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摘要集

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目 录

Strigolactone signaling pathways in rice and *Arabidopsis*

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Strigolactones (SLs) are a group of newly identified carotenoid-derived phytohormones that control many aspects of plant development, including shoot branching, leaf shape, stem secondary thickening, and lateral root growth. Although there are still many important unsolved questions in SL biosynthesis, the elucidation of SL signaling pathways attracts increasing scientists from different fields, mainly focusing on the SL perception and its regulation of the downstream target genes. SL perception requires the hormone-dependent interaction of D14, the SL receptor, with D3, an F-box component of the Skp–Cullin–F-box (SCF) E3 ubiquitin ligase complex. Treatments with artificial SL GR24 cause a rapid degradation of the repressor D53 via the proteasome in a manner that requires D14 and the SCF^{D3} ubiquitin ligase, whereas the dominant form of D53 is resistant to SL-mediated degradation. D53 can interact with transcriptional co-repressors TOPLESS-RELATED PROTEINS. Our results suggest a model of SL signaling that involves SL-dependent degradation of the D53 repressor mediated by the D14–D3 complex. Furthermore, we also showed that SL-dependent regulation of shoot branching in *Arabidopsis* requires three D53-like proteins, SUPPRESSOR OF MORE AXILLARY GROWTH2-LIKE6 (SMXL6), SMXL7, and SMXL8. The *smxl6 smxl7 smxl8* triple mutant suppresses the highly branched phenotypes of max2 and the SL-deficient mutant max3. These findings demonstrate that D53-like SMXLs in *Arabidopsis* act with TPR2 to repress transcription and so allow lateral bud outgrowth. In this meeting, recent progresses in SL signaling pathways in rice and *Arabidopsis* will be reported and discussed.

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多细胞蓝藻的形成：细胞分化信号转导和格式形成分子机理研究

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蓝藻在地球上出现发生在 35 亿年前，是一类最早进行放氧光合作用的生物。蓝藻形态多样，从单细胞到多细胞分枝丝状体都很普遍。一些多细胞丝状蓝藻在缺氮条件下部分细胞分化成为能够进行生物固氮的细胞——异型胞，这类蓝藻就是地球上最为原始的多细胞生物。我们的研究表明，细胞间交流作为多细胞生物的重要特征，是细胞分化所必需的条件。我们的研究还表明，过氧化氢是异型胞分化的最早期信号之一。关键基因 *hetR* 的表达受到过氧化氢的调节，HetR 的一个突变体可以使细胞分化绕过过氧化氢信号。多细胞蓝藻细胞分化的另一个特征是分化后的异型胞在丝状体上呈有规律分布，即格式形成（pattern formation）。有关生物界格式形成，目前大多数人赞同 Turing 模型。但是，PatA 突变体的出现对 Turing 模型提出了一定挑战。我们对 *Anabaena* 7120 的格式形成进行了较为详细的研究，并利用数学模型对生物界格式形成进行了描述。研究结果可能对更为复杂的生物的格式形成机理研究提供一定借鉴。

棉纤维伸长的调控因子研究

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棉纤维是棉花种子单细胞表皮毛，在种子发育和成熟过程中快速伸长，纤维素大量合成。迄今我们对棉纤维发育调控刚刚有了初步的认识。模式植物拟南芥的叶片和茎杆也分布有单细胞表皮毛，已知 GL1-GL3/EGL3-TTG1（又称 MBW）转录复合体及其下游的 HD-ZIP IV 转录因子 GL2 分别控制表皮毛起始与伸长。然而，遗传定位和转基因结果表明，控制棉纤维起始的关键转录因子（MYB25L）是 MIXTA 类的 MYB 转录因子，与形成 MBW 的 GL1 属于不同类型。然而，所有 MBW 复合体成员的同源基因都在棉纤维细胞中高表达，转入拟南芥后可以互补相应突变体。这些结果提示，棉纤维细胞的起始存在一个更为复杂的调控网络。

棉花 HOX3 是拟南芥 GL2 的同源基因，是控制棉纤维细胞伸长的关键因子。HOX3 可以和其他 HD-ZIP IV 因子（如 HD1）结合，形成异源二聚体后转录激活活性明显提高，而赤霉素负调控因子 DELLA 则通过竞争干扰来抑制以 HOX3 为核心的转录复合体的活性，由此介导赤霉素对纤维细胞伸长的调控。差不多同时的报道阐述了拟南芥表皮细胞伸长相似的调控机制，因此，HOX3-DELLA 作用机制的发现对认识植物生长具有普遍意义。

广泛栽培的陆地棉（*Gossypium hirsutum*）和长绒棉（*G. barbadense*）均为异源四倍体（AADD）。棉花基因组测序为研究与育种带来了大量有价值的信息。以 HOX3 为起始点，深入分析棉纤维细胞伸长的调控机制，分离新的调控因子，阐述激素和细胞自主信号介导的调控通路，将为认识植物细胞发育和伸长以及优质棉育种提供有价值的信息。

关键词：棉花，棉纤维，细胞伸长，赤霉素，转录因子

中国迁地栽培植物的编目及资源发掘利用

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近500年来，植物引种驯化及其广泛栽培深刻改变了世界农业生产的格局，对促进人类社会文明进步产生了深远的影响。无论在西方殖民地发展史还是在我国明清发展史中，每一种重要栽培植物的成功引种和驯化，都对历史进程产生了不可估量的作用。本报告综述了中国植物园迁地栽培植物的现状和特点，并系统介绍了我国迁地栽培植物编目及资源信息标准大数据的概况。我国迁地栽培植物有396个科、3,633个属、约2万余种，占我国本土植物总数约60%且多为农林、园艺、观赏、药用、工业原料等实用价值类群。迁地栽培和引种驯化传承了现代植物园几个世纪科学研究的脉络和成就，尤其是活植物收集是植物园科学研究的基础和支撑平台，也是当前和未来植物资源发掘的源头材料。迁地栽培植物编目及其专科、专属志编撰以引种栽培植物形态学描述的客观性、用途评价的适用性、基础数据的服务性为基点，立足于我国农林、医药、环保、新兴生物产业的源头资源信息和源头资源种质。一个基因可以影响一个国家的兴衰，一个物种可以左右一个国家的经济命脉，基于活植物收集的植物园研究工作具有多学科综合的特征，既对基础生物学研究具有重要意义，也与经济繁荣、社会发展和人类日常生活密切相关。

关键词: 迁地栽培, 引种驯化, 种质资源, 经济作物

Decoding the epigenetic language of life

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Epigenetics refers to the study of heritable information that is not contained in DNA sequence. An important epigenetic mark conserved in mammals and plants is DNA methylation, a chemical modification of DNA that controls gene function. Proper DNA methylation patterns are critical for development, diseases and stress responses in humans as well as in plants. Plants are excellent biological systems to study how DNA methylation patterns are generated. DNA methyltransferase enzymes that deposit the DNA methylation mark are guided to specific DNA sequences, and DNA demethylase enzymes that remove the DNA methylation mark are also guided to distinctive sequences to erase unwanted DNA methylation. I will describe work in my lab that has shed light on how DNA methyltransferases and demethylases are guided to specific sequences, and how the antagonistic actions of the enzymes are coordinated to generate proper DNA methylation patterns. I will also describe some of our recent work on how DNA methylation influences transgenerational inheritance.

Transcriptional regulation by small RNAs and Argonautes

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Small RNAs associated with Argonaute (AGO) family proteins are important components in the eukaryotic gene regulatory networks. Plants have evolved a complex system of small RNAs, among which the predominant species are herterochromatic siRNAs (hc-siRNAs) and microRNAs (miRNAs). Hc-siRNAs are associated with AGO4 and direct *de novo* DNA methylation at homologous loci through a pathway known as RNA-directed DNA methylation (RdDM), which may cause transcriptional gene silencing, whereas miRNAs are bound to AGO1 and mediate post-transcriptional gene regulation through target mRNA cleavage or translational repression. In my talk, I will present our recent findings with regard to the mechanism of DNA methylation directed by AGO4/hc-siRNA complexes and an unexpected role for AGO1 in promoting gene transcription, in addition to its role in miRNA function.

Two reference genomes of *indica* rice and their implications for rice research and breeding applications

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Asian cultivated rice consists of two subspecies: *Oryza sativa* ssp. *indica* and *O. sativa* ssp. *japonica*. *Indica* rice accounts for over 70% of total rice production worldwide and is genetically much more diverse. Although a reference genome of the *japonica* rice Nipponbare was published in 2005 and has remained the highest quality crop genome sequence for more than a decade, lack of reference genome for *indica* rice has hindered the progress of rice research. Previous studies established that *indica* rice can be divided into two major varietal groups, IndI and IndII, independently bread and widely cultivated in China and Southeast Asia. Using a BAC-by-BAC strategy and PAC Bio sequencing technique, we generated reference genome sequences for two *indica* rice lines, Zhenshan 97 and Minghui 63, which represent IndI and IndII groups, respectively. The sequences were assembled into 237 (Zhenshan 97) and 181 (Minghui 63) contigs, with an accuracy >99.99%, and covered 90.6% and 93.2% of their estimated genome sizes. Our comparative analyses of these two *indica* genomes uncovered surprising structural differences, especially with respect to inversions, translocations, presence/absence variations and segmental duplications. These analyses clearly indicated the necessity of having multiple reference genomes for understanding natural variation in rice, which also have general implications for understanding intra-specific variations of organisms with complex genomes including plants and animals. In particular, hybrids produced from crosses between these two *indica* varietal groups usually show strong heterosis, which has provided basis for successful utilization of hybrid rice in China and several other countries. Zhenshan 97 and Minghui 63 are the parents of a leading hybrid Shanyou 63 that has been widely grown in China for over 30 years. The availability of these two reference genomes, combined with data from more than 20 years of genetic analyses of the Zhenshan 97, Minghui 63, and the hybrid system, will serve as an excellent model for further characterization of the biological basis of heterosis.

Key words: *Oryza sativa* ssp. *indica*, reference genome, heterosis

红莲型杂交水稻的研究与应用

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红莲型杂交水稻细胞质来源于野生稻，配子体不育，是杂交水稻的新类型，被国际公认为三大细胞质类型之一。1972 年，武汉大学以海南红芒野生稻为母本与江西地方品种莲塘早杂交，F2 代出现不育株，用莲塘早回交，1974 年育成红莲型不育系和保持系。通过 40 多年的研究，已经形成了红莲型杂交水稻基础、应用和产业化的完整体系。克隆了不育基因 orfH79，为线粒体嵌合基因，ORFH79 蛋白与线粒体复合体 III 重要蛋白 P61 互作，从而导致细胞质雄性不育。发现了红莲型水稻 Rf5 和 Rf6 的双恢复基因模式，分别定位于水稻 10、8 号染色体。F1 植株只有 Rf5 或 Rf6 时，50%花粉恢复，当 Rf5 和 Rf6 都存在时，75%花粉恢复，结实率更稳定，这直接指导了强恢复系选育。先后克隆了恢复基因 Rf5 与 Rf6，Rf5 与包台型水稻恢复基因 Rf1a 为同一个基因，但恢复机理不同。Rf6 是个新基因，其结构域发生了串联重复，不但能恢复红莲型，也能恢复包台型。RF5 与 GRP162 互作，RF6 与 OsHXK6 互作，各自形成独立分子复合体，对不育基因转录本 atp6-orfH79mRNA 进行加工从而恢复不育性。在克隆红莲型杂交水稻不育基因 orfH79 及恢复基因 Rf5 和 Rf6 的基础上,借助分子标记辅助选择技术，创制了系列新种质，使红莲型杂交水稻不仅在籼稻，而且在粳稻和亚种间杂交稻中实现。

长期以来，围绕着红莲型杂交水稻大面积应用，以高产、优质、广适、高效为目标，不断对不育系和恢复系进行创新，上世纪 90 年代收集了国内外多种类型不育系和保持系 22 个，进行配合力、米质、开花习性和抗性等农艺性状系统比较研究，确定以红莲型粤泰 A、B 为主攻不育系，从提纯保持系入手，在夏季高温下进行成对杂交提纯，使该不育系纯度达 99.99%，选 1000 多个优良品种和恢复系进行大量测交，杨稻 6 号恢复好，优势强，选育出红莲优 6，2001 年通过湖北省破格审定推广，红莲优 6 在武汉、海南、广西各地表现很好，高产、优质、结实率高。在此基础上，进一步改进不育系和恢复系，通过辐射粤泰 B，选单株与粤泰 A 回交，选育出珞红 3A、B，通过 9311 与 E32 杂交选育新恢复系 8108，珞红 3A/8108 选育出红莲型杂交稻新组合珞优 8 号，2006 年通过湖北省品种审定，2007 年通过国家品种审定，2009 年通过农业部超级稻认定，表现高产、优质、广适性强，2007-2016 年为湖北省主推品种，2010-2016 为长江中下游主推品种，红莲优 6 号、珞优 8 号在东南亚如越南、菲律宾、孟加拉、巴基斯坦、印度尼西和非洲几内亚、马里、赞比亚、喀麦隆和尼日利亚等国家种植，增产效果非常显著，推广潜力巨大。在此基础上，从 2003 年开始，通过分子标记辅助选择将水稻抗褐飞虱基因 BPH14 和 BPH15 导入珞红 3B，通过回交选育出抗褐飞虱珞红 4A、B，并与成恢 9348 组配出珞优 9348，2016 年通过湖北省品种审定，珞优 9348 具有高产优质、抗褐飞虱和抗稻瘟病，并具节约氮肥的特点。红莲型杂交稻国内外累计推广面积上亿亩，为保障国家乃至世界粮食安全作出了巨大贡献。

作物中杂种优势的全基因组解析

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杂交水稻育种技术的成功是我国取得的一项重要科技成就。伴随着汕优 63、两优培九等一大批高产杂交稻品种的大面积推广，我国的水稻总产量在较短时间内有了大幅度的提升，为我国的粮食安全做出了巨大贡献。杂交稻的高产来自对水稻杂种优势现象的利用。杂种优势在很多物种中存在的，但其遗传机理还不完全清楚。传统的水稻杂种优势的研究是基于一套杂交重组群体的遗传分析，我们通过直接对千余份杂交稻材料及多套人工群体的基因组解析和性状考察分析，开创了新的杂种优势研究的方法。研究发现杂交稻中产量性状的表现与杂合程度相关性不高，杂交稻的高产主要来自于部分优异等位基因的聚合。研究还发现了单位点超显性的存在；但无论数目上还是效应上，杂交稻品种中杂种优势的形成更多地依赖于产量位点上正向的不完全显性。了解杂交稻品种的基因组信息及其产量优势的遗传基础，为杂交水稻的分子设计育种、杂种优势的机制研究以及通过基因组辅助的聚合育种技术培育出具有超亲优势的常规稻新品种打下了重要基础。

关键词：水稻，杂种优势，基因组学，遗传分析

稻米蛋白品质形成的分子机制研究

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水稻种子中积累大量的储藏蛋白作为种子萌发及幼苗发育的营养来源，同时稻米中的蛋白质含量和组成对稻米品质有着重要的影响。稻米中的储藏蛋白包括谷蛋白，醇溶蛋白和球蛋白，其中谷蛋白含量占 60-80%，且谷蛋白能够被人体消化吸收，是遗传改良稻米蛋白品质的首选目标。前人的研究已经克隆了几乎所有的谷蛋白结构基因，相应的表达调控研究也较为深入，但谷蛋白 57kDa 前体必须经过复杂的分选途径才能进入蛋白体 II（Protein Body II, PBII），最终以 40kDa 的酸性亚基和 20kDa 的碱性亚基的形式贮存，目前对谷蛋白翻译后的转运途径研究甚少。我们通过大规模的筛选获得了一批谷蛋白前体增加突变体（*glutelin precursor accumulation, gpa*），并采用图位克隆的策略获得多个谷蛋白转运关键因子，包括 OsPDIL1-1，OsVPE1，GPA1/OsRab5a，GPA2/OsVPS9a，GPA3 和 GPA4/OsGot1。上述因子分别作用于蛋白转运过程的各个节点：其中 OsPDIL1-1 负责内质网内谷蛋白的正确折叠；GPA4/OsGot1 负责调控谷蛋白由 COPII（Coat Protein Complex II）介导的从 ER 到 Golgi 的正向运输；GPA1/OsRab5a，GPA2/OsVPS9a 和 GPA3 三个因子形成复合体，协同调控由致密囊泡（Dense Vesicle, DV）介导的谷蛋白后高尔基体的分选；而 OsVPE1 则作用于最下游，负责 57kDa 谷蛋白前体在 PBII 中的酶切成熟。上述因子的克隆和鉴定，提高了人们对植物细胞内蛋白运输路径的认识，为最终培育蛋白质含量各异、适合不同人群的水稻新品种奠定理论基础。

关键词：水稻，品质，57H 突变体，谷蛋白运输

Pattern-recognition at the forefront of plant-pathogen interactions

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Higher plants deploy a large repertoire of Pattern-Recognition Receptors (PRRs), composed of Receptor Kinases (RKs) or Receptor Proteins (RPs) to perceive in the apoplast molecular patterns of microbe or plant origin during infection, activating pattern-triggered immunity (PTI). Previous and ongoing studies in our and other labs show that a subfamily of cytoplasmic kinases including BIK1 and PBS1-Like (PBL) proteins play a central role in PTI by acting directly downstream of multiple PRRs. They regulate important downstream signaling events including production of ROS, calcium influx, and MAPK activation, and are subject to tight regulations by heterotrimeric G proteins and ubiquitination proteasome system. A growing number of bacterial pathogen effectors are found to target PRR complexes for pathogenesis. However, these effector proteins can be recognized by intracellular NOD-Like Receptors (NLRs), thereby ″betray″ the pathogen and activate the second layer of immunity. For example, we have recently shown that the *Xanthomonas campestris campestris* effector AvrAC post-translationaly modifies BIK1 for enhanced virulence. A BIK1 homolog, PBL2, acts as a decoy to deceive AvrAC and activates the NLR protein ZAR1. In addition, the perturbation of BAK1, a co-receptor of multiple PRRs, is known to heighten host immune responses. Our recent work showed that another *Pseudomonas syringae* effector, HopB1, is a novel protease that specifically cleaves BAK1. Surprisingly, the cleavage of BAK1 by HopB1 enhances bacterial virulence but heightened immunity in plants. HopB1 cleaves BAK1 only when the latter is stimulated by bacterial molecular patterns. This constrained action of HopB1 probably has allowed the bacterium to achieve virulence at the same time minimize the likelihood of stimulating the second layer of immunity. Together the studies highlight pattern-recognition as a major battleground in plant-bacterial pathogen interactions.

Key words: pattern recognition, immunity, effectors, receptor kinases, ubiquitination

经典遗传科学问题的再研究

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遗传学一直是引领生命科学发展的引擎，是生命科学中逻辑性最强、也许是最完美的学科。尽管如此，遗传学也存在一些有待深入研究的重要科学问题。比如：形成生物多样性的原始遗传突变是如何产生的？减数分裂期间基因转换频率（和重组频率）及其遗传效应究竟如何？同源染色体间的非对称性（如 DNA 的插入/缺失和异质位点）在遗传变异中的作用？减数分裂与有丝分裂突变有何差异、对生物多样性的效应如何？一年生与多年生植物的变异与进化速率有何差异？遗传突变与自然选择是何种关系？遗传变异的法则（或过程）是否也受自然选择的影响、是否比遗传现象（或结果）的自然选择更重要？交配方式、性细胞分化与遗传多样性有何关系？…? 这些尚待解决的种种经典遗传科学问题，随着重测序和基因敲除等系列分子生物技术的重大突破，目前都可以进行试验检测。在这种背景下，我们针对上述遗传科学问题，设计和进行了种种试验研究，取得了许多意想不到的结果，从而使我们可用新的视角，重新解读经典遗传学中的种种问题，丰富、加深或矫正对遗传与进化法则的理解。为此，我将简要介绍其中几个主要的研究结果及其我们对之的解读。

关键词：遗传变异，基因转换，遗传重组，生物多样性，自然选择

Discovering and timing a high altitude extreme ecotype of *Arabidopsis thaliana* from Tibet

The Tibetan Arabidopsis thaliana Research Consortium*

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Arabidopsis thaliana illuminates the plant world for us. Studies on *A. thaliana* have unraveled the mystery of plant genetics, physiology, and adaptive evolution. After more than ten years of exploration, we discovered a wild *A. thaliana* population and named it Tibet-0 representing 4200 m above sea level in Qinghai-Tibet Plateau (QTP). Phylogenetic analysis and coalescent methods based on genome-wide resequencing both showed that Tibet-0 is the most primitive *A. thaliana* ecotypes. Using fossil calibrations and Bayesian coalescent models, we estimated that the divergence time between T4k and other ecotypes was 0.152 – 0.160 million years ago, which is the time of the last uplift of the QTP, indicating that the uplift of QTP promoted the formation of Tibet-0. Ancestor reconstruction of 5741 orthologous genes also demonstrated Tibet-0 would update our knowledge of the *A. thaliana* ancestor. In the common garden, Tibet-0 and Can_0 were characterized by more rosette leaves and later flowering time after the long exposure to the extreme environment of high altitude. Accordingly, some genes of Tibet-0 in the MAX pathway and flowering pathway showed positive selection, which may be the reason of its low height and more shoot branching. As a rare experimental material and potential genetic resource, Tibet-0 from Tibet opens a new window through which we can not only know more about the *A. thaliana* ancestor, but also understand the long-term adaptation of plants to extreme environments better.

Ranunculaceae and the evo-devo of the flower

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Ranunculaceae is a family of flowering plants with ca. 59 genera and 2500 species and shows tremendous diversity in both vegetative and reproductive organs. The family, therefore, can help answer the important evolutionary developmental (evo-devo) questions that cannot be easily addressed by using all other existing model organisms. In this talk, I will present our recent results on: 1) the molecular mechanisms underlying the parallel losses of petals in different lineages of the Ranunculaceae; 2) the molecular basis and flexibility of the floral organ identity determination program in *Nigella damascena*, a species that produces spiral rather than whorled flowers; 3) the tempo, mode, and mechanisms of character evolution in the elaboration of complex petals within the genus *Nigella*; and 4) the identification and functional studies of the genes and modules that regulate the development of the highly specialized, bilabiate petals of *Nigella*.

Key words: flower, petal, evo-devo, Ranunculaceae, *Nigella*

Comparative genomics of AA-genome *Oryza* species provides novel insights into plant speciation

Li-zhi Gao

Comparative and evolutionary genomic analyses among closely related species can greatly enhance our understanding of plant gene and genome evolution. Here, we report five *de novo*- assembled AA-genome sequences for *Oryza nivara*, *O. glaberrima*, *O. barthii*, *O. glumaepatula* and *O. meridionalis*. Our analyses reveal massive levels of genomic structural variation, including segmental duplication and rapid gene family turnover. We show, on a genomic scale, how lineage-specific expansion or contraction of gene families has led to their morphological, reproductive and adaptive diversification, thus enlightening the evolutionary process of speciation. Despite strong purifying selective pressures on most *Oryza* genes, we documented a large number of positively selected genes, especially those involved in flower development, reproduction, and resistance-related processes. These diversifying genes are expected to have played key roles in adaptations to their ecological niches in Asia, America, Africa and Australia. Extensive variation in ncRNA gene numbers, function enrichment and rates of sequence divergence might also help account for the different genetic adaptations of these rice species. Collectively, these resources provide new opportunities for evolutionary genomics, numerous insights into recent speciation, a valuable database of functional variation for sustainable utilization and tools for efficient conservation of wild rice germplasm.

四倍体棉花经历过多次杂交的分子证据

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棉属叶形呈现多样化，根据棉花叶裂的深浅可以分为阔叶和鸡脚叶，鸡脚叶又可以分为海岛型鸡脚叶、亚鸡脚叶、鸡脚叶和超鸡脚叶。阔叶和鸡脚叶由位于 D1 染色体 L_2 基因座上的等位基因控制。鸡脚叶（L_2^o）为不完全显性遗传性状，表现为裂片较长，缺刻变深。利用图位克隆方法，我们克隆出 *LATE MERISTEM IDENTITY 1* (*LMI1*)-*like* 转录因子(*GhOKRA*)调控鸡脚叶发育。利用 VIGS 技术，抑制 *GhOKRA* 基因的表达后，叶片表型由鸡脚叶变为阔叶。过表达 *GhOKRA* 的拟南芥植株，叶片出现分裂。序列分析发现，阔叶棉花 *GhOKRA* 基因发生突变，翻译提前中止，从而丧失了基因功能。通过对二倍体 D 组棉、野生及半野生棉以及驯化四倍体棉 *OKRA* 基因序列分析，发现 *OKRA* 基因存在两种提前终止形式，一种发生在异源四倍体形成之前，在二倍体 D 组供体种中，*OKRA* 已发生突变；另一种发生在异源四倍体形成之后。推测在棉花四倍体形成过程中，存在两次杂交事件，一次杂交事件形成阔叶四倍体，另一次杂交事件形成鸡脚叶四倍体棉花，为四倍体棉花经历过至少两次杂交提供了分子证据。

关键词：棉花，鸡脚叶，图位克隆，异源四倍体形成

Association analysis of whole genome re-sequencing and map-based cloning for *Arabidopsis* mutant induced by carbon ion beams

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In recent decades, heavy ion beams have been recognized as a novel powerful mutagen for their ability to induce mutations with high rate and broad spectrum. In the present study, an *Arabidopsis* variant numbered *civar* (Carbon ion beams Induced Variegated) displaying variegated stem, rosette and cauline leaves, sepals, and siliques, was induced by carbon ion beams accelerated by the Heavy Ion Research Facility in Lanzhou (HIRFL). Map-based cloning is one of the major traditional ways to isolate the mutant genes that control traits of interest in forward genetics studies. However, the process of map-based cloning is usually complicated and time-consuming. Nowadays, a direct and effective way to identify the mutations that underlying variation phenotypes of interest is the whole genome re-sequencing. To mine the genes that are responsible for the mutant phenotypes of *civar*, the association analysis of whole genome re-sequencing and rough map-based cloning were performed. Firstly, *civar* was crossed with wild type ecotype *Landsberg erecta* (Ler), and then DNA was collected from 60 F2 individuals that displayed the mutant traits. According to rough mapping, *civar* showed the lowest exchange rate at T20P8 on the chromosome 2. On the other hand, genomic DNA was extracted from leaves of *civar* by using CTAB protocol. The whole genome re-sequencing was performed based on the Illumina HiSeq2500 system. After sequence alignment and rigorous filtering, 15 SNPs (Single Nucleotide Polymorphisms), 2 small InDels (insertion-deletion) were identified. Associated with rough mapping, there were only 1 variant sites (chr2, 13175805) with deletion of a single cytosine, which led to frameshift_variant and synonymous_variant effects of VAR2 gene. Precisely, *VAR2* showed a variegated phenotype. Therefore, it showed that association analysis of rough mapping and whole genome resequencing provided crucial guides for identifying the responsible genomic regions that may contribute to mutant phenotypes.

Key words: carbon ion beams, *Arabidopsis*, whole genome re-sequencing, map-based cloning

The development and application of single cell sequencing technology in maize

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Single cell sequencing technology emerged recently, enabling high-throughput analyses of the cell lineage trees of higher organisms. In plants, single-cell sequencing is still challenging because the cell wall hinders the isolation and lysis of the nuclear contents. We have developed a simple method to isolate and sequence the whole genome of each of the four microspores from a plant tetrad which was used to study the meiotic recombination mechanism of maize male gametes (Li et al, 2015). Recently, we made further efforts to improve the method for isolating the three nuclei from one mature pollen grain of maize for whole-genome sequencing. This technique was used to study the haploid induction mechanism in maize. Production of maternal haploids via intra-specific genotypes as the haploid inducer is routine and highly efficient in maize. However, the underlying mechanism of haploid induction (HI) is unclear. We found that the aneuploidy caused by chromosome fragmentation occurring post meiosis in the gametophyte may affect embryogenesis and be the underlying cause of HI.

Key words: single cell sequencing, recombination, haploid induction, chromosome fragmentation

The rubber tree genome reveals genetic clues to producing more rubber

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The world's commercial production of natural rubber, an essential industrial raw material, is solely from one tropical tree species, *Hevea brasiliensis* (para rubber tree), owing to the high yield and good quality. A reference genome has been long awaited for this species to address important scientific questions about the biology of rubber trees, such as the physiological functions of rubber-producing laticifers and the mechanisms underlying ethylene stimulation of latex production. Here we present a high-quality genome assembly of this species (1.37 Gb, scaffold N50=1.28 Mb) that covers 93.8% of the genome (1.47 Gb) and harbors 43,792 predicted protein-coding genes. A striking expansion of the *REF/SRPP* gene family and its divergence into several laticifer-specific isoforms appear crucial for rubber biosynthesis. The REF/SRPP family has isoforms with sizes comparable to, or larger than SRPP1 (204 AA) in 17 other plants examined, but no isoforms with similar size to REF1 (138 AA), the predominant molecular variant in latex (cytoplasm of laticifers). The emergence of REF1, a protein located on the surface of large rubber particles that account for 93% of rubber in the latex despite their constituting only 6% of total rubber particles, becomes a pivotal event in rubber tree evolution and is largely responsible the modern rubber tree's capacity for high rubber production. The stringent control of ethylene synthesis under active ethylene signaling and response in laticifers resolves a longstanding mystery of ethylene stimulation in rubber production. Our study which includes the re-sequencing of five other *Hevea* cultivars and extensive RNA-seq data provides a valuable resource for functional genomics and tools for breeding elite *Hevea* cultivars.

Key words: rubber tree, reference genome, high rubber production, ethylene, *REF/SRPP* gene family, gene expansion and divergence

A fuzzy Bruijn graph approach to long noisy reads assembly

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A challenge of assembling long noisy reads from third generation sequencing (TGS) is reducing its requirement of computing resource. We present a new assembly graph termed fuzzy Bruijn graph for efficiently assembling big genomes using TGS data. The key difference between fuzzy Bruijn graph and De Bruijn graph is that the overlap length can be kilo bases in the former for long noisy reads. In experiment, it assembled human genome in 675.4 CPU.Hours and resulted in N50 contig of 22.2 Mega bases.

Key words: genome assembly, third generation squencing, fuzzy Bruijn graph

Major chromosomal rearrangements lead to the divergence of the sister genera of *Populus* and *Salix*

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Populus and *Salix* are sister genera in the Salicaceae family. In both lineages extant species are predominantly diploid. Genome sequencing of these two lineages revealed that their genomes shared a common whole genome duplication event, known as ˝Salicoid˝ duplication, which occurred ca. 58 Mya. In this study, we conducted syntenic comparison of the corresponding 19 chromosome members of the poplar and willow genomes. It revealed that almost every chromosomal segment had parallel paralogous segment elsewhere in the genomes, and the two lineages shared a similar syntenic pinwheel pattern for most of the chromosomes, which indicated that the two lineages diverged after the genome reorganization in the common progenitor. The pinwheel patterns showed distinct differences for two chromosome pairs. Further analysis detected two major inter-chromosomal rearrangements that distinguished the karyotypes of willow and poplar. Scientists have suggested that *Populus* is evolutionarily more primitive than *Salix.* Therefore, we propose that after the ˝salicoid˝ duplication event, fission and fusion of the ancestral chromosomes first give rise to the diploid progenitor of extant *Populus* species. During the evolutionary process, poplar chromosome I broke into two parts, the lower portion was joined with poplar chromosome XVI, giving rise to willow chromosome I, whereas the upper portion gave rise to willow chromosome XVI. This study provides a unique example for our understanding how chromosomal rearrangement affects life divergence in close relatives of high plants.

Key words: genome duplication, chromosomal rearrangement, genome divergence, *Salix*, *Populus*

高通量测序及芯片技术推动农业基因组学应用发展

肖瑾

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人口的指数增长和气候变化给人类维持粮食供应带来了巨大的挑战。面对这一难题，科学家们利用农业基因组学 (Agrigenomics)，即基因组学在农业中的应用，推动生产力持续发展，并为养活全世界不断增长的人口的挑战提供解决方案。高通量测序及芯片技术的发展让我们能够测序新物种，开展大数据分析，剖析复杂的性状，开展分子标记辅助选择（MAS）和 GS 等应用。在过去的几年中，这些技术已经彻底改变了牲畜和作物的育种工作，从而推动整个领域的发展。

The effect of *Arabidopsis* genome duplication on the 3D genome and gene expression

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The spatial arrangement of chromosomes in the nucleus is important for gene expression and genome function both in animals and plants. The recently developed Hi-C technology is an effective method to investigate the chromosome interaction. In order to probe the function of polyploidy levels in genome packing, Arabidopsis autotetraploid was used in our study to build three-dimensional structure of the genome with Hi-C analysis. We found that tetraploidy *Arabidopsis* shows a similar local chromatin packing compared to the wide type, most interactions are within chromosomes. However, it also has its specific conformations: intra-arm interaction frequencies reducein coincidence with that inter-arm interactions increase. In addition, increased inter-arm interactions were mainly found within the two pericentromeric domains flanking each centromere. The interaction matrix revealed that all telomeres interact with each other with reduced interaction frequency in autotetraploidy *Arabidopsis* compared to that of the wide type. These data suggested that duplicated genome prefers long-range inter-arm interactions, resulting in more compact nuclei. Further, we preformed transcriptome sequencing to address the relationship between three-dimensional structure of the genome and gene transcription. Our results indicated that gene clusters found in different interaction bins were mapped to different transcription ˝factory˝, suggesting the correlation between chromosomal organization and gene expression.

Key words: Hi-C, genome duplication, 3D genome, nuclear domain

PPR17, PPR20 and CRS1 mediate intron splicing by forming a complex in maize chloroplasts

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In mitochondria and chloroplasts of higher plants, the loss of self-splicing activity of degenerate group II introns results in recruitment of intron splicing factors encoded in the nucleus. One family of such factors is the pentatricopeptide repeat proteins (PPR) which are implicated in editing, splicing, end maturation and regulation of translation in organellar transcripts. PPR proteins belong to a large family with more than 500 members in flowering plants. Because of its large size and frequent embryo lethality in KO mutants, functions of many PPR proteins remain unknown. Here we report the function identification of PPR17 and PPR20 and their physical interactions with Chloroplast RNA Splicing 1 (CRS1) in maize. Null mutation of *PPR17* and *PPR20* showed similar arrest in embryogenesis at the transition stage. Both PPR17 and PPR20 are P-type PPR proteins with 11 and 17 PPR motifs respectively. Subcellular localization revealed that both proteins are localized in the chloroplast. *PPR17* and *PPR20* are expressed in all the tissues examined. Analysis of chloroplast transcripts revealed that loss of either the PPR17 or the PPR20 function abolishes the splicing of the *atpF* and *rpl2* introns, suggesting that PPR17 and PPR20 are required for *atpF* and *rpl2* intron splicing. Previous studies have identified that CRM protein CRS1 is also required for *atpF* intron splicing (Jenkins et al., 1997；Till et al., 2001). Yeast two hybridization, Pull-down and BiFC analyses indicated that PPR17, PPR20 and CRS1 interact physically. Domain deletion analysis showed that the first four PPR motifs of PPR17 interact with the first two CRM domains of CRS1 and PPR20, and the C-terminal region of CRS1 interacts with PPR20. These results demonstrate that PPR17, PPR20 and CRS1 mediate the *atpF* intron splicing by forming a complex in maize chloroplasts.

