huang-Huai Valley, displayed similar genotypes, whereas those populations from the Huang-Huai Valley were different.
• *Conclusions* The previously unknown population structure of the natural populations of wild soybean distributed throughout China was determined. Two evolutionarily significant units were defined and further analysed by combining genetic diversity and structure analyses from Chinese populations with representative samples from Eastern Asia. The study suggests that during the glacial period there may have been an expansion route between south-east and north-east China, via the temperate forests in the East China Sea Land Bridge, which resulted in similar genotypes of wild soybean populations from these regions. Genetic diversity and bottleneck analysis supports that both extensive collection of germplasm resources and habitat management strategies should be undertaken for effective conservation studies of these important wild soybean resources.

Key words: Wild soybean, *Glycine soja*, microsatellites, genetic diversity, population structure.

INTRODUCTION

Crop wild relatives (CWRs) have been recognized as valuable genetic resources for crop improvement (Prescott-Allen and Prescott-Allen, 1986, 1988; Feuillet *et al.*, 2008). They are also important for both applied and basic research as a means of understanding the biology of crop plants (Tanksley and McCouch, 1997; Damania, 2008; Feuillet *et al.*, 2008). However, global climate change and the destruction of the ecological balance have sped up the extinction rate of these species (Saunders *et al.*, 1991; Thomas *et al.*, 2004). More attention should be paid to the effective conservation of plant biodiversity, especially for wild relatives that have potential for the genetic improvement of cultivars (Myers *et al.*, 2000; Rao and Hodgkin, 2002). Therefore, comprehensive and extensive investigation of the population genetic structure and the phylogenetic relationship of CWRs is a requisite for identifying conservation units and developing *in situ*/*ex situ* conservation priorities for CWRs (Heywood *et al.*, 2007).

Wild soybean (*Glycine soja*) is well known as the closest wild relative of the cultivated soybean (*G. max*). It is endemic over a wide range of areas of East Asia including China, the Russian Far East, the Korean Peninsula and Japan. A long history of domestication, cultivation and breeding has narrowed the genetic basis of cultivated soybean, limiting further improvement of crop yield and quality. In contrast, wild soybeans, which inhabit a wide range of eco-geographic regions in East Asia, have diverse genetic variability in pest and disease resistance genes and other useful agricultural and ecological characteristics (Hajjar and Hodgkin, 2007; Chung and Singh, 2008). Thus wild soybeans have been explored as a very important genetic resource for cultivated soybean improvement in response to global climate change (Chung and Singh, 2008).

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RESEARCH PAPER

Introduction of apple *ANR* genes into tobacco inhibits expression of both *CHI* and *DFR* genes in flowers, leading to loss of anthocyanin

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Abstract

Three genes encoding anthocyanidin reductase (ANR) in apple (*Malus*×*domestica* Borkh.), designated *MdANR1*, *MdANR2a*, and *MdANR2b*, have been cloned and characterized. *MdANR1* shows 91% identity in coding DNA sequences with *MdANR2a* and *MdANR2b*, while *MdANR2a* and *MdANR2b* are allelic and share 99% nucleotide sequence identity in the coding region. *MdANR1* and *MdANR2* genes are located on linkage groups 10 and 5, respectively. Expression levels of both *MdANR1* and *MdANR2* genes are generally higher in yellow-skinned cv. Golden Delicious than in red-skinned cv. Red Delicious. Transcript accumulation of *MdANR1* and *MdANR2* genes in fruits gradually decreased throughout fruit development. Ectopic expression of apple *MdANR* genes in tobacco positively and negatively regulates the biosynthesis of proanthocyanidins (PAs) and anthocyanin, respectively, resulting in white, pale pink-coloured, and white/red variegated flowers. The accumulation of anthocyanin is significantly reduced in all tobacco transgenic flowers, while catechin and epicatechin contents in transgenic flowers are significantly higher than those in flowers of wild-type plants. The inhibition of anthocyanin synthesis in tobacco transgenic flowers overexpressing *MdANR* genes is probably attributed to down-regulation of *CHALCONE ISOMERASE* (*CHI*) and *DIHYDROFLAVONOL-4-REDUCTASE* (*DFR*) genes involved in the anthocyanin pathway. Interestingly, several transgenic lines show no detectable transcripts of the gene encoding leucoanthocyanidin reductase (LAR) in flowers, but accumulate higher levels of catechin in flowers of transgenic plants than those of wild-type plants. This finding suggests that the *ANR* gene may be capable of generating catechin via an alternative route, although this mechanism is yet to be further elucidated.

Key words: Anthocyanin, anthocyanidin reductase, flavonoid, *Malus*, proanthocyanidin.

Introduction

Proanthocyanidins (PAs), also known as condensed tannins, are phenolic polymers of condensed flavan-3-ols and are among the major flavonoid compounds found in higher plants (Winkel-Shirley, 2001). PAs are powerful antioxidants, and thus provide multiple health benefits to humans, including anti-inflammatory effects, immunity enhancement, as well as lowering risks of cardiovascular diseases and certain cancers (Santos-Buelga and Scalbert, 2000). PAs can also protect ruminants against pasture bloat disease and enhance ruminant nutrition (McMahon *et al.*, 2000). Moreover, PAs can interact with proteins, particularly saliva proteins such as α-amylase, resulting in the astringent and bitter sensations in many fruits and fruit juices (Vidal *et al.*, 2003; Obreque-Slier *et al.*, 2010; Renard *et al.*, 2011).

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Exogenous Glycine Betaine Ameliorates the Adverse Effect of Salt Stress on Perennial Ryegrass

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Additional index words. antioxidant enzymes, glycine betaine, ion homeostasis, perennial ryegrass, salt stress

Abstract. **Salinity stress may involve the accumulation of glycine betaine (GB). The objective of this study was to examine whether exogenous GB would ameliorate the detrimental effect of salinity stress on perennial ryegrass (*Lolium perenne*). The grass was subjected to two salinity levels (0 and 250 mM NaCl) and three GB levels (0, 20, and 50 mM). Salinity resulted in a remarkable decrease in vertical shoot growth rate (VSGR), shoot and root fresh weight, relative water content (RWC), relative transpiration rate (Tr), and chlorophyll (Chl) content, superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX) activities. Plants subjected to salt exhibited an increase in leaf electrolyte leakage (EL), lipid peroxidation (MDA), and proline content. Application of GB reduced EL, MDA, and proline content in salt-stressed plants. Perennial ryegrass subjected to salt stress plus GB had a greater level of VSGR, RWC, relative Tr, Chl content, and activities of SOD, CAT, and APX when compared with salt-stressed without GB. Salt stress increased Na^+ and decreased K^+ content, which resulted in a higher Na^+/K^+ ratio in perennial ryegrass. Application of 20 mM GB suppressed Na^+ accumulation, whereas the K^+ content was significantly increased in shoot, which led to a higher K^+/Na^+ ratio under saline conditions. These results suggested that GB-enhanced salt tolerance in perennial ryegrass was mainly related to the elevated SOD, CAT, and APX activity and alleviation of cell membrane damage by reducing oxidation of membrane lipid and improving the ion homeostasis under salt stress.**

Salinity stress is one of the common abiotic stresses that can directly or indirectly affect the physiological status of plants by disturbing their metabolism and inhibiting root and shoot growth (Zhu, 2001). Previous studies have shown that salt stress may induce osmotic and oxidative stress in plants, which leads to cellular adaptive responses such as the accumulation of compatible organic solutes and detoxification of reactive oxygen species (ROS) (Zhu, 2001).

Salt stress inhibited the growth of most plants as a result of the overproduction of ROS such as superoxide radical (O_2^-), hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$), and hydroxyl radical (OH^-) (Mittler, 2002). ROS are formed as byproducts of normal cellular metabolism and necessary for enzymatic reactions of inter- and intracellular signaling when plants are exposed to a low level of salinity stress (Foyer and Noctor, 2000). However, severe salinity stress could lead to an overproduction of ROS, which resulted in cellular damage through oxidation of membrane lipids, protein, and nucleic acids (Apel and Hirt, 2004). To alleviate detrimental effects of salt-induced oxidative stress, plant cells have evolved a complex antioxidant system (e.g., enzymatic and nonenzymatic detoxification mechanisms). Antioxidant enzymes consisted of superoxide dismutase, catalase, peroxidase (POD), ascorbate peroxidase, etc. (Apel and Hirt, 2004). SOD catalyzes the dismutation of O_2^- to molecular O_2 and H_2O_2 (Meloni et al., 2003). However, H_2O_2 is also toxic to cells and has to be further detoxified by CAT and/or POD or detoxified in the ascorbate–glutathione cycle, which involves the oxidation and re-reduction of ascorbate and glutathione through the APX (Mittler, 2002). Hoque et al. (2007) reported that exogenous glycine betaine mitigated the detrimental effects of salt stress on tobacco (*Nicotiana tabacum* 'Bight Yellow-2') suspension-cultured cells by maintaining or increasing the activity of antioxidant enzymes involved in NaCl-induced oxidative stress.

Environmental stresses including salinity can induce a significant accumulation of compatible solutes (Bohnert et al., 1995). Glycine betaine is one of several such compatible solutes that has an osmoprotective function and is known to improve salt stress tolerance in most crop plants (Demiral and Türkan, 2006; Hossain and Fujita, 2010; Yang and Lu, 2005). The mechanisms of GB-improved salt tolerance in plants have been attributed to the acceleration of ROS scavenging systems, protection of membrane integrity, activation of enzymes, and reduction in oxidation of membrane lipid under salt stress

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Growth Response and Gene Expression in Antioxidant-related Enzymes in Two Bermudagrass Genotypes Differing in Salt Tolerance

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Abstract. Plant adaptation to salt stress may be associated with morphological, physiological, and gene expression alterations. The objective of this study was to investigate the effect of salt stress on morphological and antioxidant enzyme changes and its gene expressions in bermudagrass (*Cynodon dactylon*). Salt-tolerant 'C43' and salt-sensitive 'C198', previously determined in our preliminary study, were subjected to four salinity levels: 0 mM (control), 100 mM (low), 200 mM (moderate), and 400 mM (high) NaCl for 21 days. Salt stress decreased turf quality and canopy height, especially in 'C198'. Salt stress increased root length, root number, root fresh weight, and root/shoot length ratio, to a greater extent in salt-tolerant genotype. Salt stress increased Na^+ and decreased K^+ content, which resulted in a higher Na^+/K^+ ratio in bermudagrass, to a great extent in shoot and root of 'C198'. Moderate (200 mM) and high (400 mM) salt concentration increased malondialdehyde and hydrogen peroxide content in old leaves of 'C198'. 'C43' exhibited a greater activity of superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and dehydro-ascorbate reductase (DHAR) than 'C198' in old leaves subjected to 200 and 400 mM NaCl. Antioxidant gene expressions were upregulated in new leaves and downregulated in old leaves with increasing salinity levels for both genotypes. Salt-tolerant genotypes exhibited a relatively greater antioxidant gene expression than salt-sensitive ones when exposed to the same level of salt stress. These results suggested that SOD, CAT, APX, and DHAR might be involved in scavenging salt stress-induced reactive oxygen species in bermudagrass at the level of gene expression. Salt tolerance might be attributed to the development and maintenance of a more extensive root system under saline conditions and induced antioxidant gene expressions, leading to more efficient enzyme stimulation and protection in bermudagrass.

Salt stress is one of the major abiotic factors that affects plant growth. Shoot and root growth reduction is a common response to salt stress because plant growth is one of the most important agricultural indicators of salt stress tolerance (Hulusi et al., 2007). Plant adaptation to salt is a complex phenomenon that may involve growth changes as well as physiological and biochemical processes (Hare et al., 1997). Salinity injury to plants was attributed to lower osmotic potential (ψ_S) and ion effect (Munns, 2002). Lower ψ_S reduces the ability of the plant to absorb water and induces physiological drought (Munns and Tester, 2008). The excessive uptake of Na^+ or Cl^- can limit the uptake of other nutritional ions such as K^+, Ca^{2+}, and Mg^{2+}; cause adverse effects on ion homeostasis, which lead to premature leaf aging; less cell division and elongation; and reduces leaf and root growth (Munns, 2002; Zhu, 2001).

In addition, salinity also results in oxidative stress in plants as a result of the overproduction of reactive oxygen species (ROS) such as the super oxide radical (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radical (•OH). The oxygen species are detrimental to membrane lipids, proteins, and nucleic acids (Murillo-Amador et al., 2006). To minimize the adverse effects of ROS, plants have evolved an efficient enzymatic and non-enzymatic antioxidant system (Abdul-Jaleel et al., 2006). In the enzymatic system, superoxide dismutase catalyzes the dismutation of O_2^- into H_2O_2 and O_2 (Sigaud-Kutner et al., 2002). Catalase, AOX, and DHAR decompose H_2O_2 to H_2O at different cellular locations (Edreva, 2005). The mechanisms regulating the activity and gene expression of different antioxidant enzymes are complex because the genes respond to environmental stress differentially (Sen Gupta et al., 1993). A higher level of these antioxidant enzyme activities is considered as one of the salt tolerance mechanisms in most plants (Ashraf, 2009). Previous studies demonstrated that salt-tolerant genotypes generally have a higher constitutive or an enhanced antioxidant enzyme activity under salt stress than the sensitive ones (Amor et al., 2006; Hu et al., 2012a; Mhadhbi et al., 2011). However, the response of plant antioxidant systems to salt varied for different plant species and the tissues (Mittova et al., 2003).

Bermudagrass is one of the most widely used warm-season turfgrass species in temperate and tropical regions, which has shown good tolerance to salinity and can survive in saline soil (Mancino and Pepper, 1994). To our knowledge, most previous studies examined in bermudagrass under salinity conditions were at either morphological or physiological levels (Adavi et al., 2006; Akram et al., 2006; Alshammary et al., 2008; Hameed et al., 2010; Lu et al., 2007; Marcum et al., 2005; Marcum and Pessarakli, 2006; Shahba, 2010). Little research has investigated antioxidant response to salinity at the level of gene expression and enzyme activity. The objective of this study was to investigate the effects of different salinity levels on the growth as well as the antioxidant defense system at the enzyme and gene expression levels in two genotypes of bermudagrass differing in salt tolerance.

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Responses of antioxidant gene, protein and enzymes to salinity stress in two genotypes of perennial ryegrass (*Lolium perenne*) differing in salt tolerance

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ABSTRACT

Salinity could damage cellular membranes through overproduction of reactive oxygen species (ROS), while antioxidant capacities play a vital role in protecting plants from salinity caused oxidative damages. The objective of this study was to investigate the toxic effect of salt on the antioxidant enzyme activities, isoforms and gene expressions in perennial ryegrass (*Lolium perenne* L.). Salt-tolerant 'Quickstart II' and salt-sensitive 'DP1′ were subjected to 0 and 250 mM NaCl for 12 d. Salt stress increased the content of lipid peroxidation (MDA), electrolyte leakage (EL) and hydrogen peroxide (H_2O_2), to a greater extent in salt-sensitive genotype. Salt-stressed plant leaves exhibited a greater activity of superoxide dismutase (SOD, EC 1.15.1.1), peroxidase (POD, EC 1.11.1.7), ascorbate peroxidase (APX, EC 1.11.1.11) at 4 d after treatment (DAT), but a lower level of enzyme activity at 8 and 12 d, when compared to the control. Catalase (CAT, EC 1.11.1.6) activity was greater at 4 DAT and thereafter decreased in salt tolerant genotype relative to the control, whereas lower than the control during whole experiment period for salt-sensitive genotype. There were different patterns of five isoforms of SOD, POD and two isoforms of APX between two genotypes. Antioxidant gene expression was positively related to isoenzymatic and total enzymatic activities during 12-d salt-treated leaves of two genotypes, with a relatively higher level in salt-tolerant genotype. Thus, salt tolerance could be related to the constitutive/induced antioxidant gene, leading to more efficient enzyme stimulation and protection in perennial ryegrass.

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Introduction

Salt stress can cause osmotic stress, ionic toxicity and nutritional imbalances in plants (Turkan and Demiral, 2009). In addition, salinity causes oxidative stress via over-production of reactive oxygen species (ROS), such as superoxide radical ($O_2^{\bullet}-$), hydrogen peroxide (H_2O_2) and hydroxyl radicals ($^{\bullet}OH$) (Gómez et al., 2004). ROS are the byproduct of normal cellular metabolism and have important roles in cell signaling and transduction (Foyer and Noctor, 2000). However, the excessive accumulation of ROS caused by salinity stress could lead to cytotoxic oxidative damages to membrane lipids, proteins and nucleic acids, and ultimately to cellular structure (Mittler, 2002).

To scavenge ROS, plants have a well-developed complex antioxidant defense system including enzymatic and non-enzymatic antioxidant processes (Abdul-Jaleel et al., 2006). The antioxidant enzymes include superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), ascorbate peroxidase (APX) (Apel and Hirt, 2004). Superoxide dismutase is usually considered as the first line of the antioxidant defense systems as it catalyses the dismutation of $O_2^{\bullet}-$ into H_2O_2 and O_2 in the cytosol, chloroplasts and mitochondria (Sigaud-Kutner et al., 2002). H_2O_2 is another important ROS, which can cause damages to cellular plasma membrane lipids and other biomolecules (Mittler, 2002). Therefore, rapid detoxification of H_2O_2 is essential for preventing oxidative damage. H_2O_2 is mainly detoxified by CAT in glyoxysomes and peroxisomes and by APX in chloroplasts, mitochondria, peroxisomes and apoplastic space (Shigeoka et al., 2002). Peroxidase is mainly located in the apoplastic space and vacuole and plays an important role in catalyzing H_2O_2 to H_2O and O_2 (Gratao et al., 2005). Generally, these enzymes have multiple molecular forms (isoenzymes), which may be expressed by distinct regulatory mechanisms in response to various environmental stresses and play cooperative role in protecting each organelle and minimizing tissue injury (Mittler, 2002). Four kinds of SOD have been found in plants depending on their metal cofactor: the copper zinc type (Cu/Zn SOD),

Abbreviations: APX, ascorbate peroxidase; CAT, catalase; EDTA, ethylene diaminetetraacetic acid; EL, electrolyte leakage; H_2O_2, hydrogen peroxide; MDA, malondialdehyde; NBT, nitro blue tetrazolium; PAGE, polyacrylamide gel electrophoresis; ROS, reactive oxygen species; SOD, superoxide dismutase; PCR, polymerase chain reaction; $O_2^{\bullet}-$, superoxide radical; $^{\bullet}OH$, hydroxyl radical; TBA, thiobarbituric acid; TEMED, N,N,N,N-tetramethylethylenediamine.

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ORIGINAL ARTICLE

Molecular characterization of the pentacyclic triterpenoid biosynthetic pathway in *Catharanthus roseus*

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Abstract *Catharanthus roseus* is an important medicinal plant and the sole commercial source of monoterpenoid indole alkaloids (MIA), anticancer compounds. Recently, triterpenoids like ursolic acid and oleanolic acid have also been found in considerable amounts in *C. roseus* leaf cuticular wax layer. These simple pentacyclic triterpenoids exhibit various pharmacological activities such as anti-inflammatory, anti-tumor and anti-microbial properties. Using the EST collection from *C. roseus* leaf epidermome (http://www.ncbi.nlm.nih.gov/dbEST), we have successfully isolated a cDNA (CrAS) encoding 2,3-oxidosqualene cyclase (OSC) and a cDNA (CrAO) encoding amyrin C-28 oxidase from the leaves of *C. roseus*. The functions of CrAS and CrAO were analyzed in yeast (*Saccharomyces cerevisiae*) systems. CrAS was characterized as a novel multifunctional OSC producing α- and β-amyrin in a ratio of 2.5:1, whereas CrAO was a multifunctional C-28 oxidase converting α-amyrin, β-amyrin and lupeol to ursolic-, oleanolic- and betulinic acids, respectively, via a successive oxidation at the C-28 position of the substrates. In yeast co-expressing CrAO and CrAS, ursolic- and oleanolic acids were detected in the yeast cell extracts, while the yeast cells co-expressing CrAO and AtLUP1 from *Arabidopsis thaliana* produced betulinic acid. Both *CrAS* and *CrAO* genes show a high expression level in the leaf, which was consistent with the accumulation patterns of ursolic- and oleanolic acids in *C. roseus*. These results suggest that CrAS and CrAO are involved in the pentacyclic triterpene biosynthesis in *C. roseus*.

Keywords CYP716AL1 · C-28 Oxidase · Oleanolic acid · Triterpene synthase · Ursolic acid

Abbreviations

CrAS	*Catharanthus roseus* mixed amyrin synthase
CrAO	*Catharanthus roseus* amyrin oxidase
EST	Expressed sequence tag
ORF	Open reading frame
LC–MS	Liquid chromatogram mass spectrum
P450	Cytochrome P450 monoxygenase
QRT-PCR	Quantitative reverse transcription polymerase chain reaction
RACE	Rapid amplification of cDNA ends
TAIL-PCR	Thermal asymmetric interlaced polymerase chain reaction

Introduction

Catharanthus roseus (L.) G. Don has been one of the most extensively investigated medicinal plants in the past two decades, because it is the sole commercial source of the

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Differential Expression of Benzophenone Synthase and Chalcone Synthase in *Hypericum sampsonii*

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cDNAs encoding *Hypericum sampsonii* benzophenone synthase (HsBPS) and chalcone synthase (HsCHS) were isolated and functionally characterized. Differential expressions of *HsBPS* and *HsCHS* were monitored using quantitative polymerase chain reaction (PCR). In the vegetative stage, *HsBPS* was highly expressed in the roots; its transcript level was approx. 100 times higher than that of *HsCHS*. Relatively high transcript amounts of *HsBPS* were also detected in older leaves, whereas the youngest leaves contained higher transcript amounts of *HsCHS*. In the reproductive stage, maximum *HsCHS* expression was detected in flowers, the transcript level being approx. 5 times higher than that of *HsBPS*. The inversed situation with a 10-fold difference in the expression levels was observed with fruits. High transcript amounts for both proteins were found in roots.

Keywords: Benzophenone synthase, Chalcone synthase, Gene expression, *Hypericum sampsonii*, Quantitative PCR, Sampsoniones.

Hypericum sampsonii Hance is used as a traditional Chinese herbal medicine in the treatment of numerous disorders such as hematemesis, epistaxis, menstrual irregularity, external traumatic injury, snakebite and swellings [1]. In recent years, the plant has aroused further scientific interest for its anticancer activity [2]. Extensive phytochemical studies demonstrated that *H. sampsonii* contains an array of sampsoniones (A-Q), which are polyprenylated benzophenones characterized by complex caged tetracyclic skeletons (Figure 1). These compounds possess profound cytotoxic activity [3]. Despite their pharmaceutical importance, biosynthesis of these benzophenone derivatives remains poorly understood. The nucleus of sampsoniones is benzophenone, which is biosynthesized by benzophenone synthase (BPS; EC 2.3.1.151), a new member of the type III polyketide synthase (PKS) [4]. The first published *BPS* (*HaBPS*) cDNA was from elicitor-treated *Hypericum androsaemum* cell cultures, which served as a model system for studying benzophenone metabolism [5]. Recently, the second BPS cDNA was cloned and characterized from *Garcinia mangostana* [6]. However, no information about expression of *BPS in planta* is so far available. Here we have cloned and functionally characterized a cDNA encoding *H. sampsonii* benzophenone synthase (HsBPS). The organ-specific expression pattern of *HsBPS* was analyzed by quantitative polymerase chain reaction (PCR). For comparison, the same studies were carried out for chalcone synthase (*HsCHS*), which is a well studied ubiquitous type III PKS in all higher plants.

The cDNAs for *HsBPS* and *HsCHS* were cloned by the homology-based cloning strategy. The ORF of the *HsBPS* cDNA was 1188 bp long and encoded a 42.7 kDa protein (395 amino acids) with a calculated p*I* of 5.91. The *HsCHS* cDNA contained a 1173 bp ORF encoding a 42.7 kDa protein (390 amino acids) with a calculated p*I* of 6.55. *HsBPS* and *HsCHS* shared around 57.0% identity at the nucleotide as well as at the amino acid sequence level. HsBPS and HsCHS were expressed as *N*-terminally His_6-tagged proteins in *Escherichia coli* and purified by affinity chromatography. Protein bands of approximately 43 kDa each were observed after SDS-PAGE (Figure 2).

Table 1: Substrate specificities of recombinant HsBPS and HsCHS [a]

Substrate	Enzyme activity (% of max)	
	HaBPS	HaCHS
Benzoyl-CoA	100	11
2-Hydroxybenzoyl-CoA	5	0
3-Hydroxybenzoyl-CoA	66	0
4-Hydroxybenzoyl-CoA	0	0
Cinnamoyl-CoA	0	65
2-Coumaroyl-CoA	0	0
3-Coumaroyl-CoA	0	0
4-Coumaroyl-CoA	0	100
Acetyl-CoA	0	0

[a] Data are means of two independent experiments.

HsBPS preferred benzoyl-CoA as a starter substrate (Table 1). The enzymatic product was identified as 2,4,6-trihydroxybenzophenone by liquid chromatography-UV spectroscopy (LC-UV) and liquid chromatography-mass spectrometry (LC-MS) in comparison with a sample of authentic reference compound. The pH and temperature optima were 6.5-7.0 and 40°C, respectively. There were also traces of a side product, 6-phenyl-4-hydroxy-2-pyrone. Beside benzoyl-CoA, HsBPS also accepted 3-hydroxybenzoyl-CoA as starter substrate to form 2,3',4,6-tetrahydroxybenzophenone and a small amount of 6-(3'-hydroxyphenyl)-4-hydroxy-2-pyrone as a derailment product. 4-Hydroxybenzoyl-CoA, acetyl-CoA, and CoA esters of cinnamic acids were not accepted by HsBPS as starter molecules (Table 1).

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Short Communication

Isolation and molecular characterisation of flavonoid 3′-hydroxylase and flavonoid 3′, 5′-hydroxylase genes from a traditional Chinese medicinal plant, *Epimedium sagittatum*

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ABSTRACT

The epimedii herb, a traditional Chinese medicinal plant, has significant pharmacological effects on human health. The bioactive components in the herb (*Epimedium sagittatum* (Sieb. et Zucc.) Maxim) are mainly prenylated flavonol glycosides, which are end-products of the flavonoid biosynthetic pathway. This has not been clearly elucidated until recently. The genes encoding flavonoid 3′-hydroxylase (*F3′H*) and flavonoid 3′, 5′-hydroxylase (*F3′5′H*) involved in the flavonoid biosynthetic pathway, designated as *EsF3′H* and *EsF3′5′H*, were isolated from *E. sagittatum* using a homology-based cloning method and deposited in the GenBank databases (GenBank ID: HM011054 and HM011055), respectively. EsF3′H and EsF3′5′H proteins shared high homology with other plant-specific flavonoid hydroxylases and were clustered into the CYP75B and CYP75A group, respectively. In addition, four conserved cytochrome P450-featured motifs were found in the amino acid sequences of both genes. Transcription levels of both genes were detected in all tissues tested and were high in most of the pigmented tissues. Moreover, the expression levels of both *EsF3′H* and *EsF3′5′H* correlated positively with the anthocyanin accumulation pattern in leaves from *E. sagittatum*. The cloning and molecular characterisation of *EsF3′H* and *EsF3′5′H* genes will accelerate progress in the study of the flavonoid biosynthetic pathway to elucidate the molecular mechanisms of the biosynthesis of the bioactive components in *E. sagittatum*.

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1. Introduction

Flavonoids represent a large class of secondary metabolites in plants that are best known as the characteristic red, blue, and purple anthocyanin pigments of plant tissues (Winkel-Shirley, 2001, 2002). They have a wide range of biological functions as they provide pigmentation to flowers, fruits, and seeds in order to attract pollinators and seed dispersers, protect plants from UV radiation, defend against phytopathogens, act as signal molecules in plant–microbe interactions, and are involved in auxin transport and pollen germination (Dixon and Paiva, 1995; Koes et al., 2005; Peer and Murphy, 2007). Flavonoids receive substantial public attention because of their significant effects on human health. The antioxidant activity of flavonoids plays a vital role in the prevention of neuronal and cardiovascular illnesses, cancer and diabetes (Harborne and Williams, 2000; Havsteen, 2002).

The genetics and biochemistry of the flavonoid pathway have been characterised in several model plant species such as *Arabidopsis*, maize, petunia, and snapdragon, and the main structural and regulatory genes have been cloned (Holton and Cornish, 1995; Mol et al., 1998; Winkel-Shirley, 2001). The *F3′H* and *F3′5′H* genes, both of which belong to the cytochrome P450 superfamily, catalyse hydroxylation at the 3′ and 3′, 5′ positions of the B-ring of the flavonoids. This leads to the production of the red cyanidin-based pigments and the blue/violet delphinidin-based pigments, respectively (Ayabe and Akashi, 2006; Tanaka, 2006). In addition to the hydroxylation of anthocyanidins, both genes also catalyse the hydroxylation of flavanones, flavones and flavonols. Since both the *F3′H* and *F3′5′H* genes were first isolated from petunia (Brugliera et al., 1999; Holton et al., 1993), their homologues have been subsequently isolated from many plants such as the apple (Han et al., 2010), *Arabidopsis* (Schoenbohm et al., 2000), gentian (Tanaka et al., 1996), grape (Bogs et al., 2006) and tomato (Olsen et al., 2010). Of these two genes, *F3′5′H* has evoked more interest from scientists and industries, because some important ornamental plants, such as roses, carnations and chrysanthemums lack *F3′5′H* enzyme activity and cannot produce blue or violet flowers (Tanaka, 2006).

Both *F3′H* and *F3′5′H* play important roles in flavonoid biosynthesis and regulation. They are two of the main structural genes encoding

Abbreviations: CTAB, cetyl trimethyl ammonium bromide; CYP, cytochrome P450; RACE, rapid amplification of cDNA ends; qRT-PCR, quantitative reverse transcription-polymerase chain reaction; *F3′H*, flavonoid 3′-hydroxylase; *F3′5′H*, flavonoid 3′,5′-hydroxylase; *FLS*, flavonol synthase; *DFR*, dihydroflavonol 4-reductase.

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A high-throughput apple SNP genotyping platform using the GoldenGate™ assay

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ABSTRACT

EST data generated from 14 apple genotypes were downloaded from NCBI and mapped against a reference EST assembly to identify Single Nucleotide Polymorphisms (SNPs). Mapping of these SNPs was undertaken using 90% of sequence similarity and minimum coverage of four reads at each SNP position. In total, 37,807 SNPs were identified with an average of one SNP every 187 bp from a total of 6888 unique EST contigs. Identified SNPs were checked for flanking sequences of ≥60 bp along both sides of SNP alleles for reliable design of a custom high-throughput genotyping assay. A total of 12,299 SNPs, representing 6525 contigs, fit the selected criterion of ≥60 bp sequences flanking a SNP position. Of these, 1411 SNPs were validated using four apple genotypes. Based on genotyping assays, it was estimated that 60% of SNPs were valid SNPs, while 26% of SNPs might be derived from paralogous regions.

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1. Introduction

In recent years, crop improvement efforts by capitalizing on identification and utilization of genetic diversity have made major strides with the aid of molecular marker technology. Molecular markers are highly reliable in identifying and selecting parents carrying traits of interest, advancing breeding schemes, and ultimate crop improvement via either marker-assisted selection/breeding and/or map-based cloning (Han and Korban, 2010). Molecular markers are routinely used to detect and link variations in traits of interest through Quantitative Trait Loci (QTL) mapping. Once DNA markers linked to a trait of interest are identified, and the linkage is close, these markers can be used to select for seedlings possessing the desirable character(s) in a breeding population using a particular breeding scheme, such as backcrossing (Collard and Mackill, 2008; Moose and Mumm, 2008).

Unlike phenotypic evaluation, selection based on DNA markers is not influenced by environmental factors. Moreover, marker-assisted selection can be carried out at the seedling stage, and therefore would alleviate requirements for land space, field evaluations, plant maintenance, along with associated costs, when compared to phenotypic selection (Collard and Mackill, 2008). Among various available molecular marker systems, Microsatellites or Simple Sequence Repeats (SSRs) have become the marker of choice as these are characterized by 1 to 5 bp repeat motifs, and are highly polymorphic between and among species (Korban and Tartarini, 2009). Moreover, they are multi-allelic, co-dominant, reproducible, and transferable across different species. SSR markers are preferred for QTL mapping studies, marker-assisted selection, and comparative genetic analysis (Liebhard et al., 2003). Initial costs for development of SSR markers from genomic regions are usually very high compared to other marker systems. However, for crops wherein expressed sequence tags (ESTs) are available, SSR development based on EST sequences is easy and cheap. Therefore, EST-SSRs have been recently developed in a wide range of plant species, and are being used for constructing genetic maps and for pursuing phylogenetic studies. Moreover, molecular markers developed from ESTs represent gene-coding regions, and thus are useful tools for bridging functional and structural genomics (Chagné et al., 2008; Korban and Tartarini, 2009; Varshney et al., 2005). To date, DNA sequences for many plant species, particularly those of ESTs, are publically available in large numbers in the NCBI database. Apple genetic maps have been generally constructed using a backbone of genomic SSR markers and populated with RAPDs, RFLPs, and AFLPs for QTL identification (Liebhard et al., 2003; Silfverberg-Dilworth et al., 2006). With availability of apple EST sequences, EST-SSRs have been identified *in silico* and used for constructing new genetic maps (Han et al., 2011; Naik et al., 2006). Although both genomic SSRs and EST-SSRs offer advantages over other marker systems, there are some concerns over their throughput efficacy, identification, genotyping costs, as well as

Abbreviations: ESTs, Expressed sequence tags; SNPs, Single Nucleotide Polymorphisms; QTL, Quantitative Trait Loci; RAPDs, Random Amplified Polymorphic DNAs; RFLPs, Restriction Fragment Length Polymorphisms; AFLPs, Amplified Fragment Length Polymorphisms; SSRs, Microsatellites or Simple Sequence Repeats; HRM, High-resolution Melting; ADT, Assay Design Tool; GC, GenCall; LD, Linkage Disequilibrium.

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PLOS ONE

A Multi-Population Consensus Genetic Map Reveals Inconsistent Marker Order among Maps Likely Attributed to Structural Variations in the Apple Genome

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Abstract

Genetic maps serve as frameworks for determining the genetic architecture of quantitative traits, assessing structure of a genome, as well as aid in pursuing association mapping and comparative genetic studies. In this study, a dense genetic map was constructed using a high-throughput 1,536 EST-derived SNP GoldenGate genotyping platform and a global consensus map established by combining the new genetic map with four existing reliable genetic maps of apple. The consensus map identified markers with both major and minor conflicts in positioning across all five maps. These major inconsistencies among marker positions were attributed either to structural variations within the apple genome, or among mapping populations, or genotyping technical errors. These also highlighted problems in assembly and anchorage of the reference draft apple genome sequence in regions with known segmental duplications. Markers common across all five apple genetic maps resulted in successful positioning of 2875 markers, consisting of 2033 SNPs and 843 SSRs as well as other specific markers, on the global consensus map. These markers were distributed across all 17 linkage groups, with an average of 169±33 marker per linkage group and with an average distance of 0.70±0.14 cM between markers. The total length of the consensus map was 1991.38 cM with an average length of 117.14±24.43 cM per linkage group. A total of 569 SNPs were mapped onto the genetic map, consisting of 140 recombinant individuals, from our recently developed apple Oligonucleotide pool assays (OPA). The new functional SNPs, along with the dense consensus genetic map, will be useful for high resolution QTL mapping of important traits in apple and for pursuing comparative genetic studies in Rosaceae.

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Introduction

Genetic maps are routinely constructed and exploited for identifying marker-trait associations through quantitative trait loci (QTL) mapping. These maps play a critical role in contributing to our understanding of the genetic architecture of quantitative traits by providing information on number, strength, and mode of interaction of QTLs. Such knowledge provides insights into designing strategies for potential improvement of traits of interest via marker-assisted breeding (MAB) or map-based cloning of genes [1–3]. Availability of an accurate and high-resolution genetic map, densely populated with high-throughput co-dominant and reproducible molecular markers, enhances efficiency and likelihood of success of a QTL mapping effort. Earlier, it has been suggested that QTLs with moderate effects can be identified even with maps having fairly wide marker intervals (~10 cM) [4,5]. However, to avoid linkage drag while performing marker-assisted introgression or to side-step pursuing an additional step of fine-mapping to identify genes underlying a QTL, a well-saturated map is highly recommended [6]. Additionally, to run a quick QTL scan, a dense genetic map offers a choice of polymorphic markers for developing a genetic map in a new population with well-distributed markers. A saturated and accurate map with co-dominant, reproducible, and high-throughput markers not only properly localizes a QTL, but it can also yield an accurate estimate of the power of the QTL [6] and contributes to enhanced map resolution, transferability across laboratories and mapping populations, and to efficient genotyping.

Multiple genetic and physical maps have become available for many species, but these are of limited use for pursuing comparative studies as they are often developed based on a single specific population with novel molecular markers and segregation of novel phenotypes [7]. Often, these individual maps have a common set of co-dominant markers, used as anchor points, that aid in the process of integration to establish a consensus map for the target species [8,9,10]. Such bridging or intercross markers

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Identification of genes associated with adaptation to NaCl toxicity in perennial ryegrass (*Lolium perenne* L.)