Key words: PPR, intron splicing, chloroplast, embryo and endosperm, maize

套索 RNA 作为分子海绵抑制植物 miRNA 产生

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植物miRNA的产生主要通过以DCL1 (Dicer Like 1) 为核心的复合物介导。已知miRNA的产生受到了RNA拼接过程的调控。套索RNA（lariat RNA）是RNA拼接过程中内含子形成的副产物，其在去分支酶DBR1 (RNA debranching enzyme 1) 的作用下被解套索后快速降解。动植物中*DBR1*的缺失突变体胚胎致死，表明套索RNA的累积是有毒害的，但其分子机制并不清楚。我们通过正向遗传学的手段分离到一株具有多效发育表型但纯合可育的*DBR1*的弱突变体，我们将其命名为*dbr1-2*。该突变体中套索RNA累积的同时伴随全基因组水平上的miRNA的下降。RIP实验结果表明*dbr1-2*突变体中DCL1加工复合物与初级miRNA (pri-miRNA) 的结合效率显著降低。体外竞争性结合实验（RNA-EMSA）表明套索RNA可以抑制DCL1复合物与pri-miRNA的结合。 利用MS2 RNA活细胞定位系统发现套索RNA与DCL1复合物共定位于dicing body, 与此结果一致的是，*dbr1-2*突变体中DCL1加工复合物在dicing body中的定位发生改变，可能影响了其组装和切割效率。因此，我们推测套索RNA可能作为分子海绵通过抑制DCL1复合物的结合而抑制miRNA的产生。套索RNA通过全局性地参与miRNA的调节一定程度上可以解释*dbr1*无义突变导致的胚胎致死的表型。此外，我们在野生型植物中大量套索RNA的存在，并证明DCL1复合物结合这些套索RNA，暗示套索RNA对miRNA的稳态水平起到了平衡作用。

关键词：套索 RNA，DBR1，miRNA， RNA 拼接，DCL1 复合物

Construction and characterization of a microRNA-centric gene network in *Arabidopsis*

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Despite the numerous identified roles for small RNAs in maintaining genome integrity and activity, molecular mechanisms involving small RNAs in governing environment-responsive plant metabolism and development are poorly understood. We focused on microRNAs (miRNAs), which are a class of negative gene regulators primarily acting at the post-transcriptional level. Through systematic analysis of whole genome chromatin immunoprecipitation data for 45 transcription factors, we constructed a miRNA-centric gene network in the model plant Arabidopsis. Characterization of this network revealed that three node feed-forward loops (FFLs) are extremely abundant. We performed detailed analysis on two of these FFLs. For the FFL constituted by the transcription factors *HY5* and *SPL7* and the miRNA miR408, we demonstrated that it underlies light-copper crosstalk and is tied to copper allocation to the chloroplast and photosynthesis. For the FFL consisting of the transcription factors *HY5* and *MYBL2* and the miRNA miR858, we demonstrated that it is a decision-making module for anthocyanin biosynthesis in which *HY5* mediated light induction of miR858 de-represses anthocyanin synthesis through targeting MYBL2, which acts as a negative regulator of the anthocyanin pathway. These findings are helpful to elucidate the design principle and control logic of the network that integrates transcriptional and post-transcriptional regulations and should have the corollary benefit of pinpointing function of individual miRNAs.

转录组水平基因表达调控：mRNA 选择性多聚腺苷化的贡献

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选择性多聚腺苷化（Alternative polyadenylation , APA）是一种广泛存在于真核生物中基因表达调控的生物学现象。对于单个基因，它可以选择在转录子的不同位置加 poly(A)尾巴从而产生长短不一的转录本，从而对转录本的功能如编码功能、稳定性、可翻译性等都产生重要的影响。在转录组水平 APA 极大地提高了基因转录调控的多样性。利用拟南芥遗传系统，我们对 APA 的生物学功能进行了多项研究并发现多聚腺苷化蛋白质因子的突变可以导致较专一的遗传表型。利用我们自己建立的高通量转录组测序法 PolyA-tag sequencing (PAT-Seq)并对这些突变子的 APA 情况进行分析，发现有高达 90%基因的转录子产生新的 APA 或导致腺苷化位点使用率的转换（switch）。因此，多聚腺苷化因子的实际效果是在转录组水平上对基因表达产生影响，由此引起表型的改变。

我们还对拟南芥与水稻等植物不同组织、不同发育时期的 APA 轮廓进行大量 PAT-seq，发现不同时期（如花粉发育、种子萌发等）的 APA 与相关基因表达有关。我们的研究结果表明，同样也在转录组水平上许多基因的不同 polyA 位点的利用产生 APA，由此可能调节了代谢调控基因表达，包括植物发育、抗病性、抗逆反应等。这些结果为了解转录后调控在植物生长、发育、环境响应中的作用提供基础，并为阐明 APA 的产生及其调控机制提供线索。这些 polyA 位点的信息提供大量基因 3' 末端数据，因此也很大程度上提高基因组的注释精度。

关键词：信使RNA，选择性多聚腺苷化，转录后调控，转录组调控

LlFH1-mediated interaction between actin fringe and exocytic vesicles is involved in pollen tube tip growth

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Pollen tube tip growth is an extreme form of polarized cell growth, which requires polarized exocytosis based on dynamic actin cytoskeleton. However, the molecular basis for the connection between actin filaments and exocytic vesicles is unclear. Here, we identified a *Lilium longiflorum* pollen-specific formin (LlFH1) and found it was involved in pollen tube tip growth. LlFH1 localized at the apical vesicles and plasma membrane via its N-terminus. Overexpression of LlFH1 induced excessive actin cables in the tube tip region, and downregulation of LlFH1 eliminated actin fringe. FRAP analysis revealed that LlFH1 labeled exocytic vesicles, which exhibited a clear initial accumulation at the shoulder of the apex. Meanwhile, we found that the exocytic site coincided with the leading edge of the actin fringe, indicating the correlation between actin fringe and exocytic vesicles. Time-lapse analysis of pollen tubes simultaneously expressing LlFH1-GFP and Lifeact-mRFP suggested that nascent actin filaments followed the emergence of the apical vesicles, implying that LlFH1 could initiate actin polymerization from the apical vesicles. In vitro biochemical assays showed that the FH1FH2 domain of LlFH1 could nucleate actin polymerization and bundle actin filaments. In addition, LlFH1 FH1FH2 could attach to the barbed end of actin filaments, and in the presence of lily profilin isoforms, LlFH1 FH1FH2 enhanced actin filament elongation rates. Thus, we propose that LlFH1 and profilins coordinates the interaction between actin fringe and exocytic vesicle trafficking, which provides a mechanism for the delivery of exocytic vesicles to the shoulder of the apex during the growth of lily pollen tubes.

Key words: formin, profilin, actin fringe, exocytic vesicle, pollen tube growth

MAP18 调控根毛顶端生长的新机制

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根毛是典型的进行顶端生长的细胞，在植物水分与养分的吸收过程中发挥重要作用。根毛细胞极性的建立和维持对于根毛的顶端生长和形态建成至关重要。前人的研究发现植物中特有的小 G 蛋白 ROP2 调控根毛的生长发育。然而对于这一过程中 ROP2 活性的调控还知之甚少。我们前期的研究发现，在营养组织细胞中通过去稳定微管调控细胞极性生长的微管结合蛋白 MAP18 具有 Ca^{2+}依赖的微丝切割活性，在花粉管中通过作用于微丝调控花粉管顶端生长的方向。进一步的研究发现，MAP18 在根毛的顶端生长过程中也发挥重要作用，通过调控微丝影响根毛细胞核的定位以及根毛的极性建立和顶端生长。更为有趣的是 MAP18 能与 ROP2 结合，参与了 ROP2 对根毛生长发育的调控。本研究解析了 MAP18 调控 ROP2 信号途径和根毛顶端生长的新机制。

关键词：MAP18，ROP GTPase，顶端生长，根毛

Cytoskeletal control of trichome cell shaping: from *Arabidopsis* trichomes to cotton fibers

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Plant cells assume an amazing diversity of cell shapes that enable these cells to execute unique physiological functions, and the study of plant cell shape determination has remained an intriguing part of plant biology. The plant cytoskeletal system, composed of microtubules (MTs) and actin filaments (F-actin), plays a central role in cell morphogenesis. However, the molecular mechanisms underlying cytoskeletal control of plant cell shaping remain largely unknown.

The *Arabidopsis thaliana* leaf trichome has long been a model system for investigating the role of the cytoskeleton in defining plant cell shape. Using live cell imaging, we observed the spatiotemporal organizations of MTs and F-actin in the *Arabidopsis* trichomes during development. We found that transverse MT rings encircle the elongating branches but leave a MT-depleted zone at the extreme apex, where precisely forms the transverse cortical F-actin cap. Importantly, we discovered that KCBP, a plant-unique kinesin, forms a gradient in the elongating trichome branch, with the highest density at MT-depleted zone. Further, single-molecule imaging demonstrated that the specific MyTH4 domain and FERM domain in the N-terminal tail of KCBP physically bind to MTs and F-actin, respectively. Collectively, our findings revealed that KCBP, a plant-unique kinesin, acts as a hub integrating MTs and actin filaments to assemble the required cytoskeletal configuration for trichome cell morphogenesis.

Cotton fiber cell is another intriguing model for studying cytoskeletal regulation of polarized cell elongation and cellulose biosynthesis. Through long-term efforts, we generated stable transgenic cotton lines expressing GFP-labelled actin filaments, and visualized actin architecture and dynamics in living cotton fiber cells. Our findings provide important insights into mechanisms underlying extremely polarized elongation of cotton fiber cells.

Key words: trichomes, cotton fibers, microtubules, actin filaments

Differential regulation of clathrin and its adaptor proteins, AP-2 and the TPLATE complex, during their membrane recruitment in *Arabidopsis*

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In plants, clathrin-mediated endocytosis (CME) is dependent on the function of clathrin and its accessory heterooligomeric adaptor protein complexes, Adaptor Protein 2 (AP-2) and the TPLATE complex (TPC), and is negatively regulated by the hormones auxin and salicylic acid (SA). The details for how clathrin and its adaptor complexes are recruited to the plasma membrane (PM) to regulate CME are however poorly understood. We found that SA and the pharmacological CME inhibitor tyrphostin A23 (TyrA23) reduce the membrane association of clathrin and AP-2, but not that of the TPC, whereas auxin solely affected clathrin membrane association, in *Arabidopsis thaliana*. Genetic and pharmacological experiments revealed that loss of AP2μ or AP2σ partially affected the membrane association of other AP-2 subunits and that the AP-2 subunit AP2σ, but not AP2μ, was required for SA- and TyrA23-dependent inhibition of CME. Furthermore, we show that although AP-2 and the TPC are both required for the PM recruitment of clathrin in wild-type cells, the TPC is necessary for clathrin PM association in AP-2 deficient cells. These results indicate that developmental signals may differentially modulate the membrane recruitment of clathrin and its core accessory complexes to regulate the process of CME in plant cells.

Key words: Adaptor Protein 2, auxin, clathrin, endocytosis, salicylic acid, TPLATE complex, tyrphostin A23, *Arabidopsis*

RNA polymerase II regulates stomatal development in *Arabidopsis*

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Stomata, which consist of paired guard cells, are known to have played crucial roles in the colonization of land by plants. Turgor-driven stomatal movement controls transpiration and gas exchange between plants and the environment. Several key genes and regulatory networks underlying stomatal development have been uncovered by molecular genetic analysis, however, much less is known about how signals involved in stomatal development are transmitted to RNA polymerase II (Pol II), which plays a central role in the transcription of mRNA coding genes. Herein, we reported a partial loss-of-function mutant of the third largest subunit of nuclear DNA-dependent RNA polymerase II (*NRPB3*). It exhibited an increased number of stomatal lineage cells and stomatal clusters. Similar stomatal phenotypes were observed in a weak allele of the second largest subunit of nuclear DNA-dependent RNA polymerase II (*NRPB2*). These results suggested that Pol II plays essential roles in stomatal development. Genetic analysis indicated that *NRPB3* synergistically interacted with stomatal patterning and differentiation regulators. We also found physical associations of NRPB3 with two bHLH transcription factors, FAMA and INDUCER OF CBF EXPRESSION1 (ICE1), indicating that NRPB3 serves as an acceptor for signals from transcription factors involved in stomatal development. Our findings highlight the surprisingly conserved activating mechanisms mediated by the third largest subunit of Pol II in eukaryotes.

Key words: stomata, RNA polymerase II, patterning, differentiation, *NRPB3*

Non-SMC elements 1 and 3 are required for early embryo and seedling development in *Arabidopsis*

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The structural maintenance of the chromosome 5/6 (SMC5/6) complex has a variety of functions in mitosis and meiosis in yeast and human, including facilitating homologous recombination, restarting stalled replication forks, and maintaining ribosomal DNA and heterochromatin, among other roles. In *Arabidopsis*, *AtSMC5* and *AtSMC6A/6B* are responsive to DNA damage. However, the non-SMC element 1 and 3 functions in *Arabidopsis* growth and development remain as yet unknown. In our study, it was found that the loss function of the *non-SMC element 1* and *3* led to the conversion of suspensor cell fate and caused defects in embryo pattern formation. Partially-complemented homologous mutants showed that the post-embryonic development of the mutants was inhibited, that chromosome fragments occurred during segregation of chromosomes, and that the transition from the G2 phase to the M phase was delayed, leading to endoreduplication. All of these results demonstrate that the *non-SMC element 1* and *3* are essential for cell division and function in maintaining the stability of chromosome ploidy in mitosis. Further, a large number of dead cells and DNA DSBs were observed in the their mutants, and the expression of the *non-SMC element 1* and *3* were up-regulated following treatment of the plants with DSBs inducer compounds, indicating that the *non-SMC element 1* and *3* play roles in DNA damage repair. Therefore, we conclude that the *non-SMC element 1* and *3* facilitate DSBs repair and contribute to maintaining the stability of chromosome ploidy in mitotic cells. The *non-SMC element 1* and *3* could thus be considered as required factors for maintaining proper early embryo cell differentiation and post-embryonic development.

Key words: non-SMC elements, embryo, seedling, *Arabidopsis*

The AtVPS41-mediated endocytic pathway is essential for pollen tube-stigma interaction in *Arabidopsis*

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In flowering plants, extensive male-female interactions are required for successful fertilization, in which various signaling cascades are involved. Prevacuolar compartments (PVC) and vacuoles are two types of subcellular compartments that terminate signal transduction by sequestrating signaling molecules in yeast and mammalian cells; however, the manner in which they might be involved in male-female interactions in plants is unknown. In this study, we identified the *Arabidopsis* VPS41 (AtVPS41), encoded by a single-copy gene with sequence similarity to yeast *Vps41p*, as a new factor controlling pollen tube-stigma interaction. Loss of AtVPS41 function disrupted penetration of pollen tubes into the transmitting tissue and thus led to failed male transmission. In the pollen tubes, AtVPS41 protein is associated with PVCs and the tonoplast. We demonstrate that AtVPS41 is required for the late stage of the endocytic pathway (*i.e.*, endomembrane trafficking from PVCs to vacuoles), because internalization of cell surface molecules was normal in the *vps41* deficient pollen tubes, while PVC-to-vacuole trafficking was impaired. We further show that the CHCR domain is required for subcellular localization and biological functioning of AtVPS41. These results indicate that the AtVPS41-mediated late stage of the endocytic pathway is essential for pollen tube-stigma interaction in *Arabidopsis*.

细胞自噬在花粉萌动过程中的作用

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作为种子植物的雄配子体，成熟花粉的萌发和花粉管生长是将精细胞顺利送达胚囊，完成受精作用的前提。大量研究表明水合是花粉萌发的关键过程。但水合引发的，并导致花粉正常萌发的生理与分子机制仍知之甚少。我们的研究发现，在花粉正常萌发过程中有大量自噬体的形成，且与自噬相关的基因表达水平呈相应动态变化。通过遗传学和药理学实验证实细胞自噬是花粉萌发启动的一个必不可少的关键过程，适度的自噬是花粉萌发的必要调控环节。进一步的研究结果表明花粉萌发过程中自噬活动受到 NtCYS2-NtCP10 复合体的调控。证实半胱氨酸蛋白酶抑制剂子 NtCYS2 可以通过调节花粉中蛋白酶 NtCP10 的活力来调控花粉萌动过程中自噬体形成，进而调控花粉萌发的启动。我们的结果揭示了烟草花粉通过 NtCYS2-NtCP10 的动态平衡来调控细胞中自噬活动，进而启动花粉的正常萌发。

关键词：细胞自噬，半胱氨酸蛋白酶抑制子，半胱氨酸蛋白酶，花粉萌发

AP1G-mediated vacuolar acidification participates in synergid-controlled pollen tube reception

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Double fertilization in angiosperms requires the delivery of immotile sperm through pollen tubes, which enter embryo sacs to initiate synergid degeneration and to discharge. This fascinating process, called pollen tube reception, involves extensive communications between pollen tubes and synergids, within which few intracellular regulators involved have been revealed. Here we report that vacuolar acidification in synergids, mediated by AP1G-dependent trafficking of V-ATPases, is critical for pollen tube reception. Functional loss of *AP1G*, encoding adaptor protein 1 γ subunit, impaired synergid degeneration and pollen tube discharge, resulting in partial female sterility. The proper targeting of V-ATPases requires *AP1G* and V-ATPase-mediated vacuolar acidification in synergids is critical for synergid degeneration during pollen tube reception. We propose that vacuolar acidification-mediated synergid degeneration might represent a distinct cell death mechanism specifically adopted by the plant kingdom.

Molecular regulation of pollen tube guidance in *Arabidopsis*

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Sexual reproduction requires recognition between the male and female gametes. In animals, the sperms swim to the egg attracted by the egg-derived chemotropic signals. While for angiosperms, the most prosperous species on earth, the sperm cells are immobile, thus a sperm-delivery structure—pollen tube is evolved. Through polar growth, the pollen tubes deliver the two sperm cells to the ovule-enclosed female gametophyte embedded in the pistil. During this process, the pollen tubes are attracted by the female gametophytes to reach the receptive synergid cells. The synergid cells can secret peptide signals to attract the pollen tubes, while it is long-puzzled that how pollen tubes sense and decode these signals to achieve directional growth. Another interesting phenomenon is that as one of the two gametes, the central cell also controls the attraction ability of the female gametophyte through a transcription factor CCG. But the underlining mechanism remains unclear. Here we report that a pair of pollen tube-enriched receptor like kinases MDIS1 and MIK1 function as a heterodimer in pollen tube response to the female gametophyte and transduce the signal through cytosolic protein kinases and ROP signaling pathway. Further, expression of MDIS1 in *C. rubella*, the sister species of Arabidopsis, can partially breakdown the interspecies cross barrier. And recently, we identified several components required for the maturation and transporting of the receptors like kinases. Through biochemical strategies, we identified a transcriptional complex CCG-CBP1 in the central cell required for the synergids function. CBP1 interacts with mediator components MED7 and MED9 as well as the RNA polymerase II. Transcriptome results suggest that CCG and CBP1 co-regulate a subset of genes expressed in both the synergid cells and central cell. These results suggest that active intercellular communication is essential for the full function of the female gametophyte.

Key words: pollen tube guidance, female gametophyte, central cell, synergid cells

Ubiquitin-mediated control of seed and organ size

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Seed and organ size is one of important agronomic traits in plants. The characteristic size of plant seeds and organs is coordinately determined by cell proliferation and cell expansion. However, the genetic and molecular mechanisms that set the final size of seeds and organs are largely unknown. We are focusing on understanding how plants know and determine their final seed and organ size. We have recently identified several seed and organ size regulators that are involved in ubiquitin-related activities, including the ubiquitin receptor DA1, E3 ubiquitin ligases DA2 and EOD1/BB, ubiquitin-specific proteases DA3/UBP14 and SOD2/UBP15 and F-box protein SOD3/SAP. We have built up a genetic and molecular framework for ubiquitin-mediated control of seed and organ size. Here, we will discuss the role of the ubiquitin pathway in seed and organ size control.

Key words: seed and organ size, ubiquitin, cell proliferation, cell expansion

水稻基因组结构变异介导的等位基因表达抑制及其杂种不育

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杂种不育是种间或亚种间的生殖隔离机制。籼稻和粳稻亚种间杂种具有很强的杂种优势，但严重的杂种不育妨碍了籼粳杂种产量潜力的利用。籼粳杂种不育由多个座位控制。我们以前克隆的 *Sa* 座位由 2 个相邻的等位分化基因间互作控制籼粳杂种雄性不育。我们利用图位克隆分离了一个新座位 *Sc* 的籼粳等位基因。粳稻等位基因 *Sc-j* 编码一个花粉配子体发育必需的 DUF1618 蛋白。测序分析发现，籼稻等位基因 *Sc-i* 发生了很大的结构变异：与 *Sc-j* 基因对应的基因有 2 个转座子插入成为假基因，而该假基因下游有 2 个或 3 个 28kb 片段顺向重复（不同籼稻品种拷贝数不同），每个重复含有一个 *Sc-j* 同源基因，但它们的启动子及其上游区序列与 *Sc-j* 的启动子序列完全不同。在 *Sc* 杂合体（杂种 F_1），*Sc-i* 表达产物介导了对 *Sc-j* 的表达抑制而导致 *Sc-j* 花粉的败育。含有 3 个 *Sc-i* 拷贝的籼粳杂种比含有 2 个 *Sc-i* 拷贝的产生更严重的杂种不育。我们在杂种以 CRISPR/Cas9 敲除或突变 1-2 个 *Sc-i* 拷贝，降低 *Sc-i* 基因剂量和表达水平，结果可以提升 *Sc-j* 表达并恢复花粉育性。我们提出了一种 *Sc* 等位互作控制杂种不育的分子遗传模型。本研究表明基因组结构和拷贝数变异是基因组进化和遗传性状变异的重要机制，揭示了 *Sc* 座位的分子遗传基础，还建立了利用基因编辑创建杂种亲和等位基因以利用杂种优势的技术方法。

关键词： 水稻，杂种不育，拷贝数变异，等位互作

Transcriptional repression regulates leaf development

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The developmental plasticity of leaf size and shape is important for leaf function and plant survival. The CINCINNATA (CIN)-like TEOSINTE BRANCHED 1/CYCLOIDEA/PCF (TCP) transcription factors are key regulators of leaf development. However, the mechanisms by which plants form diverse leaves in response to environmental conditions are still largely unknown. We identified the **T**CP **I**nteractor containing **E**AR motif protein **1** (TIE1), a novel transcriptional repressor, as a major modulator for TCP activity and leaf development. Overexpression of *TIE1* leads to hyponastic and serrated leaves, whereas disruption of *TIE1* causes epinastic leaves. *TIE1* encodes a transcriptional repressor containing a C-terminal EAR motif, which mediates interactions with the TPL/TPR corepressors. In addition, TIE1 physically interacts with CIN-like TCPs, thus forming a bridge to link TPL/TPR corepressors to suppress the activity of TCP transcription factor. We further identified a RING-type E3 ligase TEAR1 (**T**I**E**1-**a**ssociated **R**ING-type E3 ligase **1**), which regulated leaf development by promoting the degradation of TIE1. TEAR1 contains a typical C3H2C3-type RING domain and has E3 ligase activity. We find that TEAR1 interacts with TIE1, which is ubiquitinated *in vivo* and degraded by the 26S proteasome system. *TEAR1* is developmentally regulated in leaves and TEAR1 is co-localized with TIE1 in nuclei. We demonstrate that TEAR1 negatively regulates TIE1 protein level. Overexpression of TEAR1 rescued leaf defects caused by TIE1 overexpression, whereas disruption of TEAR1 resulted in leaf phenotypes resembling those caused by TIE1 overexpression or TCP dysfunction. We propose that the TIE1-TEAR1 module provides fine and flexible regulation of CIN-like TCP activity and thus leaf size and shape in response to internal and external signals during leaf development.

Key words: leaf development, TIE1, transcriptional repressor, TEAR1, RING-type E3 ligase, TCP transcription factors

The molecular function of SUMOylation in root development

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SUMOylation, which transfers the small ubiquitin-like modifier (SUMO) onto protein substrates, is a critical modification in regulation of protein activity, localization and stability. This conserved modification is involved in different biological processes, but its precise function in plants remains unclear. We focus on the functions of an Arabidopsis SUMO ligase AtMMS21. Our biochemical data showed that AtMMS21 has SUMO ligase activity. The T-DNA insertion mutant of AtMMS21 displays a short-root and disorganized apical root meristem phenotype (Huang et al., 2009). Further analysis showed that this gene is essential for the proper expression of stem cell niche-defining transcription factors. AtMMS21 is also a subunit of the STRUCTURAL MAINTENANCE OF CHROMOSOMES5/6 complex, an evolutionarily conserved chromosomal ATPase required for DNA repair (Xu et al., 2013). The AtMMS21 mutant plants show hypersensitivity in the DNA damaging treatments and lower frequency in homologous recombination (Yuan et al., 2014). In addition, AtMMS21 is required for normal meiosis and gametophyte development (Liu et al., 2014), as well as drought response regulation (Zhang et al., 2013). The AtMMS21 mutants have increased endoreplication levels, but the molecular mechanism is unknown. Recently, we discovered that AtMMS21 regulates cell cycle via the E2Fa/DPa pathway. DPa interacts with AtMMS21, and DPa is a substrate for SUMOylation mediated by AtMMS21. AtMMS21 interferes the E2Fa/DPa interaction and affects the localization of the E2Fa/DPa complex. Overexpression of AtMMS21 completely recovered the abnormal phenotypes of the E2Fa-DPa co-overexpressing plants. These results suggest that AtMMS21 dissociates the E2Fa/DPa complex via competition and SUMOylation in the regulation of plant cell cycle (Liu et al., 2016). Several other factors have also been identified as AtMMS21-interacting proteins in our laboratory. Further functional analysis would improve our knowledge on SUMOylation in plants.

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Understanding of remorin gene family in rice

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Remorins are a diverse family of plant-specific proteins with variable N-terminal sequence and conserved C-terminal sequence which is linked with plasma membrane. 19 remorin members in rice display diverse function in various growth and development processes. In our studies, a rice T-DNA insertion mutant gsd1-D (grain setting defect 1-Dominant) is characterized. GSD1 is a remorin gene (remorin 6.6) and expressed specifically in phloem companion cells and is localized in the plasmodesmata (PD) and plasma membrane. The study demonstrated that GSD1 plays a role in regulating photoassimilate translocation through the symplastic pathway to impact grain setting in rice. Characterization of another rice remorin gene, *OsREM4.1*, indicates that *OsREM4.1* expression is up-regulated by ABA through the transcriptional activator OsbZIP23. OsREM4.1 directly interacts with OsSERK1 to repress BR signaling output by inhibiting the formation and/or activation of the OsBRI1-OsSERK1 receptor complex. OsREM4.1 can be phosphorylated by active OsBRI1 and the phosphorylated OsREM4.1 is released from OsSERK1, thus allowing the formation of OsBRI1-OsSERK1 complex and subsequent activation of the BR signaling pathway. This finding reveals the OsREM4.1 function in coordinating the antagonistic interaction between ABA and BR signaling in rice.

Key words: remorin, plasma membrane protein, plasmodesmata, BR signaling, ABA

基于 CRISPR/Cas9 技术的水稻全基因组敲除体系的建立

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CRISPR/Cas9 作为一项强大的基因组编辑技术，已广泛的用于各种植物，特别是水稻。结合计算机技术和高效的遗传转化技术，我们建立了一套适用于水稻的高通量、自动化的基因敲除体系，包括 small-guild RNA(sgRNA)自动化设计程序、载体高通量构建技术、高效遗传转化系统和转化植株自动化鉴定分析程序。基于该体系，我们对水稻 4,200 多个基因/位点成功进行了靶向编辑，获得了超过 100,000 个转化植株。对 23,000 多个植株的 DNA 测序分析发现，该 Crispr/Cas9 体系在水稻中的整体敲除效率为 74.0%。

为了在水稻中建立一个涵盖全基因组的全面的 CRISPR/Cas9 敲除突变体库，我们设计计算机程序对水稻全基因组(NIP, MSU)进行分析，发现水稻基因组中总共含有 82,846,938 个 CRISPR/Cas9 位点。通过进一步的分析过滤，挑选出 116,969 个 sgRNA，靶向 46,659 基因/位点。将所有的 sgRNA 合成后，高通量构建至水稻 CRISPR/Cas9 载体，获得载体库，二代测序表明，该载体库的正确率为 93.8%。基于该载体库，我们以中花 11 为背景材料进行了第一阶段的遗传转化，获得超过 50,000 个转化植株，收获近 37,000 份 T0 代种子。对其中 14,000 份材料进行高通量测序发现，这部分种子总共涵盖 3,720 个基因，其中单拷贝 T-DNA 占 58.3%。对其中 43 份材料进行抽样分析发现，其敲除率为 83.7%。目前第二阶段遗传转化工作正在进行，计划获得 50,000 份材料，通过分析预计所有的材料可以涵盖超过 50%的基因。

Interactions of TOE proteins with CO and others regulate precise timing of floral induction in *Arabidopsis*

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Plants flower in an appropriate season to allow sufficient vegetative development and to position flower development in favorable environments. The *Arabidopsis CO* and *FKF1* genes promote flowering by inducing *FT* expression in the long-day afternoon. The CO protein is present in the morning but could not activate *FT* expression due to unknown negative mechanisms, referred to as the ˝morning gate˝, which prevent premature flowering. The *AP2/EREBP* genes participate in development and stress responses, including inhibition of flowering by the *TOE* genes. To probe the conservation and divergence of *AP2/EREBP* genes, we analyzed the duplication patterns of this family in Brassicaceae. Some *AP2/EREBP* duplicates generated early in Brassicaceae history were quickly lost, while others were retained in all tested Brassicaceae species, suggesting early functional divergence followed by persistent conservation. Furthermore, we used 16 representative *Arabidopsis* AP2/EREBP proteins as baits and identifies 1,970 potential AP2/EREBP-interacting proteins, with a small subset of interactions verified *in planta*. The putative AP2-interacting proteins participate in many functions in development and stress responses, including photomorphogenesis, flower development, drought and cold responses, abscisic acid and auxin signaling. In particular, TOE1 and related proteins interact with the activation domain of CO and COLs and inhibit CO activity. TOE1 binds to the *FT* promoter near the CO-binding site and reducing *TOE* function results in a morning peak of the *FT* mRNA. In addition, TOE1 interacts with FKF1 and likely interferes with the FKF1-CO interaction, resulting in degradation of the CO protein in afternoon to prevent premature flowering. Our results uncovered that TOE proteins are the molecular keepers of the ˝morning gate˝ and indicate that positive and negative regulators coordinate to precisely regulate flowering time, thereby ensuring maximum reproduction in response to environmental signals.

Key words: AP2 family, protein interactions, TOE1, CO, FKF1, flowering time

HAF1 mediates circadian accumulation of OsELF3 in determining the heading date of rice under long-day conditions

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Plant circadian clock has been provided to be involved in multiple physiology and metabolism process by monitoring external environment changes, including photoperiodic flowering regulation. Our previous study showed that *OsELF3* acts as a floral activator in the long-day photoperiodic pathway via its crosstalk with the circadian clock in rice. However, post-transcription regulation of *OsELF3* is still unclear. In this study, we showed that HAF1, an E3 ubiquitin ligase, physically interact with OsELF3 in *vivo* and in *vitro*. C-terminal domain of OsELF3 was identified as the functional motif for mediating interaction with HAF1, as well as formation of homodimer by itself. Further protein degradation assay indicated that *OsELF3* is the direct substrate of HAF1 for ubiquitination in rice. Genetically, the *oself3 haf1* double mutant headed as late as *oself3* under long-day conditions. Previous investigation revealed that *OsELF3* plays a crucial role in photoperiodic response to determine rice regional adaptability. We proposed that HAF1 plays a pivotal role in heading date modulation by ubiquitination of OsELF3 under long-day conditions.

Key words: circadian clock, photoperiodic flowering, ubiquitination, rice

Mitogen-activated protein kinase cascade MKK7-MPK6 plays important roles in plant development and regulates shoot branching by phosphorylating PIN1 in *Arabidopsis*

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Emerging evidences exhibit that mitogen-activated protein kinase (MAPK) signaling pathways are connected with many aspects of plant development. The complexity of MAPK cascades raises challenges not only to identify the MAPK module *in planta* but also to define the specific role of an individual module. Our previous study have characterized an *Arabidopsis bushy* and *dwarf1* (*bud1*) mutant, in which the MKK7 was constitutively activated, resulting in multiple phenotypic alterations and activated the mobile signal of systemic acquired resistance. To elucidate the specificity of MKK7 and its downstream MAPKs in multiple biological processes, we systemically screened the downstream substrates of MKK7 *in vitro*. We found that MPK3 and MPK6 are the substrates for phosphorylation by MKK7 *in planta*. Genetic analysis showed that MKK7-MPK6 cascade is specifically responsible for the regulation of shoot branching, hypocotyl gravitropism, filament elongation, and lateral root formation. We further demonstrated that the MKK7-MPK6 cascade controls shoot branching by phosphorylating Ser 337 on PIN1, which affects the basal localization of PIN1 in xylem parenchyma cells and polar auxin transport in the primary stem. Our findings specify the functions of the MKK7-MPK6 cascade, and explain how MKK7-MPK6 signaling pathway regulates polar auxin transport (PAT) through the specific substrate PIN1 to determine shoot branching in *Arabidopsis*, establishing a molecular link between the MAPK cascade and auxin-regulated plant development.

Key words: MKK7, MPK6, phosphorylation, PIN1, shoot branching

Regulation of a meristematic cell population acting in shoot branching in *Arabidopsis*

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Shoot branching requires the establishment of new meristems harboring stem cells; this phenomenon raises questions about the precise regulation of meristematic fate. In seed plants, these new meristems initiate in leaf axils to enable lateral shoot branching. Using live-cell imaging of leaf axil cells, we show that the initiation of axillary meristems requires a meristematic cell population continuously expressing the meristem marker *SHOOT MERISTEMLESS* (*STM*). The maintenance of *STM* expression depends on the leaf axil auxin minimum. Ectopic expression of *STM* is insufficient to activate axillary buds formation from plants that have lost leaf axil *STM* expressing cells. This suggests that some cells undergo irreversible commitment to a developmental fate. In more mature leaves, REVOLUTA (REV) directly up-regulates *STM* expression in leaf axil meristematic cells, but not in differentiated cells, to establish axillary meristems. Finally, leaf axil cytokinin signaling pulse, likely resulting from the enhanced *STM* levels, *de novo* activates local *WUS* expression to promote axillary bud formation. In particular, type-B ARABIDOPSIS RESPONSE REGULATORs (ARRs), transcriptional activators in the cytokinin signaling pathway, directly bind to the *WUS* promoter to activate its expression. Cell type-specific binding of REV to the *STM* region, and ARRs to the *WUS* region, correlate with epigenetic modifications. Our data favor a threshold model for axillary meristem initiation, in which low levels of *STM* maintain meristematic competence and high levels of *STM* lead to cytokinin signalling pulse, *WUS* expression, and axillary bud formation.

Key words: axillary meristem, stem cell, meristem

The *Arabidopsis* WRKY transcription factors WRKY12 and WRKY13 oppositely regulate flowering under short-day conditions

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In plants, photoperiod is an important cue for initiating flowering. The floral transition in *Arabidopsis thaliana* occurs earlier under long-day than short-day (SD) conditions, with flowering under the latter being mainly regulated by the plant hormone gibberellin. Here, we report two WRKY proteins with opposite functions in controlling flowering time under SD conditions. Phenotypic analysis showed that disruption of WRKY12 caused a delay in flowering, while disruption of WRKY13 induced flowering. Promoter-swap experiments proved that the negatively correlated expression profiles of these proteins established an important and precise equilibrium for the regulation of flowering time. Molecular and genetic analysis demonstrated that the floral inductive signal *FRUITFULL* (*FUL*) was a direct downstream target gene of WRKY12 and WRKY13. Through yeast two-hybrid screening, the DELLA proteins GIBBERELLIN INSENSITIVE (GAI) and RGA-LIKE1 (RGL1) were identified as interacting with WRKY12 and WRKY13. The interactions between DELLAs and the WRKYs interfered with the transcriptional activity of the WRKY proteins. Moreover, WRKY12 and WRKY13 partly mediated the effect of GA_3 on the control of flowering time. Taken together, our study indicates that WRKY12 and WRKY13 oppositely modulate flowering time under SD conditions.

Genetic identification of RGF1 receptors, RGF1 INSENSITIVE 1 to 5

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RGF1, a secreted peptide hormone, plays a key role in root meristem development in Arabidopsis. Previous studies indicated that a functional RGF1 needs to be sulfated at a tyrosine residue by a tyrosylprotein sulfotransferase (TPST) and that RGF1 regulates root meristem activity mainly via two downstream transcription factors, PLETHORA 1 (PLT1) and PLT2. How extracellular RGF1 is perceived by a plant cell, however, is not understood. Using genetic approaches, we discovered a clade of leucine-rich repeat receptor-like kinases (LRR-RLKs), designated as RGF1 INSENSITIVE 1 (RGI1) to RGI5, serving as receptors of RGF1. Two independent *rgi1 rgi2 rgi3 rgi4 rgi5* quintuple mutants display a consistent short primary root phenotype with a small size of meristem. An *rgi1 rgi2 rgi3 rgi4* quadruple mutant shows a significantly reduced sensitivity and the quintuple mutant is completely insensitive to RGF1. The expression levels of *PLT1* and *PLT2* are almost undetectable in the quintuple mutant. Ectopic expression of *PLT2* driven by an *RGI2* promoter in the quintuple mutant greatly rescued its root meristem defects. One of the RGIs, RGI1, was subsequently analyzed biochemically in detail. *In vitro* dot-blotting and pull-down analysis indicated that RGI1 can physically interact with RGF1. Exogenous application of RGF1 can quickly and simultaneously induce the phosphorylation and ubiquitination of RGI1, indicating that RGI1 can perceive and transduce the RGF1 peptide signal. Yet, the activated RGI1 is likely turned over rapidly. These results demonstrated that RGIs, acting as the receptors of RGF1, play essential roles in RGF1-PLT-mediated root meristem development in *Arabidopsis thaliana*.