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ABSTRACT

Perennial ryegrass (*Lolium perenne* L.) is a popular turfgrass species. To understand the molecular mechanisms of salinity tolerance, a suppression subtractive cDNA library was constructed for a salinity-tolerant ryegrass accession, with NaCl-treated (255 mM) plants as the tester. Differentially expressed cDNA fragments were cloned and screened. BLAST search revealed that 268 clones exhibited significant homologies to known genes. These genes could be categorized into 11 different functional groups, including metabolism, energy transfer, detoxification, compatible solute, cellular transport, transcription, signal transduction, etc. The salinity-regulated expression of selected genes was confirmed by RT-PCR analysis. The results suggested that these putatively salinity up-regulated genes may play a vital role in the salinity tolerance of perennial ryegrass. They can be used as candidate genes for creating stress-tolerant grasses and for understanding molecular mechanisms of plant adaptation to salinity stress.

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1. Introduction

Soil salinization is an abiotic factor that adversely affects growth and development of plant. The saline soil area covers about 1 billion ha all over the world (Cheong and Yun, 2007). In China, soils affected by salinity reach approximately a total of 99 million ha, accounting for nearly 10% of the total saline soil in the world. Salinity toxicity has been a major constraint for crop production and sprigging operation in cities along the coast in China.

The toxicity effects of NaCl can have a severe impact on plants. NaCl stress can alter plant metabolism, reduce the endogenous water potential and lead to ionic imbalance, which together results in growth inhibition of plant (Hasegawa et al., 2000). NaCl 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). In order to adapt to osmotic and Na^+ stress, plants have developed a set of complex adaptation mechanism from the whole plant to molecular levels.

When exposed to salt stress, plants can quickly regulate the expression of genes involved in the production of compatible osmolites (osmoprotectants) to keep osmotic potential normal, and to ensure continued positive turgor pressure within cells. These osmoprotectants can improve water and nutrient uptake from the soil solution and ensure the continuation of vital plant processes (Hasegawa et al., 2000; Munns, 2005). Furthermore, plants could stabilize ion concentrations within and outside plant cells through generation of various ion transporters, including Na^+ efflux transporters (Munns, 2005). Another consequence of exposure to salinity stress is the generation of reactive oxygen species (ROS), which can promote lipid peroxidation, membrane damage and enzyme inactivation. To reduce the toxic effects of ROS, plants usually initiates ROS scavenging system by increasing the gene expression of some detoxification enzymes (Hasegawa et al., 2000). In addition, transcription factors, signaling proteins and many other compounds are also observed to be regulated in plant under salt stress (Puranik et al., 2011). The identification and manipulation of such genes has been suggested as a promising approach towards the improvement of salinity tolerance in crop plant (Munns, 2005).

Perennial ryegrass is a self-incompatible diploid ($2n=2x=14$) cool-season forage and turfgrass species. It is one of the most widely used perennial grasses in temperate regions around the world (Kubik et al., 2001; Xing et al., 2007). Salt stress can adversely affect turf quality of ryegrass or restrain its use in more extent areas. Perennial ryegrass shows a potentially high degree of genetic diversity within the population. Under salt stress, perennial ryegrasses also exhibit a large variation in morphological and growth characteristics (our unpublished results). In addition, compared with other perennial grass species, more genetic and genomic

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Antioxidant Enzyme Activity and Gene Expression in Response to Lead Stress in Perennial Ryegrass

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Additional index words. heavy metal, pollution, physiology, tolerant, *Lolium perenne*

Abstract. **Lead pollution is an important issue in the world. Perennial ryegrass (*Lolium perenne*), as one of the widely used turfgrass and forage species, has a potential for bioremediation. The objective of this study was to investigate how antioxidant enzymes and their gene transcripts respond to Pb stress in perennial ryegrass. Ryegrass seedlings were subjected to 0, 0.5, and 3.2 mM of $Pb(NO_3)_2$ for 7 days in a hydroponic system maintained in a greenhouse. Both root and shoot growths were inhibited by Pb compared with the control. However, contents of chlorophyll (Chl) *a* and total Chl were unaffected by Pb treatment. Results from this study showed a substantial increase of malondialdehyde (MDA) content in leaf tissues when perennial ryegrass was exposed to Pb at 3.2 mM. The MDA content from plants in the 0.5 mM Pb treatment was lower than the control, indicating that an effective defense mechanism existed. Circumstantial evidence came also from the content of soluble protein in 0.5 mM Pb treatment, which was not different from the control. Furthermore, the activity of catalase (CAT) increased at 0.5 mM Pb compared with the control, indicating that CAT might play an important role in scavenging reactive oxygen species (ROS). The expression profiles of eight genes encoding antioxidative enzymes were upregulated within 24 hours of Pb treatment. In conclusion, antioxidant enzymes responded to Pb at an early stage of exposure and their gene expression profiles provided more details in time courses of the activation of those systems.**

Heavy metal pollution is a worldwide ecological problem because of its impact on plants and animals and ultimately on the health of human beings via the food chain. Lead is one of the most abundant and widely distributed heavy metals because of its various human activities, such as mining and smelting of lead ores, paint manufacturing, gasoline production, lead linings, and so on. As a result, Pb is readily enriched into ecosystems. As a nonessential element for plants, Pb impedes plant growth by affecting physiological process and metabolic pathways such as photosynthesis and nutrient acquisition (Godbold and Kettner, 1991; Kastori et al., 1992; Rashid and Popovic, 1990; Verma and Dubey, 2003).

Like many other toxic metals, Pb induces production and accumulation of free radicals and ROS in plant tissues and causes oxidative stress in plants. Excessive ROS can result in irreversible oxidization of lipids, proteins, chloroplastic pigments, and nucleic acids (Foyer et al., 1994; Malecka et al., 2001; Reddy et al., 2005; Schutzendubel and Polle, 2002; Verma and Dubey, 2003). Plants have developed both enzymatic and nonenzymatic defense systems to scavenge and detoxify ROS (Mittler, 2002). For example, enhanced activities of superoxide dismutase (SOD), peroxidase (POD), glutathione reductase (GR), and other antioxidative enzymes were detected in both horse gram (*Macrotyloma uniflorum*) and bengal gram (*Cicer arietinum*) subjected to Pb, and the levels of enzymes were dependent on the concentration of Pb (Reddy et al., 2005). Increased levels of POD and GR, in response to Pb, were also reported in *Arabidopsis thaliana* and maize (*Zea mays*) calli (Verma and Dubey, 2003; Zacchini et al., 2003). Elevated levels of antioxidant enzymes including SOD, glutathione peroxidase (GPX), ascorbate peroxidase (APX), GR, and CAT were found in coontail (*Ceratophyllum demersum*) when exposed to Pb (Mishra et al., 2006). On the contrary, there were also reports indicating decreased or unchanged activities of SOD and POD in plants subjected to Pb or other heavy metals (Islam et al., 2008; Liu et al., 2008; Sun et al., 2009). The discrepancies regarding the responses of SOD and POD to Pb stress may attribute to the different metal concentrations and/or plant developmental stages at which the investigations were conducted.

Plant responses to Pb have been studied at gene levels using DNA microarray technique, which overcame some of the disadvantages related to enzyme analysis (Magrini et al., 2008). Upregulated expressions were observed for type-2 metallothionein, aminocyclopropane carboxylic acid synthase/oxidase, and other genes induced by abiotic stress in rattlebox (*Sesbania drummondii*) subjected to Pb treatment (Srivastava et al., 2007). Using quantitative real-time polymerase chain reaction (Q-PCR)

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ORIGINAL ARTICLE

Comparative proteomic analyses reveal the changes of metabolic features in soybean (*Glycine max*) pistils upon pollination

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Abstract Siphonogamy is a critical process in plant reproductive growth, during which numerous cell–cell interaction events occur between pistil and pollen. Previous studies in *Solanaceae*, *Papaveraceae*, and *Brassicaceae* focusing on pollen–stigma recognition in self-incompatible systems have provided many important views. In this study, we profiled the proteome in soybean mature pistils before and after pollination. Comparative analyses of two-dimensional gel electrophoresis maps from un-pollinated and pollinated pistils were conducted. The results showed that 22 proteins were increased and 36 proteins decreased after pollination. Functional categorization showed that most of them were metabolism- and redox-related proteins. The enhancement of primary metabolism, biosynthesis of pollen tube guidance compounds, and adjustment of redox homeostasis system might be helpful for a successful pollination. Quantitative reverse transcript-polymerase chain reaction analysis implied that the regulation of gene expression might happen at both transcriptional and post-transcriptional levels during pollination. This study will enhance our understanding of pollen–stigma interaction in plant sexual reproductive growth.

Keywords Pistil · Two-dimensional gel electrophoresis · Proteomics · Soybean · Pollination

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Ming Li and Aihua Sha contributed equally.

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Introduction

Siphonogamy, as a key event in reproductive process of plants, has been extensively studied in past years. This process begins with a pollen grain alighting on and adhering to the stigma of pistil. Once the pollen grains adhere to the stigma, the interaction between pollen and stigma will start. If they are compatible and surrounding conditions are suitable, the pollen grain will germinate and start pollen tube growth. To the contrary, if they are incompatible, either the grain germination or the pollen tube growth will be inhibited.

Two participants of pollen–pistil interactions are equally important for the fertilization. In recent years, great progresses have been made in understanding the pollen–stigma interaction through self-incompatible and self-compatible pollination systems (Hiscock and Allen 2008). Some studies focusing on pollens revealed that pollen coat structure, lipids, and the pollen coat proteins play crucial roles during pollen–stigma recognition (Fiebig et al. 2000; Mayfield et al. 2001; Suen et al. 2003; Swanson et al. 2004; Zinkl and Preuss 2000; Zinkl et al. 1999). Other studies showed that the stigma is also critical for a successful pollination. The proteinaceous of the

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Accurate quantification of astaxanthin from *Haematococcus* crude extract spectrophotometrically*

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Abstract The influence of alkali on astaxanthin and the optimal working wave length for measurement of astaxanthin from *Haematococcus* crude extract were investigated, and a spectrophotometric method for precise quantification of the astaxanthin based on the method of Boussiba et al. was established. According to Boussiba's method, alkali treatment destroys chlorophyll. However, we found that: 1) carotenoid content declined for about 25% in *Haematococcus* fresh cysts and up to 30% in dry powder of *Haematococcus* broken cysts after alkali treatment; and 2) dimethyl sulfoxide (DMSO)-extracted chlorophyll of green *Haematococcus* bares little absorption at 520–550 nm. Interestingly, a good linear relationship existed between absorbance at 530 nm and astaxanthin content, while an unknown interference at 540–550 nm was detected in our study. Therefore, with 530 nm as working wavelength, the alkali treatment to destroy chlorophyll was not necessary and the influence of chlorophyll, other carotenoids, and the unknown interference could be avoided. The astaxanthin contents of two samples were measured at 492 nm and 530 nm; the measured values at 530 nm were 2.617 g/100 g and 1.811 g/100 g. When compared with the measured values at 492 nm, the measured values at 530 nm decreased by 6.93% and 11.96%, respectively. The measured values at 530 nm are closer to the true astaxanthin contents in the samples. The data show that 530 nm is the most suitable wave length for spectrophotometric determination to the astaxanthin in *Haematococcus* crude extract.

Keyword: *Haematococcus pluvialis*; astaxanthin quantification; spectrophotometry

1 INTRODUCTION

Haematococcus pluvialis is a unicellular green alga belonging to the Class Chlorophyceae, Order Volvocales, Family Haematococcaceae. When the environment is unsuitable, vegetative cells convert to cysts, which accumulate large quantities of carotenoids and display a red color (Hu, 2006). *Haematococcus* cysts contain 1.5%–3.0% astaxanthin (DW), making them the most astaxanthin-enriched cells in nature. Generally, astaxanthin accounts for 60%–80% of total carotenoids in a cyst (Johnson et al., 1991), but the value can be as high as 90%–95% (Boussiba et al., 1997; Aflalo et al., 2007). The mass culture of *Haematococcus* to produce natural astaxanthin is a rapidly growing field in microalgal biotechnology due to the wide range of potential uses of astaxanthin in aquatic culture and human health foods (Droop et al., 1955; Boussiba and Vonshak, 1991; Borowitzka et al., 1994; Cysewski and Lorenz, 2004).

Quantification of astaxanthin is an essential step in

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AJB Primer Notes & Protocols in the Plant Sciences

Microsatellite primers in the endangered quillwort *Isoetes hypsophila* (Isoetaceae) and cross-amplification in *I. sinensis*[1]

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- *Premise of the study:* The first microsatellite primers were developed for *Isoetes hypsophila*, an endangered quillwort species endemic to the Qinghai-Tibetan Plateau in China, to further describe its genetic variability and population structure. We also examined their cross-amplification in a congeneric species, *I. sinensis*.
- *Methods and Results:* Using the Fast Isolation by AFLP of Sequences COntaining Repeats (FIASCO) protocol, nine microsatellite loci were isolated and characterized in 32 samples from four natural populations of *I. hypsophila*. The primers amplified di- and hexanucleotide repeats with three to 11 alleles per locus. Seven of nine primers were cross-amplified in *I. sinensis* with two to seven alleles per locus.
- *Conclusion:* The microsatellite loci primers will be useful for studies of genetic diversity and gene flow in natural populations of *Isoetes* species.

Key words: cross-amplification; genetic diversity; Isoetaceae; *Isoetes hypsophila*; *Isoetes sinensis*; microsatellite.

Isoetes L., the single remaining genus of the family Isoetaceae, is a cosmopolitan genus of heterosporous lycopods comprising 200 or more species. *Isoetes hypsophila* Hand.-Mazz., an alpine quillwort endemic to the southeastern Qinghai-Tibetan (Q-T) Plateau, is a diploid species ($2n = 22$) distributed in shallow zones of plateau lakes or marshes of Sichuan and Yunnan provinces in China (Chen et al., 2005). Field surveys over the past eight years identified six geographically isolated regions with small populations, which are Hongyuan, Baiyu, Jiulong, Litang, and Daocheng counties in Sichuan Province and Shangri-La County in Yunnan Province (Chen et al., 2005; Chen et al., 2010). Like other quillwort species in China, such as *I. yunguiensis* Q. F. Wang & W. C. Taylor, *I. taiwanensis* De Vol, *I. orientalis* Hong Liu & Q. F. Wang, and *I. sinensis* Palmer, *I. hypsophila* is endangered because of habitat degradation and loss, water pollution and eutrophication, competitive exclusion, and human disturbance (Liu et al., 2005), and the genus *Isoetes* is listed in the first category of the key protected wild plants in China (Yu, 1999). Considering that *I. hypsophila* played an important role in the evolutionary history of quillworts and occupies a basal position among East Asian and eastern Australian members of the genus (Taylor et al., 2004), an appropriate conservation program is urgently needed to prevent further loss of *I. hypsophila*. Therefore, it is essential to develop molecular markers for the population genetic analysis of *I. hypsophila* and other Chinese quillworts to provide essential information for the development of a management and conservation strategy.

Among various molecular marker tools, microsatellite markers have proven to be highly efficient molecular tools for population genetic studies, due to their high reproducibility, multiallelic nature, codominant inheritance, relative abundance, and wide genome coverage. In this study, nine highly variable microsatellite loci were developed for the endangered *I. hypsophila*, and their cross-amplification in *I. sinensis* was examined.

METHODS AND RESULTS

Total genomic DNA was extracted from leaf tissue of *I. hypsophila* using a modified cetyltrimethylammonium bromide (CTAB) method (Doyle and Doyle, 1987). The isolated DNA was dissolved in 100 μL of TE buffer and stored at −20°C. A microsatellite-enriched genomic library was constructed using the Fast Isolation by AFLP of Sequences COntaining Repeats (FIASCO) protocol previously described by Zane et al. (2002), with some modifications. Approximately 250 ng of total genomic DNA isolated from a single sample in the Baiyu population (Appendix 1) was digested with *Mse*I (New England BioLabs, Beverly, Massachusetts, USA), and then ligated to the adapter (5′-TACTCAGGACTCAT-3′/5′-GACGATGAGTCCTGAG-3′) using T4 DNA ligase (TaKaRa Biotechnology Co., Dalian, Liaoning, China) in a volume of 30 μL. The digestion-ligation mixture was subsequently diluted 10 times, and 5 μL of digestion-ligation DNA fragments were amplified with 1 μL AFLP adapter-specific primer (5′-GATGAGTCCTGAGTAAN-3′, i.e., *Mse*I-N) (25 μM) for the first round of PCR in a 20 μL volume, using a thermal cycling program of initial denaturation of 3 min at 95°C, followed by 20 cycles of 30 s at

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AJB Primer Notes & Protocols in the Plant Sciences

Microsatellite primers in the Chinese dove tree, *Davidia involucrata* (Cornaceae), a relic species of the Tertiary[1]

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- *Premise of the study:* The first microsatellite primers were developed for *Davidia involucrata*, an endangered relic species of the Tertiary in China, to further describe its genetic variability and population structure.
- *Methods and Results:* Using the Fast Isolation by AFLP of Sequences Containing Repeats (FIASCO) protocol, 15 polymorphic microsatellite loci were isolated and characterized in 20 individuals from the germplasm collections of *D. involucrata* at the Hunan Forest Botanical Garden. High levels of polymorphism were revealed, with the total number of alleles per locus and the number of alleles per locus per individual ranging from two to 13 and from one to six, respectively.
- *Conclusions:* The multibanded patterns of microsatellite loci obtained in the current study confirmed that *D. involucrata* might be a polyploid species. The primers will be useful for studies of genetic diversity and for guiding conservation strategies for *D. involucrata.*

Key words: Cornaceae; *Davidia involucrata*; microsatellite markers; polyploidy.

Dove tree, *Davidia involucrata* Baill. (Cornaceae), which is endemic in western China, is not only one of the best known relict species of the Tertiary, but also a famous ornamental plant with dove-shaped flowers (Fu and Chin, 1992). Fossils from the Paleocene of North America indicate that the genus *Davidia* Baill. was more widespread in the past (Manchester, 2003). *Davidia involucrata* is only one relict species in the genus *Davidia*; other species within the genus went extinct during the ice ages of the Quaternary. This species survived only in the subtropical mountains of southwestern China because of the topographical complexity and weak impact of the glaciers (Wu et al., 2004). After the ice ages, *D. involucrata* populations spread slowly in mountains in southwestern China. Today, *D. involucrata* is distributed in more than 40 counties in Gansu, Shanxi, Hubei, Hunan, Yunnan, Guizhou, Sichuan, and Chongqing provinces (Wu et al., 2004). Additionally, as an ornamental plant, *D. involucrata* has been introduced from China to many countries since 1904. In the 20th century, the distribution and population size of *D. involucrata* decreased sharply, owing to human activities that destroyed natural forests. The species has been placed in the highest class of protected plant species in China (Fu and Chin, 1992) and listed as Vulnerable (VU) in the IUCN Red List (http://www.iucnredlist.org/). An appropriate conservation program is urgently needed to prevent further loss of *D. involucrata*. Although genetic characterization of germplasm resources is essential for the efficient conservation and utilization of the species, little is known about the genetic diversity and population structure of either wild or cultivated *D. involucrata*. Because of their codominant and hypervariable nature, microsatellite markers have been proven to be highly efficient molecular tools. Here, we describe the characterization of 15 polymorphic and two monomorphic microsatellite loci in the genome of *D. involucrata* for population and conservation genetics studies.

METHODS AND RESULTS

Total genomic DNA was extracted from leaf tissue of *D. involucrata* using a modified cetyltrimethylammonium bromide (CTAB) method (Doyle and Doyle, 1990). A microsatellite-enriched genomic library was constructed using the Fast Isolation by AFLP of Sequences Containing Repeats (FIASCO) protocol described by Zane et al. (2002). Approximately 250 ng of total genomic DNA was digested with *Mse*I (New England BioLabs, Beverly, Massachusetts, USA), and then ligated to the adapter (5′-TACTCAGGACTCAT-3′/5′-GACGATGAGTCCTGAG-3′) using T4 DNA ligase (TaKaRa Biotechnology Co., Dalian, Liaoning, China) in a volume of 30 μL. The digestion-ligation mixture was subsequently diluted 10 times, and 5 μL digestion-ligation DNA fragments were amplified with 1 μL AFLP adapter-specific primers (5′-GATGAGTCCTGAGTAAN-3′, i.e., *Mse*I-N) (25 μM) for the first round of PCR in 20 μL volume, using the following PCR program: 95°C for 3 min; followed by 20 cycles of 30 s at 94°C, 1 min at 53°C, and 1 min at 72°C; and a final extension at 72°C for 10 min. After denaturation at 95°C for

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Article

Variation of Medicinal Components in a Unique Geographical Accession of Horny Goat Weed *Epimedium sagittatum* Maxim. (Berberidaceae)

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Abstract: Herbal *Epimedium* species have been widely in Traditional Chinese Medicine for sexual enhancement, immunity improvement, anticancer and anti-aging treatment, with flavonoids and polysaccharides being the major active components. However, exhaustive depletion of wild sources warrants germplasm evaluation and quality resource exploration. A preliminarily analysis had previously indicated that a specific local geographic accession of *Epimedium sagittatum* found in Luotian (LT) county of Hubei Province (China) had a much higher content of total flavonoids and polysaccharides. In this study, we further investigated the medicinal component variation in the LT type under different light intensities and in different regions by the common-garden experiment. The results indicated a light intensity range of 40–160 μmol/m^2/s was the most suitable for the synthesis and accumulation of total flavonoids, while polysaccharide accumulation was negatively correlated with the light intensity. Icariin was the component displaying the highest content among flavonoids, and the content of major flavonoid bioactive components was relatively stable in the third year after cultivation. There was significant correlation between the major flavonol glycoside constituents and the geographic location, and Central China followed by Northern China were the highly suitable regions for cultivation of LT type *E. sagittatum*. The results revealed that there was a functional balance between flavonoids and

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Inheritance of anthocyanin content in the ripe berries of a tetraploid × diploid grape cross population

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Abstract: Variation patterns and inheritance of anthocyanin content in the ripe berries of a tetraploid × diploid table grape cross population were investigated in two successive years. The population segregated for three different ploid levels: dipolids, triploids, and tetraploids. A total of 28 different anthocyanins were detected and quantified in the progeny population. Transgressive segregation for the total anthocyanin content was observed in all the three ploid progeny populations. The total anthocyanin content increased as the ploid level increased. The broad sense heritabilities (H^2) of the total anthocyanin content were all relatively high, ranging from 0.53 to 0.98, 0.57 to 0.97 and 0.43 to 0.94 in the diploid, triploid and tetraploid population, respectively. Our results suggested that the total anthocyanin content followed an additive inheritance model in this polyploid segregation population. We also observed that the relative contribution of individual anthocyanins to the total anthocyanin content varied significantly among different ploid populations, suggesting that genetic background has important impact on the accumulation of the individual anthocyanin compounds. These results will help develop better breeding strategies in a polyploid table grape breeding program for improving the content of anthocyanins, an important class of polyphenolics possessing antioxidant activities and many other health-related benefits.

Keywords Anthocyanins · Inheritance · Transgressive segregation · Polyploid table grapes

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Introduction

Grapes (*Vitis* spp.) are one of the most important fruit crops and widely consumed as fresh fruits as well as wine, juice, raisin and other processed products. While most grape production is used for wine making, table grapes have been growing at an unprecedented pace in

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RESEARCH ARTICLE Open Access

Transcriptomic analysis of grape (*Vitis vinifera* L.) leaves during and after recovery from heat stress

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Abstract

Background: Grapes are a major fruit crop around the world. Heat stress can significantly reduce grape yield and quality. Changes at the molecular level in response to heat stress and subsequent recovery are poorly understood. To elucidate the effect of heat stress and subsequent recovery on expression of genes by grape leaves representing the classic heat stress response and thermotolerance mechanisms, transcript abundance of grape (*Vitis vinifera* L.) leaves was quantified using the Affymetrix Grape Genome oligonucleotide microarray (15,700 transcripts), followed by quantitative Real-Time PCR validation for some transcript profiles.

Results: We found that about 8% of the total probe sets were responsive to heat stress and/or to subsequent recovery in grape leaves. The heat stress and recovery responses were characterized by different transcriptional changes. The number of heat stress-regulated genes was almost twice the number of recovery-regulated genes. The responsive genes identified in this study belong to a large number of important traits and biological pathways, including cell rescue (i.e., antioxidant enzymes), protein fate (i.e., HSPs), primary and secondary metabolism, transcription factors, signal transduction, and development. We have identified some common genes and heat shock factors (HSFs) that were modulated differentially by heat stress and recovery. Most HSP genes were upregulated by heat stress but were downregulated by the recovery. On the other hand, some specific HSP genes or HSFs were uniquely responsive to heat stress or recovery.

Conclusion: The effect of heat stress and recovery on grape appears to be associated with multiple processes and mechanisms including stress-related genes, transcription factors, and metabolism. Heat stress and recovery elicited common up- or downregulated genes as well as unique sets of responsive genes. Moreover, some genes were regulated in opposite directions by heat stress and recovery. The results indicated HSPs, especially small HSPs, antioxidant enzymes (i.e., ascorbate peroxidase), and galactinol synthase may be important to thermotolerance of grape. HSF30 may be a key regulator for heat stress and recovery, while HSF7 and HSF1 may only be specific to recovery. The identification of heat stress or recovery responsive genes in this study provides novel insights into the molecular basis for heat tolerance in grape leaves.

Background

Most crop plants are exposed to heat stress during certain stages of their life cycle. Heat stress, defined as the temperature above a normal optimum, is expected to become a major issue in reducing crop production in coming years due to global warming [1]. Grape is a popular cultivated fruit throughout the world and represents one of the most important crops with highly valued products such as juices, liquors and wines [2]. The grape species *Vitis vinifera* makes up most of the grape production in the world. However, grape production and quality often fluctuate due to various environmental factors. Temperature has been broadly considered as a major determining factor. Studies show that crop production is severely limited by temperature stresses around the world [3]. In many regions, the maximum midday air temperature can reach 40°C and above, which can destroy grape berry ripening [4]. In addition, crop cultivation in sheltered conditions (e.g.,

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Frequency and distribution of *S*-alleles in a native population of Chinese cherry (*Prunus pseudocerasus* Lindl.)

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SUMMARY
Self-incompatibility (SI) has been studied extensively at the molecular level in many members of the Solanaceae, Rosaceae, and Scrophulariaceae, all of which exhibit gametophytic self-incompatibility (GSI). In this study, we analysed 66 new accessions of Chinese cherry (*Prunus pseudocerasus*) collected in Shandong, Henan, Anhui, Jiangsu, Zhejiang, and Sichuan Provinces. Their *S*-genotypes were identified using PCR with two conserved or four allele-specific primers. No novel *S*-alleles were found in these accessions. The frequencies and distributions of the *S*-alleles were significantly unequal. The frequencies of S_1 and S_8 were the highest, and those of S_4 and S_6 were the lowest. The unequal frequencies and distributions of *S*-alleles could be associated with different climates and/or habitats, or caused by other factors such as natural selection, which would accelerate the evolution of *S*-alleles. Understanding the frequency and distribution of *S*-alleles would help to develop informed orchard management strategies for Chinese cherry cultivars.

Self-incompatibility (SI) in flowering plants is a widespread genetic system that prevents inbreeding by enabling the pistil to reject pollen from genetically-related individuals. *Prunus* spp. in the family Rosaceae exhibit typical gametophytic self-incompatibility (GSI). This incompatibility is controlled by a single multi-allelic locus called the *S*-locus (Crane and Lawrence, 1929; de Nettancourt, 1977), which contains at least two linked genes, one for the pistil determinant and the other for the pollen determinant (Kao and Tsukamoto, 2004). When the *S*-allele in haploid pollen matches that of the pistil, the pollen is recognised as "self" (de Nettancourt, 2001), resulting in the cessation of pollen-tube growth (Roalson and McCubbin, 2003).

Although unequal *S*-allele frequencies have rarely been demonstrated, a few have recently been reported in several species. Different *S*-alleles, the frequencies of which were significantly unequal, were found in a random sample of 51 *Papaver rhoeas* plants from a natural population (R106), and one of the S_3 stigma alleles appeared to be a stigma partial mutant of the revertant type (Campbell and Lawrence, 1981a). The *S*-locus might also be linked to one or more genes that determine seed dormancy, which could also be responsible for the unequal *S*-allele frequencies observed in the natural population from which the wild-type parents of the progenies were obtained (Lane and Lawrence, 1995; Lawrence *et al*., 1993). Brooks *et al*. (1996) reported that the large variation in *S*-allele frequencies found in samples from populations of *P. rhoeas* was consistent with their being in a steady state, and may have an effect on selection (Lawrence and Franklin-Tong, 1994). Unequal *S*-allele frequencies might be caused by the facts that (i) the strength of the frequency-dependent selection that maintains the polymorphism must be weak, and/or (ii) a population will contain only a sub-set of the total number of genotypes (Fearon *et al*., 1994; Lawrence *et al*., 1994). Moreover, the *S*-allele frequency may be associated with selection operating on a genotype which favours any selective advantage in economic characteristics for the genetic improvement of sweet cherry (Williams and Brown, 1956; Williams and Gale, 1960). In addition, Kao *et al*. (2007) reported that unequal allele frequencies may result from extreme environmental and/or climatic pressures caused by volcanic eruptions and typhoons that cause serious damage to habitats.

Chinese cherry (*P. pseudocerasus*) is a polyploid species (Gu *et al*., 2010; Oginuma, 1988) with self-compatible flowers (Gu *et al*., 2010; Mizutani *et al*., 1995). The *S*-genotypes of ten Chinese cherry cultivars have been determined, and nine *S-RNase* alleles were identified (Gu *et al*., 2010; Huang *et al*., 2008). The results showed that different cultivars collected from the same Province had the same *S*-genotype, and that the frequencies and distributions of these nine *S*-alleles were also in disequilibrium in the different cultivars (Gu *et al*., 2010). However, the number of cultivars studied by Gu *et al*. (2010) was small (n = 9). Thus, we collected 66 accessions from six different Provinces (as many as possible) and combined these with the nine Chinese cultivars already described (total n = 75) in an attempt to identify any new *S*-alleles. Understanding the frequencies and distributions of *S*-alleles would help to develop more informed orchard management and pollination strategies for Chinese cherry cultivars.

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Yield potential of *Miscanthus* energy crops in the Loess Plateau of China

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Abstract

Growing second-generation energy crops on marginal land is conceptualized as one of the primary means of future bioenergy development. However, the extent to which marginal land can support energy crop production remains unclear. The Loess Plateau of China, one of the most seriously eroded regions of the world, is particularly rich in marginal land. On the basis of the previous field experiment of planting *Miscanthus* species in Qingyang of the Gansu Province, herein, we estimated the yield potential of *Miscanthus lutarioriparius*, the species with the highest biomass, across the Loess Plateau. On the basis of the radiation model previously developed from *Miscanthus* field trials, annual precipitation was introduced as an additional variable for yield estimate in the semiarid and semihumid regions of the Loess Plateau. Of 62 million hectares (Mha) of the Loess Plateau, our model estimated that 48.7 Mha can potentially support *Miscanthus* growth, with the average yield of 17.8 t ha^{-1} yr^{-1}. After excluding high-quality cropland and pasture and land suitable for afforestation, a total of 33.3 Mha of presumably marginal land were left available for producing the energy crop at the average yield of 16.8 t ha^{-1} yr^{-1} and the total annual yield of 0.56 billion tons. The analysis of environmental factors indicated that erosion, aridity, and field steepness were the primary contributors to the poor quality of the marginal land. The change of land uses from traditional agriculture to energy crop production may prevent further erosion and land degradation and consequently establish a sustainable economy for the region.

Keywords: bioenergy, biofuel, land-use change, marginal land, *Miscanthus lutarioriparius*, radiation model

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Introduction

The world's bioenergy potential depends heavily on how much land is available for growing energy crops. A large-scale, sustainable production of energy crops should not take place at the cost of productive cropland or natural ecosystems, because it would otherwise threaten world's food security or result in substantial carbon debt and weakened ecosystem function (Fargione *et al*., 2008; Robertson *et al*., 2008; Searchinger *et al*., 2008). One conceptually promising solution is to develop and grow second-generation energy crops on marginal land or abandoned and set-aside cropland (Somerville *et al*., 2010; Sang, 2011).

Second-generation energy crops include primarily perennial grasses and short rotate coppices that can be grown under water-limited and nutrient-poor conditions with infrequent tillage (Heaton *et al*., 2008b; Karp & Shield, 2008; Oliver *et al*., 2009). Thus, they require relatively low energy input and have a high net energy output. For marginal land with native vegetation already cleared out, growing second-generation energy crops can be beneficial for carbon sequestration and protection against erosion. This is particularly true for countries with little or no surplus cropland, but rich in marginal land.

China is typical of such a country having less than 9% of world's cropland supporting more than 20% of the world's population. With the vast area of land deforested or over-used for food production, 1–200 million hectares (Mha) of land has suffered from various degrees of erosion and degradation (Houghton & Hackler, 2003; Li & Hu, 2003; Zhou *et al*., 2007). The majority of this land is located in northern and northwestern regions of the country, where inappropriate land uses, such as over-grazing or grain production, have caused severe soil degradation, and in the worst cases, desertification (Akiyama & Kawamura, 2007).

The Loess Plateau that covers an area of more than 60 Mha from central to northwestern China is particularly rich of marginal land (34°–45°5′N, 101°–114°33′E;

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Research Article

Effects of soil moisture and floral herbivory on sexual expression in a gynodioecious orchid

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Abstract Compared to pollinator limitation and inbreeding avoidance, the role of ecological factors in sexual differentiation has received less attention in sexual dimorphic plants. The effect of soil moisture and florivory on two sexual morphs in a gynodioecious orchid, *Satyrium ciliatum,* was investigated in seven gynodioecious (with both female and hermaphrodite individuals) and 15 hermaphroditic (with only hermaphrodite individuals) populations. Our result showed that, compared to hermaphrodites, females tended to occur in drier sites in which soil water content was consistently lower than that of hermaphrodites in all gynodioecious populations. The soil water content where hermaphrodites grew was not significantly different between gynodioecious and hermaphroditic populations. We observed that females experienced less attack by insect florivores than hermaphrodites in gynodioecious populations, and hermaphroditic populations had higher insect attack than gynodioecious populations. Our results provide evidence for females being favored in stressful sites. However, the soil moisture and degree of florivory were not correlated to female frequency among populations, suggesting that the two ecological factors have not induced strong effects or other factors that may also influence the sex ratio in the facultative apomictic orchid.

Key words ecological factors, gynodioecious orchid, herbivory, *Satyrium ciliatum*, sex ratio, soil moisture.

The invasion of male-steriles (females) into hermaphrodite populations, leading to gynodioecy, has evolved many times in flowering plants. Approximate 6% of flowering plants show this dimorphic breeding system, in which both female and hermaphroditic individuals coexist in populations (Webb, 1999). Females, which save resources by not producing pollen, may obtain higher seed fitness than hermaphrodites by producing more seeds and/or higher quality seeds (reduced inbreeding depression), and can thereby be maintained in populations (see Dufay & Billard, 2012). In the past two decades, studies began to emphasize the effects of ecological context on this sexual dimorphism, including harsh environment or herbivores (Delph, 1990, 2003; Wolfe & Shmida, 1997; Ashman, 1999, 2002, 2006; Barr, 2004; Vaughton & Ramsey, 2004; Caruso & Case, 2007; Wise & Hebert, 2010).

Darwin (1877) first noted the association between sexual dimorphism and harsh environment and stated that females were more prevalent in harsh sites in gynodioecious species. A recent review showed that studies of 14 gynodioecious species all found females preferring to occur in harsh environments with lower resource availability than hermaphrodites (Ashman, 2006). To explain such an association, Delph (1990) hypothesized that the seed set of hermaphrodites would be more variable and plastic than that of females among different resource level environments. Given that hermaphrodites allocate resources to both pollen and seeds, in harsh environments resources may be inadequate for hermaphrodites to maintain both sexual functions. However, females are less costly, in that they only allocate resources to seeds. Therefore, resource-stressful environments can cause a plastic reduction of seed production in hermaphrodites, and females can be favored because of relatively higher seed fitness (Barr, 2004; Dorken & Mitchard, 2008; Bishop et al., 2010). In populations, the equilibrium frequency of females depends on their seed fitness relative to that of hermaphrodites. The effect of herbivores on sexual expression received recent attention in gynodioecious species. Several studies showed that pollen-bearing plants, such as hermaphrodites and males, are often preferred by herbivores (Marshall & Ganders, 2001; Ashman, 2002; Collin et al., 2002; Ashman et al., 2004;

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FLORAL DEVELOPMENT OF *STEPHANIA* (MENISPERMACEAE): IMPACT OF ORGAN REDUCTION ON SYMMETRY

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Stephania is the sole genus in the basal eudicot family Menispermaceae that possesses both actinomorphic and zygomorphic flowers. Variation in perianth merism can have an important impact on flower symmetry and thus eminent biological significance in evolution of Menispermaceae. Using SEM, we studied the floral development of four representative species, which present the two predominant floral patterns of the genus, namely, homomorphy of both male and female flowers (actinomorphy) or heteromorphy (actinomorphy/zygomorphy). The sepals of the male flowers are arranged mostly in two alternate whorls of three or four each, whereas in female flowers they are in a single whorl of three or four or there is only a single sepal. Petals of male flowers are in a whorl of three or four organs, whereas female flowers of some species have only two petals. Trimerous and tetramerous perianths can coexist in the same umbellets of some species. Variation in perianth merism and loss of perianth parts of the female flowers may result in flower symmetry switching from actinomorphy to zygomorphy. The two main floral patterns are consistent with a distinction of two subclades within *Stephania*. The unicarpellate genera of the Menispermaceae share a unique combination of characters, including a synandrium, unitegmic ovules, and absence of vestigial sexual organs. However, *Stephania* differs from its unicarpellate relatives by two features: a two-whorled arrangement of floral organs of the male flowers and a free perianth. The investigation provides new and valuable developmental information on flowers of the little-known Menispermaceae and provides a background for a discussion of the evolution of merism and unisexual flowers in the basal eudicots.