Key words: receptor-like kinase, RGF1, RGF1 INSENSITIVE, PLETHORA, *Arabidopsis*

BR 信号通路新的调控因子及生理功能的研究

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植物激素油菜素内酯(BRs)，在调控细胞伸长，微管分化，衰老和抗胁迫方面起着重要的作用。过去二十年的研究描绘出 BR 信号转导途径模型，BRs 受体 BRI1 以及下游元件激酶 BIN2 等通过转录因子 BES1/BZR1 来调控 BR 响应基因的表达。BES1 可以与 BIM1，IWS1 以及 MYB30 形成转录复合体绑定在 E-box 元件调控 BR 诱导基因的表达，但是 BR 抑制基因的调控模式还不是很清楚。我们的研究发现 BIN2 底物 HAT1 的稳定性与其磷酸化有关。HAT1 可以绑定在保守的 HB 位点，并且与 BES1 互作一起调控 BR 抑制的基因的表达。BRs 在提高植物非生物胁迫和生物胁迫抗性方面具有重要作用。首先 BRs 提高植物的非生物胁迫抗性与促进抗氰呼吸有关。同时 BRs 和 BR 信号参与调控植物病毒抗性的过程。BR 信号在调控病毒抗性过程中具有双面性，一方面 BRs 可以通过促进 MEK2-SIPK 途径和依赖于 RBOHB 的 ROS 爆发来增强植物对病毒的抗性，另一方面 BZR1 可以通过抑制依赖于 RBOHB 的 ROS 爆发来平衡 BR 信号对植物生长发育和植物免疫的调控。BR 介导的病毒系统性抗性与接种叶依赖于 RBOHB 的 H_2O_2 产生和系统叶依赖于 NR 途径的 NO 积累有关。

关键词：油菜素内酯，转录调控，HAT1，非生物胁迫抗性，病毒抗性

Brassinosteroids promote anthocyanin biosynthesis under low nitrogen conditions in *Arabidopsis*

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Brassinosteroids (BRs) are steroid plant hormones that regulate many developmental and physiological processes in pants including responses to stress. However, little is known about BR regulation of plant responses to mineral nutrients. Our recent studies indicate that BR can induce plant tolerance to low nitrogen (N) stress in *Arabidopsis* and enhance low N-induced anthocyanin biosynthesis, which seem to be both mediated by the BR-activated transcription factor BZR1. BZR1 physically interacts with PAP1, a key transcription factor controlling anthocyanin biosynthesis in *Arabidopsis*, and enhances PAP1′s transcriptional regulatory activity on its target genes in anthocyanin biosynthesis. BZR1 can also bind the promoters of some of the anthocyanin biosynthetic genes but the binding appears to be not specific, suggesting that BR promotes anthocyanin biosynthesis under low N mainly through BZR1-PAP1 interaction. As a conclusion, our study indicates that BR is capable of promoting low N-induced anythocyanin biosynthesis, which may help plants survive the low N stress conditions.

Key words: brassinosteroids, nitrogen stress, anthocyanin biosynthesis, *Arabidopsis thaliana*

利用蛋白质组学解析油菜素内酯信号传导的分子机理

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油菜素内酯（brassinosteroids, 简称 BR）是主要的植物生长调节激素之一，参与调节了细胞的伸长和分化等许多植物生长发育过程。为了解析 BR 信号传导的分子机理，我们利用 2D-DIGE 的蛋白质组学方法找到了一批 BR 调节的质膜蛋白，这些蛋白包括和信号传导有关的蛋白激酶 BAK1 和 BSK，以及蛋白磷酸酶 PP2A。BSK 蛋白包含一个 N 末端的蛋白激酶域和一个 C 末端与蛋白质相互作用有关的 TPR 结构域，能够介导 BR 信号从质膜往胞质的传递。 我们的研究表明，当 BR 信号通路没有被激活的时候，BSK 的 TPR 结构域能够和自身的激酶域结合，抑制 BSK 蛋白传递 BR 信号。而当胞外 BR 浓度升高时，质膜上 BR 受体 BRI1 被激活，磷酸化 BSK 的激酶域，通过抑制 TPR 结构域和激酶域结合来促进激酶域和下游信号组份 BSU1 的相互作用，激活 BR 信号通路。我们还发现蛋白磷酸酶 PP2A 能够通过将受体 BRI1 去磷酸化关闭 BR 信号途径。由于 PP2A 还能将 BR 信号传导途径中的 BZR1 家族转录因子去磷酸化从而正向调节 BR 信号途径。进一步的研究表明 PP2A 在 BR 信号传导途径中的双向调节作用可能由 PP2A 的亚细胞定位决定：胞质定位的 PP2A 主要通过将 BRI1 去磷酸化使 BR 信号失活，而核定位的 PP2A 则能通过将 BZR1 家族转录因子去磷酸化激活 BR 信号通路。因此，通过 2D-DIGE 的蛋白质组学方法，我们找到了两个 BR 信号传导通路的新组份，证明了蛋白质组学是一种非常好的，可以用于发掘植物信号传导新组份和新机制的研究方法。

关键词：油菜素内酯，信号传导，蛋白质组学，2D-DIGE

蛋白酶体调节子 PTRE1 参与生长素信号调控

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生长素在植物生长发育中发挥重要调节作用，其主要通过生长素受体 TIR1 介导负调控因子 AUX/IAAs 的降解，进而释放 ARFs 转录因子调控下游基因表达。泛素/蛋白酶体（Ubiquitin-Proteasome）途径可以有效快速地选择性的移除相关蛋白，在激素信号响应、发育及胁迫响应等过程中发挥重要作用。虽然已有研究表明了蛋白酶体在生长素信号中的重要作用，但是其调控机制以及 AUX/IAA 降解的平衡机制目前仍不清楚。我们鉴定了一个动物中蛋白酶体活性抑制蛋白 PI31 的同源基因，PTRE1 (PROTEASOME REGULATOR 1)，其编码一个脯氨酸富集蛋白。功能研究表明 PTRE1 介导了生长素调节的蛋白酶体活性过程并协调 TIR1 介导的蛋白降解以调控生长素信号。PTRE1 定位于细胞膜、细胞核及内质网，生化分析表明 PTRE1 可以促进 26S 蛋白酶体活性。突变体 *ptre1* 表现出多重发育缺陷并对生长素响应不敏感；遗传研究表明 TIR1 与 PTRE1 具有协同效应，*ptre1* 突变体中 TIR1 介导的 AUX/IAA 降解减弱，同时生长素可以通过 PTRE1 抑制蛋白酶体活性。进一步的研究表明生长素调控了 PTRE1 在细胞膜及核中的分布从而调控其活性。这些结果为了解 AUX/IAA 的降解调控及生长素作用机制提供了新的线索。

关键词：拟南芥，生长素，蛋白酶体活性，PTRE1, AUX/IAA

The key role of NF-YC genes in crosstalk between GA and ABA during *Arabidopsis* seed germination

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The antagonistic crosstalk between gibberellic acid (GA) and abscisic acid (ABA) plays a pivotal role in modulation of seed germination. However, the molecular mechanism of such phytohormone interaction remains largely elusive. The NUCLEAR FACTOR-Y C proteins (NF-YCs), structurally characterized by a histone-fold domain (HFD) and closely related to the core histone H2A, functionally act as one subunit of the NF-Y heterotrimer transcriptional factor that specifically recognizes the CCAAT-box in eukaryotes. Here we show that three *Arabidopsis* NF-YC homologues NF-YC3, NF-YC4, and NF-YC9 redundantly modulate GA- and ABA-mediated seed germination, contributing to the integration of GA and ABA signaling through directly interacting with RGA-LIKE 2 (RGL2), a key repressor of GA signaling. The NF-YC-RGL2 module targets *ABA INSENSITIVE 5* (*ABI5*), the gene encoding a core component of ABA signaling, via specific CCAAT elements and collectively regulates a set of GA- and ABA-responsive genes, thus control germination. These results illustrate a novel regulatory model that reveals a central molecular link of NF-YC-RGL2-ABI5 in integration of GA and ABA signaling pathways during seed germination, providing a significant mechanistic understanding on how interaction of GA and ABA signaling is fine-tuned in seed germination.

Key words: seed germination, NF-YC, GA, ABA, crosstalk

新型激素受体

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激素对于生物的新陈代谢、生长发育和繁衍生息等各种生命活动起重要调节作用；阐明激素的受体识别机制，对于揭示生命现象的本质、提高生物的生存和发展能力都具有重要意义。从 1880s 年代 Langley 和 Ehrlich 等科学家提出受体的概念以来，生命科学领域逐步揭示了激素活性分子（配体）可逆地结合受体、并循环地触发信号传导链的“活性分子-受体”识别规律。动植物激素活性分子都是其前体分子通过生物合成途径中的各种酶催化生成的、并遵循百年研究历程所揭示的“活性分子-受体”识别规律，调控各种生命活动。

植物科学领域阐明的生长素、赤霉素、乙烯、细胞分裂素、脱落酸、油菜素内酯、茉莉素和水杨酸共八类经典植物激素的受体，均通过非共价键可逆地结合其激素活性分子、循环地触发信号传导链，介导植物的生长发育和繁衍生息及防御反应。然而，植物激素独脚金内酯却与众不同、遵循新型的“底物-酶-活性分子-受体”识别规律，调控植物分枝并介导寄生杂草种萌发及共生真菌生长。

独脚金内酯的活性分子是一种不同于目前已知的动植物经典激素活性分子的新型活性形式；独脚金内酯的受体则具有生成和感知激素活性分子的双重功能。独脚金内酯激素活性分子 CLIM 是由结构各异的独脚金内酯分子作为底物被受体 D14 水解形成的新型活性分子，而 CLIM 则通过共价键不可逆地结合受体 D14、引发受体 D14 变构、触发独脚金内酯信号传导链，然后继续被受体 D14 水解形成没有生物活性的终产物。我们将对独脚金内酯受体 D14 和茉莉素受体 COI1 的发现进行简要介绍和比较分析。

Mediator links the jasmonate receptor to transcriptionally active chromatin

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A major progress in our understanding of the jasmonate signaling is that the perception of the active hormone by the E3 ubiquitin ligase SCF^{COI1} is tightly linked to genome-wide transcriptional reprogramming that is regulated by the master transcription factor MYC2. However, it remains unclear how the jasmonate receptor transmits regulatory information to RNA polymerase II to specifically transcribe jasmonate-responsive genes. Here we report that the MED25 subunit of the Arabidopsis Mediator provides an interface for interactions of COI1, MYC2 and the histone acetyltransferase HAC1 at the core promoters of MYC2 targets. Our results unravel a mechanism of how Mediator channels hormone-specific regulatory signals to the Pol II general transcription machinery and highlight the ability of Mediator in coordinating gene expression programs of diverse signaling pathways.

Key words: jasmonate, Mediator, transcriptional regulation, HAC1

HSB1 functions in jasmonate-mediated regulation of plant development and defence in *Arabidopsis*

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Many studies have been dedicated to the indispensable roles of jasmonate in plant growth and defence, but the molecular mechanisms preventing the overproduction or accumulation of jasmonate which often has detrimental effect on plant development and physiology are largely unknown. In this study, we found that transcript level of *HSB1* gene was greatly down-regulated by MeJA application and the loss-of-function mutants of *HSB1* gene accumulated significantly more jasmonate but less ethylene than WT plants. Mutations in HSB1 caused pleiotropic effects on various developmentally regulated processes such as seed weight, root elongation, petal size, flower timing, anthocyanin biosynthesis and leaf senescence. The mutants were more susceptible to pests and pathogens attacks as they displayed enhanced disease symptoms in response to fungal and bacterial pathogens *Botrytis cinerea* and *Pseudomonas syringae* pv. Tomato DC3000 (*Pst DC3000*), and the larvae of phytophagous insects *Spodoptera exigua* increased weight more rapidly when fed on the mutant than on WT plants. However, the mutant plants exhibited enhanced resistance to the root-infecting fungal pathogen *Fusarium oxysporum* which can produce ethylene itself. In addition, stomata of the mutant plants were greatly compromised in their ability to close in response to either *Pst* DC3000 or *Pst* DC3000 (avrB). Furthermore, mutations in *HSB1* gene caused hypersensitivity to high sugar induced anthocynin production and seedling growth arrest. We also provided evidence that HSB1 is not only critical for a tight control of jasmonate production and accumulation but also for the interaction of plant hormones including JA and ET in plant development and stress response.

Key words: environmental stress, jasmonate biosynthesis, modern agriculture, plant development and defence, regulatory node

Auxin regulates plant stem cells homeostasis in *Arabidopsis thaliana*

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The classic phytohormones play important roles in regulation of plant stem cells, and exhibit complex functional interactions. Among them, auxin was previously found to be essential in the differentiation of meristematic cells in shoot apical meristem. The biosynthesis of auxin occurred universally in the whole meristem, and then transported into the peripheral zone by PIN1 to promoter later organs initiation. Recently, several lines of new evidences suggested that auxin can be also transferred into the central zone of shoot apical meristem where harbored plant stem cells. However the functions of auxin in stem cells are still largely unknown.

Previously we have shown that the AUXIN RESPONSE FACTOR5/ MONOPTEROS (MP) transcription factor, mediated the cross talk between auxin and cytokinin in stem cells. By RNA sequencing, we have identified a new MP target, *MTA1*, which was specifically expressed in stem cells. MP can directly bond *MTA1* promoter and repress its expression, and knocking out MP cause transcriptional up regulation of *MAT1*. While MTA1 as a transcription factor, was sufficient to active *CLV3* expression in stem cells to participate in *WUS/CLV3* feedback. Our results provide a novel mechanism of auxin in regulating stem cells homeostasis in *Arabidopsis thaliana*.

Key words: plant stem cell, auxin, *MONOPTEROS*, *CLV3*, homeostasis

Molecular regulation of root growth plasticity in *Arabidopsis*

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Auxin plays an important role to mediates endogenous developmental signals and environmental cues and thus control root growth plasticity. Aluminum stress, which inhibits root growth, is one of the widespread environmental cues in acid (pH<5) soils. Our previous study showed that TAA1 was locally induced in the root-apex transition-zone thus involves in aluminum-induced *Arabidopsis* root growth inhibition. Here, we report that YUCCA (YUC), which encodes flavin monooxygenase-like proteins, regulates local auxin biosynthesis in the root apex transition zone (TZ) in response to Al stress. Al stress up-regulates the expression of *YUC*s in the root-apex TZ and YUC-regulated root growth inhibition in an ethylene signalling dependent manner. Ethylene-insensitive3 (EIN3) is involved into the direct regulation of *YUC9* transcription in this process. Furthermore, we demonstrated that PHYTOCHROME INTERACTING FACTOR4 (PIF4) functions as a transcriptional activator for *YUC5/8/9*. PIF4 promotes Al-inhibited primary root growth by regulating the local expression of *YUCs* and auxin signal in the root-apex TZ. The Al–induced expression of PIF4 in TZ is downstream of ethylene signaling. Taken together, our results highlight a regulatory cascade for YUCs-regulated local auxin biosynthesis in the root-apex TZ mediating root growth inhibition in response to Al stress.

Key words: auxin, root growth, Al stress, YUC, EIN3, PIF4

NCP1/AtMOB1A plays key roles in auxin-mediated *Arabidopsis* development

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MOB1 protein is a core component of the Hippo signaling pathway in animals, where it is involved in controlling tissue growth and tumor suppression. Plant MOB1 proteins display high sequence homology to animal MOB1 proteins, but little is known regarding their role in plant growth and development. Herein we report the critical roles of *Arabidopsis MOB1* (*AtMOB1A*) in auxin-mediated development in *Arabidopsis*. We found that loss-of-function mutations in *AtMOB1A* completely eliminated the formation of cotyledons when combined with mutations in *PINOID* (*PID*), which encodes a Ser/Thr protein kinase that participates in auxin signaling and transport. We showed that *atmob1a* was fully rescued by its *Drosophila* counterpart, suggesting functional conservation. The *atmob1a pid* double mutants phenocopied several well-characterized mutant combinations that are defective in auxin biosynthesis or transport. Moreover, we demonstrated that *atmob1a* greatly enhanced several other known auxin mutants, suggesting that *AtMOB1A* plays a key role in auxin-mediated plant development. The *atmob1a* single mutant displayed defects in early embryogenesis and had shorter root and smaller flowers than wild type plants. *AtMOB1A* is uniformly expressed in embryos and suspensor cells during embryogenesis, consistent with its role in embryo development. AtMOB1A protein is localized to nucleus, cytoplasm, and associated to plasma membrane, suggesting that it plays roles in these subcellular localizations. Furthermore, we showed that disruption of *AtMOB1A* led to a reduced sensitivity to exogenous auxin. Our results demonstrated that *AtMOB1A* plays an important role in *Arabidopsis* development by promoting auxin signaling.

Key words: NCP1/AtMOB1A, Hippo pathway, auxin, embryogenesis

A MAPK cascade modulates ABA signaling in *Arabidopsis*

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The phytohormone abscisic acid (ABA) is involved in plants′ responses to environmental stresses, and plays crucial roles in regulating stomatal movement, seed germination, vegetative growth and development. Increasing evidence suggests that mitogen-activated protein kinase (MAPK) play an important role in ABA signaling. The MAPK cascade is an evolutionarily conserved signal transduction module involved in transducing extracellular signals to the nucleus for appropriate cellular adjustment. This cascade essentially consists of three components, a MAPK kinase kinase (MAPKKK), a MAPK kinase (MAPKK) and a MAPK, connected to each other by the event of phosphorylation. Here we report the characterization of a MAPKKK, AIK1, which regulates ABA responses in Arabidopsis. T-DNA insertion mutants of *AIK1* showed insensitivity to ABA in terms of both root growth and stomatal response. AIK1 functions in ABA responses via regulation of root cell division and elongation, as well as stomatal responses. AIK1 is a positive regulator of ABA-induced stomatal closure. Genetic and biochemical analyses indicate that the intracellular signals are relayed and amplified through sequential phosphorylations of the AIK1-MKK5-MPK6 cascade in response to ABA, regulating the root architecture and stomatal responses. These findings clearly suggest that the AIK1 cascade functions in the ABA regulation of primary root growth and stomatal response.

Key words: MAPKKK, abscisic acid, root growth, stomata, *Arabidopsis thaliana*

ABA 信号途径关键负调控因子的调控机制

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ABA 信号途径在调控植物干旱胁迫响应的过程中起非常重要的作用。在 ABA 信号途径中，干旱快速诱导 ABA 的积累，ABA 与 ABA 受体 PYR1/PYLs 结合后，与关键负调控因子 PP2C 类磷酸酶结合，抑制了这些磷酸酶的活性，从而解除了对下调控蛋白激酶的抑制，这些蛋白激酶磷酸化下游的转录因子或定位于质膜上的离子通道，从而激活了转录因子或离子通道，调控干旱相关基因的表达，或促进气孔关闭，使植物适应干旱的环境。我们的研究发现，ABA 信号途径中的一个重要负调控因子 ABI1，在与 PYR1 或 PYL 受体互作后，可以被 PUB12/13 U-Box E3 连接酶识别，并被多泛素化，从而导致 ABI1 的特异性降解；同时，ABI1-ABA-PYL 形成复合体后，ABI1 或其它 PP2C 也可以与一个功能未知的蛋白 EAR1 互作，EAR1 可以增强这些 PP2C 的活性，使 ABA 信号加强。我们的研究揭示，ABA 信号途径处于动态平衡的调控之中，这些精细的调控，使植物更加适应多变的干旱环境。

关键词：ABA，干旱，气孔运动，ABA受体，PP2C

Fine regulation of ABA signaling and drought resistance by two bZIP transcription factors in rice

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Plants have evolved complicated mechanisms to survive adverse environmental conditions. Previously, we reported that transcription factors OsbZIP23 and OsbZIP46 regulates abscisic acid (ABA) signaling-mediated drought tolerance in rice. Here we present that OsbZIP23 directly regulates a large number of reported genes that function in stress response, hormone signaling, and developmental processes. Among these targets, we found that OsbZIP23 could positively regulate OsPP2C49, and overexpression of OsPP2C49 in rice resulted in significantly decreased sensitivity of the ABA response and rapid dehydration. Moreover, OsNCED4 (9-cis-epoxycarotenoid dioxygenase 4), a key gene in ABA biosynthesis, was also positively regulated by OsbZIP23. These results suggest that OsbZIP23 acts as a central regulator in ABA signaling and biosynthesis, and drought resistance in rice. Meanwhile, we found that the activation of OsbZIP46 is strikingly repressed by an intrinsic domain D. An OsbZIP46-in teracting protein MODD was found to be a mediator of OsbZIP46 deactivation and degradation. MODD negatively regulates ABA signaling and drought stress tolerance, and inhibits the expression of the OsbZIP46 target genes. MODD represses the activity of OsbZIP46 via interaction with the OsTPR3-HDA702 co-repressor complex and down-regulation of the histone acetylation level at the target genes of OsbZIP46. MODD promotes OsbZIP46 degradation via interaction with an ubiquitination E3 ligase OsPUB70. Interestingly, the D domain is required for both the deactivation and degradation of OsbZIP46 via interaction with MODD. These findings exemplify that plants employ elaborate transcriptional regulatory mechanisms to fine-tune the drought responses.

Key words: abscisic acid, drought, *Oryza sativa*, transcriptional regulation

The mechanism of abscisic acid signaling and stress response in rice

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Abscisic acid (ABA) is an essential phytohormone that not only regulates seeds dormancy, germination and seedling growth, but also is involved in responses to environmental stresses such as drought, high salinity and chilling. The identified ABA receptors, PYR/PYL/RCAR was found to directly bind and regulate the activity of the protein phosphatase 2C (PP2C). The Sucrose Non-fermentation Kinase Subfamily 2 (SnRK2s) protein kinases, a central signaling complex (ABA-PYR-PP2Cs-SnRK2s) that is responsible for ABA signal perception and transduction is supported by abundant genetic, physiological, biochemical and structural evidence. 10 PYLs orthologs with the special structure of ABA receptor were identified in rice. Though the common signal transduction pathway was characterized among the members of PP2C family, the function and stress response of the OsPYLs were based on their ABA binding activity in rice. The basic leucine zipper (bZIP) factors are involved in ABA signaling pathway and play an important regulator during environmental stress response. There are 10 clades of bZIPs in rice, from clade A to clade J. The function of several rice bZIP-type transcription factors was analyzed and they are involved in the regulation of the adaptive stress response and plant fertility of rice. The result of phosphorylation by OsSnRK2s show that members from both clade A and clade G are involved in ABA signal pathway, but the signal transduction pathway of members from clade G may be different from those from clade A.

Key words: rice, ABA receptor, stress response, signal transduction

An R2R3 MYB transcription factor is critical for cold stress tolerance in apple

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Cold stress is one of the adverse effects on apple distribution and production. However, the molecular mechanisms underlying apple cold stress tolerance are not clear. In this study, we cloned a novel R2R3 MYB transcription factor, MdMYB5523, and its close homolog MdMYB0970 from Golden Delicious (*Malus × domestica*). Expression of *MdMYB5523* and *MdMYB0970* was slightly induced by cold stress. Sub-cellular localization revealed that both proteins are localized in the nucleus. With Affinity Purification-Mass Spectrometry (AP-MASS) and yeast two-hybrid screening, we obtained two interacting partners of MdMYB5523 and MdMYB0970, MdHYL1 (HYPONASTIC LEAVES 1) and MdSERRATE. Chromatin Immunoprecipitation-PCR showed that MdMYB5523 and MdMYB0970 target genes involved in cold stress, including *MdSIZ1* and *MdCSP3* (*COLD SHOCK DOMAIN PROTEIN 3*). RNAi lines of *MdMYB5523* and *MdMYB0970* in Gala-3 (*Malus × domestica*) showed increased sensitivity to cold stress, while over-expression lines of *MdMYB5523* or *MdMYB0970* showed increased tolerance to cold stress, indicating that MdMYB5523 and MdMYB0970 are two positive regulators of cold stress in apple. Taken together, our results indicate that MdMYB5523 and MdMYB0970 played important roles in cold stress tolerance of apple.

Key words: *Malus × domestica*, MdMYB5523 and MdMYB0970, cold stress

Transcriptional analysis of drought-responsive genes in the desert plant *Eremopyrum triticeum*

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Eremopyrum triticeum is a kind of ephemeral plants which belongs to the wild relative of wheat. It lives mainly on the edge of Zhungeer desert in Xinjiang, China. To survive in extreme environment, *E. triticeum* has developed the characteristics of drought tolerance, leanness-resistant and high photosynthesis efficiency etc. Study on molecular mechanism of *E. triticeum* drought-tolerance is very important for increasing crop stress tolerance via gene transfer. At present, the detailed transcriptomic and genomic data for *E. triticeum* are still insufficient in public databases. To investigate changes of drought-responsive genes and explore the mechanisms of drought tolerance in *E. triticeum*, approximately 2.5 GB sequencing data were obtained using Illumina sequencing technology. After de novo assembly 44,655 unigenes were generated with an average length of 1026 bp. Among these unigenes, 33,324 were annotated with gene descriptions, conserved domains, gene ontology terms, and metabolic pathways. The results showed that a great number of unigenes were significantly affected by drought stress. About 22,507 unigenes were identified as reliable differentially expressed genes (DEGs). The main functional categories enriched in these DEGs were metabolic process, response to stresses, plant hormone signal transduction, protein processing, and plant-pathogen interaction pathway. The associated genes primarily encoded transcription factors (such as NAC, MYB/MYC, AP2/ERF family and LEA), protein kinases, and other regulatory proteins.The expression patterns of eight randomly-selected genes were confirmed by quantitative RT-PCR. The results of qRT-PCR analysis agreed with transcriptional profile data. This is the first large-scale reference sequence data of *E. triticeum*, which enlarge the genomic resources of this species. Our findings will provide a valuable resource for further investigation into the molecular adaptation of desert plants under drought stress and facilitate the exploration of drought-tolerant candidate genes.

Key words: *Eremopyrum triticeum*, drought-responsive genes, transcriptome sequencing, quantitative RT-PCR, transcriptome

Genetic variation in *ZmVPP1* contributes to drought tolerance in maize seedlings

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Maize production is threatened by drought stress worldwide. Identification of the genetic components underlying drought tolerance in maize is of great importance. Here, we report a genome-wide association study (GWAS) of maize drought tolerance at the seedling stage that identified 83 genetic variations which were resolved to 42 candidate genes. The peak signal of GWAS uncovered that the natural variation in *ZmVPP1*, encoding a vacuolar-type H^+-pyrophosphatase, most significantly contributes to the trait. Further analysis found that a 366-bp insertion in the promoter, containing three MYB *cis*-elements, confers drought-inducible expression of *ZmVPP1* in drought-tolerant genotypes. Introgression the tolerant allele of *ZmVPP1* into drought sensitive genetic background greatly improved seedling drought stress tolerance. Transgenic maize with enhanced *ZmVPP1* expression exhibits improved drought tolerance, most likely due to enhanced photosynthetic efficiency and root development. Taken together, this research provides important genetic insights into the natural variation of maize drought tolerance. The identified loci/genes can serve as direct targets for both genetic engineering and selection for the trait improvement of maize.

Key words: drought stress, maize, GWAS, H^+-pyrophosphatase

Deletion of an endoplasmic reticulum stress response element in a *ZmPP2CA* gene facilitates drought tolerance of maize seedlings

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Drought is a major abiotic stress that causes the yearly yield loss of maize, a crop cultured worldwide, thus breeding drought tolerant maize cultivars is a priority target of the world agriculture. Clade A PP2C phosphatases (PP2CA) are conserved in plants and are important for ABA signaling and plant drought response. However, the natural variations of *PP2CA* genes that directly associated with levels of drought tolerance remain to be elucidated. Here, we conducted a candidate gene association analysis of *ZmPP2CA* gene family in a maize panel consisting of 368 varieties collected worldwide, and identified a drought responsive gene *ZmPP2CA10* that is tightly associated with drought tolerance. We found that the degrees of drought tolerance of maize cultivars negatively correlate with the expression levels of *ZmPP2CA10*. ZmPP2CA10, like its Arabidopsis orthologs, interacts with ZmPYL ABA receptors and ZmSnRK2 kinases, suggesting that ZmPP2CA10 functions in mediating ABA signaling in maize. Transgenic studies in maize and Arabidopsis confirmed that *ZmPP2CA10* plays a negative role in regulating drought tolerance. Further, a causal natural variation, InDel-338 (causing a deletion of ERSE, Endoplasmic Reticulum Stress response Element) in the 5′UTR region of *ZmPP2CA10* was detected, and this deletion causes loss of ER stress induced expression of *ZmPP2CA10,* leading to increased plant drought tolerance. Our findings provide direct evidence linking ER stress signaling with drought tolerance, and the results from this study provide genetic recourses that can be directly used in breeding of drought tolerant maize cultivars.

Key words: drought response, clade A PP2C, natural variation, ER stress signaling, maize (*Zea Mays*)

Ubiquitin receptor OsDSK2a regulates salt tolerance through affecting gibberellin metabolism in rice

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Increasing investigations reveal that ubiquitin-proteasome system plays important roles in modulating plant growth and abiotic stress response. And ubiquitin receptors, UBL/UBA (ubiquitin-like/ubiquitin-associated), play a role in protein degradation through ubiquitin-proteasome pathway in yeast and human, however, the regulatory function of UBL/UBA proteins in rice is largely unclear. To address the roles of UBL/UBA proteins in rice salt response, genes encoding UBL/UBA proteins were identified from rice genome on the basis of bioinformatics. And the loss of function mutant *osdsk2a*, one of UBL/UBA genes, displays the increase of salt tolerance in seedling, indicating that OsDSK2a may be an important regulator for rice salt response. Importantly, OsDSK2a can be combined with polyubiquitin and interacted with RPT4, a subunit of 19S lid in the ubiquitin proteasome, indicating that OsDSK2a is typical UBL/UBA protein as ubiquitin receptor that might be involved in the regulation of protein degradation. To reveal the regulation of OsDSK2a in rice salt tolerance, further studies in our laboratory identified about 290 possible targets of OsDSK2a through SWATH (non-labeled proteomic analysis), including EUI (elongated uppermost internode), which is a regulator that greatly affects plant height development through deactivation of gibberellins. Further investigations reveal that OsDSK2a interacts with EUI. More importantly, the knockout mutants of EUI decreased the tolerance to salt, evidencing that the interaction of OsDSK2a and EUI modulates salt response. Meanwhile, the semi-dwarf phenotype of *osdsk2a* mutant was recovered by the application of GA_3, further demonstrating the role of OsDSK2a in gibberellin metabolism. Thus we speculate that the modulation of OsDSK2a in plant salt response might be mediated by the metabolism of gibberellins. These results unravel the role of UBL/UBA protein OsDSK2a in the control of the plant salt tolerance through gibberellin metabolism.

Key words: ubiquitin receptor, OsDSK2a, salt tolerance, gibberellin, protein degradation

Ethylene signaling in rice

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Ethylene as a gas phytohormone plays significant roles in the whole life cycle of plant, ranging from growth and development to stress responses. A linear ethylene signaling pathway has been established in dicotyledonous model plant *Arabidopsis*. The ethylene signaling mechanism in rice is limited. Based on the distinct phenotype of root inhibition but coleoptile promotion in rice etiolated seedlings upon ethylene treatment, we isolated a set of rice ethylene-response mutants (*mao huzi, mhz*) and identified the corresponding genes through map-based cloning. Among these, MHZ7 and MHZ6 are similar to EIN2 and EIN3 in Arabidopsis. MHZ6/OsEIL1 and OsEIL2 regulate ethylene responses of roots and coleoptiles respectively. MHZ7, MHZ6/OsEIL1 and OsEIL2 negatively affect salt tolerance in rice seedlings. We further revealed novel interactions between ethylene and ABA in regulation of root and coleoptile growth. Ethylene can induce expression of MHZ5 (carotenoid isomerase) and MHZ4 (OsABA4) gene and drive the metabolic flux from carotenoid biosynthesis pathway to the ABA biosynthesis pathway to affect root growth. A few novel genes have been further identified and the possible functions were investigated and the relevant mechanism was discussed. Manipulation of the corresponding genes may help to improve stress tolerance and other agronomic traits in rice and/or other crops.

Key words: ethylene, rice, roots, signaling, stress response

Spatio-temporal analysis for phosphatidic acid at plasma membrane in plant cells using a FRET-based biosensor

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Phosphatidic acid (PA) is a structurally simplest membrane phospholipid, which plays key roles in membrane trafficking, stress responses and cytoskeletal dynamics. However it remains unknown about spatio-temporal distribution of PA produced in living plant cells. Here we generated PA biosensor PPB (Plant Phosphatidic acid Biosensor) to determine PA changes at plasma membrane using Förster resonance energy transfer (FRET) approaches. A PA-binding domain of RbohD (0-250 amino acids) was sandwiched with the cyan fluorescent protein and yellow fluorescent protein and was tagged with the plasma membrane-targeting sequence of K-Ras4B-CT. The transgenic lines with PPB vectors were generated for both wild-type and *pldα1 Arabidopsis*. The cell imaging of PA dynamics in transgenic *Arabidopsis* plants was performed using confocal laser scanning microscopy. Using these toolkits, we identified temporally distinct PA responses to different lipids, NaCl, and ABA. The PPB biosensor also revealed the different accumulation of PA between WT and *pldα1* in these processes. In conclusion, we have set a method to detect spatial and temporal characteristics of PA signatures in plant cells.

Key words: plasma membrane, PA biosensor, FRET, spatio-temporal dynamics,

作物疫病菌效应子研究进展与育种应用探索

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疫霉属(*Phytophthora*)病原菌有 120 余种，寄主广，所致病害蔓延速度快且易爆发成灾，因此生产上将其统称为作物“疫病”。在互作过程中疫霉菌产生近千种效应子，它们不但是侵染寄主引起病害的重要武器，而且能被寄主抗病基因识别诱导抗病，还能通过分子变异逃脱识别导致作物抗病性丧失和病害爆发成灾,因此研究其功能与作用机制有助于揭示病原菌致病和植物抗病机理，开发病害控制新技术。我们将汇报以下几个方面的研究进展：

1. 疫霉菌效应子的转运途径与机理。疫霉菌有 RxLR 和 CRN 两类效应子在寄主胞内起作用，其穿透寄主细胞膜的转运过程一直是领域内关注的重要科学问题，但进展缓慢。我们近期发现疫霉菌可能合成三磷酰肌醇，结合效应子，介导其嵌合到细胞膜表面，促进转运过程。

2. 致病关键效应子的鉴定和毒性作用机理。疫霉菌有近千个效应子，尽管功能上存在冗余，但目前已明确多个效应子对致病不可或缺，它们作用于寄主靶标(蛋白质或 DNA)，利用不同的生化机制干扰寄主的免疫反应。

3. 无毒效应子的克隆和 ETI 发生机制。疫霉菌与其寄主的互作符合基因对基因学说，克隆无毒效应子是明确寄主 ETI 发生机制的前提，我们发展了基于 RxLR 效应子序列特征的克隆方法，获得了一批疫霉菌无毒效应子，开发了其田间变异规律的检测方法。

4. 基于效应子的作物疫病控制技术。在掌握效应子转运，作用机制，以及无毒效应子变异规律的基础上，提出和发展了作物疫病控制的多种新技术和方案。

大丽轮枝菌致病机理和棉花抗黄萎病应用研究

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大丽轮枝菌是一种土传病原真菌，通过侵染植物的根进入维管束定殖，在世界范围内引起严重的黄萎病害；在我国，棉花黄萎病素有“棉花癌症”之称。由于大丽轮枝菌从根部侵染，侵染时是否发育侵染结构一直存在争议，其侵染过程的调控机制更是不清楚。我们对大丽轮枝菌的侵染结构和侵染机制进行研究，鉴定了大丽轮枝菌侵染结构—附着枝—的存在，发现少量大丽轮枝菌在紧密接触到宿主根表皮细胞时分化形成顶端膨大的菌丝分枝（即附着枝），并发育出侵染钉；进一步实验发现依赖于四跨膜蛋白 tetraspanin VdPls1 的 NADPH 氧化酶 VdNoxB 所产生活性氧是侵染结构发育和穿透植物根所必需的。我们的结果证明了附着枝特异的依赖于 VdNoxB/VdPls1 的活性氧-钙离子信号途径是调控大丽轮枝菌侵染结构发育的重要途径。

大丽轮枝菌在土壤中形成微菌核存活时间长，化学农药难于防治，严重制约我国棉花生产。利用寄主诱导基因沉默（HIGS）技术，我们证明了棉花产生的小 RNA 能够进入菌丝体诱导目标基因沉默；利用纯和转基因株系通过新疆实地病圃抗病检测，证明了 HIGS 在棉花抗黄萎病上应用的可行和有效性。我们的结果将为培养抗棉花黄萎病、解决实际生产中棉花缺乏抗病资源的困境开拓新的策略和研究方向。

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The orosomucoid proteins contribute to sphingolipid homeostasis and stress responses in *Arabidopsis*

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Serine palmitoyltransferase (SPT), a pyridoxyl-5′-phosphate-dependent enzyme, catalyzes the first and rate-limiting step in sphingolipid biosynthesis. In humans and yeast, the orosomucoid proteins (ORMs) negatively regulate SPT and thus play an important role in maintaining the levels of sphingolipids. Despite the importance of sphingoid intermediates as bioactive molecules, the regulation of sphingolipid biosynthesis through SPT is not well understood in plants. In the present study we identified and characterized the *Arabidopsis thaliana* ORMs, AtORM1 and AtORM2. Loss-of-function of both *AtORM1* and *AtORM2* (*orm1 amiR-ORM2*) stimulated *de novo* sphingolipid biosynthesis, leading to strong sphingolipid accumulation, especially of long-chain bases and ceramides. Yeast two-hybrid, bimolecular fluorescence complementation, and coimmunoprecipitation assays confirmed that ORM1 and ORM2 physically interact with the small subunit of SPT (ssSPT), indicating that ORMs inhibit ssSPT function. We found that *orm1 amiR-ORM2* plants exhibited an early-senescence phenotype accompanied by H_2O_2 production at the cell wall and in mitochondria, active vesicular trafficking, and formation of cell wall appositions. Strikingly, the *orm1 amiR-ORM2* plants not only showed increased the expression of genes related to ER stress and defenses, but also enhanced resistance to oxidative stress and pathogen infection. Our results indicate that AtORMs function to regulate sphingolipid biosynthesis in maintaining sphingolipid homeostasis and play a role in response to abiotic and biotic stresses.