Keywords: floral development, floral symmetry, Menispermaceae, merism, *Stephania*, unicarpellate gynoecium.

Introduction

Stephania is one of the few unicarpellate genera in the pantropical climbing Menispermaceae (Kessler 1993). The family, comprising 70~75 genera and 450~520 species (Jacques et al. 2007; Ortiz et al. 2007), is a member of a "core Ranunculales" sister to a Ranunculaceae + Berberidaceae clade (Hoot et al. 1999; APG II 2003; Kim et al. 2004; Wang et al. 2009; Soltis et al. 2011). Within the order, Menispermaceae are distinctive in being dioecious and having small flowers, with floral parts usually in whorls of three (Kessler 1993).

The genus *Stephania*, with ~30–60 species, is primarily found in tropical and subtropical Asia, parts of Africa, and Oceania (Diels 1910; Kessler 1993; Luo et al. 2008). It is a relatively large genus within Menispermaceae (Cronquist 1981). In his eight-tribe classification, Diels (1910) placed *Stephania* in the tribe Menispermeae which was subdivided into three subtribes: Cocculinae (2–6 carpels), Cissampelinae (1 carpel, with asymmetrical perianths of female flowers), and Stephaniinae (1 carpel, with symmetrical perianths of female flowers). Among these, the Stephaniinae consisted of only *Stephania*, whereas the Cissampelinae consisted of *Cissampelos*, *Cyclea*, and *Antizoma*. Forman (1956, 1968) considered this classification inappropriate because he found monosymmetric female flowers with asymmetric perianth in Malesian *Stephania*. However, pollen morphology (Harley and Ferguson 1982) supported the division between Stephaniinae (triporate) and Cissampelinae (tricolporate).

Whether the two subtribes are monophyletic is controversial, as the molecular phylogenies of the Menispermaceae are incongruent, based on different samplings and different genes. A preliminary phylogenetic study of Hong et al. (2001, on internal transcribed spacers [ITSs]) revealed that within the Menispermeae, *Stephania* was monophyletic and sister to *Cyclea* and *Cissampelos*. The trees of Ortiz et al. (2007, studying *ndh*F) and Wang et al. (2007, studying *mat*K, *trn*L-F, and ITS) showed that the two unicarpellate subtribes are monophyletic while Cocculinae is paraphyletic. However, Hoot et al. (2009, studying *rbc*L and *atp*B) and Jacques et al. (2011, studying *rbc*L and *atp*B) indicated that Cissampelinae is polyphyletic, and Stephaniinae is paraphyletic because the African species *Stephania laetificata* and the sampled Asian species are placed in different clades. *Stephania laetificata* was placed in a separate section by Diels (1910) and is characterized by triple pseudo-racemes of conglomerate cymes and rough hairs on the stems. From a strict

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Genetic Diversity and Characterization of a Core Collection of *Malus* Germplasm Using Simple Sequence Repeats (SSRs)

Sarah M. Potts · Yuepeng Han · M. Awais Khan · Mosbah M. Kushad · A. Lane Rayburn · Schuyler S. Korban

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Abstract Simple sequence repeats (SSRs) were used to assess genetic diversity and study genetic relatedness in a large collection of *Malus* germplasm. A total of 164 accessions from the *Malus* core collection, maintained at the University of Illinois, were genotyped using apple SSR markers. Each of the accessions was genotyped using a single robust SSR marker from each of the 17 different linkage groups in *Malus*. Data were subjected to principal component analysis, and a dendrogram was constructed to establish genetic relatedness. As expected, this diverse core collection showed high allelic diversity; moreover, this allelic diversity was higher than that previously reported. Cluster analysis revealed the presence of four distinct clusters of accessions in this collection.

Keywords Apple · Microsatellite markers · Genetic diversity · Genetic relatedness · Germplasm characterization

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Introduction

Availability of diverse *Malus* germplasm is critical for pursuing successful apple breeding efforts, as it increases genetic diversity and allows for development of new apple cultivars with enhanced and/or desirable traits. This also aids in diversifying the gene pool and preserving those unique genetic traits available in this material.

When characterizing plant germplasm, incidence of multiple clones of genetic material as well as mislabeling of accessions may occur, which are both costly and undesirable (Garkava-Gustavsson et al. 2008). Moreover, proper identification and characterization of plant germplasm will protect intellectual property as well as aid in identifying parents carrying genes of interest for breeding efforts (Goulão et al. 2001; Dávila et al. 1998). By selecting diverse parents and increasing genetic diversity through germplasm collections, progress can be made in apple plant breeding efforts towards developing new cultivars with economically valuable traits including those with enhanced fruit quality and disease and pest resistance.

As in vivo maintenance and management of *Malus* germplasm are labor-intensive, costly, and require commitment of land resources for germplasm conservation efforts, determining genetic identity and genetic relatedness among accessions also impacts efficiency and utilization of such germplasm collections in breeding programs (Kresovich and McFerson 1992; Russell et al. 1997). The constraints of management of the *Malus* germplasm collection have led to development of strategies for germplasm evaluation. One of these strategies is the development of a core collection consisting of accessions having high levels of genetic diversity that could serve as representatives of the entire genetic

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Molecular evolution of psbA gene in ferns: unraveling selective pressure and co-evolutionary pattern

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PLOS ONE

Analysis of Natural Variation in Bermudagrass (*Cynodon dactylon*) Reveals Physiological Responses Underlying Drought Tolerance

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Abstract

Bermudagrass (*Cynodon dactylon*) is a widely used warm-season turfgrass and one of the most drought tolerant species. Dissecting the natural variation in drought tolerance and physiological responses will bring us powerful basis and novel insight for plant breeding. In the present study, we evaluated the natural variation of drought tolerance among nine bermudagrass varieties by measuring physiological responses after drought stress treatment through withholding water. Three groups differing in drought tolerance were identified, including two tolerant, five moderately tolerant and two susceptible varieties. Under drought stress condition, drought sensitive variety (Yukon) showed relative higher water loss, more severe cell membrane damage (EL), and more accumulation of hydrogen peroxide (H_2O_2) and malondialdehyde (MDA), while drought tolerant variety (Tifgreen) exhibited significantly higher antioxidant enzymes activities. Further results indicated that drought induced cell injury in different varieties (Yukon, SR9554 and Tifgreen) exhibited liner correlation with leaf water content (LWC), H_2O_2 content, MDA content and antioxidant enzyme activities. Additionally, Tifgreen plants had significantly higher levels of osmolytes (proline level and soluble sugars) when compared with Yukon and SR9554 under drought stress condition. Taken together, our results indicated that natural variation of drought stress tolerance in bermudagrass varieties might be largely related to the induced changes of water status, osmolyte accumulation and antioxidant defense system.

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Introduction

Drought is one of the most serious world-wide problems and largely affects plant growth, development and survival rate, leading to enormous crop yield loss. Plants are greatly restricted in growth field under stress condition, and have evolved many mechanisms to rapidly adapt to drought stress condition to keep growth and productivity [1,2]. Recently, more attentions have been paid to mechanisms of plant drought stress tolerance, including physiological and biochemical metabolisms, gene expression regulation, proteomic profiling and cross-talks between several hormones etc, which further helps us develop different genetic approaches to improve plant drought tolerance and prevent yield loss.

In response to drought stress, turfgrass has developed complex mechanisms such as physiological, biochemical, molecular and cellular changes to cope with limited water supply [3,4]. Comparatively, bermudagrass (*Cynodon dactylon*) is one of the most drought tolerant turfgrasses [5,6]. As a warm-season perennial grass, bermudagrass is widely used as turfgrass on sport fields, golf courses and home lawns. Drought stress is a world-wide problem, and different grass species may develop different strategies to tolerate, escape, or avoid drought stress condition [4,7,8,9,10].

To date, a lot of research groups have paid attentions to the natural variations in biotic and abiotic stress tolerances in many plant species, such as *Arabidopsis*, rice, bermudagrass, *brachypodium ditachyon*, and perennial ryegrass (*Lolium perenne*) [3,9,11,12,13,14,15]. Using these germplasms differing in drought tolerance, the major quantitative trait locis (QTL) contributing to drought tolerance and genetic networks controlling important stress processes were further dissected [4,5,9,10,15]. Previous studies have suggested that drought tolerance of bermudagrass varieties might be correlated with plant development such as leaf firing, root and shoot systems, mass production [16,17,18,19], accumulation of dehydrin [9,20], evapotranspiration [21], leaf water content (LWC), chlorophyll content, proline content, and antioxidant enzyme activities [10,22,23]. Physiological and biochemical mechanisms in response to water deficit stress have been largely studied in some bermudagrass varieties, however, most of these studies have focused on physiological level. Correlations between the natural variation and the detailed mechanisms among different species are still largely unknown.

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Molecular Characterization of Pear 1-Aminocyclopropane-1-Carboxylate Synthase Gene Preferentially Expressed in Leaves

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Abstract

1-Aminocyclopropane-1-carboxylate (ACC) synthase catalyzes the conversion of S-adenosyl-L-methionine to ACC in the ethylene biosynthetic pathway. Lots of important ACC synthase genes have been isolated and characterized from the plant kingdom. In this study, a cDNA clone encoding putative ACC synthase was isolated from a cDNA library produced using mRNA from pear (*Pyrus pyrifolia*). The cDNA clone, designated *PpACS1* (GenBank accession No. JQ284383), comprised an open reading frame of 1, 341 bp encoding a protein of 446 amino acids that shares high similarity with the known plant ACSs. Using PCR amplification techniques, a genomic clone corresponding to *PpACS1* was isolated and shown to contain two introns with typical GT/AG boundaries defining the splice junctions. The *PpACS1* gene product shared 97% identity with an ACC synthase from pear (*Pyrus communis*), and phylogenetic analyses clearly placed the gene product in the ACC synthase cluster of the plant ACS superfamily tree. RT-PCR analysis indicated that the *PpACS1* gene was preferentially expressed in pear leaves. The transcript of *PpACS1* gene was accumulated at relatively high levels in anthers. The expression signal was detected in shoot at relatively low levels, but none signal was detected in developing fruit of pear. These results suggested that the *PpACS1* may participate in the regulation of ethylene production in pear leaves and anthers.

Keywords: Pear (*Pyrus pyrifolia*), Ethylene, 1-Aminocyclopropane-1-carboxylate (ACC) synthase, Gene expression

1. Introduction

Ethylene is a plant hormone regulating a wide range of physiological processes such as germination, growth, development, and senescence (Klee and Tieman, 2002). As a senescing hormone, it promotes leaf-yellowing, flower and leaf abscission, climacteric fruit ripening (Yang and Hoffman, 1984). The production of ethylene in higher plants is from S-adenosyl-L-methionine (SAM) via the intermediate 1-aminocyclopropane-1-carboxylic acid (ACC). The conversion of SAM to ACC is catalysed by ACC synthase, and the subsequent oxidation of ACC to ethylene is catalysed by ACC oxidase (Yang and Hoffman, 1984; Ververidis and John, 1991).

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ORIGINAL RESEARCH

Whole Genome Duplication of Intra- and Inter-chromosomes in the Tomato Genome

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ABSTRACT

Whole genome duplication (WGD) events have been proven to occur in the evolutionary history of most angiosperms. Tomato is considered a model species of the Solanaceae family. In this study, we describe the details of the evolutionary process of the tomato genome by detecting collinearity blocks and dating the WGD events on the tree of life by combining two different methods: synonymous substitution rates (*Ks*) and phylogenetic trees. In total, 593 collinearity blocks were discovered out of 12 pseudo-chromosomes constructed. It was evident that chromosome 2 had experienced an intra-chromosomal duplication event. Major inter-chromosomal duplication occurred among all the pseudo-chromosome. We calculated the *Ks* value of these collinearity blocks. Two peaks of *Ks* distribution were found, corresponding to two WGD events occurring approximately 36−82 million years ago (MYA) and 148−205 MYA. Additionally, the results of phylogenetic trees suggested that the more recent WGD event may have occurred after the divergence of the rosid-asterid clade, but before the major diversification in Solanaceae. The older WGD event was shown to have occurred before the divergence of the rosid-asterid clade and after the divergence of rice-*Arabidopsis* (monocot-dicot).

KEYWORDS: Whole genome duplication; Collinearity block; Phylogenetic tree

1. INTRODUCTION

Gene duplication has played an important role in evolution (Ohno, 1970). Genome doubling (polyploidy), often referred to whole genome duplication (WGD), has played a dramatic role in the diversification of most, if not all, eukaryotic lineages. This diversification is perhaps most impressively seen within the angiosperms (Cui et al., 2006; Soltis et al., 2009). The plant genomes have experienced comprehensive sequence diversification (Bowers et al., 2005), such as small fragment insertions, deletions, inversions, translocations, duplication (Navajas-Perez and Paterson, 2009), chromosomal rearrangement and fusion (Simillion et al., 2004) from an ancient WGD event. These processes eventually led to species diversification.

The complete sequencing of plant genomes has dramatically increased the investigation of WGD in angiosperms. Analysis of the *Arabidopsis thaliana* genome revealed a number of duplicated genes and suggested that two or three rounds of WGD had occured (Blanc et al., 2000, 2003; Paterson et al., 2000; Bowers et al., 2003; Simillion et al., 2004). The complete rice genome also suggested an ancient polyploidy event (Paterson et al., 2004; Yu et al., 2005). In addition, the recently completed sequencing of the *Sorghum bicolor* genome confirmed WGD in a common ancestor of cereals (Paterson et al., 2009). Sequencing of the complete *Populus* genome suggested that an independent WGD event occurred before the divergence of *Salix* and *Populus* and that an older duplication was shared by both the *Populus* and

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High genetic diversity in remnant natural populations of *Myricaria laxiflora*, a species once considered to be extinct in the wild

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ABSTRACT

Myricaria laxiflora was once considered to be restricted to the Three Gorges Reservoir Area (TGRA), China, and was thought to be the only species to become extinct due to the construction of the Three Gorges Dam (TGD). Recently, three natural populations of this species were found along the Yangtze River valley downstream of the TGD. We studied the genetic diversity and genetic differentiation of three remnant populations and nine extirpated populations, the material of which were collected from natural populations before the impoundment of the Three Gorges Reservoir, using inter-simple sequence repeat (ISSR). High levels of genetic diversity were revealed within populations, both at the species [percentage of polymorphic bands (PPB) = 95.9%] and population level (PPB varied from 58.5% to 80.3%). For the three remnant populations, the Yidu (MYD) and Yichang (MYC) populations showed comparatively higher genetic diversity among the 12 populations, whereas the Zhijiang (MZJ) population showed lower genetic variation. 97.96% of total genetic variation of the species was contained in the three remnant populations. The MZJ population appeared to be distinct from the other 11 populations, whereas the MYD and MYC populations had a close genetic relationship with the nine extirpated populations. A moderate level of genetic differentiation was detected among populations by different estimation approaches. The high genetic diversity of the three natural remnant populations indicates that *in situ* conservation of this species may enable it to successfully maintain its evolutionary potential, especially in the present case that the habitat of transplanted populations are not suitable for individuals' survival.

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1. Introduction

The construction of the world's largest hydroelectric dam across the Yangtze River, the Three Gorges Dam (TGD), has brought forth major concerns from conservation biologists (*e.g.* Wu et al., 2003; Liu et al., 2006). Many riparian plants have adapted to the natural dynamics of seasonal fluctuations over a long period of evolution (Poff et al., 1997; Stromberg, 2001; Imbert and Lefèvre, 2003) and are highly susceptible to environmental change. The construction of hydroelectric dams may destroy living habitats directly or produce dramatic environmental stress on riparian plants, which will shrink their distribution range and/or reduce the genetic diversity. Hence, habitat loss and fragmentation due to construction of hydroelectric dams could promote the increased long-term risk of species decline and extinction of many riparian plants (Wu et al., 2003; Liu et al., 2006).

The genus *Myricaria* (Tamaricaceae) contains 13 species, the majority of which occur in high altitudes (1000–5200 m above sea level). The only exception to this is *Myricaria laxiflora*, an evergreen shrub that is narrowly distributed in the low-altitude riverbank and shore habitats within the water-level fluctuation zone of the Yangtze River valley in China (Zhang and Zhang, 1984; Wang et al., 2003). The riparian zone is normally flooded from May to October at which time all of the *M. laxiflora* plants are submerged. *M. laxiflora* is highly tolerant to river flooding with a lifestyle of summer dormancy and winter growth (Wu et al., 1998; Wang et al., 2003; Liu et al., 2006; Chen and Xie, 2009). At present, *M. laxiflora* is an endangered plant species with most of its natural habitat lost due to the construction of TGD.

Wang et al. (2003) carried out an exhaustive survey by extensively collecting data on the geographic distribution, natural habitat and community structure of *M. laxiflora*, and documented a total of 31 populations of approximately 90,000 individuals occurring in 12 counties within the Three Gorges Reservoir Area (TGRA). Now, all the sites of the 31 populations located have been submerged and *M. laxiflora* individuals are unable to survive in these places as the TGD was completed and the water level has risen to 175 m

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PLOS ONE

Identification of Conserved and Novel microRNAs from *Liriodendron chinense* Floral Tissues

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Abstract

Background: *Liriodendron chinense* (*L. chinense*) is an endangered basal angiosperm plant in China because of its low reproductive efficiency. Recently, miRNAs have obtained great attention because they can play important roles. Through high throughput sequencing technique, large amount of miRNAs were identified from different plant species. But there were few studies about the miRNAs in the basal angiosperms especially in the sexual reproduction process.

Results: Deep sequencing technology was applied to discover miRNAs in *L. chinense* flowers at different stages. After bioinformatic analysis, 496 putative conserved miRNAs representing 97 families and 2 novel miRNAs were found. Among them, one is previously regarded as gymnosperm specific. Their expressions were further validated by Real-time PCR for 13 selected miRNAs. Putative targeting genes were predicted and categorized with gene ontology (GO) analysis. About ten percents of the targets are involved in the reproduction process. Further expressional analysis showed that many of these miRNAs were highly related to the reproductive growth.

Conclusions: This is the first comprehensive identification of conserved and novel miRNAs in *L. chinense*. The data presented here might not only help to fill the gap of miRNA registered about basal angiosperm plants but also contribute to understanding the evolution of miRNAs. The differential expression of some of the miRNAs and the prediction of their target genes are also helpful in understanding the regulation of *L. chinense* sexual reproduction.

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Introduction

MicroRNAs are endogenous 21–24 nt small non-coding RNAs (sncRNAs) that have been found in a wide variety of organisms ranging from prokaryotes to eukaryotes [1,2]. They negatively regulate gene expression on transcriptional and post-transcriptional level [3], and play pivotal roles in many aspects of plant growth and development [4,5,6] including floral organ identity, female gamete formation and reproductive development [7,8]. In plant, most of the miRNA encoding genes are intergenic and rarely clustered in tandem. To broaden the knowledge of miRNAs, high throughput sequencing techniques have been applied and large amount of miRNAs from different plants were identified. Currently, 19 724 mature miRNAs belonging to 153 species are deposited in miRBase (release 17.0 version, April 2011) [9]. Among them, a total of 3362 are from 46 plant species. The majorities of these identified miRNAs are from monocots *Oryza sativa* and eudicots *Populus trichocarpa* and *Arabidopsis thaliana*. Other plants include algae *Chlamydomonas reinhardtii* [10] and *Porphyra yezoensis* [11], moss *Physcomitrella patens* [12], conifer *Pinus taeda* [13] and *Picea abies* [14], monocot *Brachypodium distachyon* [15], *Triticum aestivum* [16] and *Zea mays* [17], basal eudicot *Eschscholzia californica* [18], core eudicot *Aquilegia coerulea* [19], *Arachis hypogaea* [20], *Populus euphratica* [21], *Medicago truncatula* [22], *Glycine max* [23] and Euphorbiaceous plants [24]. However there is little information on the miRNAs from basal angiosperm plants except a study on the miRNAs from *Selaginella moellendorffii* [25].

L.chinense is one of the two species in Liriodendron genus which belongs to basal angiosperm. It is the early branching angiosperm lineages (Fig. 1). Because it occupied pivotal positions in the phylogenetic tree, the plant of Liriodendron genus was always used to study the evolution of flowering plants. Although basal angiosperms only represent 3% of whole angiosperm species, most of the diversity in floral structure and organization was found among them [26]. In addition, *L. chinense* is ideal for landscaping because of its beautiful flowers and leaves. But it is endangering in China, which is mainly resulted by its low sexual reproductive efficiency. The molecular mechanism underlying its sexual reproduction barrier is totally unknown. Many internal factors including miRNAs have been found to play regulatory roles in the reproductive growth of plants. To profile the miRNAs in the flowers of *L. chinense* may help to explore the mechanisms that control the sexual reproductive process in this species, and hence to overcome its barrier in reproduction.

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Construction of a high-density genetic map for grape using next generation restriction-site associated DNA sequencing

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PLoS one

AFLP Genome Scan to Detect Genetic Structure and Candidate Loci under Selection for Local Adaptation of the Invasive Weed *Mikania micrantha*

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Abstract

Why some species become successful invaders is an important issue in invasive biology. However, limited genomic resources make it very difficult for identifying candidate genes involved in invasiveness. *Mikania micrantha* H.B.K. (Asteraceae), one of the world's most invasive weeds, has adapted rapidly in response to novel environments since its introduction to southern China. In its genome, we expect to find outlier loci under selection for local adaptation, critical to dissecting the molecular mechanisms of invasiveness. An explorative amplified fragment length polymorphism (AFLP) genome scan was used to detect candidate loci under selection in 28 *M. micrantha* populations across its entire introduced range in southern China. We also estimated population genetic parameters, bottleneck signatures, and linkage disequilibrium. In binary characters, such as presence or absence of AFLP bands, if all four character combinations are present, it is referred to as a character incompatibility. Since character incompatibility is deemed to be rare in populations with extensive asexual reproduction, a character incompatibility analysis was also performed in order to infer the predominant mating system in the introduced *M. micrantha* populations. Out of 483 AFLP loci examined using stringent significance criteria, 14 highly credible outlier loci were identified by Dfdist and Bayescan. Moreover, remarkable genetic variation, multiple introductions, substantial bottlenecks and character compatibility were found to occur in *M. micrantha*. Thus local adaptation at the genome level indeed exists in *M. micrantha*, and may represent a major evolutionary mechanism of successful invasion. Interactions between genetic diversity, multiple introductions, and reproductive modes contribute to increase the capacity of adaptive evolution.

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Introduction

Why some species become successful invaders is an important issue in invasive biology. When species are introduced into a new region, they face two fates. Some species quickly go extinct, whereas others persist and finally become highly competitive invaders, posing a serious threat to native diversity and ecosystems [1,2]. Successful invasions involve three major phases: introduction, naturalization, and invasion [3,4]. In the initial introduction phase, invasive species often contain low levels of genetic diversity due to bottleneck and founder effects [5–8]. Then, invaders produce pre-adapted genotypes in response to the abrupt environmental changes during naturalization [9–12]. Finally, exotic species become broadly invasive in the extended period [9,13]. The rapid population expansion of invaders is expected to promote adaptive evolution, since it has been shown that the rapidly increasing population size is conducive to withstanding (and responding to) strong directional selection [14,15]. A substantial time lag is involved during the transition from introduction via naturalization to invasion [4]. The occurrence of a lag phase allows populations to adapt to new environmental factors such as ecological niche, temperature, precipitation, soils, frost, and wind speed or growing season length [11,12,16–18]. On the other hand, neutral or deleterious alleles, which become favored in new ecological contexts, will contribute to adaptive changes of invasive populations [19]. These changes may increase the survival rate of invasive species [12], making them become gradually dominant in the introduced range [20]. Therefore, pre-adaptation to novel environments is often counted as a premise for successful invasion [3,4,21].

Genomic scans are useful to identify potential adaptive loci under selection at the genomic level [22,23]. All loci across the genome are anticipated to possess similar demography and neutral evolution history of populations, including genetic drift and gene dispersal [24]. If variation of a locus is beyond the genomic pattern with an unusual frame of higher genetic differentiation, it is deemed an "outlier locus" under natural selection [24,25]. The outlier locus can be identified explicitly in the genes under selection and also in neutral flanking regions due to hitchhiking effects [26,27]. In model organisms for which whole genomic information is available, it is easy to track the "outlier locus" under selection [28]. However, for non-model organism such as invasive

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Population Genetic Variation in the Tree Fern *Alsophila spinulosa* (Cyatheaceae): Effects of Reproductive Strategy

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Abstract

Background: Essentially all ferns can perform both sexual and asexual reproduction. Their populations represent suitable study objects to test the population genetic effects of different reproductive systems. Using the diploid homosporous fern *Alsophila spinulosa* as an example species, the main purpose of this study was to assess the relative impact of sexual and asexual reproduction on the level and structure of population genetic variation.

Methodology/Principal Findings: Inter-simple sequence repeats analysis was conducted on 140 individuals collected from seven populations (HSG, LCH, BPC, MPG, GX, LD, and ZHG) in China. Seventy-four polymorphic bands discriminated a total of 127 multilocus genotypes. Character compatibility analysis revealed that 50.0 to 70.0% of the genotypes had to be deleted in order to obtain a tree-like structure in the data set from populations HSG, LCH, MPG, BPC, GX, and LD; and there was a gradual decrease of conflict in the data set when genotypes with the highest incompatibility counts were successively deleted. In contrast, in population ZHG, only 33.3% of genotypes had to be removed to achieve complete compatibility in the data set, which showed a sharp decline in incompatibility upon the deletion of those genotypes. All populations examined possessed similar levels of genetic variation. Population ZHG was not found to be more differentiated than the other populations.

Conclusions/Significance: Sexual recombination is the predominant source of genetic variation in most of the examined populations of *A. spinulosa*. However, somatic mutation contributes most to the genetic variation in population ZHG. This change of the primary mode of reproduction does not cause a significant difference in the population genetic composition. Character compatibility analysis represents an effective approach to separate the role of sexual and asexual components in shaping the genetic pattern of fern populations.

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Introduction

Almost all ferns are homosporous, producing only one type of spore that germinates to form a bisexual gametophyte [1]. In homosporous ferns, there are three possible modes of sexual reproduction [2–4]: (i) intragametophytic selfing - the union of sperm and egg from the same gametophyte resulting in a completely homozygous sporophyte; (ii) intergametophytic selfing - the union of sperm and egg from different gametophytes arising from spores from the same parental sporophyte (analogous to selfing in seed plants); and (iii) intergametophytic crossing - the union of sperm and egg from gametophytes arising from spores of different sporophytes (analogous to outcrossing in seed plants). In addition to these modes of sexual reproduction, many ferns also have the ability to propagate vegetatively in either the gametophyte or the sporophyte generation [5–7]. Numerous studies have been performed to evaluate the predominant mode of reproduction in natural populations of homosporous ferns [8–10].

Life-history traits, such as reproductive system and frequency of sexual versus asexual reproduction, are considered to be particularly important for shaping the level and structure of genetic variation in plant populations [11,12]. In seed plants, it has been generalized that inbreeding potentially reduces within-population genetic variation but increases within-population genetic subdivision and among-population genetic differentiation, while outcrossing functions to enhance population genetic variation but impedes population subdivision and structuring [11,13]. This generalization is upheld in some fern populations, such as populations of *Hemionitis palmata* [8], *Pleopeltis* species [14], *Polystichum otomasui* [15], *Asplenium trichomanes* subsp. *quadrivalens* [16], and *Dryopteris aemula* [17]. Yet notable exceptions do exist. For example, populations of *Botrychium virginianum* show little interpopulation

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Phenotypic Characterization and Simple Sequence Repeat Identification of Red-fleshed Kiwifruit Germplasm Accessions

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Additional index words. Actinidia, red-flesh, SSR marker, phenotype, fruit character

Abstract. **Big fruit size and nice red pigmentation combined with good flavor should be the major target for red-fleshed kiwifruit (*Actinidia* spp.) breeding programs. Genetic diversity and plant characteristics were evaluated on a set of kiwifruit accessions with predominantly red flesh to identify the superior individuals for further breeding or study of commercial application. The leading phenotypic characters varied widely among the accessions. Accession R reached average fruit weight ≈100 g, whereas it ranged from 43.15 to 84.71 g for the other accessions. Fruits of L and Q were flatter in shape than the others. The core volume accounted for fruit proportions ranging from 2.33% to 11.42%. 'Chuhong', 'Honghua', and K exhibited a round fruit apex, whereas most others showed a depressed apex. R, L, and Q had the highest a* values in the inner pericarp and also the most appealing visual coloration. Results revealed significantly higher soluble solid content (SSC), total sugar, and sugar/acid ratio in Q, R, and L. The 12 pairs of simple sequence repeat (SSR) markers were successfully used to characterize the genetic variability and confirm true-to-type identity for four accessions. However, the limited number of markers had no ability to discriminate among the other 11 accessions. Based on additional 28 SSRs, six of the indistinguishable accessions were confirmed to be genetically different, and three seemed to belong to the same clone vine. The results demonstrated that application of SSR data could improve the efficiency of identifying red-fleshed kiwifruit germplasm.**

Red flesh was first found in the fruit of *A. chinensis* from Hubei (Liang, 1982) and described by Cui (1993) again. In the late 1990s, the first red-fleshed kiwifruit cultivar in the world, 'Hongyang', was developed and firstly released from Cangxi County of Sichuan Province in China (Wang, 2003; Wu, 1992), which now has the largest cultivation acreage of red-fleshed kiwifruit in China. Red-fleshed kiwifruit are novel fruits that have unique and attractive appeal and make consumers willing to buy, although the green- and yellow-fleshed cultivars still represent the main body of the kiwifruit traded internationally (Ferguson and Seal, 2008). To date, 'Hongyang' has been introduced into at least eight Provinces in China. Other cultivars or selections with red flesh or red core include 'Chuhong', 'Hongmei', 'Honghua', 'Yuanhong', 'Xiangjihong', 'Tianyuanhong', and 'Longshanhong', which were later registered in China (Wang et al., 2005a, 2005b, 2006; Wu and Wu, 1998). However, their comprehensive characteristics (especially the red coloration, fruit size, sweetness, or flavor) did not meet 'Hongyang' standards. However, as the predominant cultivar in red-fleshed kiwifruit production (≈5000 ha in China), besides its small fruit size, 'Hongyang' is susceptible to *Pseudomonas syringae* pv. *Actinidiae* (*Psa*), one of the most dangerous bacterial pathogens of kiwifruit plants worldwide, which causes devastating damage to phyllosphere organs (Balestra et al., 2009; Feng and Li, 2009; Rossetti and Balestra, 2008). Moreover, its fruit coloration is badly inhibited by high day temperatures, which results in an obvious reduction or almost disappearance in pigmentation of the inner pericarp (Zhong et al., 2007; unpublished data). Larger fruit, higher intensity of the red pigments, and their high stability under warmer climates combined with strong resistance to *Psa* and good flavor should be the major targets for breeding and improvement of red-fleshed cultivars.

Kiwifruit cultivars on the world market are mostly selected from wild germplasm, bud-sports, seedlings, and controlled crosses (Chen et al., 2009; Ferguson and Huang, 2007). The kiwifruit industry in Cangxi of Sichuan Province began with a batch of mixed seeds of wild *A. chinensis* from the Funiu Mountains, Henan Province in 1978, from which 'Hongyang' was developed. Large variations in flesh color, size, shape, flavor compounds, storage life, and

Table 1. Accessions origins used in this study.

Cultivar name or code of accession	Origins
Cultivars	
Hongyang	One seedling from mixed wild-collected seeds of *A. chinensis* var. *chinensis* and *A. chinensis* var. *deliciosa* from the Funiu Mountains (Wu, 1992)
Chuhong	One wild plant of *A. chinensis* var. *chinensis* from the mountainous region of Xupu County in the western part of Hunan Province (Wang et al., 2005b)
Hongmei	One seedling from wild-collected seeds of *A. chinensis* var. *deliciosa* in the northern mountains of the Sichuan Basin (Wang et al., 2005a)
Honghua	One hybrid of 'Hongyang' and *A. chinensis* var. *deliciosa* male plant (Wang et al., 2006)
Accessions	
AB, AF, H, K, L, O, Q, R	Selections from the seedlings of mixed wild-collected seeds of *A. chinensis* var. *chinensis* and *A. chinensis* var. *deliciosa* from the Funiu Mountains
C, I, J	Phenotypic variations from 'Hongyang' orchards

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Genetic and cytological analysis of a new spontaneous male sterility in radish (*Raphanus sativus* L.)

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Abstract A male sterile plant appeared in the radish breeding program at the Hubei Academy of Agricultural Sciences, Hubei, China. In its progeny, a two-type (half of plants male sterile, the other half male fertile) line 01GAB was established. An F_2 population of 260 plants from a cross of male-sterile 01GAB and a male fertile line 9802H segregated for male fertility in a 3:1 ratio indicating that fertility was restored by a single dominant gene, here designated *RsMs*. A PCR-based DNA marker specific to the male fertility *Rfob* gene in 9802H was absent in 01GAB. Linkage analysis placed the *RsMs* locus 10.7 cM away from the *Rfo* locus. In an F_2 population of hybrids between 01GAB and male fertile 9802B, a co-dominant DNA marker for the *RSultr3.2A* (a radish sulfate transporter gene) locus was linked to the *RsMs* locus at 1.5 cM suggesting that fertility restoration in 01GAB was located in the region with known male sterility restorers in radish. However, no maintainer for the 01GAB source of male sterility has been identified so far. Cytological observations have shown that the abnormalities in male sterile anthers first appeared in tapetum at the tetrad stage, followed by a hypertrophy of the tapetal cells at the vacuolate microspore period. These results suggest that male sterility in 01GAB is likely to be genetic in nature, or it may represent a new type of the cytoplasmic male sterility.

Keywords Cytological characteristics · Fertility restorer · Male sterility · Molecular marker · Radish

Lei Gao and Chang Ping Xiang contributed equally to this study.

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Introduction

Male sterility, defined as the failure of a plant to produce functional pollen, has been reported in many higher plants involving at least 43 families, 162 genera, and 320 species (Kaul 1988). Utilization of this trait in breeding of numerous crops eliminates labor-intensive hand emasculation, and thereby

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Maternal inheritance of sugars and acids in peach (*P. persica* (*L.*) Batsch) fruit

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Abstract Maternal inheritance of sugars and acids in peach fruit were investigated during two successive years using reciprocal populations derived from 'low acid' (LA) flat peach 'Zaolupan' and non-LA round peach 'Zaoxing'. The reciprocal populations segregated into LA round, LA flat, non-LA round and non-LA flat-fruited offspring. Generally, the reciprocal populations had similar range and mean values of sugar and acid. Mean values were to different degree lower than or similar to mid-parental values. Maternal inheritance did not show significant effects on sugars and acids. Broad sense heritability of sugars and acids was high, ranging from 0.61 to 0.90. The correlations among sugars and acids were studied, and positive correlations were always found between glucose and fructose, and quinate and shikimate. Generally, mean glucose, fructose, sorbitol, quinate and shikimate contents did not show significant difference among LA round, LA flat, non-LA round and non-LA flat-fruited progenies. Mean sucrose and total sugar contents of flat-fruited progenies tended to be higher than round-fruited progenies, while mean malate, citrate and total acid contents did not significantly differ with fruit shape (round vs. flat).

Keywords Correlations · Fruit quality · Principal component analysis · Quantitative traits Reciprocal populations · Round/flat peach

J. B. Zhao contributed equally to this study.

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Introduction

Soluble sugars and organic acids are important components of taste, together with aroma. They have an impact on the overall organoleptic quality of fruit. In mature peach fruit, the main soluble sugars are sucrose, glucose, fructose and sorbitol, and the main organic acids are malate, citrate and quinate, with traces of shikimate (Esti et al. 1997; Quilot et al. 2004). Our knowledge of sugar and acid metabolism and the genetic bases determining sugar and acid contents in fruit has increased considerably over time. However, the effect of maternal inheritance on sugar and acid contents in fruit, which would benefit fruit breeding programs, are still limited.

Developmental Cell
Article

Mitochondrial GCD1 Dysfunction Reveals Reciprocal Cell-to-Cell Signaling during the Maturation of *Arabidopsis* Female Gametes

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SUMMARY

Cell-to-cell communication in embryo sacs is thought to regulate the development of female gametes in flowering plants, but the details remain poorly understood. Here, we report a mitochondrial protein, GAMETE CELL DEFECTIVE 1 (GCD1), enriched in gametophytes that is essential for final maturation of female gametes. Using *Arabidopsis gcd1* mutants, we found that final maturation of the egg and central cells is not required for double fertilization but is necessary for embryogenesis initiation and endosperm development. Furthermore, nonautonomous effects, observed when GCD1 or AAC2 function is disrupted, suggest that mitochondrial function influences reciprocal signaling between central and egg cells to regulate maturation of the partner (egg or central) cell. Our findings confirm that cell-to-cell communication is important in functional maturation of female gametic cells and suggest that both egg and central cells sense and transmit their mitochondrial metabolic status as an important cue that regulates the coordination of gamete maturation.

INTRODUCTION

Well-developed egg and central cells, which act as female gametes, are prerequisites for double fertilization in angiosperms (Ma and Sundaresan, 2010; Yadegari and Drews, 2004). During double fertilization, the two female gametic cells fuse with two sperm cells, producing an embryo and endosperm. Successful interaction with the two sperm cells during double fertilization requires simultaneous maturation of the egg and central cells. The mechanism controlling the coordinated development of the female gamete cells remains unknown but is thought to involve cell-to-cell communication, which usually involves signal transduction among neighboring cells via a physical connection or secreted extracellular cue.