Key words: orosomucoids (ORMs), early senescence, sphingolipids, ER stress, plant defense

Biosynthesis of flavonoids protects wheat from powdery mildew infection

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Biogenic flavonoids were widely reported in wheat resistance to fungal pathogens with some of them having antimicrobial functions in other cereal crops. However, in wheat, their specific functions have not yet been defined. In this study, we built a spring Durum wheat EMS mutant library and screened for mutants with altered flavonoids contents. Mutants with both significantly increase or decrease flavonoids were isolated firstly by an intact high-through-put screening. These mutants were then verified by a colorimetric method. HPLC analyses showed a wide array of changes in flavonoids profiles, such as increase or decrease in levels of most compounds, addition or disappearance of one group of compounds. The change in flavonoids was most likely independent of lignin deposition in the cell wall, as over 70% of the high flavonoids mutants are indistinguishable from control plants in their total lignin contents. Unexpectedly, more than 85% of the mutants with increased flavonoids had less powdery mildew in field, while 2/3 mutants with decreased flavonoids had more powdery mildew than control plants, suggesting a negative correlation between flavonoids contents and powdery mildew growth rates *in planta*. In the Pearson correlation analysis, 3 flavonoids compounds displayed very significant positive correlation with powdery mildew resistance, with luteolin 6-*C*-glucoside as a potent protective compound *in planta*. Taken together, our data demonstrate that flavonoids play an important role in wheat against powdery mildew, and therefore, these mutants with high flavonoids contents are excellent genetic resources for fungal resistant wheat breeding.

Key words: durum wheat, EMS mutant, flavonoids, lignin, powdery mildew, luteolin

OsLRR-RLK1，水稻与害虫互作过程中的一个早期调节因子

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富含亮氨酸类受体蛋白激酶（Leucine-rich repeat receptor-like kinases, LRR-RLKs）是植物类受体蛋白激酶中最大的一个亚家族，通过感受与传递各种内部与外部环境信号，在调控植物的生长、发育以及逆境反应中发挥着重要作用。然而，有关 LRR-RLKs 在植物虫害诱导防御反应中的作用还所知甚少。我们在水稻中克隆了一个新的定位于细胞质膜的 LRR-RLK 基因 *OsLRR-RLK1*, 其转录水平受二化螟（the striped stem borer, SSB; *Chilo suppressalis*）为害诱导但却不受褐飞虱（the brown planthopper, BPH; *Nilaparvata lugens*）为害的影响。OsLRR-RLK1 作用于促细胞分裂原活化蛋白激酶（mitogen-activated protein kinase, MPK）上游；沉默 *OsLRR-RLK1*降低水稻中 SSB 诱导的3个 MPKs 和6个 WRKY 转录因子的转录水平以及茉莉酸（jasmonic acid, JA）、茉莉酸-异亮氨酸偶联物（jasmonoyl-isoleucine, JA-Ile）和乙烯（ethylene, ET）含量，并由此导致水稻胰蛋白酶抑制剂（trypsin protease inhibitors）活性以及对 SSB 抗性的下降。另一方面，沉默 *OsLRR-RLK1*显著提高 BPH 诱导的3个 MPK 和3个 WRKY 转录因子的转录水平，增加水稻中 BPH 诱导的 JA、JA-Ile、水杨酸（salicylic acid, SA）、ET 和 H_2O_2含量，并增强对 BPH 的抗性。这些研究结果表明，*OsLRR-RLK1* 是水稻诱导防御反应中的一个早期识别与调节元件，可以通过调节 MPK 级联途径、WRKY 转录因子以及防御相关的 JA、JA-Ile、SA、ET 和 H_2O_2等信号转导途径，调控水稻对不同害虫为害作出特异性的响应。

关键词：水稻，害虫，富含亮氨酸类受体蛋白激酶，防御反应，防御相关信号途径

叶绿体信号转导

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叶绿体不仅是植物进行光合作用的场所，还是脂肪酸、维生素、氨基酸、四吡咯、赤霉素等重要化合物的合成场所。高等植物的叶绿体被认为是经内共生演化而来。在内共生过程中，叶绿体自身的大部分基因转移到核基因组，只有不到 100 个基因被保留下来，因此叶绿体要维持正常的发育和光合功能需要细胞核与叶绿体基因组之间的协调。叶绿体也可以将自身的发育和代谢状况反馈给细胞核而调节核基因组编码的叶绿体定位的光合和胁迫相关基因的表达，从而来维持叶绿体光合功能和发育代谢状态的正常进行，这种信号的传递被称为质体信号。质体信号通过协调细胞核与叶绿体基因表达调控植物多种生长发育过程，包括光形态建成、叶片发育等。近些年来，对质体信号源的研究已经取得了相当大的进展。但是目前为止，对质体信号分子的本质以及它究竟是在细胞内如何传递仍然是不清楚的。不仅如此，质体信号如何协调外界环境信号和内源信号共同调控植物生长发育的分子机制还不清楚。我们主要介绍质体信号激活叶绿体被膜结合的转录因子 PTM，其剪切后转移到细胞核激活 AP2 类转录因子 ABI4 的转录（Sun et al. Nature Comm, 2011）；研究还发现质体信号参与瞬时细胞质钙震荡，促进钙结合蛋白 14-3-3ω 和 MKK4/MKK5-MPK3/MPK6 的互作引起 MPK3/MPK6 的被高效磷酸化进而磷酸化 ABI4。（Guo et al. Nature Comm, 2016）这样， ABI4 转录水平和翻译后磷酸化水平的双重激活 ABI4 导致 *Lhcb* 的抑制。进一步研究揭示质体信号和光信号等交互作用调控光形态建成（Xu et al. Nature Plants, 2016）和调节高光下开花（Feng et al. PNAS, 2016）等发育过程的分子机制。

关键词：质体**信号**，生长发育，信号转导，叶绿体

A rice novel gene *ALBL* encoding a PPR protein functions in plastid gene translation

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Plastid gene translation is carried out in its ribosome which RNAs undergo posttranscriptional processing. Several pentatricopeptide repeat (PPR) proteins are involved in the processes. However, the mechanism underlying the regulatory mRNA processing for plastid ribosome genes remains to be elucidated. Here, we report that a rice PPR protein ALBL plays crucial role in plastid gene expression by participating in the maturation of the precursor of plastid 23S-4.5S rRNA. We isolated *ALBL* gene from a rice (*Oryza sativa*) mutant, nearly *albino phenotype* (*albl*) by map-based cloning method, and identified ALBL as a novel nucleus-encoded PPR protein with a C-terminal DYW domain, and localized to the chloroplast. Mutation of *ALBL* resulted in the dramatic decrease of photosynthetic proteins and abnormal chloroplasts in the seedling, plastid ribosome deficiency. *albl* was defected the maturation of 23S-4.5S rRNA, while significantly increased in the amount of its precursor transcripts. Based on these results, we propose that ALBL is required for plastid gene expression, participating in the maturation process of 23S-4.5S rRNA.

Key words: PPR protein, rRNA processing, plastid rRNA, chloroplast

The plastid terminal oxidase is at the center balancing the redox state of thylakoid membrane

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Mitochondria possess oxygen-consuming respiratory electron transfer chains (RETC), and the oxygen-evolving photosynthetic electron transfer chain (PETC) resides in chloroplasts. However cyanobacteria harbor both RETC and PETC on their thylakoid membranes. It is proposed that chloroplasts could possess a RETC on the thylakoid membrane, in addition to PETC. Identification of a plastid terminal oxidase (PTOX) in the chloroplast from the Arabidopsis variegation mutant *immutans(im)* demonstrated the presence of a RETC in chloroplasts, and the PTOX is the committed oxidase. PTOX is distantly related to the mitochondrial alternative oxidase (AOX), which is responsible for the CN-insensitive alternative RETC. Similar to AOX as a ubiquinol oxidase, PTOX is a plastoquinol (PQH2) oxidase on the chloroplast thylakoid membrane.

Lack of PTOX, Arabidopsis *im* showed a light-dependent variegation phenotype; and mutant plants will not survive the mediocre light intensity during its early development stage. PTOX is involved in carotenoid biosynthesis, and the phytoene desaturation is blocked in the white sectors of Arabidopsis *im* mutant. PTOX is a stress-related protein, and can protect plants from various environmental stresses, especially high light stress. PTOX also plays significant roles in chloroplast development and plant morphogenesis.

Physiological roles played by could be a direct or indirect consequence of its PQH2 oxidase activity to maintain the PQ pool redox state on the thylakoid membrane. The PTOX- dependent chloroplast RETC (so called chlororesipration) does not contribute significantly when chloroplast PETC is normally developed and functions well. However, PTOX-mediated RETC could be the major force to regulate the PQ pool redox balance in the darkness, under conditions of stress, in non-photosynthetic plastids, especially in the early development from proplastids to chloroplasts.

Key words: photosynthesis, plastid terminal oxidase (PTOX), plastoquinone (PQ) pool, variegation, carotenoid biogenesis

衣藻叶绿体 CPN60 晶体结构解析与亚基功能分化研究

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分子伴侣是维持生物体内蛋白稳态的重要分子家族。它们参与生命体的发育、生长、成熟的各个阶段，辅助蛋白折叠组装、跨膜运输、指导降解等。分子伴侣素（Chaperonin）是最早发现的分子伴侣之一，由分子量 60 kD 的亚基组装为双环桶状寡聚体，中央空腔用于容纳底物蛋白。依据亚基在桶状结构中的空间位置，每个亚基可分为赤道区，中间区和顶端区三个结构域。叶绿体分子伴侣素（Cpn60）辅助折叠光合作用固碳关键酶 Rubisco，由 α 和 β 两种类型组成，而且，在一个物种中每种类型包含多个亚基。莱茵衣藻（*Chlamydomonas reinhardtii*）含有 CPN60α，CPN60β1 和 CPN60β2 三种亚基。对同源寡聚体 CPN60β1 生化功能的分析表明，CPN60β1 在 CPN20 或线粒体 Hsp10 帮助下，可以行使分子伴侣功能。我们首次解析了叶绿体 CPN60β1 晶体结构，分辨率达 3.8 Å。与真细菌的 GroEL 对比发现，CPN60β1 空腔直径多出 6 Å，亚基排列疏松及 ATP 结合口袋更宽，其整体分子构象类似于 GroEL 的变构态。分析结构域交换重组嵌合体显示，赤道区决定了寡聚体的形成和结构稳定性，而且亚基间的协作依赖于 CPN60α 的赤道区和部分中间区，且这种协作的发生高度依赖 CPN60α 的第 461 位谷氨酸。进一步对亚基间的分工研究发现，顶端区不仅参与决定了分子伴侣素的 ATP 酶活差异，且 CPN60α 和 CPN60β 顶端区在底物结合能力和辅伴侣互作两方面都存在显著差异。高分辨率晶体结构分析 CPN60α 和 CPN60β1 两种类型的顶端区，发现 203，235 和 241 三个氨基酸位点影响其功能分化。分子伴侣结构的解析将为我了解其折叠 Rubisco 提供理论基础。

关键词：Rubisco，碳反应，分子伴侣，蛋白结构，莱茵衣藻

Dissecting the genetic basis and signaling network of shade avoidance response in *Arabidopsis* and maize

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Increasing the planting densities has been used as an effective approach for increasing crop yield per unit land area. However, plants compete with neighboring vegetation for light when planting at high densities. The shade of nearby vegetation reduces the ratio of red to far-red light, triggering a series of responses known collectively as shade avoidance syndrome (SAS): including accelerated elongation of stems, elevated leaf angles to the horizontal, reduced branching and early flowering. Although the SAS is thought to provide an adaptive advantage by increasing a plant′s ability to compete for limited resources in natural settings, it is often accompanied by reduced investments in other organs such as roots and leaf blades, and other potentially deleterious effects on a plant′s fitness, disease resistance, and yield.

Over the past few decades, much has been learned about the genetic networks regulating SAS in the model dicotyledenous plant Arabidopsis thaliana. It has been shown that in Arabidopsis, SAS is mainly controlled by the red/far-red photoreceptor-phytochrome B (phyB), and that in the light (high R/FR), photoactivated phyB represses hypocotyl and petiole growth by targeting a group of phyB-interacting PIF proteins (PIF1, PIF3, PIF4 and PIF5, bHLH transcription factors) for proteolytic degradation, whereas shade environments (low R/FR) induce a rapid increase in PIF protein abundance, which promotes shade-induced growth. Moreover, recent studies documented that members of the SQUAMOSA-PROMOTER BINDING PROTEIN-LIKE (SPL) family of transcription factors have emerged as pivotal regulators of divergent important biological processes in plants, including the timing of vegetative and reproductive phase change, leaf development, tillering/branching, plastochron, panicle/tassel architecture, fruit ripening, fertility, and response to stresses. The transcripts of a subset of SPLs are targeted for cleavage and/or translational repression by microRNA156s (miR156s). Our preliminary studies suggested that phyB mediated SAS may be converged with the miR156/SPL pathway to regulate different aspects of SAS in both Arabidopsis and maize. Current undergoing work is to substantiate the working model that phyB regulates SAS through the PIF-miR156-SPL genetic pathway in both *Arabidopsis* and maize. Our results may facilitate the breeding of shade-tolerant maize by attenuation or refinement of SAS in maize.

Key words: phytochrome, shade avoidance syndrome, PIFs, SPLs

Molecular mechanism for plant seedling emerging from the soil

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Low The survival of seed plants in natural environments requires the successful emergence from the soil. In this process, the ethylene signaling pathway is utilized by plants to sense and respond to the mechanical resistance of the soil. Here, we report that CONSTITUTIVE PHOTOMORPHOGENESIS 1 (COP1), a central repressor of light signaling, is a key component required for seedlings to sense the depth of soil overlay. Mutation in COP1 causes severe defects in penetrating soil, due to decreased level of EIN3, a master transcription factor in ethylene pathway that mediates seedling emergence. We show that COP1 directly targets the F-box proteins EBF1 and EBF2 for ubiquitination and degradation, thus stabilizing EIN3. As seedlings grow towards the surface, the depth of soil overlay decreases, resulting in a gradual increase of light fluences. COP1 channels the light signals while ethylene transduces the information on soil mechanical conditions, which cooperatively control EIN3 protein levels to promote seedling emergence from the soil. The COP1-EBF1/2-EIN3 module reveals a mechanism by which plants sense the depth to surface and uncovers a novel regulatory paradigm of an ubiquitin E3 ligase cascade.

Key words: seedling emergence, COP1, ethylene signaling, phyB, EBF1 and EBF2, EIN3/EIL1

Exploring the diversity of crop metabolism

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Plants produce a vast array of chemically and biologically different compounds. These compounds are not only important for plant themselves and their interactions with the environment, but also provide indispensable resources for humans as sources for nutrition, energy, and medicine. Understanding the genes involved in metabolism and dissection of the metabolic pathway are essential to improve plant adaptation to environmental stresses, to improve food quality, and to increase the yield of major crops such as rice and maize through metabolomics-based breeding. Here we report the genetic studies of crop metabolomes combining both metabolic quantitative trait locus (QTL) mapping and metabolic genome-wide association study (GWAS). Hundreds of loci with high resolution and large effects were uncovered. Data mining revealed a large number of candidate genes underlying metabolites that are of physiological and agronomical importance. Our forward-genetics-based strategy provides a powerful tool for large-scale gene-metabolite annotation and identification, pathway elucidation and knowledge about crop improvement.

Key words: metabolites, metabolic diversity, genetics, major crops, crop improvement

苔类植物黄酮类化合物生物合成途径系统解析

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苔藓是植物由水生向陆生、低等向高等演化的重要过渡(先锋)类群,合成丰富的次级代谢产物。苔类植物是最早产生黄酮的植物，虽然高等植物黄酮生物合成途径已经研究清楚，但是在苔类植物中尚无该方面的研究。我们对 6 种苔类植物进行了转录组测序，从中克隆鉴定了苯丙氨酸裂解酶 (L-phenylalanine ammonia-lyase, PAL)、桂皮酸-4 羟化酶 (cinnamic acid 4-hydroxylase, C4H)、4-羟基肉桂酰辅酶 A 连接酶 (*p*-coumarate CoA ligase, 4CL)、查尔酮合酶 (chalcone synthase, CHS)、查尔酮异构酶 (chalcone isomerase, CHI)、黄酮合酶 (flavone synthase, FNS)和黄酮甲氧基转移酶 (flavonoid *O*-methyltransferase, FOMT)等关键酶，系统解析了苔类植物黄酮类化合物生物合成途径。发现苯丙氨酸裂解酶、桂皮酸-4 羟化酶、4-羟基肉桂酰辅酶 A 连接酶和查尔酮合酶与高等植物中的酶序列保守，功能类似。首次发现苔类植物含有以前认为仅存在于豆科植物的黄酮生物合成途径，包括 II 型查尔酮异构酶和查尔酮还原酶。提出植物黄酮生物合成途径的演化起源过程中，II 型 CHI 基因出现早，高等植物通过基因复制产生 I 型 CHI 基因，而且丢失了 II 型 CHI 基因，只有豆科植物同时保留 I 型和 II 型 CHI 基因。首次证明苔类植物的 I 型黄酮合酶 (FNSI)有脱氢和羟基化的双功能。对苔类植物黄酮甲氧基转移酶的研究发现甲氧基转移酶具有底物广谱性，可作为工具酶实现黄酮类化合物特定位置羟基上的甲氧基化。

关键词：苔类植物，黄酮，查尔酮异构酶，黄酮合酶，甲氧基转移酶

The nitrate transporter NRT1.5/NPF7.3 functions as a H^+/K^+ antiporter for K^+ loading into the xylem in *Arabidopsis*

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Potassium and nitrogen are essential macronutrients for plant growth and crop yields. Although the previous studies have indicated that the absorption and translocation of K^+ and NO_3^- are correlated each other, the molecular mechanism for the coordination between K^+ and NO_3^- transport remains unknown. In this study, using a forward genetic approach, we isolated a low-K^+-sensitive *Arabidopsis* mutant *lks2* that exhibits a defect in K^+ translocation from root to shoot. Under low-K^+ conditions, the K^+ contents in *lks2* shoot were reduced, while the K^+ contents in *lks2* root were significantly increased compared with wild-type plants. Map-based cloning revealed that *LKS2* encodes the nitrate transporter NRT1.5/NPF7.3, a member of the NRT1/PTR family. Similar to K^+, the NO_3^- translocation from root to shoot was also impaired in *lks2* mutant. It is suggested that NRT1.5 not only participates in NO_3^- translocation but also regulates K^+ transport from root to shoot. Using *Xenopus* oocytes, we confirmed that nitrate transporter NRT1.5 can also act as an H^+/K^+ antiporter and directly mediates K^+ release out of cells. In this study, our results demonstrate that NRT1.5 functions as a dual-role transporter for K^+ and NO_3^- loading from root parenchyma cells into the xylem in *Arabidopsis* root, as well as serving as a key coordinator for K^+/NO_3^- distribution in plants.

Key words: NRT1.5, K^+ transporter, NO_3^- transporter, xylem loading, *Arabidopsis thaliana*

拟南芥 *NRG2* 基因调控 NO_3^- 信号的研究

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氮素是植物生长发育所必需的大量元素之一。研究植物吸收利用 NO_3^- 的规律和机制，对于有效提高作物的氮素利用率具有重要的理论指导意义。本研究通过正向遗传学的方法克隆了一个新的 NO_3^- 调控基因 *NRG2*。研究发现，*nrg2* 突变体中 NO_3^- 响应基因的诱导量明显受到抑制，全苗和根中的 NO_3^- 含量显著低于野生型，通过进一步研究发现突变体中 NO_3^- 含量的变化可能主要与 *NRT1.1* 基因表达量降低及 *NRT1.8* 基因表达量增加有关。分子和遗传学分析发现，在 NO_3^- 信号调控通路中 *NRG2* 位于 *NRT1.1* 基因的上游并调控 *NRT1.1* 的表达。转录分析结果表明，*nrg2* 突变体中有 4 个与氮素响应、转运等有关的基因簇发生了改变，多个与氮素相关的下游基因受到 *NRG2* 与 *NRT1.1* 的共同调控，进一步表明 *NRG2* 与 *NRT1.1* 在同一条通路中调控 NO_3^- 信号。另外，研究发现 NRG2 与 NLP7 在细胞核中存在互作关系。

拟南芥中 NRG2 家族共有 15 个成员，都含有 DUF630 和 DUF632 两个未知功能的结构域。对该家族的 *NRG2.10* 和 *NRG2.15* 基因的研究结果表明，这两个基因的突变体中 NO_3^- 响应基因的诱导量同样受到抑制，根中 NO_3^- 的含量与野生型相比显著降低，说明 NRG2 的家族成员可能在 NO_3^- 信号通路的调控中也发挥重要作用。

关键词：拟南芥，硝态氮信号，调控基因，NRG2，NRT1.1

Mechanisms of heavy metal accumulation in rice

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Approximately one fifth of the agricultural soils in China is contaminated, mainly by heavy metals or metalloids such as cadmium (Cd) and arsenic (As), which are the class-one carcinogens. Rice produced in some areas of southern China often exceeds the maximum permissible limits of Cd and As, posing a significant risk to food safety. Contamination in soil and irrigation water, soil acidification and growing cultivars with a high accumulation ability are the main reasons for high levels of heavy metals in rice grain. There are large genetic variations among rice cultivars and germplasms in both grain Cd and As concentrations. OsHMA3 is a Cd transporter on the tonoplast functioning to sequester Cd into the vacuole of rice roots. We have recently identified a new loss-of-function allele of *OsHMA3* associated with high Cd accumulation in rice grain. This allele has a predicted amino acid mutation at the 380th position from Ser to Arg and is present only in some Japonica cultivars. Cultivars possessing this allele have markedly increased root to shoot translocation of Cd. Paddy rice also accumulates As because of the elevated availability of As in flooded paddy soil. We have previously discovered that arsenite is taken up by silicon transporters in rice. In contrast, arsenate is taken up by phosphate transporters. Plants are able to reduce arsenate to arsenite efficiently and to extrude arsenite to the external medium, thus avoiding excessive buildup of As in plant tissues. We have recently characterized two arsenate reductases in rice (OsHAC1;1 and OsHAC1;2). Mutations in the two genes result in decreased arsenate reduction and arsenite efflux, and increased As accumulation in rice plants. Overexpression of either gene increases arsenate reduction and arsenite efflux, leading to lower As accumulation in rice plants and increased tolerance to arsenate.

Key words: arsenic, cadmium, food safety, heavy metal contamination, rice

微滴式数字 PCR 技术及其在植物学研究中的应用

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微滴式数字聚合酶链式反应（ddPCR）采用数字化终点检测方式和分子计数的手段，实现对核酸分子的准确定量检测，无需标准曲线和参照，对影响 PCR 效率的抑制物不敏感，大大提高了检测灵敏度、精确度、准确度和重复性，并实现了真正意义上的绝对定量。在植物学研究领域，ddPCR 可用于基因表达分析，包括编码或非编码 RNA 的绝对定量，mRNA 剪接体定量；拷贝数多态性分析（CNV）；低丰度稀有序列的定量检测；甲基化程度分析；病原微生物检测，病理学研究，转基因研究等。ddPCR 可为植物学研究提供有力的实验技术和方法学的支持。

GAD1 控制水稻穗粒数、粒长及芒的发育

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亚洲栽培稻（*Oryza sativa* L.）是从普通野生稻（*Oryza rufipogon* Griff.）驯化而来的，在驯化过程中，形态性状和生理特性上都发生了巨大的变化。野生稻通常拥有较少的每穗粒数，籽粒较长，且籽粒顶端长有长芒。而栽培稻每穗粒数较多，籽粒较短，且籽粒顶端无芒或者短芒。这些性状的改变都是水稻驯化过程中的重要事件。我们在普通野生稻（W2014）与无芒的籼稻品种 93-11（NA9311）构建的渗入系中，筛选到了一个具有长芒的渗入系（OIL31），该系还表现较少的穗粒数和较长的籽粒。利用 OIL31 与轮回亲本 NA93-11 构建的分离群体，采用图位克隆技术，克隆了控制穗粒数、粒长及芒的发育基因 *Grain number, grain length and Awn Development 1*（*GAD1*）。该基因位于水稻第 8 染色体长臂，编码一个预测的富含半胱氨酸的小分子分泌肽，并与拟南芥中的 EPIDERMAL PATTERNING FACTOR-LIKE（EPFL）家族有较高的同源性。GAD1 蛋白在 N 端具有一个信号肽位点，其成熟肽在 C 端具有保守的半胱氨酸残基。栽培稻中 *gad1* 基因在编码区的移码突变破坏了保守半胱氨酸结构，导致了功能丧失，使穗粒数增加，籽粒变短，芒的发育受阻。序列分析表明，该移码突变导致的保守半胱氨酸残基的结构的变化与栽培稻芒的有无高度相关。该基因在水稻驯化过程中受到了强烈的人工选择，并引起附近 ~900-kb 的基因组区域遗传多样性的剧烈下降。我们的研究表明，除了大部分已知的编码转录因子和酶的基因，*GAD1* 编码的小分子分泌肽参与了水稻驯化过程。*GAD1* 的克隆不仅揭示了信号肽分子在植物发育中的新功能，也为揭示水稻驯化的分子机制提供了新线索。

棉花纤维发育的分子机制解析

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棉花是关系到国计民生的重要经济作物。生产上种植的棉花主要是两个异源四倍体，即具有很好的生态适应性、丰产性好的陆地棉（95%面积）和只能在特定生态环境下种植、纤维品质优良的海岛棉（约占 5%面积）。我国只有南疆阿克苏地区有海岛棉种植，而大面积种植的是陆地棉。海岛棉的纤维品质好于陆地棉，有海岛棉改良陆地棉纤维品质是提升棉花生产品质的重要途径。我们以品质优良的海岛棉纤维发育机制研究为切入点，解析了棉花纤维伸长机制，克隆了优质纤维基因，创制了一批优质纤维材料。利用全基因组鸟枪法完成了海岛棉 3-79 基因组测序，拼接出的基因组大小为 2.45 Gb，绘制了高质量的基因组图谱，预测得到 80876 个编码蛋白质的基因。发现 Dt 亚基因组的 CesA 在纤维伸长期高量表达，然而在次生壁加厚期 At 亚基因组的 CesA 表达量占主导地位。基于此，提出海岛棉纤维发育过程中 “CesA 基因家族的接力赛模型”。基于基因组测序，利用 SmalI RNA 测序，总共检测到参与纤维发育的 47 个保守的 miRNA 家族和 7 个新的纤维特异的 miRNAs。特异抑制纤维细胞 miRNA156/157 的表达导致成熟纤维变短。通过整合高覆盖度的转录组数据鉴定了 30,550 个基因间区域的 lncRNA 位点和 4,718 个反义 lncRNA 位点。鉴定出突变体和野生型之间纤维起始期差异表达的 lncRNA，发现一对可能调控纤维的起始发育的长链非编码 RNA 是 miRNA397 的前体。多组学数据整合分析绘制出棉花纤维发育过程中 DNA 甲基化和染色质重塑的动态图谱，并且揭示了其对于细胞伸长和次生壁加厚的重要作用。基于海岛棉转录组数据，筛选了纤维发育相关基因，通过转基因棉花验证了基因功能。下调 GbPDF1 纤维起始和早期伸长滞后；适当上调转录因子 GbTCP 表达促进纤维伸长，其通过影响 JA 信号途径调控纤维发育，并发现合适浓度的 JA 促进纤维伸长；超表达小肽激素磺钠肽 PSK 上调 ROS 及改变 K^+促进纤维变长变细；发现第二信使 GhCaM7 上调活性氧 ROS 促进纤维的早期伸长；超表达海岛棉特异基因 GbEXPATR 促进纤维又细又长；抑制转基因棉花 GhF3H 的表达纤维变短，柚皮素（NAR）和二氢山柰酚（DHK）是抑制纤维发育的主要类黄酮，解释了彩棉品质差的分子机理。对这些重要的功能基因的解析，为棉花纤维品质改良提供了理论基础，并积累优质的育种材料。

miR172c-NNC1 module controls nodule number in soybean

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MicroRNAs (miRNAs) are noncoding RNAs that are master regulators of various biological processes by post-transcriptionally repressing their target genes. It has been shown that miRNAs are involved in nodulation in legumes, however, the molecular mechanism through which miR172 regulates nodulation remains elusive. Recently, we have demonstrated that miR172c is a central positive regulator of both rhizobia infection and nodule organogenesis in soybean. miR172c is induced by either compatible *B. japonicum* or lipo-oligosaccharide Nod Factor, and continues to be upregulated during nodule development. Alteration in miR172c results in dramatic changes in nodule initiation and nodule number. Furthermore, miR172c regulates nodule formation by repressing its target gene, *Nodule Number Control 1* (*NNC1*), which encodes a protein that directly targets the promoter of the early nodulin gene, *GmENOD40*. Moreover, we proved that miR172c functionally depends on *GmNFR1α/5α* and its activity is negatively regulated by autoregulation of nodulation (AON). Furthermore, we found that NNC1 directly binds to the promoter of the *RIC1* and *RIC2* genes that encode CLE peptides to activate AON. Intriguingly, NNC1 competes with Nodule Inception (NIN), the transcriptional activator of *RIC1/2*, for *RIC1/2* promoter binding to repress the expression of *RIC1/2*. Thus, we elucidate a miR172c-mediated regulatory mechanism that modulates soybean nodulation and identify a node that links NF and AON signaling pathways to fine tune ultimate nodule number in soybean.

Key words: soybean, nodulation, microRNA172c, NNC1

The soybean-specific maturity gene E1 family of floral repressors controls night-break responses through down-regulation of *FLOWERING LOCUS T* orthologs

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Photoperiodism is a rhythmic change of sensitivity to light, which helps plants to adjust flowering time accor to seasonal changes in day length and to adapt to growing conditions at various latitudes. To reveal the molecular basis of photoperiod is min soybean (*Glycine max*), a facultative short-day plant, we analyzed the transcriptional profiles of the maturity gene E1 family and two FLOWERING LOCUS T (FT) orthologs (FT2a and FT5a). E1, a repressor for FT2a and FT5a, and its two homologs, E1-like-a (E1La) and E1Lb, exhibited two peaks of expression in long days. Using two different approaches (experiments with transition between light and dark phases and night-break experiments), we revealed that the E1 family genes were expressed only during light periods and that their induction after dawn in long days required a period of light before dusk the previous day. In the cultivar Toyomusume, which lacks the E1 gene, virus-induced silencing of E1La and E1Lb up-regulated the expression of FT2a and FT5a and led to early flowering. Therefore, E1, E1La, and E1Lb function similarly in flowering. Regulation of E1 and E1L expression by light was under the control of E3 and E4, which encode phytochrome A proteins. Our data suggest that phytochrome A-mediated transcriptional induction of E1 and its homologs by light plays a critical role in photoperiodic induction of flowering in soybean.

Key words: photoperiodism, soybean, flowering, E1 gene

油菜高产油量形成的分子解析

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油脂是人类赖以生存的三大类营养素之一，维护食用油供给安全是国家安全战略的核心之一。但目前我国食用植物油自给率仅为 35%左右，严重威胁着食用油供给安全。我国油菜常年种植面积约 1.1 亿亩，菜籽油占国产油料作物产油量的 55%以上，是国产植物油的第一大来源。然而，与加拿大等菜籽出口国相比，我国油菜产业存在着产油量不高的问题，制约了其国际竞争力。因此，提高油菜产油量对维护国家食用油供给安全具有重要意义，而油菜高产油量的形成主要是由籽粒产量和种子含油量共同决定的。油菜油脂积累发生在胚胎，但胚胎的油脂合成和积累并非是孤立的事件，整个过程依赖于其它母体组织如角果皮和种皮等。课题前期遗传学实验也证实油菜含油量受母体器官和细胞质效应影响显著。课题重点分析了角果皮光合作用和种皮蔗糖转运对种胚含油量的贡献和作用机制；比较具有显著细胞质效应品系间的线粒体和叶绿体基因组序列差异，发掘出影响含油量的细胞质基因。油菜单株产量由单株角果数、每角果籽粒数以及粒重三个产量构成因子决定。课题利用千粒重差异显著的甘蓝型油菜品系 zy72360 和 R1 以及关联群体，通过连锁分析、关联分析、亲本序列比对和转基因验证，最终确定 *ARF18* 是调控粒重和角果长度的目标基因，该基因的过量表达可使粒重变异 15%，而角粒数保持不变。研究表明，*ARF18* 是调控生长素反应基因表达的一种转录因子，具有转录抑制活性。

关键词：油菜，含油量，千粒重，遗传调控

Molecular genetics of blood-fleshed peach reveals activation of anthocyanin biosynthesis by NAC transcription factors

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Anthocyanin pigmentation is an important consumer trait in peach (*Prunus persica*). In this study, the genetic basis of the blood-flesh trait was investigated using the cultivar Dahongpao, which shows high levels of cyanidin-3-glucoside in the mesocarp. Elevation of anthocyanin levels in the flesh was correlated with the expression of an R2R3 MYB transcription factor, *PpMYB10.1*. However, *PpMYB10.1* did not co-segregate with the blood-flesh trait. The blood-flesh trait was mapped to a 200-kb interval on peach Linkage Group (LG)5. Within this interval, a gene encoding a NAC domain transcription factor (TF) was found to be highly up-regulated in blood-fleshed peaches when compared with non-red-fleshed peaches. This NAC TF, designated BLOOD (BL), acts as a heterodimer with PpNAC1 which shows high levels of expression in fruit at late developmental stages. We show that the heterodimer of BL and PpNAC1 can activate the transcription of *PpMYB10.1*, resulting in anthocyanin pigmentation in tobacco. Furthermore, silencing the *BL* gene reduces anthocyanin pigmentation in blood-fleshed peaches. The transactivation activity of the BL-PpNAC1 heterodimer is repressed by a SQUAMOSA promoter-binding protein-like transcription factor, PpSPL1. Low levels of *PpMYB10.1* expression in fruit at early developmental stages is likely attributed to lower levels of expression of *PpNAC1*, plus the presence of high levels of repressors such as PpSPL1. We present a mechanism whereby *BL* is the key gene for blood-flesh trait in peach, via its activation of *PpMYB10.1* in maturing fruit. Partner TFs such as bHLHs and NAC1 are required, as are the removal of transcriptional repressors.