Early in female gametophyte development, multiple plasmodesmata link the functional megaspore and the surrounding nucellar cells, which are thought to be important for the differentiation of female gametophytes (Bajon et al., 1999). Genetic analysis has suggested that cross-communication occurs between the female gametophyte and sporophytic tissues in the ovule, highlighting the important role of the chalaza and integument in mediating the exchange of information necessary for development of the embryo sac (Bencivenga et al., 2011). Experiments in which fluorescent morpholino antisense oligomers (Okuda et al., 2009) and small fluorescent tracers were injected into the cytoplasm of *Torenia fournieri* central cells have indicated the presence of a symplastic connection between the egg cell, synergids, and central cell (Han et al., 2000), implying molecular transportation among these cells. However, when the 27 kDa green fluorescent protein (GFP) molecule (Liarzi and Epel, 2005) is expressed in *Arabidopsis*, no diffusion of the fluorescent signal from one cell to the other in the embryo sac is observed (Punwani et al., 2007; Steffen et al., 2007). Thus, additional investigations are required to confirm that communication between the gametic cells occurs via physical connections.

Communication between the central and antipodal cells was recently reported by Kägi et al. (2010), who examined central cells defective for *FIONA*, a gene normally expressed in the central cell but not in antipodals. *FIONA* is required for the final maturation of the central cell. The *fiona* defect disrupted the timing of antipodal cell degradation, providing convincing evidence that the central cell determines the life span of antipodal cells. It has been also shown in maize that *ZmEAL1* encoding a secreted, non-cell-autonomous peptide specifically expresses in the egg cell and functions in preventing antipodal cells from adopting central cell fate (Krohn et al., 2012). Other researchers have suggested that central cell–egg cell communication is involved in cell fate patterning during embryo sac development (Chevalier et al., 2011), thereby influencing female gametic development and fate determination. Clearly, this fascinating hypothesis warrants further investigation.

As the central and egg cells reach maturation, they become functionally specialized such that their fates diverge after fertilization. However, the mechanism that regulates the final maturation and functional specification of female gametes remains poorly understood. In animals, active transcription occurs as

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Invited Expert Review

The Maternal-to-Zygotic Transition in Higher Plants

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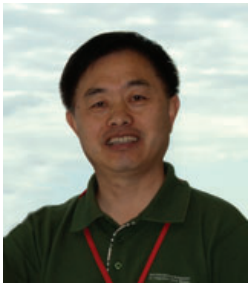

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Abstract

During early embryogenesis in mammals and higher plants, the maternal-to-zygotic transition (MZT) marks the turnover of developmental control from maternal products to *de novo* zygotic genome transcripts. Intensive studies in animals indicate that early embryonic development is largely maternally controlled. In recent years, the MZT has drawn the attention of botanists, as it is important for understanding the mechanism of embryogenesis and hybrid vigor. In this study, we present a brief overview of some aspects of the MZT in flowering plants. Based on what we have learned from *Nicotiana tabacum*, we hypothesize that the MZT occurs before zygotic cell division and that the development of the fertilized egg cell in flowering plants can be divided into two phases: the zygote stage, which is mainly controlled maternally, and the one-celled proembryo stage, in which zygotic genome activation (ZGA) occurs and is required for zygote division.

Keywords: Embryogenesis; maternal-to-zygotic transition; *Nicotiana tabacum*; zygote.

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Introduction

The maternal-to-zygotic transition (MZT) is characterized by two combined processes: the degradation of maternal mRNAs and proteins deposited in the egg before fertilization, and the onset of transcription from the zygotic genome, which has been defined as zygotic genome activation (ZGA, Schier 2007). The differences between the MZT and ZGA have been well defined by Baroux et al. (2008). ZGA could be one of the requirements for the MZT, whereas the MZT not only implies transcriptional activity of the zygotic genome but also the controlled fate of the embryo by the zygotic genome (Baroux et al. 2008).

The timing and scale of the MZT has been extensively studied in animals (reviewed by Tadros and Lipshitz 2009). The results show that the first few cell divisions are mainly controlled by maternally-deposited products during the early stages of embryogenesis. The MZT occurs after several cell divisions, although the time course is species-specific and ranges from a few hours to several days after fertilization (Tadros et al. 2007).

Compared with that in animals, investigations into the MZT in flowering plants are just beginning. Inaccessibility of the egg cells and early embryos, which are deeply embedded in the ovule, has hampered research in this field. However, relevant studies have progressed rapidly in recent years due to well-established methods for isolating gametes and embryos in higher plants (Sun et al. 1993; Fu et al. 1996). Increasing evidence indicates that the MZT also occurs in higher plants, similar to that in animals (Baroux et al. 2008). The results from

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Variability and adaptability of *Miscanthus* species evaluated for energy crop domestication

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Abstract

A growing body of evidence indicates that second-generation energy crops can play an important role in the development of renewable energy and the mitigation of climate change. However, dedicated energy crops have yet to be domesticated in order to fully realize their productive potential under unfavorable soil and climatic conditions. To explore the possibility of domesticating *Miscanthus* crops in northern China where marginal and degraded land is abundant, we conducted common garden experiments at multiple locations to evaluate variation and adaptation of three *Miscanthus* species that are likely to serve as the wild progenitors of the energy crops. A total of 93 populations of *Miscanthus sinensis*, *Miscanthus sacchariflorus*, and *Miscanthus lutarioriparius* were collected across their natural distributional ranges in China and grown in three locations that represent temperate grassland with cold winter, the semiarid Loess Plateau, and relatively warm and wet central China. Evaluated with growth traits such as plant height, tiller number, tiller diameter, and flowering time, the *Miscanthus* species showed high levels of genetic variation within and between species. There were significant site × population interactions for almost all traits of *M. sacchariflorus* and *M. sinensis*, but not *M. lutarioriparius*. The northern populations of *M. sacchariflorus* had the highest establishment rates at the most northern site owing to their strong cold tolerance. An endemic species in central China, *M. lutarioriparius*, produced not only the highest biomass of the three species but also higher biomass at the Loess Plateau than the southern site near its native habitats. These results demonstrated that the wild species harbored a high level of genetic variation underlying traits important for crop establishment and production at sites that are colder and drier than their native habitats. The natural variation and adaptive plasticity found in the *Miscanthus* species indicated that they could provide valuable resources for the development of second-generation energy crops.

Keywords: biomass, cold tolerance, lignocellulosic energy crops, *M. lutarioriparius*, *M. sacchariflorus*, *M. sinensis*

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Introduction

Miscanthus is considered to be a promising candidate for second-generation energy crops (Clifton-Brown *et al*., 2004, 2007; Karp & Shield, 2008; Oliver *et al*., 2009). As a C4 perennial grass capable of producing high biomass in cool climates, *Miscanthus* is especially

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Characterization of 39 novel EST-SSR markers for *Liriodendron tulipifera* and cross-species amplification in *L. chinense* (Magnoliaceae)[1]

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- *Premise of the study:* A novel set of simple sequence repeat (SSR) markers were developed and characterized from the expressed sequence tag (EST) database of *Liriodendron tulipifera* for application in population genetic studies of *Liriodendron*.
- *Methods and Results:* Thirty-nine polymorphic EST-SSR loci were identified among 27 individuals sampled from a cultivated population of *L. tulipifera*. The number of alleles per locus ranged from three to 18. The average observed heterozygosity and expected heterozygosity were 0.684 and 0.778, respectively. Of the 39 loci, 32 showed interspecific transferability and polymorphism in a related species, *L. chinense*. The number of alleles per locus ranged from two to 11, and the average observed heterozygosity and expected heterozygosity were 0.475 and 0.736, respectively.
- *Conclusions:* The developed EST-SSR markers will be useful for investigating adaptive genetic differentiation in *Liriodendron*.

Key words: adaptive genetic differentiation; cross-species amplification; EST-SSR; *Liriodendron chinense*; *Liriodendron tulipifera*; Magnoliaceae.

The tree genus *Liriodendron* L. (Magnoliaceae) comprises only two species, *L. tulipifera* L. (yellow poplar) and *L. chinense* (Hemsl.) Sarg. Yellow poplar is a commercially valuable tree species and is widely distributed in North America. However, the morphologically similar *L. chinense* is rare, occurring in small and isolated populations in China and northern Vietnam (Hao et al., 1995). This species was listed in the IUCN Red List of Endangered Plants in China (Fu and Jin, 1992), and is currently classified as a lower risk or near-threatened species (http://www.iucnredlist.org/). To develop appropriate conservation measures for *L. chinense*, molecular markers are needed to investigate the adaptive genetic diversity of extant populations.

Population genome scans have been used recently to detect genetic markers associated with natural selection (Nosil et al., 2009). A locus that is linked to adaptive genes under selection is expected to exhibit aberrant patterns of variation relative to the rest of the genome, and these outlier loci can aid the discovery of genes conferring adaptations that are currently under selection (Storz, 2005). Expressed sequence tag–simple sequence repeat (EST-SSR) markers derived from expressed sequences may be associated with functional genes. Genome scans using a large number of EST-linked microsatellites have proven useful in examining functional diversity related to adaptive variation (Vasemagi et al., 2005). To date, only about 110 EST-SSR markers have been reported for *L. chinense* (Xu et al., 2006, 2010); additional markers are needed. We present a novel set of polymorphic EST-SSR markers developed and characterized for *L. tulipifera* and report their transferability to *L. chinense*.

METHODS AND RESULTS

A total of 14 601 newly published ESTs for *L. tulipifera* were downloaded from the dbEST of GenBank (http://www.ncbi.nlm.nih.gov/dbEST), converted into FASTA format, and screened for the presence of SSRs using Simple Sequence Repeat Identification Tool (SSRIT) software (Temnykh et al., 2001; http://www.gramene.org/db/markers/ssrtool/). Four hundred unique sequences with at least one SSR were yielded with the criteria of 12, 10, 8, 8, and 8 repeat units for di-, tri-, tetra-, penta-, and hexanucleotide motifs, respectively. A subset of 169 SSRs with sufficient primer-designated sites was selected. Primers ranging from 20 to 22 base pairs, and with moderate GC content flanking the SSR region, were designed using Primer3 (Rozen and Skaletsky, 2000; http://frodo.wi.mit.edu/primer3/).

Microsatellite polymorphism was initially assessed in eight individuals of *L. tulipifera* from one cultivated population (Jurong, Jiangsu, China; 32°06′36″N, 119°13′12″E). Total genomic DNA was extracted from young leaves using the cetyltrimethylammonium bromide (CTAB) method (Doyle and Doyle, 1987). PCR amplification was performed in a 10-μL reaction solution, containing 40 ng genomic DNA, 1× *Taq* Buffer with $(NH_4)_2SO_4$ (supplied with *Taq*), 2.0 mM $MgCl_2$, 0.2 mM dNTPs, 0.25 μM each primer, and 0.5 unit *Taq* polymerase (Fermentas, Vilnius, Lithuania). A touchdown PCR protocol was applied for all the primers and performed on GeneAmp PCR System 9700 (Applied Biosystems, Foster City, California, USA). Touchdown PCR was performed as follows: an initial denaturation at 95°C for 4 min, followed by 35 cycles of 30 s at

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Genetic linkage maps for Asian and American lotus constructed using novel SSR markers derived from the genome of sequenced cultivar

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RESEARCH ARTICLE

Genetic diversity in *Eucommia ulmoides* (Eucommiaceae), an endangered traditional Chinese medicinal plant

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Abstract *Eucommia ulmoides*, the only species of Eucommiaceae, has been used as Chinese medicinal plant for more than 2,000 years, and is endangered as a consequence of long-term overexploitation. In this study, genetic diversity within and among the semi-wild and cultivated populations of *E. ulmoides* collected from its main production area was investigated using two cpSSR and 227 AFLP loci. A moderate level of within-population diversity was observed ($hS = 0.549$ for cpSSR and $H_{eB} = 0.183$ for AFLPs), which could be explained by the dioecious breeding system, longevity, and human mediated seed exchange. Compared to the semi-wild population, the cultivated population possessed relatively low genetic diversity, which suggests cultivation practice may have reduced the genetic diversity of *E. ulmoides*. Populations were low to moderately differentiated from one another ($G_{ST} = 0.281$ for cpSSR, and $\theta^B = 0.100$ for AFLPs), which was also supported by the AMOVA analysis that 62.52 and 83.48 % total variation resided within populations based on cpSSR and AFLP analysis, respectively. An isolation by distance analysis revealed that geographical distances was not significantly correlated with genetic distance. Human activities such as seed selection and seed exchange could account for the genetic structure of cultivated populations. In addition, two genetic clusters were detected by the Structure analysis. For conservation purpose, an *ex situ* conservation measure for conserving the genetically distant populations to maximize genetic diversity of the species is recommended.

Keywords Genetic diversity · Domestication and cultivation · *E. ulmoides* · Medicinal plant · Conservation

Xiaohong Yao and Jianyun Deng contributed equally to this study

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Introduction

Anthropogenic destruction of natural medicinal plant populations has dramatically increased in recent decades (Gu 1998), and the threat to resource security of Chinese traditional medicine is well articulated (Fu and Jin 1992). There are over 11,146 indigenous plant taxa in China that are recorded as having medicinal use (Chinese Pharmacopoeia Committee 1990). As a result of the increasing demand for medicinal plants, most of which is still met by wild collection, the natural resources of medicinal plants have been declining dramatically due to over-harvesting and loss of habitat over the past several decades (Huang et al. 1999). In certain instances, plant species can become threatened with extinction as a result. Conservation of medicinal plants is therefore one of the most important cultural and ecological issues faced today in China.

Eucommia ulmoides Oliver ('du-zhong' in Chinese), the single extant species of Eucommiaceae (related to Ulmaceae), is a dioecious, wind-pollinated tree evenly distributed in the mixed mesophytic forest habitats of valleys, hills, and low mountains in central and eastern China (Cronquist 1981; Zhang et al. 2003). *Eucommia ulmoides* is also an economically important tree species for both herbal medicine and the

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A ballistic pollen dispersal system influences pollination success and fruit-set pattern in pollinator-excluded environments for the endangered species *Synaphea stenoloba* (Proteaceae)

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The critically endangered *Synaphea stenoloba* (Proteaceae) has numerous scentless flowers clustered in dense inflorescences and deploys a ballistic pollen ejection mechanism to release pollen. We examined the hypothesis that active pollen ejection and flowering patterns within an inflorescence influence the reproductive success (i.e. fruit formation) of individual flowers within or among inflorescences of *S. stenoloba* in a pollinator-excluded environment. Our results showed that: (1) no pollen grains were observed deposited on the stigma of their own flower after the pollen ejection system was manually activated, indicating self-pollination within an individual flower is improbable in *S. stenoloba*; (2) fruit set in the indoor open pollination treatment and the inflorescence-closed pollination treatment indicated that *S. stenoloba* is self-compatible and pollen ejection can potentially result in inter-floral pollination success; (3) fruit set in the inflorescence-closed pollination treatment was significantly lower than that of indoor open pollination, indicating within- and between-flower pollination events in an inflorescence are most likely limited, with pollination between inflorescences providing the highest reproductive opportunity; and (4) analysis of the spatial distribution of cumulative fruit set on inflorescences showed that pollen could reach any flower within an inflorescence and there was no functional limitation on seed set among flowers located at various positions within the inflorescence. These data suggest that the pollen ejection mechanism in *S. stenoloba* can enhance inter-plant pollination in pollinator-excluded environments and may suggest adaptation to pollinator scarcity attributable to habitat disturbance or competition for pollinators in a diverse flora. © 2012 The Linnean Society of London, *Botanical Journal of the Linnean Society*, 2012, **170**, 59–68.

ADDITIONAL KEYWORDS: diverse flora – flowering patterns – pollinator scarcity.

INTRODUCTION

Flowers and inflorescences are the functional units of angiosperm mating and exhibit a tremendous diversity of size, shape, colour, scent, rewards, symmetry, placement of sexual organs, number of flowers open concurrently, arrangement in inflorescences and gender (Barrett, 1998, 2003). Since Darwin, most floral traits have been interpreted as mechanisms that passively encourage cross-pollination by preventing or discouraging self-pollination, thereby allowing more opportunities for ovules to be outcrossed (Barrett & Harder, 1996). It is now widely recognized that many features of flowers and inflorescences influence patterns of pollen dispersal and pollination success (Harder & Wilson, 1994; Barrett, 2003).

Most plant species require vectors to transfer pollen to achieve pollination success, and some species have evolved active pollen release mechanisms to enhance pollination success to cope with various pollinator-poor environments. For example, a sudden movement of the anther walls of *Ricinus communis* L. launches

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Genetic footprints of habitat fragmentation in the extant populations of *Sinojackia* (Styracaceae): implications for conservation

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Sinojackia, a member of the family Styracaceae, is an endangered genus endemic to China. The number of populations and population size of *Sinojackia* have decreased sharply because of habitat fragmentation and destruction. We studied the genetic diversity of extant populations in two different cohorts (adult and seedling) using eight microsatellite markers to investigate the genetic footprints of habitat fragmentation in four recognized *Sinojackia* spp. and to develop appropriate conservation measures. Data on intrapopulational genetic diversity suggest that *Sinojackia* populations have maintained relatively high levels of genetic diversity and low levels of genetic differentiation despite severe fragmentation. The high genetic diversity may be explained by the outcrossing mating system and high longevity of *Sinojackia* spp. The amount of genetic variation is not associated with population size, which was also supported by bottleneck analysis. In the species studied, there was no significant difference in the genetic diversity between the two cohorts analysed. However, inbreeding increased from adult trees to seedling populations, suggesting that the higher proportion of biparental inbreeding in the recent generations of seedlings is the result of restricted current genetic flow caused by habitat fragmentation. Average seed set per population was not significantly correlated with either population size or genetic diversity. Conservation management should aim to monitor inbreeding and outbreeding depression carefully to ensure the *in situ* and *ex situ* conservation of *Sinojackia* spp. © 2012 The Linnean Society of London, *Botanical Journal of the Linnean Society*, 2012, **170**, 232–242.

ADDITIONAL KEYWORDS: China – conservation strategies – genetic diversity – life stages.

INTRODUCTION

As the growth of the human population has continued, more and more natural areas and resources have been utilized to develop the agricultural industry. Thus, continuous populations of many species have been transformed into small and isolated populations (i.e. habitat fragmentation). Habitat fragmentation and deforestation have been generally recognized as the main threats to biodiversity (Fahring & Meriam, 1994). Theory predicts that habitat fragmentation should lead to increased inbreeding and genetic divergence among populations and reduced levels of genetic diversity through random genetic drift and limited gene flow (Young, Boyle & Brown, 1996). In the long term, genetic erosion is expected to limit the ability of a species to respond to environmental change and to increase the likelihood of its extinction (Young *et al*., 1996; Newman & Pilson, 1997; Jump & Penuelas, 2006). Therefore, more attention should be paid to the genetic effect of habitat fragmentation in order to provide optimum management strategies for the conservation of endangered species.

Many studies have focused on the genetic consequences of habitat fragmentation of plant species in recent decades. Although most experimental and field

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Effects of alkali stress on growth, free amino acids and carbohydrates metabolism in Kentucky bluegrass (*Poa pratensis*)

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Abstract Soil alkalization is one of the most prominent adverse environmental factors limiting plant growth, while alkali stress affects amino acids and carbohydrates metabolism. The objective of this study was conducted to investigate the effects of alkali stress on growth, amino acids and carbohydrates metabolism in Kentucky bluegrass (*Poa pratensis*). Seventy-day-old plants were subjected to four pH levels: 6.0 (control), 8.0 (low), 9.4 (moderate) and 10.3 (severe) for 7 days. Moderate to severe alkali stress (pH >9.4) caused a significant decline in turf quality and growth rate in Kentucky bluegrass. Soluble protein was unchanged in shoots, but decreased in roots as pH increased. The levels of amino acids was kept at the same level as control level at 4 days after treatment (DAT) in shoots, but greater at 7 DAT, when plants were subjected to severe (pH 10.3) alkali stress. The alkali stressed plants had a greater level of starch, water soluble carbohydrate and sucrose content, but lower level of fructose and glucose. Fructan and total non-structural carbohydrate (TNC) increased at 4 DAT and decreased at 7 DAT for alkali stressed plants. These results suggested that the decrease in fructose and glucose contributed to the growth reduction under alkali stress, while the increase in amino acids, sucrose and storage form of carbohydrate (fructan, starch) could be an adaptative mechanism in Kentucky bluegrass under alkali stress.

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Keywords Alkali stress · Free amino acids · Carbohydrate · Kentucky bluegrass

Introduction

Inappropriate farming systems resulted in the large-scale development of salt-alkaline soil and substantial losses of arable lands, especially in the arid and semi-arid areas of most countries. Salinization and alkalization in soils have detrimental effects on the growth, development and differentiation of plants and causes lower productivity of agricultural crops and grasses. However, the high soil pH (>8.0) due to alkaline salts (Na_2CO_3 and $NaHCO_3$) was more destructive than that of soil salinization caused by neutral salts such as NaCl and Na_2SO_4, because most plants favor a slightly acidic to slightly alkaline soil (pH 6–7) (Clark and Zeto 1996). The severe effects of alkalinity can directly damage plant roots, disrupt the balance of ions and mineral nutrition (Shi and Zhao 1997). In addition, alkalinity alters the availability of micronutrients and cause micronutrient deficiency in plants (Fageria and Baligar 1999). The degree of these effects depends on its impact on the plant physiological and biochemical process and the capability of plant to adapt to alkali stress (Takahashi et al. 2001). To survive in alkaline environments, plants have evolved a number of physiological and metabolic adaptations to alkali stress, which include the changes of carbohydrates and amino acids metabolism.

Growth and development of plants depends substantially on carbohydrate metabolism. Carbohydrates are synthesized from a complex series of reactions involving photosynthesis in plants, which include monosaccharides (glucose and fructose), disaccharides (sucrose), oligosaccharides and polysaccharides (Smith 1972). The biochemical breakdown

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Evaluation of Genetic Diversity in Chinese Wild Apple Species Along with Apple Cultivars Using SSR Markers

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Abstract China, one of the primary centers of genetic diversity for the genus *Malus*, is very rich in wild apple germplasm. In this study, genetic diversity in 29 *Malus* accessions, including 12 accessions from 7 Chinese *Malus* species, 4 Chinese landraces, and 13 introduced apple cultivars, was assessed using a set of 19 single-locus simple sequence repeat (SSR) markers distributed across all 17 linkage groups of the apple genome. The number of alleles detected at each locus ranged from 2 to 11, with an average of 5.3 per SSR marker. In some accessions, 16 unique alleles were identified. Ten out of these 16 unique alleles (62.5%) were detected exclusively in wild species, indicating that these Chinese wild apple species have considerable genetic diversity and can be used in breeding programs to increase the genetic diversity of apple cultivars. Using 19 SSRs, an unweighted pair-group method with arithmetic average cluster analysis was conducted, and the resulting dendrogram revealed that all cultivars, except for EфpeMeBckoe, were clustered together in the same group. The Russian cultivar EфpeMeBckoe was closely related to the Chinese crabapple Baihaitang (*M. prunifolia*), with a high similarity coefficient value of 0.94. Of the two *M. sieversii* accessions used, one accession showed a close relationship to apple cultivars, while the other accession was closely related to wild apple species, suggesting the presence of a wider genetic diversity in Chinese *M. sieversii* species. The influence of SSR marker selection on genetic diversity analysis in this *Malus* collection was also discussed.

Keywords *Malus* · Genetic diversity · SSR markers · Cluster analysis

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Introduction

Apple (*Malus* × *domestica* Borkh.) is one of the most economically important cultivated fruit crops that have been subjected to heavy selection and breeding. Except for a few selected apple breeding programs, such as the Purdue–Rutgers–Illinois program in the USA and some European programs wherein a few selected *Malus* species were used in breeding apple for disease resistance (Korban and Tartarini 2009), most of the parents used in breeding efforts have relied on a narrow genetic base, often involving crosses among popular commercial cultivars (Kumar et al. 2010). For example, several cultivars such as "Red Delicious," "Golden Delicious," and "Jonathan" have been frequently used in the parentages of large numbers of modern cultivars (Noiton and Alspach 1996). Moreover, the selection and release of mutants of popular cultivars have also accelerated the trend toward genetic uniformity in commercial apple cultivars (Brooks and Olmo

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Identification, characterization, and utilization of genome-wide simple sequence repeats to identify a QTL for acidity in apple

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ORIGINAL ARTICLE

Floral development of *Phyllanthus chekiangensis* (Phyllanthaceae), with special reference to androecium and gynoecium

Zi-gang Zhang · Ai-ping Meng · Jian-qiang Li · Qi-gang Ye · Heng-chang Wang · Peter K. Endress

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Abstract The floral development of *Phyllanthus chekiangensis* has been studied by scanning electron microscopy. The perianth organs are initiated in two whorls, dimerous in male flowers and trimerous in female flowers, with a longer plastochron between whorls than between the organs within a whorl. Male flowers have two stamens. The prominent connective protrusions begin development simultaneously with the floral disk. The disk is two-lobed in male flowers but continuous in female flowers. In female flowers, the developing gynoecium remains open relatively long, so the developing ovules are visible from the outside for some time. The direction of the hemitropous ovules in the carpels is antitropous (epitropous). Two small obturators are formed per carpel, one above each ovule. The prominent nucellar beak extends far beyond the "micropyle". A micropyle in the classical sense formed by integuments closing over the nucellus apex is not present at any stage of development. Thus, it is not correct to say that the nucellar beak "grows through the micropyle". The exposed nucellar beak continues the curvature of the antitropous (epitropous) ovule and becomes contiguous with the obturator. The unusual length of the nucellar beak may be a potential synapomorphy of the enlarged *Phyllanthus* clade as inferred from molecular phylogenetics.

Keywords Euphorbiaceae sensu lato · Floral development · Nucellar beak · Ovule · Phyllanthaceae · *Phyllanthus*

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Introduction

Phyllanthus is the largest genus in Phyllanthaceae (Euphorbiaceae sensu lato) (Webster 1994; Govaerts et al. 2000; Radcliffe-Smith 2001). With the inclusion of *Breynia*, *Glochidion*, *Reverchonia*, *Sauropus*, and *Phyllanthodendron* it contains ca. 1,270 species, encompassing more than half of the number of species in the family (Hoffmann et al. 2006; Kathriarachchi et al. 2006). Within Malpighiales, Phyllanthaceae are sister to Picrodendraceae (Wurdack et al. 2004; Wurdack and Davis 2009).

Despite the extensive embryological data (revised in Kapil and Bhatnagar 1994; Sutter and Endress 1995; Tokuoka and Tobe 1995, 2001), there is a relative lack of knowledge on floral development in the large assemblage of Euphorbiaceae sensu lato (Prenner and Rudall 2007; Prenner et al. 2008; De-Paula et al. 2011). In *Phyllanthus*, despite a few embryological and seed developmental studies (Mukherjee and Padhye 1964; Singh 1972), floral development is practically unknown. In embryological studies it was found that the ovules in some Euphorbiaceae sensu lato produce long nucellar beaks and that these beaks are exceedingly long in *Phyllanthus* (see review papers cited at the beginning of this paragraph). However, their development relative to the surrounding structures,

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‘Jinyan’, an Interspecific Hybrid Kiwifruit with Brilliant Yellow Flesh and Good Storage Quality

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Additional index words. new cultivar, hybridization, *Actinidia eriantha*, *Actinidia chinensis* var. *chinensis*

In the world kiwifruit industry, there are both green-fleshed and yellow-fleshed kiwifruit in the marketplace (Huang, 2009). The dominant position of the green-fleshed kiwifruit is changing. The green-fleshed kiwifruit, represented by ‘Hayward’, dominate the international kiwifruit market because of good marketability and outstanding storage quality. However, during the past two decades, yellow-fleshed kiwifruit such as ‘Hort16A’ in New Zealand and ‘Jintao’ in China have also been developed. These new yellow-fleshed cultivars are sweeter and more aromatic than ‘Hayward’ and have attracted much attention from consumers. The purchase price for ‘Hort16A’ or ‘Jintao’ is at least 30% higher than that for ‘Hayward’. However, in general, the storage life of yellow-fleshed cultivars is not as good as that of ‘Hayward’. This limited storage life has resulted in considerable fresh fruit losses. Therefore, it is necessary to breed new yellow-fleshed cultivars with long storage and shelf life.

Hybridization, especially interspecific hybridization, is a very important method to develop new kiwifruit cultivars, which can combine the good traits from different species in the genus *Actinidia*. The hybrid can be intermediate types or types with combinations of the dominant traits of its parents or can even obtain new traits and variation, which the parents do not possess. For example, ‘Kiri’, a selection developed from the hybridization between *A. arguta* (Sieb. & Zucc.) Planch. ex Miq. and *A. chinensis* Planch. var. *deliciosa* Cheval, and backcrossing to *A. chinensis* var. *deliciosa*, had large fruit averaging 100 g with smooth and edible fruit skin, resulting in a successful combination of the good traits of *A. arguta* and *A. chinensis* var. *deliciosa* (White and Beatson, 1993). However, its fruit skin was easily damaged and its storage life was short. Unfortunately, up to now, there have been no kiwifruit cultivars arising from interspecific hybridization that are cultivated widely or are important in the kiwifruit industry.

‘Jinyan’, a new yellow-fleshed, late-season kiwifruit cultivar with large fruit size, good taste, and excellent storage quality and shelf life (Fig. 1), was developed by the Wuhan Botanical Garden, Chinese Academy of Sciences (Wang et al., 1989, 1994, 2000). ‘Jinyan’ is the first good kiwifruit cultivar originating from interspecific hybridization in China. This article describes its origin, the cultivar characteristics, and commercialization.

Origin

In 1984, the cross was conducted with *Actinidia eriantha* Benth. as the female parent and *A. chinensis* var. *chinensis* Planch. as the male parent. The female plant of *A. eriantha* was a selection with large fruit size originated from its natural distribution in Fujian Province, whereas pollens were used from several male plants of *A. chinensis* var. *chinensis* originally collected from its natural range in Jiangxi Province. In 1985, the hybrid seeds were sown after stratification in a greenhouse. From 1987 to 1989, a total of 69 F_1 seedlings were obtained after an initial screening for desirable traits. These hybrid plants have a wide spectrum of flower colors ranging from white color similar to the male parent to various red colors similar to the female parent. Only seven female plants with white flowers produced fruit normally and a superior plant whose fruit stored best was selected and labeled as M_3. The M_3 produced fruit with shape, hairness, skin, and flesh color similar to the male parent and flesh texture and maturity similar to those of its female parent. Five mitochondrial DNA markers (*atp*, *nad2-31-1*, *nad24F*, *nad24R,* and *rps*) were used to test its hybrid origin, revealing a complete sequence consistency of all mitochondrial DNA markers between M_3 and its female parent, *A. eriantha* (data not shown).

From 1990 to 1996, many clonal plants propagated from the M_3 were evaluated for genetic stability of biology characteristics by top-grafting. During1996 to 2006, the adaptability of M_3 plants to different ecological environments was tested in Hubei, Jiangxi, Yunnan, Sichuan, and Shaanxi Provinces. The results indicated that the M_3 was stable in genetic traits, including outstanding storage life, and other excellent fruit traits. M_3 was authorized by Hubei Provincial Forest Cultivar Appraisal Committee in 2006 and named ‘Jinyan’ (cultivar No. E.S-SC-AC-002-2006). The application made to the Ministry of Agriculture, China, for cultivar protection was approved in 2009 (Variety Right Number CNA20070118.5). In 2010, ‘Jinyan’ was also authorized as a new kiwifruit cultivar in China by the National Forestry Cultivar Approval Committee (national cultivar No. S-SV-AE-019-2010).

The propagation and production of ‘Jinyan’ was licensed domestically to China New Agricultural Science & Technology Co. Ltd (Sichuan, China) in 2007. This company has established kiwifruit orchards of 700 ha in Chengdu, Sichuan Province, and also subcontracted many kiwifruit growers in main kiwifruit production areas of Sichuan and Shaanxi Provinces. ‘Jinyan’ is now widely commercialized for kiwifruit production in China. F1

Description

‘Jinyan’ is tetraploid with strong vine vigor. One-year-old shoots are dark brown and 2-year-old shoots are reddish brown. Internodes are 5 to 15 cm long. Lenticels are elliptical and brown. The leaf blade is large, round, and chartaceous (19 cm × 15 cm long and wide, respectively). The leaf margin has serrulations, leaf basal lobes are slightly apart, and the leaf apex is round. The leaf upper surface is hairless and dark green, but the lower surface is densely covered with greenish hairs. The petiole is 4.3 to 11 cm long, 0.28 cm in diameter, and yellowish brown in color.

The flower has five to six green sepals and five to six milky white petals. The arrangement of petals is separated at the base. The corolla diameter is large, ≈5.5 cm long. The flower has 32 to 35 upright stigmas and 56 to 60 yellowish brown anthers, which do not produce viable pollen as a result of the functional dioecism. The flowers occur in cymes from nodes 1 to 6; the majority has three flowers (63%), two flowers (15%), or a single flower (21%), and a few cymes have four flowers, which cost more in labor for thinning flowers under orchard management. The fruit is cylindrical and slightly depressed at the stylar end and squared at the stalk end. The fruit surface is yellowish brown color with sparse short hairs. The fruit skin is thick with conspicuous lenticels. The transverse

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ORIGINAL ARTICLE

Site-specific deletions in the tomato genome by the CinH-*RS2* and ParA-*MRS* recombination systems

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Abstract We have tested the CinH-*RS2* and ParA-*MRS* site-specific deletion systems in tomato (*Solanum lycopersicum* L.). The ParA-*MRS* system is derived from the broad-host-range plasmid RK2, where the 222 aa ParA recombinase recognizes a 133 bp multimer resolution site (*MRS*). The CinH-*RS2* system is derived from *Acinetobacter* plasmids pKLH2 and pKLH204, where the 188 amino acid CinH recombinase recognizes a 113-bp recombination site known as *RS2*. In this study, target lines containing a DNA segment flanked by recombination sites were crossed to recombinase-expressing lines producing CinH or ParA recombinase. CinH-mediated recombination of *RS2* substrates was detected in 2 of 3 F_1 plants that harbor both the target and recombinase loci. On the other hand, recombination mediated by ParA was not detected among F_1 plants, but was found among 13 of 47 F_2 plants. These data show that both systems can mediate site-specific DNA deletion in the tomato genome, and, upon further refinement, can provide additional molecular tools for tomato improvement through precise genome manipulation. As the target construct also contains additional recombination sites for site-specific integration by other recombination systems, these tomato lines could be used for future testing of gene stacking through site-specific integration.

Keywords Site-specific recombination · Marker removal · Transgenic plants · Tomato transformation · Gene stacking

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Introduction

Tomato is one of the most important vegetable crops and has been a model plant for fruit research. Since its appearance as the first transgenic food crop created through modern biotechnology (Calgene Flavr Savr tomato), the genetic modification of tomato has continuously expanded in recent years to include a variety of improved traits, such as enhanced tolerance to biotic and abiotic stresses and improved nutrition or tastes of the fruit (Cantu et al. 2008; Zhang and Blumwald 2001; Butelli et al. 2008; Davidovich-Rikanati et al. 2007). The recent completion of its whole genome sequence (Mueller et al. 2009) will provide deeper understanding of its genetic background as well as greater opportunities for improving commercial traits through genetic modifications.

With current gene transfer methods, transgenes invariably integrate at random locations and often as multiple copies. As far as research is concerned, multiple copy insertions are not necessarily a problem, so long as the scientific question can be addressed. For commercial development, however, where long-term stability is required, developers and regulators prefer single copy

ORIGINAL ARTICLE

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PHYLOGENETIC ANALYSES UNRAVEL THE EVOLUTIONARY HISTORY OF NAC PROTEINS IN PLANTS

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NAC (NAM/ATAF/CUC) proteins are one of the largest groups of transcription factors in plants. Although many NAC proteins based on *Arabidopsis* and rice genomes have been reported in a number of species, a complete survey and classification of all NAC genes in plant species from disparate evolutionary groups is lacking. In this study, we analyzed whole-genome sequences from nine major lineages of land plants to unveil the relationships between these proteins. Our results show that there are fewer than 30 NAC proteins present in both mosses and lycophytes, whereas more than 100 were found in most of the angiosperms. Phylogenetic analyses suggest that NAC proteins consist of 21 subfamilies, most of which have highly conserved non-NAC domain motifs. Six of these subfamilies existed in early-diverged land plants, whereas the remainder diverged only within the angiosperms. We hypothesize that NAC proteins probably originated sometime more than 400 million years ago and expanded together with the differentiation of plants into organisms of increasing complexity possibly after the divergence of lycophytes from the other vascular plants.

KEY WORDS: **Angiosperm, NAC, phylogenetic analysis, transcription factors.**

Transcription factors are a group of proteins that control cellular processes by regulating the expression of downstream target genes. A large proportion of the plant-specific proteins are transcription factors, indicating the importance of these proteins for the evolution of the plant lineage (Olsen et al. 2005). The NAC (NAM/ATAF/CUC) domain is a highly conserved amino acid motif that defines one of the largest groups of plant-specific transcription factors. The first characterized NAC proteins were petunia NAM (no apical meristem) (Souer et al. 1996) and *Arabidopsis* ATAF and CUC (cup-shaped cotyledon) (Aida et al. 1997), after which "NAC" was named. NAC was later identified in protein sequences of other plant species, including rice, wheat, tomato, potato, and pumpkin (John et al. 1997; Ruiz-Medrano et al. 1999; Xie et al. 1999; Kikuchi et al. 2000; Collinge and Boller 2001). Proteins that contain the NAC domain are involved in diverse regulatory processes during the plant's development, such as mediating lateral root formation and auxin signaling (Xie et al. 2000); regulating senescence, cell division, and wood formation (Kubo

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