Key words: peach, anthocyanins, gene mapping, PpMYB10.1, PpNAC1

水稻白背飞虱抗性基因的遗传分析与育种利用

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水稻白背飞虱是远距离迁飞性害虫，自上世纪 80 年代以来，随着我国高产杂交稻技术的推广及应用，白背飞虱的为害已成为影响我国水稻稳产、高产的主要害虫之一。白背飞虱的为害引起水稻植株黄化、矮缩、甚至枯死，而导致出现大面积的 “虱烧”。我们从种质资源的筛选着手，结合建立的水稻白背飞虱抗性检测体系，鉴定出高抗白背飞虱的特异种质春江 06。通过构建 DH 群体、染色体片段置换系群体及 QTL 分析，克隆了水稻白背飞虱拒食抗性主效 QTL——*qSI4*，精细定位了水稻白背飞虱杀卵抗性主效 QTL——*qWL6*，分析了白背飞虱侵害水稻的全基因组表达谱，确定了部分候选基因群。同时，利用业已构建的分子育种技术平台，开展水稻白背飞虱抗性的育种改良。

Genetic mechanism of OsmiR396 regulates rice inflorescence architecture and grain yield

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How to increase rice productivity to meet the serious challenge of the world population expansion bothers breeders around the world. Here, we report that a new microRNA named miR396b was identified involving regulation of outgrowth and elongation of the secondary branches of rice. Over expression of miR396b led to aggregation of the inflorescence secondary branches and male sterility, expression of target mimicry of miR396 (MIM396) increased rice yield by modulating development of the secondary branch and the inflorescence architecture. MIM396 transgenic lines showed great increase in number of the secondary branches and spikelets, leading to an increase of yield up to 16.8% in a field trial. Growth regulation factor 6 (*GRF6*), was determined as the direct target of miR396b to mediate the function of miR396 in modulating rice yield. GRF6 coordinately regulated transcription factors *OsMADS34* and *OsTAWAWA1*, which are involved in the branch and spikelet development, and *OsYUCCA1*, *OsARF2, OsARF7* and *OsARF11*, which are related to AUX/IAA signaling. Our results suggest that miR396b/*OsGRF6* pathway act as a key player in shaping rice inflorescence architecture, and provide a novel way to engineer high yield rice.

Key words: rice, *OsmiR396*, *OsGRF6*, inflorescence architecture, grain yield

Engineering nitrogen use efficiency in wheat

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Developing wheat varieties with improved ability to use nitrogen (N) efficiently is very desirable, and may offer a sustainable solution to improve crop yields with less fertilizer application. Roots are the main site for nutrient uptake; their size and distribution in soil profiles, and uptake activity largely determine nutrient uptake efficiency. However, at seedling stage, low temperature inhibits root development of winter wheat and nutrient bioavailability in soils; and during grain filling root senescence is not able to meet the increased N demand of high yield potential of modern wheat varieties. Here, we developed transgenic wheat lines with improved NUE by increasing roots′ ability in acquiring N at seedling stage and N uptake after flowering. We showed that a nitrate-inducible NAC transcription factor TaNAC2-5A could directly bind to the promoter regions of the genes encoding nitrate transporter and glutamine synthetase (GS). Overexpression of *TaNAC2-5A* in wheat enhanced root growth and nitrate influx rate, and hence increase root ability to acquire nitrogen at seedling stage. Further, we found that *TaNAC2-5A*-overexpressing transgenic wheat lines had higher grain yield and higher nitrogen accumulation in aerial parts, and allocated more nitrogen in grains in a field experiment. We further analyzed the haplotypes of the plastic GS isoform (GS2) genes in wheat. The transgenic wheat lines of the favorable *TaGS2* haplotypes *TaGS2-A1bpro::TaGS2-A1b* had higher N uptake after flowering and grain yield than did the wild type under both low and high N conditions in field experiments.

Key words: *Triticum aestivum* L., NAC2, glutamine synthetase, nitrate uptake, lateral root, grain yield, grain nitrogen concentration, nitrogen use efficiency.

Gene duplication at the 27-kDa γ-zein locus underlies successful breeding of Quality Protein Maize

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Quality Protein Maize (QPM) has the potential to benefit millions of people in developing countries who consume maize as their sole protein source. To do this, the scientists have made great efforts to identify the genetic locus or gene responsible for the high amount of lysine in maize endosperm. More than half a century ago, Oliver Nelson and Edwin Mertz at Purdue University found that the maize *opaque2* (*o2*) mutant produces doubling of the endosperm lysine content, suggesting *o2* as a potential target gene for QPM breeding. However, breeding such a QPM hybrid based on *o2* takes longer than regular hybrids, primarily because of the complex and unknown components of *o2*-mediated endosperm modification pathway.

Previous studies have shown that enhanced expression of 27-kDa γ-zein in QPM is essential for endosperm modification. Taking advantage of genome-wide association study analysis of a natural population, linkage mapping analysis of a recombinant inbred line population, and map-based cloning, we identified a quantitative trait locus (*qγ27*) affecting the expression of 27-kDa γ-zein. *qγ27* was mapped to the same region as the major *o2* modifier (*o2* modifier1) on chromosome 7 near the 27-kDa γ-zein locus. *qγ27* resulted from a 15.26-kb duplication at the 27-kDa γ-zein locus, which increases the level of gene expression. The elevated level of 27-kDa γ-zein is very critical to facilitate the formation of numerous small protein bodies surrounding the starch granules, which is able to partially rebuild the proteinaceous matrix for restoration of kernel vitreousness in QPM. Thus, these findings not only improve our understanding of genetic variation and artificial selection in maize, but also provide a potential genetic resource for QPM breeding.

Carbon transfer sustains arbuscular mycorrhizal symbiosis

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Arbuscular mycorrhiza (AM) is an important and widespread symbiosis that forms between plant roots and soil fungi of the phylum Glomeromycota. The partnership is founded on a nutrient exchange that takes place through highly branched hyphal structures inside the plant root cells known as arbuscules. The plant receives inorganic nutrients and water absorbed by the mycelium extending into the soil, and in return provides the fungus with its only source of carbon. It is widely believed that the bulk of the carbon is supplied as sugars, transported across the periarbuscular and fungal plasma membranes. However, within the fungus lipids are the major carbon store and are transported throughout the mycelium. Here we show that the arbuscular mycorrhizal fungus *Rhizophagus irregularis* relies on its host plant to supply it with fatty acids via that activation of a specialised lipid export pathway. This pathway employs *REDUCED ARBUSCULAR MYCORRHIZATION2*, which encodes an *sn-2* regioselective glycerol-3-phosphate palmitoyltransferase/phosphatase from a land-plant-specific gene family, previously shown to be involved in cutin biosynthesis. Given the abundance of lipids in arbuscular mycorrhizal fungi, we suggest that the majority of the carbon provided by their host plant is supplied in the form of fatty acids, rather than sugars. Our findings have broad ramifications for understanding the physiology, ecology and evolution of one of the biosphere's most influential symbiotic relationships.

P1

Genome-wide SNP discovery and phylogenetic analysis in wheat using genotyping-by-sequencing

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Genotyping-by-sequencing (GBS) is a new powerful method for affordably acquiring dense genome wide marker data for large sample size populations and has been successfully utilized for genetic studies in a variety of species. In this study, 1637 wheat accessions from China and other countries were sequenced using GBS method. On average, 5,765,333 reads per line were generated, which is equivalent to ~0.02-fold coverage of the wheat genome for each accession. About 15,000 homogeneous SNPs were identified per line. Phylogenetic tree of all accessions inferred from SNPs showed that the collection mainly has six divergent groups, and the accessions with similar geographical origin or hybrid progenies from the same ancestors (such as progenies of *Agropyron elongatum*×*wheat*) usually cluster to the same group. These data shed light on the underlying relationship between different wheat accessions and provided phylogenetic reference for future potential breeding utilization.

Key words: Genotyping-by-sequencing (GBS), wheat, phylogenetic tree

P2

Genetic polymorphism among *Arabidopsis thaliana* mutants induced by carbon ion beam irradiations

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Arabidopsis thaliana is a member of crucifer family and has become an important model plant in plant sciences, which is widely used in genetics and molecular biology. As a novel mutagen, heavy ion beams play an important role in mutation breeding. In the present study, in order to explore the effects of heavy ion beam irradiations on *Arabidopsis thaliana*, the wild-type (ecotype *Columbia*, *Col*) and *A. thaliana* M_3 plants induced by carbon ion beam irradiations (with the energy of 43.3 MeV/u and linear energy transfer (LET) of 50 keV/μm) were used for analyzing genetic polymorphisms by inter-simple sequence repeat (ISSR) markers and random amplified polymorphic DNA (RAPD) markers. Compared with wild-type, either ISSR or RAPD patterns, the major forms of genetic polymorphism variations of the M_3 plants were appearance of new bands and disappearance of normal bands. The total polymorphism rates were 17.06% (ISSR) and 21.24% (RAPD) among M_3 plants, and the genetic similarity coefficient between the M_3 plants and wild-type distributed from 0.65 to 0.96 (ISSR) and 0.74 to 0.91 (RAPD). Dendrograms constructed by UPGMA method divided the genotypes into two clusters by ISSR and RAPD techniques, respectively. In addition, the connection between genetic polymorphism and phenotypic changes of carbon-ion beam induced *A. thaliana* mutants in laboratory conditions were investigated. In summary, the present results showed that carbon ion beam irradiations are efficient mutagens on account of its ability to induce heritable genetic polymorphism which lead to diversified mutations, and the results could provide theoretical foundations for irradiations mutation breeding to improve better genotypes and produce better varieties with higher yield or other characters.

Keywords: carbon ion beams, *Arabidopsis thaliana*, Inter-simple sequence repeat (ISSR), random amplified polymorphic DNA (RAPD), genetic polymorphism

P3

构建莲线粒体基因组图谱揭示其进化特征

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莲（*Nelumbo nucifera*）是冰期后子遗植物的代表之一。莲线粒体基因组序列测定与其基因组图谱的构建对研究基部双子叶植物的进化特征有重要意义。本研究利用第三代基因组单分子测序技术（SMRT）对中国莲的线粒体基因组进行了测序、组装和注释，得到了莲线粒体基因组序列和完整基因组图谱。结果表明：莲线粒体基因组总长 524,797-bp，GC 含量为 48.2%，共包含 63 个基因，其中包括 40 个蛋白编码基因，3 个 rRNA 基因(*rrn*5，*rrn*18，*rrn*26)，以及 20 个 tRNA 编码基因，其中 13 个来自线粒体自身编码的 tRNA，7 个是叶绿体起源的 tRNA。对线粒体基因组特征分析，表明莲线粒体基因组存在 8 个大的重复片段,占总基因组的 9.3%，包括 4 个正向重复序列（DR1/DR1，DR4/DR4，DR5/DR5，DR6/DR6）和 4 个反向重复序列（DR2/IR2，DR3/IR3，DR7/IR7，DR8/IR8），最大的重复为 DR1，长达 31,512kb。基因共线性分析发现莲线粒体中有 15 个与其他植物共线性的保守基因簇；RNA 编辑分析鉴定出了其蛋白编码基因区的 700 个 RNA 编辑位点；基因选择压力分析表明，莲线粒体基因中有两个基因（*cox1* 和 *sdh4*）为正选择基因。这些结果表明莲线粒体基因组具有进化上的保守性特征，即保留着古老的基因簇和基因数目，高频率的 RNA 编辑现象，以及低频率的叶绿体片段的插入。莲线粒体基因组图谱的构建将有助于更加深入地了解基部双子叶植物的进化特征，并为从基部双子叶植物到高等双子叶植物的进化提供新线索。

关键词: 莲，线粒体基因组，分子进化，RNA编辑

P4

水稻 Trihelix 转录因子家族的全基因组学分析

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Trihelix 转录因子家族是植物中所特有的，因其保守结构域均含有 3 个连续的 α 螺旋而得名。相关研究表明该家族部分基因在植物抗逆境胁迫和生长发育等方面发挥了重要作用，但对该家族基因的系统分析目前在植物中还未见相关报道。在本研究中，我们对 Trihelix 转录因子家族进行了进化和功能等的系统分析。来自于水稻、拟南芥、二穗短柄草以及高粱的 120 个 Trihelix 基因家族成员被分成了 5 个亚家族。此外，我们还重点分析了 Trihelix 转录因子家族的结构特征、染色体分布情况、在水稻自身及水稻和其他物种之间局部染色体复制情况、组织表达、基因对不同激素处理响应以及 Trihelix 家族蛋白互作网络。这些结果为进一步揭示植物 Trihelix 转录因子家族的进化规律和基因的生物学功能奠定基础。

关键词：水稻，Trihelix，转录因子家族，进化

P5

Genome-wide identification of circular RNAs in *Arabidopsis thaliana* across the lifespan

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Circular RNAs (circRNAs) are a newly identified class of non-coding RNA with the presence of a covalent bond linking the 3′ and 5′ ends generated by backsplicing. Large amount of circRNAs has been identified in humans, mammals, fish, insects, worms, fungi, and protists, whereas little is known about plant circRNAs especially in tissue/developmental-stage- specific expression. Here, we conducted a genome-wide identification of circRNAs in *Arabidopsis thaliana* across lifespan and presented the profile of complete circRNAs via deep sequencing of RNA samples with rRNA and other linear RNA removed. In total, 1217 circRNAs were identified from the mixed samples which covered seven developmental stages in *A. thaliana*, using the strict threshold of at least two unique back-spliced supporting reads. The identified circRNAs showed a non-random distribution in all of the chromosomes, and most were exonic circRNAs or located within a transcript. Functional annotation analysis indicated that many circRNAs were related to photosynthesis. In addition, large proportions of circRNAs were potential miRNA targets, and almost half of the identified circRNAs had more than two different miRNA-binding sites, which possibly affecting the post-transcriptional regulation of genes by acting as miRNA sponges. Overall, our data provide an important resource for the emerging circRNA research in *A. thaliana* and should contribute to enhanced understanding of circRNAs in plants.

Key words: Circular RNA, *Arabidopsis*, deep sequence, back-splice

P6

Maize pan-transcriptome provides novel insights into genome complexity and quantitative trait variation

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Gene expression variation largely contributes to phenotypic diversity and constructing pan-transcriptome is considered necessary for species with complex genomes. However, the regulation mechanisms and functional consequences of pan-transcriptome is unexplored systematically. By analyzing RNA-seq data from 368 maize diverse inbred lines, we identified almost one-third nuclear genes under expression presence and absence variation, which tend to play regulatory roles and are likely regulated by distant eQTLs. The ePAV was directly used as ˝genotype˝ to perform GWAS for 15 agronomic phenotypes and 526 metabolic traits to efficiently explore the associations between transcriptomic and phenomic variations. Through a modified assembly strategy, 2,355 high-confidence novel sequences with total 1.9 Mb lengths were found absent within reference genome. Ten randomly selected novel sequences were fully validated with genomic PCR, including another two NBS_LRR candidates potentially affect flavonoids and disease-resistance. A simulation analysis suggested that the pan-transcriptome of the maize whole kernel is approaching a maximum value of 63,000 genes, and through developing two test-cross populations and surveying several most important yield traits, the dispensable genes were shown to contribute to heterosis. Novel perspectives and resources to discover maize quantitative trait variations were provided to better understand the kernel regulation networks and to enhance maize breeding.

Key words: *Zea mays*, pan-transcriptome, ePAV, novel sequences

P7

Distant eQTLs and non-coding sequences play critical roles in regulating gene expression and quantitative trait variation in maize

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A detailed understanding of genetic architecture of mRNA expression by millions of genetic variants is important for studying quantitative trait variation. In this study, we identified 1.25M SNPs with a minor allele frequency greater than 0.05 by combining reduced genome sequencing (GBS), high-density array technologies (600K), and previous deep RNA-sequencing data from 368 diverse inbred lines of maize. The balanced allelic frequencies and distributions in a relatively large and diverse natural panel helped to identify expression quantitative trait loci (eQTLs) associated with more than 18000 genes (63.4% of tested genes). We found that distant eQTLs were more frequent (~75% of all eQTLs) across the whole genome. Thirteen novel associated loci affecting maize kernel oil concentration were identified using the new dataset, among which, one intergenic locus could affect the kernel oil variation by controlling expression of three other known oil related genes. Altogether, this study provides resources for expanding our understanding of cellular regulatory mechanisms of transcriptome variation and the landscape of functional variants within the maize genome, thereby enhancing the understanding of quantitative variations.

Key words: eQTL, RNA-seq, GBS, GWAS, non-coding regulation, *Zea mays*

P8

Intraspecific variation of residual heterozygosity and its utility for quantitative genetics studies in maize

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Residual heterozygosity (RH) in advanced inbred progenies of plants benefits quantitative trait locus (QTL) mapping studies. However, knowledge of factors affecting its genome-wide distribution remains limited. In this study, a collection of maize heterogeneous inbred family (HIF) lines was assembled, comprising a set of 12 recombinant inbred line (RIL) populations with 2,196 lines that were genotyped using a Maize50K commercial SNP chip. Each population revealed 505 to 2095 RH intervals which collapsed to 18,615 unique RH intervals across 12 populations, covering 94.8% of the maize genome. On average, a given RH region was present in 5 different individuals. We found that some genomic regions were enriched in RH segments and seven RH hotspots were identified in the genome. The RH patterns varied significantly across populations, presumably reflecting differences in genetic backgrounds and 8 QTLs were identified for the RH hotspots in and of themselves. QTL fine mapping of kernel tocopherol content demonstrated a potential of the HIF library to efficiently map QTL locations, down to approximately ⩽ 1Mb-resolution based on publicly available information for the 12 populations. The library of HIF lines gave us new insights into the RH landscape and its intraspecific variation and provides a useful resource for QTL cloning of agronomic important traits in maize.

Key words: maize, residual heterozygosity, heterogeneous inbred family, QTL mapping, recombinant inbred lines

P9

A reliable high-density variant map of a maize synthetic population

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Maize (*Zea mays*) is an essential crop and a great model for plant research. However, the great genetic diversity and complex genome structure makes it ever challenging in variant calling and following genomic studies. In present study, we provided an integrated strategy in variant calling and imputation for large maize population (1404 individuals) at such low sequencing coverage (~1x). Over 53M SNPs, 2.8M InDels, 660K SVs, 600M novel sequences were finally obtained, which constituted the highest density and the most diverse maize variant map to date. The SNP set was found >99% consistency compared with genotypes derived from array- and assembly- based methods, while the percent of validated large structural variants was considered as ~60%. This variant map was further shown to be excellent in association mapping, taking plant height as an example, some known genes and potentially novel loci have been identified simultaneously. In brief, the variant map, together with the methodological practices, would be a great valuable resource for the plant community.

Key words: variant; whole-genome-resequencing; synthetic population; genomics; *Zea mays*.

P10

Patterns of allele specific expression in an elite rice hybrid under different environmental conditions revealed by comparative transcriptional analyses

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Allele specific expression (ASE), whereby an allele from one parent was preferentially expressed in the hybrid over the allele from the other parent, has been assumed to play a role in heterosis. In this study, we investigated the extent and patterns of ASE by comparing the transcriptomes of Zhenshan 97 (ZS97) and Minghui 63 (MH63), and their hybrid, which is the *indica* cross of Shanyou 63, the most widely grown hybrid in China over the past three decades. The hybrid, and the parents were grown under four different conditions (long day/high temperature (LDHT), long day/low temperature (LDLT), short day/high temperature (SDHT) and short day/low temperature (SDLT)). Shoot at seedling stage, flag leaf and panicle at heading stage were sampled by RNA-seq technology. ASE was identified by aligning the RNA-seq reads to the high quality reference genomes of ZS97 and MH63 recently released from our lab. In three tissues (shoot/flag leaf/panicle), 1536/2161/2375, 1344/3072/2028, 1530/1714/2821 and 1297/3380/3517 genes showed differential expression between the hybrid and the parents at LDHT, LDLT, SDHT and SDLT conditions respectively. Moreover, 1172/1325/1216, 992/1060/1189, 1115/1026/1056 and 987/1099/1152 genes exhibited ASE at LDHT, LDLT, SDHT and SDLT conditions respectively. Subsequent analysis of expression regulation of allelic genes shows dynamic patterns affected by developmental stages and growth conditions. Our results suggested that dynamic patterns of ASE may play a role in the manifestation of heterosis.

Key words: allele specific expression, comparative transcriptional analyses, heterosis

P11

Strigolactones are required for nitric oxide to induce root elongation in response to nitrogen and phosphate deficiencies in rice

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The response of the root system architecture to nutrient deficiencies is critical for sustainable agriculture. Nitric oxide (NO) is considered a key regulator of root growth, although the mechanisms remain unknown. Phenotypic, cellular and genetic analyses were undertaken in rice to explore the role of NO in regulating root growth and strigolactone (SL) signaling under nitrogen-deficient and phosphate-deficient conditions (LN and LP). LN-induced and LP-induced seminal root elongation paralleled NO production in root tips. NO played an important role in a shared pathway of LN-induced and LP-induced root elongation via increased meristem activity. Interestingly, no responses of root elongation were observed in SL d mutants compared with wild-type plants, although similar NO accumulation was induced by sodium nitroprusside (SNP) application. Application of abamine (the SL inhibitor) reduced seminal root length and *pCYCB1;1::GUS* expression induced by SNP application in wild type; furthermore, comparison with wild type showed lower SL-signalling genes in *nia2* mutants under control and LN treatments and similar under SNP application. Western blot analysis revealed that NO, similar to SL, triggered proteasome-mediated degradation of D53 protein levels. Therefore, we presented a novel signaling pathway in which NO-activated seminal root elongation under LN and LP conditions, with the involvement of SLs.

Key-words: nitric oxide, nitrogen, phosphate, rice, root, strigolactone

P12

Pinoresinol-lariciresinol reductase with the conversion of pinoresinol to secoisolariciresinol directly from *Camellia sinensis*

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(+)-Pinoresinol/(+)-lariciresinol reductase has been reported for the first time that it can catalyze the sequential reduction of (+)-pinoresinol into (-)-secoisolariciresinol via (+)-laririciresinol in *Forsythia intermedia*. The intermediacy of lariciresinol in secoisolariciresinol biosynthesis was also clarified in *Lp*PLR, *Lu*PLR and *La*PLR. However, the biosynthesis of lignan is still remained uncomplete. In this study, we have cloned two PLRs from tea plants, named *CsPLR1* and *CsPLR2*. The cDNAs are 1325bp and 1126bp, respectively, and both of them contain an ORF of 936bp encoding 312 amino acids. It was noteworthy that there was a huge difference between CsPLR1 and CsPLR2 or even PLRs in other species through enzymatic analysis *in vitro*. when (+)/(-)-pinoresinol and racemic lariciresinol were used as substrate, CsPLR2 could convert lariciresinol into secoisolariciresinol, while CsPLR1 barely carried out this reduction step, but a significant conversion of pinoresinol into secoisolariciresinol was observed. Moreover, the time course of CsPLR1 demonstrated that there was no correlation between the depletion of lariciresinol and the formation of secoisolariciresinol. So, the possible biosynthetic routes may be elucidated as follows: CsPLR2 could convert (+)-pinoresinol enantioselectively into secoisolariciresinol through lariciresinol, while CsPLR1 reduced both (+)/(-)-pinoresinol to lariciresinol and secoisolariciresinol immediately, but lariciresinol not acted as an intermediate or optimal substrate. Taken together, these results showed that the formation of secoisolariciresinol could be independent of the catalyzation of lariciresinol. This study shed light on the biosynthesis of lignan, but the mechanism needs further study.

Key words: pinoresinol-lariciresinol reductase, tea plants, biosynthetic routes

P13

The calcium-dependent protein kinase (CDPK) and CDPK-related kinase gene families in *Hevea brasiliensis*: comparison with five other plant species in structure, evolution, and expression

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Calcium-dependent protein kinases (CDPKs or CPKs) play important roles in various physiological processes of plants, including growth and development, stress responses and hormone signaling. Although the CDPK gene family has been characterized in several model plants, little is known about this gene family in *Hevea brasiliensis* (the Para rubber tree). Here, we characterize the entire *H. brasiliensis* CDPK and CDPK-related kinase (CRK) gene families comprising 30 *CDPK* genes (*HbCPK1* to *30*) and 9 *CRK* genes (*HbCRK1* to 9). Structure and phylogeny analyses of these *CDPK* and *CRK* genes demonstrate evolutionary conservation in these gene families across *H. brasiliensis* and other plant species. The expression of *HbCPK* and *HbCRK* genes was investigated via Solexa sequencing in a range of experimental conditions (different tissues, phases of leaf development, ethylene treatment, and various abiotic stresses). The results suggest that *HbCPK* and *HbCRK* genes are important components in growth, development, and stress responses of *H. brasiliensis*. Parallel studies on the *CDPK* and *CRK* gene families were also extended to five other plant species (*Arabidopsis thaliana, Oryza sativa, Populus trichocarpa, Manihot esculenta* and *Ricicus communis*). The *CDPK* and *CRK* genes from different plant species that exhibit similar expression patterns tend to cluster together, suggesting a co-evolution of gene structure and expression behavior in higher plants. The results serve as a foundation to further functional studies of these gene families in *H. brasiliensis* as well as in the whole plant kingdom.

Key words: *Hevea brasiliensis*, calcium-dependent protein kinase (CDPK), CDPK-related kinase (CRK), structure and evolution, gene expression

P14

Myo-inositol content determined by myo-inositol biosynthesis and oxidation in blueberry fruit

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Myo-inositol metabolism in plant edible organs has become the focus of many recent studies because of its benefits to human health and unique functions in plant development. In this study, MI contents in fruits were analyzed in two blueberry cultivars, cv 'Bluecrop' and cv 'Berkeley', along with the expression profiles of the key genes involved in MI biosynthesis and catabolism. Besides, the change patterns of the corresponding enzyme activities were analyzed. The results showed that some differences exist in the change profiles of the MI levels between the two cultivars. Furthermore, two *VcMIPS* genes, one *VcIMP* gene and one *VcMIOX* gene were isolated for the first time from blueberry fruits. Specifically, *VcMIPS 1/2* and *VcMIOX* encoded the rate-limiting enzymes in MI biosynthesis and oxidation, respectively. The expression patterns of *VcMIPS2*, *VcIMP* and *VcMIOX* genes showed a relationship with the change profiles of MI content during fruit ripening. The results were further confirmed by the analyses of the enzyme activities. Overall, the results indicated that both MI biosynthesis and oxidation played important roles in determining MI levels in the development of blueberries. The current results will provide new information for us to understand the mechanisms regulating MI accumulation and the roles of MI in AsA biosynthesis during the development of blueberries.

Key words: blueberry, fruit ripening, gene expression, Myo-inositol

P15

Dissecting the function of *BnaMPKa* in modulating ROS accumulation and in regulating JA induced leaf senescence

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Reactive Oxygen Species (ROS) mediate many different processes such as growth, development, biotic and abiotic stress response. The homeostasis of ROS is tightly controlled by ROS producing proteins and scavenging systems. Mitogen-activated protein kinase (MAPK) signaling components can modulate the ROS production and on the other hand it can be activated by ROS. We previously observed that *BnaMPKa* and its possible downstream target *WRKYa* could induce ROS accumulation and cell death in *Nicotiana benthamiana.* However, its underlying molecular mechanism remains to be investigated. Here we report the characterization of its downstream pathway of *MPKa* in inducing ROS production. Firstly, malondialdehyde (MDA) accumulation, DNA fragmentation, and ion leakage accompanied by the ROS production further confirmed the function of BnaMPKa in eliciting ROS and cell death. Secondly, the expression of a few genes related to ROS homeostasis including *NbGPX2* and *NbGST* was regulated by transient expression of *BnaMPKa* or *BnaWRKYa*. Further co-expression of *BnaMPKa* and *BnaWRKYa* significantly induced *NbGPX2* and *NbGST* than expressing *BnaMPKa* or *BnaWRKYa* alone. Thirdly, BnaWRKYa bound to the W-box elements in the promoters of *NbGPX2* and *NbGST* by i*n vitro* electrophoretic mobility shift assay (EMSA). However, whether MPKa could strengthen the binding ability of BnaWRKYa is still undergoing. More importantly, leaves of Arabidopsis lines expressing *BnaMPKa* exhibited precocious senescence compared to wild type while mutant *mpka* showed delayed leaf senescence after JA (Jasmonic acid) treatment. This finding revealed a possible role of BnaMPKa in mediating JA induced leaf senescence. The molecular mechanism of MPKa involved in JA induced leaf senescence needs to be further elucidated.

Key words: *Brassica napus*, *MPKa*, *WRKYa*, ROS, leaf senescence

P16

Genome and comparative transcriptomics of African wild rice *Oryza longistaminata* provide insights into molecular mechanism of rhizomatousness and self-incompatibility

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Oryza longistaminata is a close wild relative of the African cultivated rice and has several hallmark biological traits, such as rhizomatousness, self-incompatibility and high resistances to biotic and abiotic stresses. Deciphering genome of *O. longistaminata* has been hindered by the high heterozygosity of this species, which is a result of its inherent self-incompatibility. Here, we present the reference genome of *O. longistaminata* based on a large quantity of combined sequencing data (~396× Illumina short reads and ~5.9× Roche GS FLX+ long reads) and compared genomics revealed more resistance (R) genes/families. By comparing transcriptomes of 4 pairs of tissues related to the presence/absence of rhizomes or self-incompatibility, we identified candidate genes related to rhizomatousness and self-incompatibility. In aggregate, the draft genome and large quantity of transcriptome data of *O. longistaminata* provide a basic evidentiary foundation for targeted studies into the genes underlying its valuable phenotypic traits, future gene mining or breeding efforts, and further study into the evolution of African rice and the *Oryza* genus.

Key words: *Oryza longistaminata*, genome, rhizomatousness, self-incompatability

P17

基于 SSSL 的水稻种子休眠性 QTL 的鉴定与精细定位

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水稻种子休眠性（Seed dormancy）是一个复杂而重要的农艺性状，与穗发芽和粮食安全密切相关。本研究选用 388 份以‘华粳籼 74’为受体亲本的水稻单片段代换系（Single segment substitution lines，SSSLs）对种子休眠性 QTL 进行了鉴定。这些 SSSLs 的代换片段来源于包括 Basmati370 在内的 16 个供体亲本，平均长度为 18.74 cM，对水稻基因组的覆盖率为 82.5%。通过四年七季的田间试验，采用染色体片段代换作图法（Substitution mapping）共定位了 25 个水稻种子休眠性 QTLs，分布在除第 5 号染色体外的 11 条染色体上。这些 QTLs 的加性效应范围是-0.33 ~ -0.13，加性效应百分率范围是-45.3 ~ -16.7%。其中，*qSD3-2*，*qSD4-1*，*qSD7-1*，*qSD7-2*，*qSD7-3* 和 *qSD11-2* 等 6 个 QTLs 的加性效应百分率≥30%，*qSD3-3*，*qSD7-1*，*qSD9-1* 和 *qSD10-1* 等 4 个 QTL 为本研究首次报道。我们通过构建 *qSD7-1* 的 F_2 群体并发展次级单片段代换系，将其精细定位在大约 71 Kb 的区间，基因注释表明该区间存在 9 个候选基因，其中 3 个最有可能是目标基因，目前正在构建候选基因的互补载体进行验证。本研究结果为进一步开展水稻种子休眠性基因的克隆和抗穗发芽分子育种奠定了基础。

关键词：水稻，种子休眠性，穗发芽，单片段代换系，QTL，图位克隆

P18

RNA binding proteins HLP1 and HLP2 coordinately regulate alternative polyadenylation in *Arabidopsis*

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Alternative polyadenylation (APA) is a widespread mechanism for gene regulation and has been implicated in development and the pathophysiology of diseases. Pre-mRNA can generate transcript isoforms with different coding sequence or alternative 3′-UTR via APA, to enlarge the diversity and complexity of transcriptome. The molecular mechanisms of APA is intricated and remains to be elucidated in plants. Our previous work identified HLP1 as a novel 3′-end processing factors and HLP1 mutation caused thousands of poly(A) sites shifts. Here we report HLP2, the ortholog of HLP1, can also regulates APA. Phenotype analysis shows HLP2 regulate flowering time in a *FLC*-dependent way, and *hlp1hlp2* double mutant are embryo lethal, implying the significance of HLP1 and HLP2 in plant development. Both HLP1 and HLP2 are located in nucleus and have the same expression pattern. The mutation of HLP2 leads to similar APA shift with that of *hlp1* mutant, particularly, a distal-to-proximal poly(A) site shift in *FCA*, a direct target of HLP1 and HLP2, leads to upregulation of *FLC* and delayed flowering. Biochemistry assay prove HLP1 and HLP2 can form a heterodimer, suggesting that HLP1 and HLP2 regulate APA in a synergetic manner. Interestingly, HLP1 negatively modulates HLP2 expression by regulating *HLP2* transcript APA. Taken together, our results demonstrate that HLP1 and HLP2 bind directly to certain RNA targets to control the selection of poly(A) sites in a synergetic mode to regulate flowering time. The results in this study shed new light on the role of RBPs-directed RNA processing in plant development.

Key words: alternative polyadenylation, HLP1, HLP2, embryo lethal

P19

组蛋白修饰与两系杂交稻育性转化关系的初探

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随着两系杂交水稻研究的日益深入，人们对其不育机理的认识也不断加深，两系不育系育性主要受到光照和温度的影响而改变。通常情况下在长日照、高温条件下表现为不育，而在短日照、低温条件下表现为可育，这是一种典型的表观遗传现象。但是温度及光照是怎样调控两系水稻育性转化的分子机制仍有待研究。本课题以杂交水稻两系不育系武香S为材料，从组蛋白修饰中最主要的组蛋白甲基化及组蛋白乙酰化角度研究探索武香S的育性转化机制。

通过western blot对不同光、温条件下处理得到的武香S可育（WXS-F）和不育（WXS-S）材料的组蛋白修饰情况进行半定量分析，经过对比分析，观察到组蛋白H3K9ac和H3K4me2在WXS-F和WXS-S间有显著差异，而H3K9me2和H3K27me2则没有显著差异。对有显著差异的两种组蛋白修饰进行免疫染色，得到它们在间期细胞核上的大概分布情况，且免疫染色荧光信号强弱和western blot的结果是一致的。我们进一步选用其中两个组蛋白修饰的材料进行染色质免疫沉淀，并结合高通量测序，得到了与这两个组蛋白修饰相互作用的DNA序列的具体分布信息。对WXS-F和WXS-S这两个样品进行差异分析,确定样品间差异修饰的区间，结果表明H3K9ac修饰下有9个差异基因；H3K4me2修饰下有402个差异基因。初步找到一些与育性相关的基因，为进行后续的研究分析提供了科学依据。

关键词：两系杂交稻，不育机制，组蛋白修饰，染色质免疫沉淀

P20

花生 HDA1 互作蛋白 AhPGRF1 在花生旱后恢复生长过程中功能研究

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课题组前期从花生中克隆了组蛋白去乙酰化酶基因(*AhHDA1*), 发现其在花生植株或毛状根响应干旱过程中发挥作用。超表达 *AhHDA1* 拟南芥植株在干旱胁迫后复水的生长过程中, *AhHDA1* 表达降低，同时叶绿素合成关键酶叶绿素酸酯氧化还原酶基因 *AhPORA* 表达也明显升高, 推测 *AhHDA1* 介导的组蛋白去乙酰化修饰影响着植株旱后（复水）恢复生长。

通过 AhHDA1 蛋白互作酵母库，筛选并克隆到一个与花生旱后恢复生长相关因子，命名为 *AhPGRF1* 基因，其全长 1209 个碱基，包含 6 个外显子，编码 403 个氨基酸；进一步研究表明 AhHDA1 与 AhPGRF1 存在互作. AhPGRF1 蛋白含有 MYB 保守域，其氨基酸序列与大豆 GsGLK、拟南芥 AtGLK 等同源性较高；定位于细胞核, 具有转录激活作用. AhPGRF1 主要在花生叶片中表达. 花生幼苗在 30%PEG 条件下，植株生长被抑制, *AhPGRF1* 表达持续下降. 花生生长的土壤脱水 30 天, 植株枯萎, 叶片发黄萎蔫, 叶片 *AhPGRF1* 基因表达极低; 花生植株再复水用水生长 3 天, 叶片返绿, *AhPGRF1* 基因快速上调, 叶绿素合成基因 *AhPORA* 表达增强, 并发生新芽, *AhPGRF1* 基因表达程度与叶片额度发育形态一致. 进一步用原生质体瞬时表达分析的结果显示，AhPGRF1 蛋白 激活 *AhPORA* 基因启动子的转录活性. 推测 *AhPGRF1* 可能通过影响叶绿素合成促进花生旱后叶片的恢复生长. 将深入研究 AhPGRF1 可能作为这一过程的功能性转录因子的功能。

关键词：花生，*AhHDA1*互作，*AhPGRF1*，旱后恢复生长，功能, 叶绿素酸酯氧化还原酶

P21

Identification of regulatory DNA elements using genome-wide mapping of DNase I hypersensitive sites during tomato fruit development

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Development and ripening of tomato fruit are precisely controlled by transcriptional regulation, which depends on the orchestrated accessibility of regulatory proteins to promoters and other *cis*-regulatory DNA elements. This accessibility and its effect on gene expression play a major role in defining the developmental process. To understand the regulatory mechanism and functional elements modulating morphological and anatomical changes during fruit development, we generated genome-wide high-resolution maps of DNase I hypersensitive sites (DHSs) from the fruit tissues of the tomato cultivar ˝Moneymaker˝ at 20 days post anthesis as well as break stage. By exploring variation of DHSs across fruit development stages, we pinpointed the most likely hypersensitive sites related to development-specific genes. By detecting binding motifs on DHSs of these development-specific genes or genes in the ascorbic acid biosynthetic pathway, we revealed the common regulatory elements contributing to coordinating gene transcription of plant ripening and specialized metabolic pathways. Our results contribute to a better understanding of the regulatory dynamics of genes involved in tomato fruit development and ripening.

Key words: DNase I hypersensitive sites, cis-regulatory element, gene expression, fruit development, tomato

P22

拟南芥 AtHAP5s 影响组蛋白甲基化修饰参与开花调控

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开花是植物关键的发育过程，与许多重要农艺性状相关。在拟南芥中，表观遗传调控在开花过程中起着重要的作用。然而，表观遗传因子如何参与植物开花过程的具体分子机制仍需深入探讨。研究表明，AtHAP5c（又称核因子 Y 的亚基 C，NF-YC）家族基因参与拟南芥的开花调控。我们前期工作发现，拟南芥 AtHAP5c 直接调控开花整合因子基因的表达，并可能通过与 PRC2 亚基 CLF 互作影响相关基因的染色质组蛋白甲基化沉积，调控植物的开花过程。在此基础上，我们通过蛋白互作实验研究发现，AtHAP5s 的四个同源蛋白与 PRC2 的亚基蛋白 CLF 直接相互作用，且两者共同调控相关开花基因的表达；遗传上，*CLF* 上位于 *AtHAP5s*；AtHAP5s 与 CLF 互作并介导开花基因染色质组蛋白 H3K27me3 修饰变化。这些工作初步预示了 AtHAP5s 与 PRC2 互作参与开花基因组蛋白甲基化调控的分子机制。本研究将进一步解析 AtHAP5s 如何通过影响组蛋白甲基化修饰参与开花基因的转录调控，为植物发育的表观遗传调控机制提供理论基础。

关键词：开花，表观遗传，蛋白互作，转录调控

P23

Ancient two expansions of GYPSY-LTR transposons underlie the large genome of *Norway spruce*

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DNA methylation plays important roles in many biological processes, such as silencing of transposable elements, imprinting and regulating gene expression. Many studies of DNA methylation have shown its essential roles in angiosperms (flowering plants). However, few studies have examined the roles and patterns of DNA methylation in gymnosperms. Here, we present the first genome-wide single-base resolution methylation maps of Norway spruce (*Picea abies*) from both needles and culture tissues through whole genome bisulfite sequencing. Dense methylation was observed globally across the whole genome, which differed from other plants that only had increased methylation present in their pericentromeric regions. On average, DNA methylation levels of CG and CHG were much higher than most other model plants. Consistent with other plants, CHH methylation was relatively low and at least one copy of RdDM pathway genes was found in Norway spruce. In comparison to needles, culture tissue showed lower CG and CHG methylation levels but higher CHH methylation, which is inconsistent with other regenerated tissue studies. Similar to other genic DNA methylation studies, global DNA methylation levels were also positively correlated with genome size in plants. Two rounds of GYPSY-type LTR expansions were identified in the history of Norway spruce. This could partly explain the large genome size of the gymnosperms, like Norway spruce, which differ from most of angiosperms in that they have not undergone recent whole genome duplication (WGD). Altogether, the secret of the large genome of Norway spruce could not be explained by reduced DNA methylation levels and reactivation of transposons or whole genome duplication, but it can be partly explained by ancient GYPSY expansions.

Key words: DNA methylation, Norway spruce, whole genome duplication.

P24

Integrated epigenetic maps of cotton fiber provide novel insights into staged single-cell differentiation

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DNA methylation is highlighted for its great importance in regulating plant development, but its function associated with single-cell differentiation remains undetermined. Here, we used cotton fiber, specifically, the epidermal hairs on the cotton ovule, as a model to investigate the regulatory role of DNA methylation in cell differentiation. Global disruption of DNA methylation was first demonstrated to re-activate a large number of genomic loci and depress cotton fiber development. We constructed single-base resolution maps of DNA methylation dynamics representing each fiber developmental stage. The CHH (H=A, T, or C) DNA methylation level was increased during fiber development, accompanied by a decreased pattern of RNA-directed DNA methylation (RdDM). Examination of nucleosome positioning revealed a gradual eu- to hetero-chromatin transition for chromatin reprogramming in developing fibers, coupled with increased DNA methylation. Compared with cotton ovules, increased DNA methylation in fibers was demonstrated to be predominantly mediated by an active H3K9me2-dependent pathway instead of the inactive RdDM pathway, especially in heterochromatic regions. We examined how asymmetric DNA methylation contributed to homoeologous gene expression bias, illustrating a sub-genomic collaborated regulation of allotetraploid cotton fiber development. Furthermore, integrated multi-omics analyses revealed that dynamic DNA methylation could play a role in the regulation of lipid biosynthesis and spatio-temporal modulation of reactive oxygen species for staged fiber differentiation. Our study established a framework for understanding the role of DNA methylation and chromatin reprogramming during fiber development and also provided novel insights into the epigenetic regulation of single-cell differentiation in plants.

Key words: DNA methylation, cotton fiber, single-cell

P25

Comparison of DNA methylation in developing seeds of yellow- and black-seeded *Brassica napus* by MSAP

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DNA methylation is a crucial modification process implicated in epigenetic regulation of gene expression, which may be responsible for variation of agronomic traits. However, the epigenetic regulation of yellow- and black-seeded character in *Brassica napus* has not been reported. In this study, methylation-sensitive amplification polymorphism (MSAP) analysis was performed to investigate target regions of seed coat variation in *B. napus* by using yellow-seeded rapeseed line derived from the somatic hybrids of *B. napus*-*Sinapis alba* and black-seeded rapeseed as materials. Extensive methylation changes were observed between yellow- and black-seeded *B. napus*. In particular, ~10% of demethylation and ~5% of hypermethylation were detected in yellow rapeseeds compared with black seeds. Nonetheless, this variation was barely identified among different developing stages. Relative expression value and 20 polymorphic fragments in MSAP profiles were analyzed. The gene expression of demethylated fragments in yellow rapeseeds was upregulated. For instance, bHLH, a transcription factor regulating flavonoid biosynthesis, was upregulated at three to five weeks after flowers (WAF) of yellow seeds. In general, epigenetic changes among rapeseed lines with different seed colors likely helped elucidate the formation of yellow seed character.

Key words: *Brassica napus*, yellow seed, DNA methylation, flavonoid biosynthesis

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DNA METHYLTRANSFERASE1 involved in shoot regeneration is regulated by cytokinin-induced cell cycle in *Arabidopsis*

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DNA methylation plays critical roles in diverse developmental processes of plants. Mutation of *Arabidopsis DNA METHYLTRANSFERASE1* (*MET1*) represses shoot regeneration by modulation of the organizing center regulator WUSCHEL (WUS). However, the regulation of MET1 during this process is unclear. Here, we analyzed upstream signals that regulate *MET1* expression during *de novo* shoot regeneration in *Arabidopsis*. *MET1* was initially expressed throughout the callus but was restricted to the outer cell layers of the shoot meristem at the advanced stages during shoot regeneration. Expression of *WUS* was involved in callus regions showing loss of *MET1* expression. Furthermore, mutation of the cell-cycle transcription factor *E2FA* reduced the *MET1* transcript level, whereas *E2FA* overexpression increased the *MET1* transcript level. E2FA directly bound to the *MET1* promoter through the E2F element to promote *MET1* expression. Moreover, *E2FA* and *MET1* expression co-localized in callus during shoot regeneration. Promotion of *MET1* expression by E2FA was dependent on the cell-cycle regulator CYCD3. Exogenous cytokinin promoted *MET1* expression through enhancing the *CYCD3* transcript level. Thus, our study provides new insights for understanding the regulation of cell proliferation and differentiation in shoot regeneration.

Key words: *Arabidopsis*, shoot regeneration, cytokinin, cell cycle, DNA methylation, *WUSCHEL* expression

P27

AtPRMT5 regulates shoot regeneration through mediating histone H4R3 dimethylation on *KRP1* and pre-mRNA splicing of *RKP* in *Arabidopsis*

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Protein arginine methylation plays essential roles in diverse biological processes, but its role in regulating shoot regeneration remains elusive. In this study, we analyzed the function of the protein arginine methyltransferase AtPRMT5 during *de novo* shoot regeneration in *Arabidopsis*. AtPRMT5 encodes a type II PRMT that methylates proteins, including histones and RNA splicing factors. Mutation of AtPMRT5 decreased the frequency of shoot regeneration and number of shoots per callus in the *atprmt5* mutant compared with those of the wild type. Chromatin immunoprecipitation analysis indicated AtPRMT5 targets *KIP-RELATED PROTEIN*, which encodes a cyclin-dependent kinase that inhibits the cell cycle. During shoot regeneration, the *KRP1* transcript level increased in the *atprmt5* mutant, which resulted from the reduced histone H4R3 methylation in the *KRP1* promoter. Overexpression of *KRP1* significantly reduced the frequency of shoot regeneration and shoot number per callus. Furthermore, an abnormal pre-mRNA splicing in the ubiquitin E3 ligase RELATED TO KPC1 (RKP) was detected in the *atprmt5* mutant. RKP functions in regulating KRP protein degradation. Mutation of RKP inhibited shoot regeneration. Thus, AtPRMT5 regulated shoot regeneration through histone modification-mediated *KRP1* transcription and RKP pre-mRNA splicing. Our findings provide insights into the function of protein arginine methylation in *de novo* shoot regeneration and establish a novel link between pre-mRNA splicing and shoot induction.

Key words: shoot regeneration, protein arginine methylation, cell cycle, pre-mRNA splicing

P28

水稻印记基因的鉴定和功能研究

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哺乳动物和被子植物受精后，大多数来自母本和父本的等位基因都会得到相同程度的表达。但一部分基因的表达会受到其亲本来源的影响，这部分基因被称为印记基因。已有的研究表明植物的印记主要在种子的胚乳中，并且 DNA 甲基化是产生基因组印记的重要原因。我们以水稻中花 11 开花后的胚乳和胚为材料，在测定不同发育时期全基因组 DNA 甲基化谱和基因表达谱的基础上，选择优先在胚乳表达且在胚乳中的甲基化程度低于胚的基因作为候选印记基因开展研究。我们共筛选获得了逾 200 个候选印记基因，进一步利用日本晴和 9311 正反交得到杂交种子，利用相关基因父母本之间存在的 SNP，对正反交胚乳中同源等位基因的表达情况进行测序分析，共验证确认了 27 个印记基因。对随机挑选的 10 个基因构建了 CRISPR 基因敲除突变体，初步的表型观察发现其中两个基因突变体的结实率下降。目前正在对这些基因的功能及其印记的机制进行深入研究。

关键词：水稻，印记基因，DNA甲基化，结实率

P29

水稻磷酸肌醇磷酸酶的定位克隆和功能研究

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磷脂酰肌醇作为生物膜中的重要组分之一，根据肌醇分子上磷酸基团的数量及相对位置的不同可以分为 7 种， 分别为 PtdIns(3) P，PtdIns(4) P，PtdIns(5) P，PtdIns(3,4) P，PtdIns(3,5) P，PtdIns(4,5) P，PtdIns(3,4,5) P。其中 PtdIns(4,5) P 作为经典的 IP3/DAG 信号通路中信号分子的前体而最为人所知。但动物中经典的 IP3/DAG 通路的重要组分：IP3 受体、与 DAG 互作的 PKC 等在植物中并没有被发现，暗示植物中的磷脂酰肌醇相关信号转导通路与动物中在进化中有一定分化。近年来，有越来越多的研究发现其它种类的磷脂酰肌醇也可以作为独立的信号分子在许多生物学过程中发挥重要的作用。而对于磷脂酰肌醇在植物，尤其是水稻中的功能以及具体的机制研究十分有限。我们以粳稻品种 CB 和籼稻品种桂朝 2 号为亲本进行杂交，通过图位克隆的方法找到了一个控制水稻株高、穗粒数、分蘖数等多个性状的基因。基因注释信息显示，该基因编码一个磷脂酰肌醇磷酸酶。将其在昆虫 sf9 细胞中进行表达并进行酶活检测发现，该桂朝 2 号来源的目的基因可以特异性对 PtdIns(4) P 进行去磷酸化，而 CB 来源的目的基因则丧失了酶活。同时构建了以 CB 为轮回亲本，桂朝 2 号位供体亲本的近等基因系（NIL），对含有 CB 片段来源的 NIL-CB 和含有桂朝 2 号片段来源的 NIL-GC 进行 PtdIns(4) P 含量测定，结果显示 NIL-CB 中 PtdIns(4) P 含量明显高于 NIL-GC 植株。细胞学观察还显示，NIL-CB 的根尖细胞中，细胞骨架表现出明显的不规则排列，细胞内囊泡也出现更多的积累。对二者体内各种植物激素含量测定发现，NIL-CB 中水杨酸（SA）发生显著的积累，表明目的基因的突变可能也参与植物抗逆过程中。目前正在构建互补过表达以及 CAS9 敲除的转基因植株从遗传学验证表型。同时并通过寻找 PtdIns(4) P 的结合蛋白从机理上进行阐述。

关键词：水稻，PtdIns(4) P，磷酸酶，细胞骨架，囊泡运输

P30

微丝细胞骨架参与植物重力感知和响应的机制研究

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重力是重要的地球环境因素之一，在长期的进化中植物形成了特有的重力感知和响应机制调控着植物发育和形态建成；如根的向地性生长和地上部背地性直立生长，从而保障了植物可以有效地利用营养、水分和光能。已有较多的研究结果表明，微丝细胞骨架在植物响应重力变化中起到重要作用；但是由于以往研究中所用的微丝抑制剂、研究材料、器官的不同，至今还没有明确的有关微丝细胞骨架如何参与植物重力响应的精细机制。根据“淀粉体-平衡石”假说，植物感重细胞（如根尖小柱细胞和茎内皮层细胞）内淀粉体在感知重力变化发生沉降，可迅速将物理信号转化为生物化学信号。由于感重细胞内存在着复杂的亚细胞结构（如细胞骨架，内膜系统等）造成了淀粉体运动复杂性。我们首次应用流体力学微流变方法分析了拟南芥根尖中央小柱细胞内淀粉体的运动特性，发现在重力刺激（旋转 90 度）下野生型感重细胞内的淀粉体运动具有明显的“牢笼-逃逸（cage-escape)”和协同运动的力学效应。在 ARP2/3 微丝相关蛋白复合体突变体（*dis1-1*, *dis2-1*）的中央小柱细胞中，由于淀粉体被异常形成的粗微丝束所束缚和分离，缺少明显的淀粉体“牢笼-逃逸”和协同运动；而微丝解聚剂（Latrunculin B）预处理可以显著地打破微丝突变体中存在的淀粉体运动的“牢笼”效应。我们进一步的研究结果还表明，ARP3/DIS1 亚基不仅参与感重细胞内重力感知，还参与了作为重要的重力信号生长素在胞间的极性运输；在 *dis1-1* 突变体中，多个生长素运输载体 PIN 家族蛋白（PIN2， PIN3， PIN7）胞内运转的发生异常，影响了生长素在根上、下两侧细胞内不对称分布的迅速建立，造成根的向地弯曲生长延迟。此研究结果揭示了微丝细胞骨架在植物重力感知、信号传递中的功能，对于进一步揭示植物发育和形态建成的调控机制，以及改良作物株型、抗倒伏等农艺性状提供了新的研究方法和理论依据（Zheng et al., 2015, Molecular Plant；Zou et al., 2016, JXB）

关键词：细胞骨架，ARP2/3，重力，生长素，根

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内质网 SNARE 因子对种子储藏蛋白质运输的调控

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膜泡运输是细胞各项生命活动的基础。种子储藏蛋白质在粗面内质网合成之后运输到蛋白质储藏型液泡进行储存需要经过很多分选、运输的步骤。我们在前期研究中发现拟南芥内质网拴留因子 MAG2 与 MIP1、MIP2 和 MIP3 形成拴留复合体，在 ER-Golgi 间的膜泡运输中调控运输囊泡与内质网膜的融合过程。这些拴留因子缺失致使新合成的蛋白质从内质网运离的步骤受阻，造成储藏蛋白质前体蓄积在内质网腔中。为了进一步揭示 MAG2 相关的 ER-Golgi 膜泡运输机制，我们对内质网定位的 SNARE 因子进行了探讨，包括 Qa-SNARE、Qb-SNARE 和 Qc-SNARE，以及它们参与膜泡运输的机制。我们的研究结果表明，内质网定位的 SNARE 对种子储藏蛋白质的膜泡运输有重要的调控作用。当这些因子缺失时，不仅膜泡运输受到阻碍，而且在一定程度上植物生长发育受到影响。

关键词：种子储藏蛋白质，膜泡运输，SNARE因子

P32

An ARF-GEF independent pathway recycles non-basal plasma membrane proteins

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The endomembrane system is an interconnected network required to establish signal transduction, cell polarity and cell shape in response to developmental or environmental stimuli. In the model plant *Arabidopsis thaliana*, there are numerous markers to visualize polar localized plasma membrane proteins utilizing endomembrane trafficking. Previous studies have shown that the large ARF-GEF GNOM plays a key role in the establishment of basal polarity, whereas the apically polarized membrane proteins undergo sorting via different routes. However, the mechanism to maintain apical polarity is largely unknown. Here, we used a chemical genomic approach and identified the compound endosidin 16 (ES16), which perturbed apically localized plasma membrane proteins without affecting basal polarity. We demonstrated that ES16 is an inhibitor for recycling of apical, lateral and non-polar plasma membrane proteins as well as biosynthetic secretion, leaving the basal proteins as the only exception independent from ES16 action. Further evidences from pharmaceutical and genetic data revealed that ES16 defines a unique trafficking pathway distinct from ARF-GEF mediated basal recycling and secretory processes that functions through the inhibition of small GTPase RabA proteins. Our results reveal that ES16 defines a distinct pathway for endomembrane sorting routes and is essential for the establishment of cell polarity.

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复合药用植物提取液的抑菌性能及其作用机制初探

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以白念珠菌菌株（CCTCC AY 206001 *Candida albicans*） 为实验菌，来研究天然植物抗菌液(PAMs) 对白念珠菌的抑菌效果与作用机理。通过倍比稀释法及牛津杯（管碟）法确定天然植物抗菌液对白念珠菌的最小抑菌浓度(MIC)与最小杀菌浓度(MBC)，结合抑菌活力、真菌生长曲线、细胞膜完整性、膜通透性与真菌超显微结构观察等，综合评价不同浓度天然植物抗菌液在不同处理时间内对白念珠菌的作用效果。结果表明，天然植物抗菌液对白念珠菌的(MIC)与(MFC)分别为75%与80%，随着处理时间的延长，天然植物抗菌液明显抑制白念珠菌的生长，造成该真菌菌体细胞壁通透性增大，细胞结构的完整性受到破坏，菌液电导率值显著升高，菌体电解质等内容物外泄，从而影响细胞内环境和细胞膜的稳定性，菌体皱缩变形，表面粗糙，细胞壁塌陷或破裂，细胞质外泄渗出，导致菌体死亡。

关键词: 复合药用植物提取液; 白色念珠菌；抑菌性能；机理

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OsBBX14 调节水稻抽穗期的机理研究

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B-box 蛋白是一类包含有一个或两个 B-box 结构域的锌指蛋白，这类蛋白在拟南芥生长发育过程中起到重要的作用。然而迄今为止关于水稻中 BBX 蛋白功能的报道还非常少。在本研究中，我们鉴定到一个仅含有两个 B-box 结构域的水稻 BBX 蛋白 *Oryza sativa* BBX14 (OsBBX14)，属于 B-box 蛋白第 IV 亚家族，我们对该蛋白进行了进一步的研究。

OsBBX14 基因在 *phyB* 突变体中表达上调，在叶片中大量表达。在光周期条件下以及随后的连续光照条件下，*OsBBX14* 在转录水平上表现出明显的昼夜节律性，该基因转录本在黄昏后开始积累，在黎明时转录水平达到最高值，随后开始迅速下降。OsBBX14 蛋白定位于细胞核中，而且至少在酵母中具有转录激活活性，其 N 端 B-box 结构域对于该蛋白的上述功能至关重要。

进一步研究显示 *OsBBX14* 过表达转基因株系（*OsBBX14*-OX）在长日照及短日照条件下表现出延迟的抽穗期，通过对转基因水稻取材进行 RT-pcr 分析发现，不论是在长日照条件下还是在短日照条件下，开花素基因 *Hd3a* 和 *RFT1* 的表达水平明显下调，这与 *OsBBX14*-OX 株系表型是一致的。随后我们又对开花素上游调控基因进行分析发现，在长日照条件下 OsBBX14 有可能是通过上调 *Hd3a* 的负调控因子 *Hd1* 在 *OsBBX14*-OX 株系中的表达水平延迟水稻抽穗。而在短日照条件下，OsBBX14 通过降低 *Ehd1* 表达水平延迟水稻抽穗。接下来我们将解析 OsBBX14 调控的下游基因网络，揭示 OsBBX14 调节水稻抽穗时间的分子机制。

关键词：水稻，BBX14，B-box 锌指蛋白，抽穗期，光周期途径

P35

To be a flower or fruiting branch: insights revealed by mRNA and small RNA transcriptomes from different cotton developmental stages

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The architecture of the cotton plant, including fruit branch formation and flowering pattern, is the most important characteristic that directly influences light exploitation, yield and cost of planting. Nulliplex branch is a useful phenotype to study cotton architecture. We used RNA sequencing to obtain mRNA and miRNA profiles from nulliplex- and normal-branch cotton at three developmental stages. The differentially expressed genes (DEGs) and miRNAs were identified that preferentially/specifically expressed in the pre-squaring stage, which is a key stage controlling the transition from vegetative to reproductive growth. The DEGs identified were primarily enriched in RNA, protein, and signalling categories in *Gossypium barbadense* and *Gossypium hirsutum*. Interestingly, during the pre-squaring stage, the DEGs were predominantly enriched in transcription factors in both *G. barbadense* and *G. hirsutum*, and these transcription factors were mainly involved in branching and flowering. Related miRNAs were also identified. The results showed that fruit branching in cotton is controlled by molecular pathways similar to those in *Arabidopsis* and that multiple regulated pathways may affect the development of floral buds. Our study showed that the development of fruit branches is closely related to flowering induction and provides insight into the molecular mechanisms of branch and flower development in cotton.

Key words: cotton, architecture, branch, mRNA, small RNA

P36

SPIKE1 activates ROP GTPase signaling to modulate petal growth and shape in *Arabidopsis thaliana*

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The diverse morphological features displayed by plants, including shape and size of organs are regulated by the polarity and extension events of specially endowed cells. However, information regarding the functional proteins involved in the regulation of petal shape has not been extensively elucidated. Here, we investigate the mechanism that determines petal shape formation in *Arabidopsis thaliana* owing to its relatively short life span and structure simplicity. Current evidence showed that in Plants Rho (ROP) which is a kind of small GTPase protein that regulates the organization of cortical microtubules and vesicle trafficking under direct SPIKE1 activation, SPIKE1 belongs to DOCK family of proteins encoding a guanine nucleotide exchange factor (GEF). In this study, we respectively identified the interaction of SPIKE1 with ROP2 and ROP6 in our pull-down assays in vitro. Furthermore, analysis of *rop2, rop6, rop2rop6* and *rop2rop4irop6* mutants generated in this study exhibited lesser incidence of zigzag coupled with a reduction in the size of petal abaxial cell simultaneously, suggests ROP2, ROP4 and ROP6 synergistically modulate the development of petal shape, also, more transversely ordering microtubules were observed in late petal cells. More so, all the phenotypic characteristics observed in *rop2rop4irop6* could be mirrored in *spk1-4* point mutants. In conformity with these results, we posited that, ROP2, ROP4 and ROP6 activated by SPIKE1 and functions synergistically in modulating petal cell elongation and polarity in tandem with microtubules orientation and thus, influence growth and morphological development in *Arabidopsis thaliana*.

Key words: ROP GTPase, SPIKE1, petal shape, microtubules, Arabidopsis

P37

Suppressor of rid1 (SID1) coupled with RID1 initiate floral transition by inducing expression of florigen genes in rice

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Transition from the vegetative to reproductive growth is a critical process in the life cycle of higher plants. Previously, we cloned *Rice Indeterminate 1* (*RID1*), which acts as the master switch for the transition from the vegetative to reproductive phase in rice. Although the photoperiod pathway of *RID1* inducing expression of the florigen genes *RFT1* and *Hd3a* through *Ehd1* has been established, the alternative pathways for the essential flowering transition need to be further examined. Here, we identified a *Suppressor* of *rid1* (*SID1*), which rescues the never-flowering phenotype of *rid1*. *SID1* encodes an INDETERMINATE DOMAIN (IDD) transcription factor. Mutation in *SID1* showed the delayed flowering phenotype. Gain-of-function of *SID1* could restore the *rid1* to normal flowering. Further analysis indicates that SID1 physically interact with RID1 and directly targeting with the promoter regions of *Hd3a* and *RFT1*, two florigen genes in rice. Taken together, our results uncover an autonomous flowering pathway mediated by RID1, thereby controlling the phase transition from vegetative to reproductive development in rice.

Key words: floral transition, *RID1*, *Suppressor* of *rid1*, florigen genes

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ICK5, a gene encoding a CDK inhibitor is involved in promotion of leaf senescence in *Arabidopsis*

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ICK5 is one of the ICK family genes encoding CDK (cyclin-dependent kinase) inhibitor, which are negative regulators of cell division. *AtNAP* is a NAC family transcription factor gene that plays a key role in leaf senescence, but its mechanisms are not well elucidated. Here we report that *AtICK5* is a direct target gene of the AtNAP transcription factor, and expression of *AtICK5* was induced by leaf senescence. Leaf senescence in one T-DNA insertion line of *AtICK5* is significantly delayed. The T-DNA knockout plants are otherwise normal. The mutant phenotype can be restored to wild-type by the intact *AtICK5*. Overexpression of *AtICK5* resulted in dwarf plants. When *AtNAP* was chemically induced, *AtICK5* was also induced, and the expression of *AtICK5* was significantly reduced in *atnap*. These data suggest that the expression of *AtICK5* is predominantly dependent on AtNAP. Yeast one-hybrid experiments showed that AtNAP could physically bind to *AtICK5* promoter in vitro. These data suggest that *AtICK5* as a direct target gene of AtNAP plays an important role in leaf senescence in Arabidopsis.

Key words: *AtICK5*, leaf senescence, *AtNAP*, Arabidopsis

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ZYG1 regulates zygote division in *Arabidopsis* through regulating cyclin B1 degradation

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As the starting point of a new life cycle, activation of the first division of the zygote is a critical event in both plants and animals. Due to poor accessibility of plant zygotes, it has remained poorly understood how this process is regulated. Through genetic analyses in Arabidopsis we identified a *zygote-arrest 1* (*zyg1*) mutant that exhibited zygote-lethality and over-accumulation of cyclin-GUS phenotypes. Map-based cloning showed that *ZYG1* encodes the anaphase promoting complex/cyclosome (APC/C) subunit 11 (APC11). Live-cell imaging showed that *APC11* is expressed in both egg and sperm cells, and that expression continued in zygotes and during early embryogenesis. Using an *APC11-GFP* fusion construct, we showed that *APC11-GFP* expression persisted throughout the mitotic cell cycle, and localized to cell plates during cytokinesis. Expression of non-degradable cyclin under the control of a zygote-specific *DD45* promoter led to a *zyg1*-like zygote-lethal phenotype. Biochemical studies showed that APC11 is able to ubiquitinate cyclin, thus promoting cyclin degradation. These results together suggest that APC11-mediated cyclin degradation in Arabidopsis is critical for the first division of the zygote.

Key words: zygote activation, cell cycle, the anaphase promoting complex, protein ubiquitination

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Control of grain size by *GS1* in rice

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Grain size is one of the most important agronomic traits in crops. Several factors that regulate grain size have been identified in rice , but the genetic and molecular mechanisms of grain size control remain almost unknown. Here, we describe the *GS1* gene that encodes an unknown protein. Elevated expression of GS1 significantly increases grain size and weight by increasing cell proliferation in spikelet hulls and accelerating the grain milk filling rate, while downregulation of GS1 decreases grain size and weight. To understand the molecular mechanism of GS1 in grain size control, we have identified GS1-interacting proteins (GIP). Biochemical analyses show that GIP1 interacts with GS1 in vitro and in vivo and modulates the stability of GS1. GS1 also physically interacts with the transcription factor GIP2 to regulate its transcription activity. Thus, our findings define a novel genetic and molecular mechanism by which GIP1-GS1-GIP2 function to control grain size and weight in rice, suggesting this pathway is a promising target for rice grain yield improvement.

Key words: rice, *GS1* gene, grain size, interacting proteins

P41

Functions of *Thick Aleurone1* (*TA1*) in regulating aleurone and starchy endosperm differentiation in rice

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Rice endosperm consists of an outer aleurone layer and an inner starchy endosperm. In mature rice seeds, the aleurone consists of one layer of living cells that accumulate mainly storage proteins, lipids, iron and zinc, while the starchy endosperm comprising dead cells that accumulates mainly starch. In this study, we report the screening and identification of *thick aleurone 1* (*ta1*) mutant in rice. Instead of one layer of aleurone cells in the wild type, the mature *ta1* seeds had 3 to 5 layers of aleurone cells. Nutritional analyses showed that the *ta1* mature seeds had increased levels of storage proteins, lipids, iron and zinc content. Then we located *TA1* at chromsome 5 through map based cloning approach. qRT-PCR result showed that *TA1* is expressed mainly in mature pollens, shoot apical meristem, embryos and aleurone. Further genetic and biochemical analyses of *TA1* may help to develop nutrient-rich rice varieties and to establish a molecular network behind the differentiation of aleurone and starch endosperm in rice.

Key words: aleurone, starcy endosperm, differentiation, *TA1*

P42

弯曲碎米荠光周期敏感性自然变异的分子基础

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光周期和温度是调控植物开花、决定植物分布的关键环境因素。许多植物通过光周期来调控开花时间从而更好地适应环境。多倍体植物弯曲碎米荠（*Cardamine flexuosa*）与拟南芥（*Arabidopisis thaliana*）同属十字花科，主要分布在亚洲东部，澳大利亚和北美洲。作为广泛分布的田间杂草，不同地区的弯曲碎米荠在表型上具有明显的差异性。我们从中国大陆等地区采集到 39 份弯曲碎米荠材料。通过对这些弯曲碎米荠在不同光周期条件下的开花时间进行统计分析，我们发现不同地区的弯曲碎米荠对光周期的敏感性存在巨大差异：长日照能够显著促进部分弯曲碎米荠的开花，而另一些弯曲碎米荠则对光周期不敏感。进一步的研究表明，蓝光受体基因 *CRYPTOCHROME 2*（*CRY2*）的突变是导致弯曲碎米荠光周期敏感性自然变异的主要原因。许多植物在不同光周期条件下的开花时间都存在着自然变异，那么包括 *CRY2* 在内的光受体基因的突变是否也是导致这些自然变异的原因呢？*CRY2* 决定的光周期敏感性对弯曲碎米荠的地理分布和环境适应有着怎么样的影响？光周期敏感性的自然变异与春化途径又是如何共同作用影响弯曲碎米荠的开花时间呢？这些问题还需要我们进一步的研究。

关键词：弯曲碎米荠，开花时间，光周期，自然变异

P43

Tuning growth cycles of *Brassica* crops *via* natural antisense transcripts of *BrFLC*

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Several oilseed and vegetable crops of *Brassica* are biennials that require a prolonged winter cold for flowering, a process called vernalization. *FLOWERING LOCUS C* (*FLC*) is a central repressor of flowering. Here, we report that the overexpression of natural antisense transcripts (NATs) of *Brassica rapa FLC* (*BrFLC*) greatly shortens plant growth cycles. In rapid-, medium- and slow-cycling crop types, there are four copies of the *BrFLC* genes, which show extensive variation in sequences and expression levels. In Bre, a biennial crop type that requires vernalization, five NATs derived from the *BrFLC2* locus are rapidly induced under cold conditions, while all four *BrFLC* genes are gradually down-regulated. The transgenic Bre lines overexpressing a long NAT of *BrFLC2* do not require vernalization, resulting in a gradient of shortened growth cycles. Among them, a subset of lines both flower and set seeds as early as Yellow sarson, an annual crop type in which all four *BrFLC* genes have nonsense mutations and are non-functional in flowering repression. Our results demonstrate that the growth cycles of biennial crops of *Brassica* can be altered by changing the expression levels of *BrFLC2* NATs. Thus, *BrFLC2* NATs and their transgenic lines are useful for the genetic manipulation of crop growth cycles.

Key words: *Brassica rapa*, *BrFLC*, natural antisense transcripts, flowering time, growth cycle, vernalization.

P44

楸树体细胞胚胎发生过程中 4 种同工酶分析

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楸树(*Catalpa bungei* C.A.Mey.)是我国特有的珍贵优质用材和园林观赏树种，具有较高经济价值。楸树自花不育，自然结实率低，发芽率极低。体细胞胚胎发生具有快速、高增殖率及高再生率等优点，已成为扩繁优良种质资源的重要技术手段。本研究在建立楸树高效、稳定发生的体细胞胚胎发生技术体系基础上，采用不连续聚丙烯酰胺凝胶电泳技术和酶活性测定，对楸树体胚发生过程中的酯酶(EST)、过氧化物酶(POD)、淀粉酶(AMY)和 ATP 酶 4 种同工酶进行分析。结果表明，EST 及 POD 同工酶酶带在楸树体细胞胚胎发生的不同阶段呈现规律性变化，胚性愈伤组织中 EST、POD 同工酶谱带较非胚性愈伤组织多且有特异性条带出现，酶活性达到最高点，表明这一时期细胞内代谢旺盛。EST、POD、AMY 及 ATP 同工酶在楸树胚性与非胚性愈伤组织中谱带差异明显，在黄色愈伤组织与绿色子叶胚中的酶活性较高，表明在这两类组织中存在旺盛的生理生化代谢。通过对同工酶谱带和酶活性分析，表明这 4 种同工酶与体胚发生具有密切关系，可以作为楸树胚性愈伤组织和体细胞胚胎发生的重要标记，有助于开展楸树体胚发生机制在生理生化水平上的研究。

关键词：楸树，体细胞胚胎发生，同工酶

P45

Homologs of SCAR/WAVE complex components are required for epidermal cell morphogenesis in rice

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Filamentous actins (F-actins) play a vital role in epidermal cell morphogenesis. However, a limited number of studies have examined actin-dependent leaf epidermal cell morphogenesis events in rice. In this study, two recessive mutants were isolated: less pronounced lobe epidermal cell2-1 (lpl2-1) and lpl3-1, whose leaf and stem epidermis developed a smooth surface, with fewer serrated pavement cell (PC) lobes, and decreased papillae. The lpl2-1 also exhibited irregular stomata patterns, reduced plant height, and short panicles and roots. Molecular genetic studies demonstrated that LPL2 and LPL3 encode the PIROGI/Specifically Rac1-associated protein 1 (PIR/SRA1)-like and NCK-associated protein 1 (NAP1)-like proteins, respectively, two components of the suppressor of cAMP receptor/Wiskott-Aldrich syndrome protein-family verprolin-homologous protein (SCAR/WAVE) regulatory complex involved in actin nucleation and function. Epidermal cells exhibited abnormal arrangement of F-actins in both lpl2 and lpl3 expanding leaves. Moreover, the distorted trichomes of Arabidopsis pir could be partially restored by an overexpression of LPL2. A yeast two-hybrid assay revealed that LPL2 can directly interact with LPL3 in vitro. Collectively, the results indicate that LPL2 and LPL3 are two functionally conserved homologs of the SCAR/WAVE complex components, and that they play an important role in controlling epidermal cell morphogenesis in rice by organising F-actin.

Key words: epidermal cell, F-actin, lobe, morphogenesis, rice, SCAR/WAVE

P46

Repressed function of the C terminal domain of GS3 in grain size regulation

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Grain yield in many cereal crops is largely determined by grain size. Our group previously established that GS3-4 allele showing stronger effect on reducing grain size due to its deficiency of the C terminal cysteine-rich domain, compared with the GS3-1 allele. Here show that the cysteine-rich domain cloud not interact with the N terminal (OSR) domain, excluding the possibility of head–to-tail interaction to block the function of OSR. We detected the protein level of the GS3-1 and GS3-4 transgenic plant which under ubiquitin promoter and fused with Flag tag in the C terminal. As expect, the enhanced phenotype mediated by overexpressing GS3-4 was correlated with the increased protein level but not mRNA level. Accumulation of GS3-1 protein but not GS3-4 protein was detected compared with the untreated control when treated with the proteasome inhibitor MG-132, suggesting that GS3-1 might degrade via C terminal domain through the proteasome-mediated pathway. These findings added to the understanding of the molecular mechanism with respect to GS3 in grain size regulation.

Key words: grain size, *GS3* gene, OSR, cysteine-rich domain, protein level

P47

Immature Pollen Exine (*IPE*) is required for pollen wall development in maize

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Exine, the outer layer of pollen wall, is more structured than any other plant walls due to its vital role in protecting genetic material from hostile environment. It is composed of sporopollenin, an un-deciphered complicated lipidic compound. Tapetum, the innermost layer of sporophytic anther wall, serves as the provider for sporopollenin precursor. We reported here a male sterile mutant *immature pollen exine* (*ipe*) in maize. It showed shrunk anther and immature pollen wall at large vacuole stage. We cloned the gene by map based cloning. It encoded a fatty acyl carrier protein (ACP) reductase which was localized in plasmid mediated by N-terminal transit peptide. Expression analysis indicated that *IPE* was expressed mainly in tapetum and microspore after the microspore was released from tetrad. Functional complementation of homologous *Arabidopsis* mutant demonstrated that *IPE* is conserved in monocot and dicot, and may even in flowering plants.

Key words: male sterile, exine, *IPE*, tapetum, maize

P48

starchy endosperm cell factor 1 (*sec1*) is a new imprinted transcription factor in maize endosperm

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In *Zea mays*, endosperm is a terminal organ that is differentiated into four cell types, i.e. aleurone layer, starchy endosperm cells, transfer cell layers and embryo surrounding cells. Starchy endosperm is the most prominent part in seed, fulfilling the main role of synthesis and storage of starch and protein. Our project is to identify the endosperm-specific transcription factors and study their biological functions in the endosperm development and synthesis of storage products. We have identified a new factor, *starchy endosperm cell factor 1* (*sec1*), which is specifically expressed in the endosperm. Its expression could be detected by RT-PCR as early as 6 DAP and it reached the peak at 8 DAP. Sec1 was shown to localize into the nucleus, consistent with the prediction as a transcription factor. RNA in situ hybridization showed that *sec1* is expressed across the whole endosperm at 6 DAP, but its expression is only restricted to the starchy endosperm cells after the completion of endosperm differentiation. We sequenced *sec1* gene from more than 100 inbred lines and found *sec1* is highly conserved and only a few inbred lines bear a couple of SNPs. We made the reciprocal crosses between inbred lines A619 and Fangyin, which have a restriction enzyme polymorphism in the coding sequence. It revealed by enzyme digestion and cDNA sequencing that the maternal allele is much more preferentially expressed in the 18-DAP endosperm cells compared to the paternal allele, resulting in the ratio of the female and male alleles significantly higher than the expectation value. Comparison of the methylation status by a methylation sensitive enzyme demonstrated that the male allele promoter is more highly methylated, indicating that *sec1* is subject to the imprinted regulation. Now we are taking advantage of CRISPR/Cas9 to create null mutants to study its biological function.

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Molecular regulation of bract suppression during panicle development in rice

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The reproductive transition in rice is characterized by a number of dramatic changes. Two of the most striking features of these changes are the suppression of bract, and the change of the lateral organ identity from tiller branching into panicle branching. Both of these features indicate suppression of the vegetative programs for panicle development. However, the underlying mechanisms are not well known. We identified a mutant *panicle with ectopic leaf* (*pel*) that failed to suppress bract outgrowth. *PEL* had two paralogs in rice known as *PEL-like 1* and *2* (*PEL1*, *2*). All three *PEL*s were expressed in the bract primordium revealed by *in situ* hybridization, and PEL proteins formed homodimers and heterodimers *in vitro* and *in vivo*. We used CRISPR/Cas9 to generate single gene and triple-gene mutants. Much more bracts were formed in the triple mutant *pel pel1 pel2* than any single gene mutant. Moreover, the panicle branches were also completely supplanted by the vegetative shoots in the *pel pel1 pel2* plants. Therefore, *PEL*s redundantly terminated the vegetative programs after reproductive transition. Y2H was used to screen the interacting proteins of PELs, and one protein PEL Interacted Protein 1 (PIP1) was identified. Pull-down, BiFC and LCI further substantiated the physical interactions between PIP1 and PELs *in vitro* and *in planta*. Moreover, *PIP1* was co-expressed with the *PEL*s based on the global transcriptomic analysis, suggesting that *PEL*s might regulate *PIP1* or *vice versa*. The results of Y1H, EMSA and ChIP-qPCR demonstrated that PELs directly regulated *PIP1* in rice. Genetic analysis showed that *PIP1* also repressed bract outgrowth. All of these genes encoded transcription factors, suggesting that they should have vital roles in reprogramming the transcriptomes following the reproductive transition. Several genomic approaches will be used to address this question, and the related works are underway. According to these results, a complex network of genes required for terminating the vegetative programs after reproductive transition is emerging.

Key words: transcription factor, reproductive transition, inflorescence architecture

P50

GSNOR 在拟南芥侧根中的调控机理

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亚硝基谷胱甘肽还原酶（S-nitrosoglutathione reductase, GSNOR）在生物体中调控信号分子一氧化氮（NO）的代谢和平衡。GSNOR 完全缺失突变体 *hot5-2*（Col 背景）与 *hot5-4*（Ws 背景）均表现出极度缺乏侧根原基，且回补系能完全恢复突变体的侧根表型。外源施加 NO 减少侧根数目。而同时施加 NO 清除剂 cPTIO，则减弱了 NO 对侧根的抑制。表明 NO 对侧根的发生起到重要的调控作用。通过对生长素报告蛋白 DR5::GUS 的观察发现，突变体侧根原基中没有明显的生长素累积，缺乏侧根发生及发育的生长素浓度梯度。然而，外源施加 IAA,2,4D 不能恢复突变体中侧根缺失表型，但施加不需运输载体的 NAA 能恢复部分突变体侧根。相应地，外源施加 SNP 对侧根的抑制可以通过外源施加 NAA 减弱。表明生长素极性运输缺陷是造成突变体侧根急剧降低的主要原因。进一步实验观察生长运输载体 PINs-GFP，我们发现 PIN1, PIN3 参与侧根起始，在突变体中显著降低，外源 SNP 处理也能使 PIN1 和 PIN3 的表达降低。同时，在实验中发现 PIN1::PIN1-GFP 和 PIN3::PIN3-GFP 转入突变体 *hot5-2* 中得到的拟南芥植株，其根长有显著恢复，相较 *hot5-2* 空载分别提高了 36% 和 39%。证明了 NO 确实对生长素极性运输载体有抑制作用。因此，我们认为，GSNOR 通过对 NO 平衡的调控，保障生长素极性运输，以便生长素积累形成有效的浓度梯度，使侧根正常发生及发育。

关键词：一氧化氮，亚硝基谷胱甘肽还原酶，侧根，生长素，生长素极性运输载体，拟南芥

P51

Arabidopsis MMD1/DUET ensures the progression of male meiotic chromosome condensation and directly regulates the expression of condensin gene *CAP-D3*

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Chromosome condensation mediated by the condensin complex is essential for proper chromosome segregation during cell division. Unlike the rapid mitotic chromosome condensation, meiotic chromosome condensation occurs over a relative long prophase I and is unusually complex due to the coordination with chromosome axis formation and homolog interaction. The molecular mechanisms that regulate meiotic chromosome condensation progression from prophase I to metaphase I are unclear. We show here that the *Arabidopsis* meiotic PHD-finger protein MMD1/DUET is required for progressive compaction of prophase I chromosomes to metaphase I bivalents. The MMD1 PHD domain is required for its function in chromosome condensation and binds to methylated histone tails. Transcriptome analysis and qRT-PCR showed that several condensin genes exhibit significantly reduced expression in *mmd1* meiocytes. Furthermore, MMD1 specifically binds to the promoter region of the condensin subunit gene *CAP-D3* to enhance its expression. Moreover, *cap-d3* mutants exhibit similar chromosome condensation defects, revealing a novel MMD1- dependent mechanism for regulating meiotic chromosome condensation in part by promoting condensin gene expression. Together, these discoveries provide strong evidence that the histone reader MMD1/DUET defines an important step for regulating the progression of meiotic prophase I chromosome condensation.

Key words: MMD1, meiotic chromosome condensation, condensin

P52

参与叶片衰老调控的 E3 泛素连接酶 *KPIL* 基因的功能研究

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衰老（Senescence）是植物叶片发育的最后一个阶段。做为一个受遗传程序严格调控的生命过程，衰老不仅对调节植物营养分配和增强环境适应性意义重大，更跟作物产量和品质息息相关。叶片衰老的严格有序调控表现在多种层次，其中蛋白质的翻译后修饰过程，如磷酸化与泛素化，近年来正被越来越多的研究证实为调控该生命过程的重要因素。在拟南芥中，占整个基因组约 6%的 1400 多个基因参与编码泛素-蛋白酶体介导的蛋白质降解路径的相关组分，其中 90%的基因负责编码具备底物特异性的各种 E3 连接酶。含有 RING 结构域的 E3 连接酶分子量相对较小，但成员数却有几百个之多。我们在前期实验中筛选得到一个参与调控拟南芥叶片衰老过程的 RING type E3 连接酶基因并命名为 *KPIL*。30μM 地塞米松处理可诱导型启动子控制 *KPIL* 表达的 *GVG:KPIL* 转基因植株，可引起植株叶片明显早衰。幼苗中诱导 *KPIL* 过表达也可以引起子叶黄化、根毛密集，暗示该基因过表达影响乙烯的合成和/或响应。通过对该基因的表达模式以及 *KPIL:GUS* 转基因植株的染色结果分析，我们发现 *KPIL* 是一个衰老上调基因。上述研究结果表明 *KPIL* 可能是拟南芥叶片衰老的正调节因子，对 *KPIL* 参与叶片衰老调控的分子机制进行深入研究，将有助于我们更好地理解蛋白质泛素化降解路径在叶片衰老控制中的角色与功能。

关键词：拟南芥，叶片衰老，E3泛素连接酶，乙烯

P53

Morphological structure and transcriptome comparison of the cytoplasmic male sterility line in *Brassica napus* (SaNa-1A) derived from somatic hybridization and its maintainer line SaNa-1B

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SaNa-1A is a novel cytoplasmic male sterility (CMS) line in *Brassica napus* derived from progenies of somatic hybrids between *B. napus* and *Sinapis alba*, and SaNa-1B is the corresponding maintainer line. In this study, some considerable differences of floral organs between CMS and the maintainer lines were observed. By comparing different anther developmental stages between the two lines through microscope observation, we found that anther development in SaNa-1A was abnormal since the tetrad stage, and the development of microspores ceased during the uninucleate stage. Subsequently, we conducted a genome-wide high-throughput transcriptomic sequencing for young floral buds of sterile and fertile plants to elucidate gene expression and regulation caused by the alien chromosome and cytoplasm from *S. alba* during the development of young floral buds. After filtering the low-quality data, a total of 36,539,702 and 43,903,006 clean tags remained in the 2 libraries. The reads were assembled into 195,568 unigenes composing the transcriptome of the floral bud. A total of 7,811 unigenes, which were mainly distributed in the metabolic and protein synthesis pathways, were identified with significant expression differences between the two libraries. We also observed that genes participating in carbon metabolism, tricarboxylic acid cycle, oxidative phosphorylation, oxidation–reduction system, pentatricopeptide repeat, and anther development were downregulated in the sterile line. Several of these genes may be candidates for future research on the sterility mechanism in the CMS line, fertility restoration, and improvement of agronomic traits in the maintainer line. Further study on the unknown tags specifically expressed in the fertile line will help in the exploration of desirable agronomic traits from wild species of rapeseed.

Key words: *Brassica napus*, cytoplasmic male sterility (CMS), somatic hybridization, morphological structure, transcriptomic analysis

P54

NRPB3, the third largest subunit of RNA polymerase II, is essential for stomatal patterning and differentiation in *Arabidopsis*

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Stomata are highly specialized epidermal structures that control transpiration and gas exchange between plants and the environment. Signal networks underlying stomatal development have been previously uncovered but much less is known about how signals involved in stomatal development are transmitted to RNA polymerase II (Pol II or RPB), which plays a central role in the transcription of mRNA coding genes. Here, we identify a partial loss-of-function mutation of the third largest subunit of nuclear DNA-dependent Pol II (NRPB3) that exhibits an increased number of stomatal lineage cells and paired stomata. Phenotypic and genetic analyses indicated that NRPB3isnot onlyrequiredforcorrect stomatalpatterning,butisalso essential for stomatal differentiation. Protein-protein interaction assays showed that NRPB3 directly interacts with two basic helix-loop-helix (bHLH) transcription factors, FAMA and INDUCER OF CBF EXPRESSION1 (ICE1), indicating that NRPB3 serves as an acceptor for signals from transcription factors involved in stomatal development. Our findings highlight the surprisingly conserved activating mechanisms mediated by the third largest subunit of Pol II in eukaryotes.

Keywords: stomata, RNA polymerase II, patterning, differentiation, Arabidopsis

P55

玉米次生壁相关 NAC 转录因子 (ZmSWNs) 的功能研究

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植物次生细胞壁主要由木质素、纤维素及半纤维素组成，是植物中含量最为丰富的生物质材料。次生壁对于植物直立生长，水分运输等具有重要作用，因此次生壁的发育对于植物而言非常重要。目前，对于植物次生壁相关组分生物合成的途径及相关基因研究较为清晰，然而这些基因转录调控的分子机制仍不清楚。研究表明，一类与次生壁相关的 NAC 转录因子（SWNs）在次生壁发育过程中具有重要作用。利用同源比对，我们找到了玉米中相关的 SWN 基因，并对其中两个基因（*ZmNST3/4*）进行了功能的深入分析。实验表明 ZmNST3/4 具有转录激活活性，定位于细胞核，体外能特异的结合 SWN 识别元件 SNBE，原位杂交结果表明 ZmNST3/4 特异的在次生壁积累的细胞中表达。表型回补实验结果显示异源表达 *ZmNST3/4* 能回复拟南芥 *nst1 nst3* 双突变体不能直立生长的表型，突变体中束间纤维细胞无次生壁积累，异源表达 *ZmNST3/4* 后，次生壁正常积累，表明 *ZmNST3/4* 能促进植物次生壁的发育，从而回复突变体的表型。过表达 ZmNST3/4 可引起转基因拟南芥拟南芥束间维管次生壁加厚，木质素、纤维素含量增加。转基因玉米表现出与转基因拟南芥相似的表型，即次生壁发育程度发生变化，同时木质素、纤维素的含量改变。进一步分析发现，*ZmNST3/4* 通过调控 *ZmMYB109* 等次生壁相关的 MYB 转录因子来调控木质素、纤维素及半纤维素的生物合成及积累。这些结果表明 ZmNST3/4 是玉米中调控次生壁发育的关键转录因子，能正调控次生壁的沉积。

关键词：玉米，次生细胞壁，NAC 转录因子

P56

Involvement of autophagy in regulating chloroplast development in *Arabidopsis*

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Autophagy plays multiple roles in the plastid degradation during senescence and/or stresses, particularly in the chloroplast. However, relatively little is known about the role of autophagy in the developmental process of chloroplasts. Here, we characterized the relationship between autophagy and a chloroplast protein (CP1), which was identified to interact with Atg8 from a yeast two hybrid screen. Our results further revealed that CP1 also interacts with Atg8 *in planta*, and Atg5 and Atg7 are required for the autophagy-mediated degradation of CP1. Nevertheless, our results also indicated that the interaction between Atg8 and CP1 was controlled by a specific Atg8-interacting motif (AIM). So far, there are two mechanisms, ATI1-PS and Rubisco-containing body (RCB), controlling the plastid proteins trafficking from plastids to the vacuole in the autophagy-dependent manner. In this study, we found that autophagy-mediated transport of CP1 from plastid to the vacuole is independent on both ATI1-PS and RCB pathways, suggesting a novel unknown mechanism underlying this process. In addition, two other proteins CP2 and CP3 that has been found to regulate chloroplast development were also evident to be turned over by autophagy. Therefore, we propose that autophagy may be involved in the chloroplast development through the regulation of the homeostasis of a subset of chloroplast proteins in plant.

Key words: autophagy, ATG8, chloroplast development, ATI1, RCB

P57

Characterization and fine mapping of the rice gene *OsARVL4* regulating leaf morphology and leaf vein development

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Leaf morphology and chlorophyll content are closely related to the photosynthetic efficiency, which would potentially contribute to crop yield. In this study, we isolated an EMS-mutagenized rice mutant displaying abaxial rolling and vein-albino leaves, and thus designated it as Osarvl4. Compared to the wild type 'Nipponbare', *Osarvl4* mutant had abnormal development of clear cells, parenchyma cells, sclerenchymatous cells, mesophyll cells, bulliform cells and vascular bundles. As a result of the defective leaf development, the chlorophyll content and photosynthetic efficiency were significantly affected in the mutant. Genetic analysis using map-based cloning indicated that the mutation was controlled by a single recessive karyogene localized within a 44 kb region on the long arm of chromosome 4. Sequence analysis and alignment indicated that the three candidate genes in this region showed no difference at the DNA level. However, quantitative realtime PCR analysis showed that the expression of the LOC_Os04g33580 gene in the mutant was significantly lower than that of wild type, while expression of the other two candidate genes (LOC_Os04g33560 and LOC_Os04g33570) exhibited no significant difference. Therefore, we speculate that LOC_Os04g33580 might be the target gene which regulates leaf vein development and leaf morphogenesis in rice and this locus might be subject to epigenetic regulation, such as DNA methylation, Thus, our finding suggests that the *OsARVL4* gene is involved in the regulation of chlorophyll content and photosynthetic efficiency in plants, and provides a genetic basis for the future study of genes related to leaf development in rice.

Key words: leaf morphology; parenchyma cells; pleiotropism; photosynthetic efficiency

P58

RNA-Seq identifies genes associated with aberrant meiotic prophase I of a novel TE5A genic male sterility in *Brassica napus*

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Genic male sterility (GMS), as a promising pollination control system, has already been extensively utilized for hybrid rapeseed production. TE5A is a novel thermo-sensitive dominant GMS line in Brassica napus. Analysis of paraffin-cross sections of anthers and TEM showed that the male gamete development was arrested at meiosis prophase I, eventually, meiotic PMCs were degenerated, leaving an empty locule, and no pollen grains were generated. Chromosome spreads and FISH showed that homologous chromosomes could not pair, synapse, condense and form bivalents in TE5A. EdU uptake of S-phase meiocytes revealed that TE5A mutant could accomplish DNA replication. We then analyzed the transcriptome differences between young floral buds of sterile plants and its near-isogenic fertile plants. RNA-Seq analysis identified 3,841 differentially expressed genes (DEGs), some of which were associated with homologous chromosome behavior and cell cycle control during meiosis. These DEGs represented a set of potential candidate genes associated with GMS in the TE5A. Dynamic expression changes of candidate DEGs were then detected at different developmental stages of anthers. The present study provided a global assessment of the differences between GMS sterile plants and its near-isogenic fertile plants, as well as identified new fertility-associated genes and elucidated the mechanisms of GMS.

Key words: genic male sterility, RNA-Seq, prophase I, meiotic cycle, meiosis

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基于转录组的黄芪种子萌发过程的生化分析

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种子萌发开始于吸涨，结束于胚根突出，是植物生长的第一步。种子的成功萌发不仅是建立幼苗的关键，也关系到作物的产量问题。黄芪是豆科植物蒙古黄芪（*Astragalus membranaceus* var. *mongholicus*）或膜荚黄芪（*Astragalus membranaceus* ）的干燥根，可药食兼用。蒙古黄芪和膜荚黄芪虽在分类学地位上相似，但种子外形相近，肉眼难以区分。光学显微镜下观察，两种黄芪种子表面纹理有明显差异；蒙古黄芪硬实率比膜荚黄芪种子高 3.5 倍，蒙古黄芪萌发不整齐，萌发高峰滞后。前人多关注因硬实率高而导致黄芪种子的萌发率低的问题，而对黄芪种子萌发过程中生理生化物质的变化关注甚少。

我们对蒙古黄芪和膜荚黄芪在 25°C 恒温培养箱中萌发 12 h、24 h 和 48 h 从萌发到幼苗建立过程中的种子进行生理生化指标进行测定，并结合转录组分析两种黄芪种子萌发过程的差异。通过组装并去冗余后得到 92,359 个 Unigene，将 Unigene 比对到 7 大功能数据库进行注释，最终分别有 NR: 63.11%，NT: 66.15%，Swissprot: 41.13%，COG: 23.50%，KEGG: 46.44%，GO: 17.22%以及 Interpro: 43.37%的 Unigene 获得功能注释。将各萌发阶段进行对比，注释到 KEGG 中，最多的是糖代谢。两个品种的黄芪种子的总多糖含量均随萌发时间先增加后减少，膜荚黄芪略高于蒙古黄芪。注释到 KEGG 的差异表达基因较多的还有转录和运输及代谢，萜类和黄酮类代谢。黄芪种子总黄酮含量均随着萌发时间也持续增加。黄芪种子赤霉素均随萌发时间逐渐减少，且在萌发初期蒙古黄芪略高于膜荚黄芪，萌发后期差异不大；脱落酸也随萌发时间逐渐减少，但蒙古黄芪减少更迅速；玉米素在萌发前期和中期均保持较高水平，萌发后期有所下降。差异表达基因注释到 GO 通路中最多的是代谢途径，其次是催化活性和细胞过程。综上所述，两种黄芪种子在萌发到幼苗建立过程中，总多糖和总黄酮呈增长趋势，而激素类呈下降趋势。可以看出，种子在萌发过程中的细胞分裂迅速，代谢活跃。

关键词：蒙古黄芪，膜荚黄芪，转录组，总黄酮，总多糖，内源激素

P60

芸苔属作物表皮毛发育的分子机制研究

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芸薹属蔬菜作物的叶在大小、形状、叶色、夹角、卷曲和表面特征等形态表现上呈现多样性。其中，表皮毛的有无和多少是这些蔬菜作物商品性状的重要标志。结球大白菜（*B. rapa* ssp. *pekinensis* cv. Bre）的叶着生表皮毛，而不结球乌塌菜（*B. rapa* ssp. *chinensis* cv. Wut）叶没有表皮毛。我们以白菜和乌塌菜为亲本构建重组自交系，基于基因组重测序数据进行基因分型获得高分辨率的遗传图谱，结合表型数据的统计完成表皮毛性状的初定位，鉴定了控制表皮毛的候选基因 *GL1-1*。我们分别克隆了大白菜和乌塌菜中的 *GL1-1* 等位基因，发现乌塌菜 *GL1-1* 两个 SNP 造成氨基酸序列第 93 位和第 135 位的非同义突变。通过构建定点突变的表达载体并在拟南芥无毛突变体 *gl1* 中转化，转基因株系表型回复的实验结果表明，第 93 位的色氨酸突变成精氨酸后一律无法回复表型，是控制大白菜表皮毛的关键氨基酸位点。通过拟南芥原生质体双分子荧光互补实验（BiFC）和蛋白体外免疫共沉淀实验，我们验证了 *GL1-1* 基因第 93 位的色氨酸是 GL1 与 GL3 相互作用的关键氨基酸，该色氨酸突变后 GL1 与 GL3 的相互作用减弱，造成 GL1 与 GL3 参与的 MBW 蛋白复合物无法正常形成，影响下游 *GL2* 基因起始表达。这些结果表明，白菜 *GL1-1* 等位基因上一个氨基酸决定着表皮毛的形态发生。此外，白菜 *GL1-1* 同源基因 *GL1-2* 有长片段插入，是功能缺失的，而乌塌菜 *GL1-2* 可以正常编码蛋白，可以在叶片边缘区域回复拟南芥 *gl1* 突变体无毛的表型。扫描电镜结果显示在乌塌菜叶片边缘部位有极少数的单分支表皮毛，目前，我们正在构建 GL1-2 启动子序列驱动 GUS 表达的载体，期望通过 GUS 染色实验验证在芸苔属蔬菜植物中 GL1-2 在表皮毛发育中具有空间特异性。

关键词：芸薹属，表皮毛，*GL1*，等位基因，同源基因，形态发生

P61

Albino Leaf 1 that encodes the sole octotricopeptide repeat protein is responsible for chloroplast development in rice

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Chloroplast, the photosynthetic organelle in plants, plays a crucial role in plant development and growth through manipulating the capacity of photosynthesis. However, the regulatory mechanism of chloroplast development still remains elusive. Here, we characterized a mutant with defective in chloroplasts in rice, termed *albino leaf 1* (*al1*), which exhibits distinct albino phenotype in leaves, eventually leading the *al1* seedling to lethal. Electronic microscopy observation demonstrated that the numbers of thylakoids was reduced and the structure of thylakoids was disrupted in the *al1* mutant during rice development, which eventually led to the breakdown of chloroplast. Molecular cloning revealed that *AL1* encodes the sole Octotricopeptide Repeat Protein (RAP) in rice. Genetic complementation of the Arabidopsis *rap* mutants indicated that AL1 protein is a functional RAP. Further analysis illustrated that three transcript variants were present in the *AL1* gene and the altered splices were occurred at the 3′UTR of *AL1* transcript. In addition, our results also indicate that disruption of *AL1* gene results in the altered expression of chloroplast associated genes. Consistently, proteomic analysis demonstrated that the abundances of photosynthesis associated proteins are significantly altered, as well as a group of metabolism associated proteins. More specifically, we found that the loss of *AL1* resulted in the altered abundances of ribosomal proteins, suggesting that RAP likely also regulates the homeostasis of ribosomal proteins in rice in addition to the rRNA. Taken together, we conclude that the *AL1*, particularly the *AL1a* and *c* isoform, plays an essential role in chloroplast development in rice.

Key words: chloroplast development, RAP, *AL1* gene, OPR

P62

XRN2 and XRN3 interact with HYL1 for pri-miRNA processing

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MicroRNAs (miRNAs) play important role in plant development by post-transcriptional regulation of target genes. In *Arabidopsis thaliana*, HYPONASTIC LEAVES1 (HYL1) is a double-stranded RNA-binding protein that forms a complex with DICER-LIKE1 (DCL1) and SERRATE (SE) to process primary miRNA (pri-miRNA) into mature miRNA and forms homodimers to ensure the correct selection of cleavage sites in primary miRNA. Here, we found that HYL1 coordinated with XRN2 and XRN3, the 5′ to 3′ exoribonucleases, in cleavage of pri-miRNA. HYL1 directly interacted with XRN2 and XRN3, which were co-localized with SE and DCL1 as well. The accumulations of mature miRNA were slightly reduced in *xrn3* mutants, but considerably increased in *p35S::XRN3* plants. In *xrn2* and *xrn3* mutants, the accumulations of pri-miRNA were increased, but decreased in *p35S::XRN2* and *p35S::XRN3* plants. The in vitro miRNA processing experiments revealed that the concentration of XRN2 and XRN3 in HYL1-DCL1 complexes affected the cleavage of pri-miRNA and the efficiency of miRNA processing. We suggest that XRN2 and XRN3 enhance miRNA processing through cleavage of pri-miRNA.

Key words: HYL1, XRN2, XRN3, pri-miRNA

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Cytokinin as a positional cue regulating lateral root spacing in *Arabidopsis*

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The root systems of plants have developed adaptive architectures to exploit soil resources. The formation of lateral roots (LRs) contributes to root system architecture. Roots of plants with a lower cytokinin status form LR primordia (LRP) in unusually close proximity, indicating a role for the hormone in regulating the positioning of LRs along the main root axis. Data obtained from cytokinin-synthesis mutants of *Arabidopsis thaliana* combined with gene expression analysis indicate that cytokinin synthesis by IPT5 and LOG4 occurring early during LRP initiation generates a local cytokinin signal abbreviating LRP formation in neighbouring pericycle cells. In addition, IPT3, IPT5, and IPT7 contribute to cytokinin synthesis in the vicinity of existing LRP, thus suppressing initiation of new LRs. Interestingly, mutation of *CYP735A* genes required for *trans*-zeatin biosynthesis caused strong defects in LR positioning, indicating an important role for this cytokinin metabolite in regulating LR spacing. Further it is shown that cytokinin and a known regulator of LR spacing, the receptor-like kinase ARABIDOPSIS CRINKLY4 (ACR4), operate in a non-hierarchical manner but might exert reciprocal control at the transcript level. Taken together, the results suggest that cytokinin acts as a paracrine hormonal signal in regulating root system architecture.

Key words: *Arabidopsis thaliana*, cytokinin, lateral root, lateral root spacing, root branching, root system architecture.

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ABI5 家族保守区 II 的结构与功能研究

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ABI5 及其亚家族成员是一类碱性亮氨酸拉链类转录因子，它们参与了脱落酸（ABA)介导的植物体对许多生物及非生物胁迫的应答。其家族中共有 7 个保守区，保守区 II 在酵母细胞和植物细胞中均具有明显的转录激活活性。然而，存在于保守区 II 两侧的保守区 I 和保守区 III 均对保守区 II 的活性有抑制。本研究利用拟南芥叶肉原生质体瞬时表达分析发现：没有 ABA 存在时，保守区 I-II-III 在植物细胞中没有转录激活活性；而在 ABA 存在时，保守区 I-II-III 在植物细胞中具有转录激活活性。利用酵母单杂交技术进一步分析保守区 II 中 α-螺旋结构对保守区 II 活性的影响时发现，当 α-螺旋结构单独存在时也能激活报告基因的转录。当 α-螺旋 N 端的前 5 个氨基酸残基中的任意一个被丙氨酸替换或 α-螺旋结构本身被破坏时，保守区 II 的活性完全丧失。上述结果表明,α-螺旋结构 N 端的前 5 个氨基酸残基以及 α-螺旋结构本身对保守区 II 的活性都是必需的。

关键词：ABI5 转录因子，转录激活，保守区 II，酵母单杂交

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AtSARK 关键自磷酸化位点的鉴定与功能研究

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蛋白激酶和蛋白磷酸酶所催化的蛋白质可逆磷酸化反应常常作为调控植物特定发育信号通路的“开关”，也是调控叶片衰老的重要因素。我们实验室在前期研究中分离鉴定了一个通过生长素和乙烯的协同作用正向调控拟南芥叶片衰老的 LRR 型双底物特异性类受体蛋白激酶 AtSARK（Senescence-Associated Receptor-like Kinase），并进一步分离鉴定了一个与之直接互作、负调控拟南芥叶片衰老的 PP2C 型蛋白磷酸酶 SSPP（Senescence Suppressed Protein Phosphatase）。AtSARK 具有很强的自磷酸化活性；SSPP 可与 AtSARK 直接互作并对其自磷酸化的胞内域进行脱磷酸化作用，过表达该基因可以明显抑制 AtSARK 诱导的拟南芥早衰。在此基础上，我们通过质谱分析鉴定了 AtSARK 序列中 27 个可能的磷酸化氨基酸残基位点，并分别利用对上述位点的模拟磷酸化和非磷酸化的点突变来细致分析各位点的功能。我们发现其中的 9 个点突变显著改变了 AtSARK 的自磷酸化状态，进一步 pull-down 实验表明，这 9 个位点中有 6 个点的突变改变了 AtSARK 与 SSPP 之间的互作。相应的转基因拟南芥表型分析结果表明：AtSARK 自磷酸化活性降低会导致该蛋白激酶部分生物学功能丧失，而磷酸化状态的 AtSARK 是其保持其生物学活性的重要条件。对这一对调控叶片衰老的蛋白激酶和蛋白磷酸酶的深入研究，将有助于我们深入理解叶片衰老调控的分子机制。

关键词：拟南芥，自磷酸化，类受体蛋白激酶，蛋白磷酸酶，叶片衰老

P66

AUX/IAA 和 MYB 在姜花花香代谢中作用

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白姜花(*Hedychium coronarium*)具有浓郁的芳香，其花朵挥发性物质主要为沉香醇、β-罗勒烯、金合欢烯、苯甲酸甲酯等萜类和苯丙烷类物质。生长素是调控花发育的重要激素，但对花香物质代谢的调控报道不多。本研究从白姜花花瓣中克隆了均定位于细胞核属 A 亚族的 HcIAA2、IAA-B 亚族的 HcIAA4 和 R2R3 型的 MYB2。*HcIAA2*、*HcMYB2* 主要在具香味的白姜花和金姜花花器官中表达，不香的红姜花和原瓣姜花中不表达；*HcIAA2*、*HcIAA4*、*HcMYB2* 在不同花发育时期的表达量均为在蕾期较低，盛开期达到最高，衰老期降低，与花香释放的时空相一致。外源激素 IAA 处理明显提高白姜花罗勒烯、沉香醇和苯甲酸甲酯的挥发量，相关合成酶基因 *HcBSMT1* 和 *HcBSMT2* 的相对表达量显著上调，*HcIAA4*、*HcMYB2* 表达量上调，而 *HcIAA2* 表达量下调。生长素转运抑制剂 TIBA 处理显著降低沉香醇、金合欢烯和苯甲酸甲酯的挥发量，*HcIAA4*、*HcMYB2* 表达量降低，*HcIAA2* 升高，*HcTPS8*、*HcTPS10*、*HcBSMT1* 相对表达量显著下调。在白姜花花瓣中 BSMV-VIGS 病毒瞬时沉默 *HcIAA2* 后，沉香醇、别罗勒烯、苯甲酸甲酯的挥发量增加，*HcTPS3*、*HcTPS8* 表达量升高，瞬时沉默 *HcIAA4* 和 *HcMYB2* 后，沉香醇、别罗勒烯、金合欢烯和苯甲酸甲酯的挥发量有不同程度的减少，*HcTPS3*、*HcTPS8*、*HcTPS10* 有不同程度的降低。在酵母体中 HcIAA2 和 HcIAA4， HcMYB2 与 HcIAA4 能够互作。本研究为姜花花香代谢与生长素调控网络之间的关联性进行了有益的探索，为进一步深入解析其调控机制提供了参考。

关键字：白姜花，花香，AUX/IAA，生长素，MYB

P67

Ethylene promotion of lettuce seed germination under salinity may act through modulation of proline biosynthesis

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Diverse genotypes of lettuce were assessed for their seed germination responses to salt stress. The most tolerant genotype, PI251246, a primitive accession of *Lactuca sativa*, can germinate well at 250 mM NaCl, while the most sensitive genotypes are greatly inhibited at 225 mM NaCl. The inhibitory effect of salinity on germination was due to the osmotic stress rather than ionic toxicity. The inhibition of seed germination by salinity could be greatly alleviated by ethylene or partly by 1-aminocyclopropane carboxylic acid (ACC, ethylene precursor). Application of aminoethoxyvinylglycine (AVG, an ethylene biosynthetic inhibitor) and silver thiosulfate (STS, an ethylene receptor inhibitor) can completely block seed germination of PI251246 seeds at 250 and 200 mM NaCl, respectively. Ethylene production is seven times higher from PI251246 seeds than from UC96US23 seeds imbibed in 200 mM NaCl, although there is no significant difference between them when imbibed on water. Furthermore, free proline content of PI251246 seeds is significantly higher than that of UC96US23 seeds at 200 mM NaCl. Application of AVG or STS dramatically reduced free proline content of PI251246 seeds at 250 and 200 mM NaCl, respectively. Real-time PCR analysis showed that the transcript levels of ethylene biosynthetic gene *LsACS1* and *LsACO1* and of *LsPDH1*, a proline catabolic gene, are more abundant in PI251246 seeds imbibed in 200 mM NaCl than in other lettuce genotypes; STS greatly reduced their transcript levels in PI251246 seeds at 200 mM NaCl. However, seedling biomass and root length analyses revealed that the tolerance of PI251246 to salinity is specific to germination; seedling growth was not tolerant to salt stress. Thus, tolerance to osmotic stress during germination does not necessarily imply tolerance of seedling growth to salinity.

Key words: lettuce, germination, salinity, ethylene, proline salinity

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拟南芥磷脂酰肌醇锚定蛋白 LORELEI/LLG1 作为分子伴侣和共同受体参与 FERONIA 受体激酶的信号通路

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拟南芥受体激酶FERONIA参与到众多重要的植物生长发育过程中，包括生长素、脱落酸和快速碱性因子的信号转导以及生殖发育过程等。前人的工作表明，雌性器官特异表达的磷脂酰肌醇锚定蛋白LORELEI的突变体表现出同*feronia*突变体相似的花粉管感受雌性信号的缺陷。在本研究中，我们发现营养器官特异性表达的LORELEI-like GPI-anchored Protein 1 (LLG1)的突变体具有同*feronia* 突变体相似的各种激素反应的缺陷。LLG1/LRE同FERONIA直接在细胞膜上和内质网中相互作用，而且，在*llg1*缺失突变体中FERONIA蛋白被阻滞在内质网中而无法正确定位在细胞膜上，从而确认FERONIA和LLG1/LRE的复合体是在内质网中形成的。进一步的结果表明，LLG1存在于FERONIA调控的RAC/ROP信号途径复合体中并作为FERONIA感受快速碱性因子的共同受体起作用。另外，我们还发现FERONIA通过其胞外区的Juxtamembrane区段同LLG1结合，其缺失导致FERONIA突变体蛋白停留在内质网中。综上所述，我们的结果表明LLG1/LRE作为FERONIA的分子伴侣和共同受体参与其信号通路，并且提出了磷脂酰肌醇锚定蛋白协助受体激酶完成生物学功能的新机制 (Li et. al, *Elife,* 2015)。

关键词：受体激酶，磷脂酰肌醇锚定蛋白，生长素，快速碱性因子，信号传导

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Molecular regulation of age-dependent JA response decay in *Arabidopsis*

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The JA signaling pathway plays important roles in plant defense against herbivorous insects. Jasmonate ZIM-domain (JAZ) proteins, which are the repressors of JA signaling, have two conserved domains: the N-terminal ZIM domain and the C-terminal Jas domain. The Jas domain is a protein-protein interaction surface required for binding to either the transcription factors such as MYC2, or CORONATINE INSENSITIVE1 (COI1), a component of the ubiquitin E3 ligase SCFCOI1. In normal conditions, the relatively high level of JAZs represses the activity of transcription factors. External stimuli, such as wounding or insect attack, cause rapid rise of JA-Ile concentration in plant cell, which triggers COI1-JAZ interaction and degradation of JAZs by the 26S proteasome, releasing the transcription factors to activate downstream defense genes. Therefore, the abundance of JAZ proteins in cells, determined largely by the rates of protein degradation, controls the output of JA response. Here we reported that JA response decayed in an age dependent manner and this was controlled by miR156-SPL module. miR156 targeted SPLs dampens JA responses in leaves of adult plant by stabilizing JAZ proteins. However, leaves from adult plant actually have enhanced resistance to insect herbivore. We found contrary to JA response, the major defense compounds in plants of Brassicaceae, glucosinolates (GLSs) in leaves increase sequentially, a tendency independent of the miR156-SPLs or the JA signaling. Thus, the two pathways change in reverse directions: while the JA-mediated inductive defense fades away, secondary metabolites deposit cumulatively. Our data provide new insight into the balanced regulation over defense systems.

Key words: JA response, insect resistance, JAZ proteins, miR156-SPL module

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Genetic identification of RGF1 receptors, RGF1 INSENSITIVE 1 to 5

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RGF1, a secreted peptide hormone, plays a key role in root meristem development in Arabidopsis. Previous studies indicated that a functional RGF1 needs to be sulfated at a tyrosine residue by a tyrosylprotein sulfotransferase (TPST) and that RGF1 regulates root meristem activity mainly via two downstream transcription factors, PLETHORA 1 (PLT1) and PLT2. How extracellular RGF1 is perceived by a plant cell, however, is not understood. Using genetic approaches, we discovered a clade of leucine-rich repeat receptor-like kinases (LRR-RLKs), designated as RGF1 INSENSITIVE 1 (RGI1) to RGI5, serving as receptors of RGF1. Two independent *rgi1 rgi2 rgi3 rgi4 rgi5* quintuple mutants display a consistent short primary root phenotype with a small size of meristem. An *rgi1 rgi2 rgi3 rgi4* quadruple mutant shows a significantly reduced sensitivity and the quintuple mutant is completely insensitive to RGF1. The expression levels of *PLT1* and *PLT2* are almost undetectable in the quintuple mutant. Ectopic expression of *PLT2* driven by an *RGI2* promoter in the quintuple mutant greatly rescued its root meristem defects. One of the RGIs, RGI1, was subsequently analyzed biochemically in detail. *In vitro* dot-blotting and pull-down analysis indicated that RGI1 can physically interact with RGF1. Exogenous application of RGF1 can quickly and simultaneously induce the phosphorylation and ubiquitination of RGI1, indicating that RGI1 can perceive and transduce the RGF1 peptide signal. Yet, the activated RGI1 is likely turned over rapidly. These results demonstrated that RGIs, acting as the receptors of RGF1, play essential roles in RGF1-PLT-mediated root meristem development in *Arabidopsis thaliana*.

Key words: receptor-like kinase, RGF1, RGF1 INSENSITIVE, PLETHORA, Arabidopsis

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Paper-based electroanalytical devices for analysis of phytohormones *in situ*

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Phytohormones are a group of naturally occurring trace substances which regulate multiple physiological processes of plants, including growth, development, and response to biotic and abiotic stresses. Detection of phytohormones *in situ* has gained significant attention due to their critical roles in regulating developmental processes and signaling for defences in plants at low concentration. Here we report the application of paper-based electroanalytical devices for sensitively *in situ* detection of salicylic acid in tomato leaves with the sample volume of several microliters. Specifically, disposable working electrodes were modified with multiwall carbon nanotubes and Nafion. Then, by integrating the paper-based electroanalytical devices on the tomato leaves, salicylic acid can be determianted *in situ*. The results also demonstrated that the amounts of salicylic acid differed statistically in normal, phytoene desaturase (*PDS*) silent and diseased (infected by *Botrytis cinerea*) tomato leaves. The study might also provide a sensitive method with spatiotemporal resolution for mapping of other phytohormones in the plant body in *situ*.

Key words: phytohormones, salicylic acid, *in situ* electrochemical detection, paper-based analytical devices

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Functional study of a putative zinc finger protein in brassinosteroid-regulated plant growth in *Arabidopsis*

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Brassinosteroids (BRs) are a group of steroidal hormones that regulate a wide range of developmental processes in plants, including cell expansion, cell division, senescence, male fertility, fruit ripening, and responses to environmental stresses. BR signaling pathway is mediated by the BR receptors BRI1/BAK1 and the transcription factors BZR1/BES1. As a positive regulator and a key transcription factor in BR signaling, BZR1 has been shown to mediate BR crosstalk with many other signaling pathways in plants. To explore whether BZR1 works together with other transcription regulators to regulate different BR responses, we performed a BZR1 yeast two-hybrid screen and identified a BZR1-interacting protein BZF1 (BZR1-interacting Factor 1) in *Arabidopsis*.BZF1 is a putative transcription factor because it contains a CCCH-type zinc finger motif. BZF1 interacts with BZR1 *in vitro* and *in vivo* and localizes in nucleus and cytoplasm and its accumulation is BR-inducible. However, while BZR1 enhances plant growth and cell elongation, BZF1 inhibits these processes when it is overexpressed. The inhibitory effect of BZF1 seems due to its inhibition to BZR1 activity because overexpression of BZF1 suppresses BZR1's hypersensitivity to BR. These results suggest that BZF1 is likely a negative regulator of BR signaling and plant growth. Further studies will be undertaken to understand how BZF1 modulates BR signaling and plant growth.

Key words: *Arabidopsis thaliana*, brassinosteroids, plant growth.

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ABCA9 抑制 BRI1 蛋白降解过程的分子机制研究

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油菜素内酯（Brassinosteroid，BR）属于植物固醇类激素，通过结合位于细胞膜上的类受体激酶 BRI1（BR Insensitive1)传递信号，调控植物生长发育过程。BRI1 不同位点的突变可致其蛋白活性降低、蛋白量的减少或表达定位的改变，从而导致 BR 信号的减弱或缺失。其中，*bri1-5* 是 BRI1 弱突变体，植株矮小，BRI1 蛋白减少，且主要表达在内质网上。ABCA9 蛋白属于 ABC 蛋白家族的 A 亚族，是一类定位于内质网负责运输的转运体。本项目前期研究发现 ABCA9 的突变能部分恢复 *bri1-5* 表型及对 24-表油菜素内酯的敏感性，BR 信号通路恢复正常，且 bri1-5 亚细胞定位没有改变，但受体蛋白量明显增加。本项目预期通过对 ABCA9 的进一步研究，分析其对 BRI1 蛋白的作用机理及调控机制，完善调控 BR 信号的途径，为今后深入研究 BR 信号提供理论依据及实验基础。

关键词：油菜素内酯，*BRI1*（Brassinosteroid Insensitive 1），*bri1-5*，ABCA9

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拟南芥转录因子 HAT1 对黄瓜花叶病毒的响应

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拟南芥同源域亮氨酸拉链蛋白 HAT1 属于 HD-Zip Ⅱ家族，转录因子 HAT1 在植物生长和发育中起到重要作用。为了进一步阐明 HD-Zip Ⅱ转录因子在植物防御中的作用，黄瓜花叶病毒（CMV）感染拟南芥缺失突变体 *hat1*，*hat1hat3*，*hat1hat2hat3* 和 *HAT1* 过表达植株（HAT1OX）。结果显示，HAT1OX 植株对 CMV 更加易感，*HAT1* 功能缺失突变体显示较低的易感性。和缺失突变体 *hat1*（或 *hat1hat3*）相比，*hat1hat2hat3* 对 CMV 病毒的抗性增加，*HAT1* 和其同源基因 *HAT2*，*HAT3* 在病毒防御中存在功能冗余。此外，与野生型植株相比抗氧化系统（抗氧化酶活性和抗氧化酶基因的表达）和防御基因的表达在 HAT1OX 植株中均下调，而在缺失突变体三突（*hat1hat2hat3*）中上调。进一步研究表明，*HAT1* 参与对 CMV 病毒的防御反应可能依赖于水杨酸（SA）而非茉莉酸（JA）。CMV 病毒感染后，SA 含量及 SA 合成相关基因的表达在 HAT1OX 植株中下降而在 *hat1hat2hat3* 中升高，而 JA 含量及 JA 合成相关基因的表达在 HAT1OX 和 *hat1hat2hat3* 植株中不存在显著差异。进一步研究发现 *HAT1* 的表达依赖于 SA 含量的积累。综上所述，我们的结果显示 HAT1 在抵抗 CMV 防御反应中起到负调控作用。

关键词：拟南芥，CMV，HAT1，转录因子，病毒抗性，水杨酸

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The rhythmic expression of clock genes and stress responding genes of Tibetan hulless barley under low temperature

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Tibetan hulless barley (*Hordeum vulgare* L. *var. nudum. hook. f.*) has been cultured as main cereal crops by the inhabitants on Tibetan plateau for thousands of year. Compared to the normal cultivated barley and all other plants within the genus Hordeum, the hulless barley has developed stronger endogenous resistance systems to survive and grow under the general environmental stresses in the Tibetan Plateau. However, little of the mechanism of these stress defense systems has been reported. The low temperature acclimation process but non-freezing temperatures would trigger rapid signaling events in target plants which often leads to the responding expression of cold-regulated genes, as well as their transcription factors, for an anticipation of the coming environmental freezing tolerance. In order to find out whether the circadian rhythmic expression profiles of clock genes in hulless barley, such as *TOC1, CCA1, LHY, etc.* and the cold responding genes, either *CBF*, *LEA* in ABA pathway or *NHX, CBL* and *CIPK* in non-ABA pathway are affected by the non-freezing low temperature stress, qPCR method was employed to detect the rhythmic expression patterns of those above-mentioned genes in time course hulless barley seedlings which had been stressed by non-freezing low temperatures under LL condition. The primary results demonstrated that in the control group (under 25°C), the expression profiles of the circadian clock genes showed robust circadian rhythmic, as well as which of those non-ABA pathway cold-stress responding genes displayed obvious circadian rhythmic profiles, while none of the stress responding genes' expression involved in the ABA pathway were proved to be clock controlled. Under the low temperature (4°C) treated condition, the rhythmic expression patterns of most clock genes maintained their robust rhythmic though that of *GI* and *LUX* showed slight phase shifting and dimmed amplitudes. In terms of the stress responding genes in non-ABA pathway, their expression profiles were severely disturbed by environmental low temperature. A few of these stress responding genes showed very dim amplitudes or shorted periods, such as NHX1, NHX2, NHX3, but most of them lost their rhythmic phenotype completely. The above results indicated that low temperature signal could disturb the output of the circadian clock in Tibetan hulless barley.

Key words: hulless barley, circadian clock, stress responding genes, low temperature signal

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Physiological and antioxidant enzyme gene expression analysis reveals the improved tolerance to drought stress of the somatic hybrid offspring of *Brassica napus* and *Sinapis alba* at vegetative stage

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Drought is a major constraint of agriculture development. The intergeneric somatic hybrids between *Brassica napus* and *Sinapis alba* were created by electrofusion to obtain materials with enhanced drought tolerance.The drought tolerance of *B. napus* cv. Yangyou 6 (Y6) and one offspring line (W146) of the somatic hybrids was evaluated by morphological observation. Physiological parameters were determined in this study. Moreover, the activities of a few antioxidant enzymes and the transcript level of the antioxidant enzyme encoding genes were analyzed by qPCR. W146 and Y6 showed apparent wilting after drought stress for 7 days. However, Y6 wilted more severely than W146. The result of the physiological analysis showed that the relative electronic conductivity and malondialdehyde content of Y6 were higher than that of W146. The relative water content, net photosynthesis rate, proline content, and the activities of superoxide dismutase (SOD) and peroxidase in W146 were higher than that in Y6 after drought stress for 12 days. The DNA and nitrotetrazolium blue chloride staining analysis revealed less accumulation of O_2- and H_2O_2 in W146 than that in Y6 after drought stress.Moreover, the transcript level of some antioxidant enzyme encoding genes,such as Cu/ZnSOD, MnSOD, ascorbate peroxidase, glutathione reductase, and glutathione peroxidase in W146, was higher than that in Y6 under drought stress. Results revealed that line W146 showed more drought stress tolerance than Y6 because line W146 could reduce oxidative damage by efficient antioxidant systems.

Keywords: antioxidant enzymes, *Brassica napus* L., drought stress, gene expression, somatic hybrid progeny

P77

Reversion of hyperhydricity in pink (*Dianthus chinensis* L.) plantlets by $AgNO_3$ and its associated mechanism during *in vitro* culture

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Hyperhydricity occurs frequently in plant tissue culture and can severely affect commercial micropropagation and genetic improvement of the cultured plantlets. Hyperhydric shoots are charaterzized by high water content, but how this occurs is still a subject of investigation. Silver ion (Ag^+) can reduce the extent of hyperhydricity in plants, but its effect on the reversion of hyperhydric plantlets and the underlying mechanism of reversion has not been clarified. In this study, about 67% of the hyperhydric *Dianthus chinensis* L. plantlets were found to revert to normal condition when the plantlets were cultured in medium supplemented with 29.4 μmol $L^{-1}AgNO_3$. Water content and hydrogen peroxide (H_2O_2) production in the guard cells of these plantlets were reduced, while stomatal aperture and water loss rate were increased. $AgNO_3$ also reduced the content of endogenous ethylene and expression of ethylene synthesis and ethylene signal transduction-associated genes. Reduced accumulation of ethylene consequently led to an increase in stomatal aperture mediated by decreased H_2O_2 production in the guard cells. These results adequately verified the role of $AgNO_3$ in the reversion of hyperhydricity in *D. chinensis* L. and also provided clues for exploring the cause of excessive water accumulation in hyperhydric plants.

Key words: *Dianthus chinensis* L., hyperhydricity, silver nitrate, reactive oxygen species, stomatal aperture, ethylene.

P78

BR 以 BZR1 不依赖的方式调节热激信号通路

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作为一种重要的植物激素，油菜素内酯(Brassinosteroids,简称 BR)在种子萌发、器官分化、细胞伸长、根部发育、气孔形成、光形态建成、应激反应、植物衰老等过程上起着重要作用。BR 信号被质膜上的受体 BRI1 (BRASSINOSTEROID INSENSITIVE 1)感知后，能磷酸化下游蛋白激酶 BSK3 (BR-SIGNALING Kinase 3), 并通过 BSK3 激活下游蛋白磷酸酶 BSU1 (*bri1*-SUPPRESSOR1)。BSU1 能将蛋白激酶 BIN2(BR INSENSITIVE2) 去磷酸化，并将 BIN2 失活，解除 BIN2 磷酸化对下游 BZR1 (BRASSINAZOLE RESISTANT1) 家族转录因子的活性抑制，调节 BR 相关的基因的表达。我们的研究发现，在 45°C 热激的条件下，拟南芥 BR 信号途径中受体 *AtBRI1* 的功能部分缺失突变体 *bri1-301* 和 *bri1-5*、*AtBSK3* 的 T-DNA 插入突变体幼苗的死亡率高于野生型，表现为对热激的超敏表型；而 *AtBSK3* 的过表达植物和 *AtBIN2* 及其同源基因的 T-DNA 插入突变体 *bin2bil1bil2* 三突的死亡率则低于野生型，表现为对热激的不敏表型。有意思的是，同样的热激条件下，BZR1 家族转录因子的功能获得型突变体 *bzr1-1d*, *bes1-1d* 以及 T-DNA 插入突变体 *bes1beh1beh3beh4* 四突变体的存活率和野生型相比没有显著差异。Western 的结果也显示，热激后 BZR1 的磷酸化状态以及受 BZR1 调节的基因和野生型相比没有显著变化。综合以上的研究结果，我们认为 BR 信号传导途径和植物热激信号途径存在交叉互作，而且这种互作可能是单向的：即 BR 信号能够通过 BZR1 不依赖方式调节植物的热激响应，而植物的热激响应不会对 BR 信号传导途径产生显著的影响。

关键词：BR，热激响应，单向调节，拟南芥

P79

玉米 PIFs 转录因子参与 ABA 依赖途径的抗旱调控机制

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光敏色素作用因子 PIFs 属于转录因子 bHLH 家族中的一个亚家族成员，作为细胞内的一个信号中心，PIFs 转录因子汇集了多个信号通路，在植物生长发育中起到重要作用。目前关于 PIFs 转录因子在抗旱中作用的研究还比较少，在其他植物中还没有 PIFs 转录因子参与抗旱机制的报道。本研究克隆了两个玉米 PIFs 转录因子基因 ZmPIF1 和 ZmPIF3。定量表达分析表明 ZmPIF1 和 ZmPIF3 基因能够被 PEG 和 ABA 处理诱导表达。推测 ZmPIF1 和 ZmPIF3 可能参与干旱胁迫应答和 ABA 信号途径。为了进一步验证 ZmPIF1 和 ZmPIF3 的基因功能，分别构建了 ZmPIF1 和 ZmPIF3 水稻过表达纯合植株。通过营养液和土壤中分别胁迫处理转基因水稻，发现 ZmPIF1 和 ZmPIF3 基因能够通过调节气孔开闭，降低蒸腾速率，从而显著增强转基因水稻的节水耐旱性。萌发和萌发后幼苗分别用 ABA 处理，结果发现无论是萌发还是萌发后转基因水稻对 ABA 超敏感，且转基因水稻内源 ABA 含量没有变化，推测 ZmPIF1 和 ZmPIF3 参与了 ABA 信号通路。对转基因水稻收获时农艺性状进行初步的统计分析，统计结果显示 ZmPIF1 和 ZmPIF3 基因对水稻的产量性状并没有产生不良的影响。上述结果表明玉米 ZmPIF1 和 ZmPIF3 基因具有抗旱功能，ZmPIF1 和 ZmPIF3 转录因子参与 ABA 依赖途径的信号通路，调节气孔开闭，降低蒸腾速率，产生节水抗旱功能，且基因具有抗旱功能的同时不会降低作物产量，具有应用价值。

关键词：PIFs，转录因子，抗旱；

P80

Functional characterization of diacylglycerol kinase gene family in maize (*Zea Mays*)

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Diacylglycerol kinase (DGK) is a kind of phosphokinase involved in the formation of signaling molecule phosphatidic acid (PA) in plants. In this study, seven maize(*zea mays*) DGK gene family members were identified by bioinformatics analysis, and designated as ZmDGK1-7. The proteins encoded by *ZmDGKs* had a molecular weight (MWs) between 54.6 and 80.2 kDa, and predicted PI values of 6.08 to 8.74, respectively. Phylogenetic analysis revealed that ZmDGKs fall into three clusters. While all *ZmDGKs* have a conserved catalytic domain DGKc, only *ZmDGK1*, *ZmDGK4* and *ZmDGK5* have the DAG/PE binding domain. Gene expression of *ZmDGKs* were detected in roots, stem, leaves, and endosperm tissues. *ZmDGKs* genes were also significantly up- or down-regulated in response to drought, salt and low temperature treatment. We identified maize DGK genes, and conducted bioinformatics and gene expression analysis under several abiotic stresses, which will help us to decipher the functions of maize diacylglycerol kinase family and their roles in maize growth, development and responses to environmental changes.

Key words: maize (*zea mays*), diacylglycerol kinase, bioinformatics analysis, abiotic stress

P81

HEAT TOLERANT DEFECT 1 acts as a regulator of *HsfA2* in response to heat stress in Arabidopsis

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Unlike animals, plants are not able to escape from the stressful growth conditions such as heat stress, and a variety of adaptation mechanisms have been evolved in plants to cope with these unfavorable conditions. With the increasing global warming effect, plants face much more serious challenges than before. However, the mechanisms by which plants tolerate the heat stress remain unclear. Here we report that the *heat tolerant defect 1* (*htd1*) mutant of Arabidopsis showed defects in thermotolerance and growth, such as delayed flowering time. The heat-responsive expression of *HsfA2*, a key player involved in acquired thermotolerance, was strongly repressed under 38°C heat treatment for 2h compared with wild type. Moreover, HTD1, encoding a putative DNA-binding protein, can be induced by heat treatments, further implying that *HTD1* acts upstream of heat stress signaling pathway, regulating the expression of *HsfA2*. In addition, we also examined the basal and acquired thermotolerance of the *htd1* mutant. The detached leaves of *htd1* were much more sensitive to the heat treatment of 37°C for 7.5 h. Similarly, the four-week-old *htd1* plants also exhibited defects in acquired thermotolerance when treated with 37°C for 1h, 22°C for 2h, and then 45°C for 10 h, following by 7-day-recovery at 22°C. Taken together, *HTD1* can be induced by heat and acts as a regulator in modulating the heat-responsive expression of *HsfA2*. Detailed molecular and biochemical analysis of HTD1 in regulation of the *HsfA2* expression is ongoing. We believe that these findings would provide a deep insight in better understanding the *HTD1*-dependent heat stress signaling transduction and heat tolerance in plants.

P82

Overexpression of *Sddt* enhances drought tolerance in *Arabidopsis* and *Brassica napus* by regulating microRNA activity

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In plants, high root density and larger root surface area can be maintained or expanded to promote water uptake, a process that plays crucial role in drought stress tolerance. In our study, a gene named *Sddt* (a semi-dwarfism and drought tolerance gene) was found to regulate the development of lateral root growth in *Arabidopsis* and *Brassica napus*. Compared with wild type, transgenic plants overexpressing *Sddt* showed increased lateral roots. Using yeast-2-hybrid, we demonstrated that Sddt interacted with DCL1. Hybridization of *Sddt*-overexpressing plants with miRNA activity reporter plants indicated that *Sddt* greatly enhanced miRNA activity. Furthermore, by small RNA sequencing, we identified 96 known differentially expressed miRNA genes. Among them, the miRNA160 family was up-regulated and its target genes, ARF10, -16, and -17, were down-regulated. Our results suggest that the overexpression of *Sddt* enhances microRNA activity via its interaction with DCL1, decreases expression of ARF genes through miRNA160, promoting both lateral root development and increased drought stress tolerance. These findings will help us to better understand the mechanism of root development during drought stress, and implies that *Sddt* has potential applications in genetic engineering to improve crop tolerance.

Key words: *Arabidopsis*, *Brassica napus*, drought tolerance, miRNA activity, Sddt, miRNA160

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Characterization of *GsCHX19.3*, a member of cation/H^+ exchanger, from wild soybean suggests a positive role in salt and alkaline stresses

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Cation/H^+ exchanger (CHX) transporters are characterized to play vital roles in plant growth, development and defense. Although soybean genome sequencing has been complete, the CHX family genes haven't been analyzed systematically in soybean. Here, in this study, 40 putative CHX genes were identified and clustered into five distinct subfamilies. We further investigated their chromosome distribution and gene duplication, protein structure and phylogenetic relationship. According to the online database (Phytozome, http://phytozome.jgi.doe.gov/pz/portal.html), the expression of *GmCHXs* varied among different tissues. Based on our previous RNA-seq data, only six *GmCHXs* were up-regulated in soybean root in response to salt-alkaline stress. Among them, *GsCHX19.3* exhibited maximum expression values and fold changes under salt-alkaline stress. Subsequently, we confirmed that *GsCHX19.3* expressed diversely in different tissues and was induced by both salt and alkaline stresses. Green fluorescent protein-tagged GsCHX19.3 expressed in *Arabidopsis* protoplasts was localized to plasma membrane. GsCHX19.3 conferred resistance to low K^+, high pH, hygromycin B and carbonate stress, but not to high salt (Na^+ and Li^+) stresses. However, *GsCHX19.3* overexpression in *Arabidopsis* increased salt and alkaline tolerant, whereas *atchx19* T-DNA insertion mutant displayed opposite phenotype. Moreover, we also demonstrated that *GsCHX19.3* overexpression could promote K^+ uptake and helped maintain K^+/Na^+ homeostasis in transgenic plants. As expected, K^+/Na^+ homeostasis was disrupted in *atchx19* knockout mutants. Taken together, our results demonstrated that GsCHX19.3 is involved in ion balance and pH homeostasis, functioned as a specific K^+/H^+ antiporter in yeast cells.

Key words: cation/H^+ exchanger, *GsCHX19.3*, *Glycine soja*, salt-alkaline stress, K^+ homeostasis

P84

Identification and characterization of R2R3-MYB transcription factor gene family in rapeseed (*Brassica napus* L.)

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MYB (v-myb avian myelobastosis viral oncogene homolog) transcription factor family is one of the largest families in plants. Members of this family are divided into four subgroups including R1/2-MYB, R2R3-MYB, 3R-MYB and 4R-MYB, with R2R2-MYB being the subgroup harboring the largest members and playing diverse functions in plants. Previous studies have demonstrated that members of R2R3-MYB subgroup play important roles in biotic and abiotic stress, secondary metabolism, meristem formation, cell cycle control and cell morphogenesis and so on. However, we still have very limited understanding of R2R3-MYB genes in rapeseed (*Brassica napus* L.) In the present research, we mined the expressed sequence tag (EST) of rapeseed and identified 76 R2R3-MYB genes (excluding alleles). Then we successfully cloned 44 R2R3-MYB genes. And we analyzed transcriptional activities of these BnaMYB proteins in yeast. We also investigated the subcellular localizations of some R2R3-MYB proteins through GFP fusion. Besides we have identified the transcript abundance levels of 15 R2R3-MYB genes during abiotic stress conditions and ABA treatment and found that a group of *R2R3-MYB* genes responded to one or more treatments. In the meantime, we identified one functionally unknown MYB gene-*BnaMYB78*, which regulates reactive oxygen species (ROS) accumulation and hypersensitive response(HR)-like cell death. We further found it could regulate the transcription of a few ROS- and defence-related genes and modulate the ROS-dependent cell death in *Nicotiana benthamiana*. Taken together, this research has provided a solid foundation to understand the functions and regulatory mechanisms of R2R3-MYB genes in the oil crop-rapeseed.

Key words: rapeseed, MYB, abiotic stress, ROS, PCD

P85

Identification of flavonoids and expression of anthocyanin biosynthetic genes in leaf color mutant induced by carbon ion beam in Wandering jew (*Tradescantia fluminensis*)

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Leaf color mutants are ideal materials for studying physiological processes in plants. Here, a thermos-sensitive leaf-color mutant of Green wandering jew was isolated after carbon ions irradiation, which was designated as mt. The color of young mutant leaves was more sensitive to variations of temperature, however, the young leaves of wild type remained green under low - temperature conditions (6°C-20°C). To elucidate the characteristics of pigmentation in mutant leaves under room temperature conditions (25°C) and low - temperature conditions (7°C), the ultra-structural, pigment composition, molecular mechanisms and anthocyanin accumulation involved in this phenomenon have been investigated in four independent experiments. The results showed that chloroplasts of mutants exhibited abnormal morphology and distribution at 25°C, and under low - temperature conditions(7°C), the chloroplasts converted into leucoplast in leaves on mutants. Temperature change affected the rate of color transition, chlorophyll and anthocyanin concentrations in leaves on mutants. Molecular analysis indicated that all the anthocyanin biosynthetic genes and regulatory genes were constitutively up-regulated in mutant leaves at 7°C. The other anthocyanin biosynthesis and regulatory genes showed similar expression levels between mutant and wild type except PAL, CHS, ANS were up-regulated at 25°C. HPLC analysis of anthocyanins in mutant and wild type leaves revealed that the contents of cyanidin, petunidin and delphinidin were significantly lower than those of under low-temperature conditions (7°C) except pelargonidin was not detected in mutant leaves under room - temperature conditions(25°C). The HPLC profile also indicated the highest levels of flavonols in mutant leaves at 7°C significantly. Overall, above results indicated a close similarity among ultra-structures, pigment compositions, transcript amount and anthocyanin accumulation were tightly associated with temperature variation in leaves of leaf color mutant. These findings would provide better understanding of the mechanism of pigmentation changes in mutant leaves under different temperature conditions in Wandering jew.

Key words: Wandering jew, anthocyanin, flavonols, gene expression, HPLC

P86

Proteogenomic analysis reveals alternative splicing and alternative translation elements of the abscisic acid response in *Arabidopsis* seedlings

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In eukaryotes, mechanisms such as alternative splicing (AS) and alternative translation initiation (ATI) contribute to organismal protein diversity. Specifically, splicing factors play crucial roles during stress, in responses to hormones and development cues. However, the underlying mechanisms are not well investigated in plants. Here, we report the parallel employment of RNA sequencing and proteomic identification by constructing specific libraries to unravel AS isoforms and novel proteins in response to abscisic acid (ABA) treatment. Our results suggest that approximately 78% of intron-containing genes were alternatively spliced. Amongst a total of 141,419 identified AS events, two newly defined AS types, which are referred to as alternative first exon (TSS) and alternative last exon (TTS), were more abundant than intron retention (IR) in *Arabidopsis*. However, by contrast to AS events detected under normal conditions, ABA-regulated AS isoforms were more likely to be translated into proteins. Subsequent bioinformatic and structural analysis indicates that the alternation of splicing factor AS isoforms was crucial in response to ABA treatment. ABA extensively regulates the AS pattern in *Arabidopsis* by increasing the number of non-conventional splicing sites. This work is also the first instance in which thousands of new proteins encoded from the same mRNA through ATI or 3′ to 5′ translation were detected using a self-constructed library on the sense and antisense strand of the *Arabidopsis* genome. The combined results thus enhance our understanding the regulation of AS and translation mechanisms under normal condition and in response to ABA treatment.

Key words: alternative spling, alternative translation, ABA, proteogenomic

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干旱胁迫对防风幼苗生长及抗氧化性的影响

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干旱胁迫严重影响植物的生长发育，也是限制干旱、半干旱地区农作物产量的主要因素。为了适应干旱环境，植物进化出多种机制，其中抗氧化系统在保护植物免受干旱损伤中起到重要作用。防风（*Saposhnikovia divaricata*）是一味传统中药材，《中国药典》（2015 版）记载其主要药效成分为升麻素苷和 5-O-甲基维斯阿密醇苷。内蒙古地处我国干旱、半干旱地区，是防风道地产区之一。本试验研究干旱胁迫下防风幼苗生物量及一些生理指标的变化，探讨其对水分亏缺的适应机理。

试验于 2016 年 3~6 月在内蒙古大学南区温室进行，采用盆栽试验（容器规格：10.5 cm×18 cm×25.5 cm）。3 月 19 日播种，滴灌供水，待幼苗长出 7~8 片真叶后，于 6 月 25 日开始处理。对照组每 4 天浇水 1 次，使土壤含水量维持在 27%左右；干旱组饱和浇水后不再浇水，干旱至 20 d 后恢复浇水，期间每 4 天取样 1 次，测其根、叶相关生理指标。结果表明，随着干旱处理时间延长，土壤含水量及防风幼苗叶片相对含水量明显下降，20 d 分别降至 4.3%、42.1%，复水 4 d 后土壤含水量恢复到正常水平，而叶片相对含水量则因干旱程度过重无法恢复。根干重在干旱 8~16 d 内有略微增加，但未达到显著水平，复水后有所减少；叶片干重在 8~20 d 内减少，复水并未恢复。根系、叶片丙二醛（MDA）、过氧化氢（H_2O_2）、抗坏血酸（ASA）含量随干旱胁迫时间延长逐渐增加，复水后含量减少；根系谷胱甘肽（GSH）含量随干旱胁迫时间延长持续增加，复水后减少不明显，而叶片 GSH 含量从干旱胁迫至复水都持续增加。研究发现防风叶片含水量变化首先响应干旱胁迫，随后土壤含水量的下降导致防风幼苗水分亏缺，细胞内产生大量 H_2O_2，并伴随着膜脂质过氧化，这些变化触发了抗氧化保护，一些抗氧化剂随之增加。相关文献表明这些生理指标的变化可能是受植物内部信号转导通路调控的，DREB、AP2/ERF、NAC、ZFP、bZIP、WRKY 等家族是近几年研究较多的抗旱转录因子。同时，基因调控介导着内源激素的变化，这种交叉作用为我们接下来的工作提供了一种思路。

关键词：干旱胁迫，复水，丙二醛，过氧化氢，抗氧化剂

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大豆低磷响应 *GmPHR1-7*控制根系磷平衡的功能研究

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磷是植物所必需的大量元素之一，土壤有效磷量低严重抑制作物的生长。以往的研究表明，在长期的进化过程中，大豆形成了一系列适应低磷胁迫生理和分子机制。但是，调控大豆磷信号网络的重要因子有待进一步明确，尤其是研究大豆转录因子 P*HR1* 的功能鲜有报道。本研究首先在大豆全基因组水平上，通过 BLAST 序列比对分析发现大豆 *GmPHR1* 家族有 10 个成员，分为两个亚家族。表达模式分析结果表明，10 个 *GmPHR1* 基因成员在大豆叶部、根部、花、幼嫩种子和豆荚中的表达丰度不同，且不同程度地受到缺氮、磷或缺钾处理的调控表达。在此基础上，选取了在叶部、根部和根瘤中都受低磷显著上调的 *GmPHR1-7*进行功能分析。研究结果表明，在正常供磷条件下，超量表达 *GmPHR1-7* 的转基因大豆离体毛根和复合植株的生长受到显著抑制，磷浓度明显增加。而且，超量表达 *GmPHR1-7* 显著加强了 11 个大豆高亲和磷转运子以及 5 个缺磷胁迫响应基因在毛根中的表达。综上所述，大豆 *GmPHR1-7*是大豆磷信号网络中的重要调控因子，参与调控了大豆的磷平衡。

关键词：大豆，基因家族，磷平衡，*GmPHR1-7*

P89

Role of vacuolar invertase in regulation of stomatal behavior

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There are several hypotheses that explain stomatal behavior. These include the concept of osmoregulation mediated by potassium and its counter-ions malate and chlorine and the more recent starch–sugar hypothesis. Here we report that the activity of the sucrose cleavage enzyme, vacuolar invertase (VIN), is significantly higher in guard cells than in other leaf epidermal cells and its activity is correlated with stomatal aperture. Changes in VIN activity was speculated to be affected by the pH within the vacuole through potential modulation of the activities of H+/ sugar antiporters on the tonoplast or other transporters for potassium, chloride, and malate, across guard cell membrane. To this end, it has been reported that guard cell vacuolar pH increases during stomatal closing, but it decreases during stomatal opening. Further more, we examined whether VIN indeed controls stomatal movement under normal and drought conditions by transforming *Arabidopsis* with a tobacco vacuolar invertase inhibitor homolog (*Nt-inhh*) under the control of an abscisic acid-sensitive and guard cell-specific promoter (*AtRab18*). The data obtained showed that guard cells of transgenic Arabidopsis plants had lower VIN activity, stomatal aperture and conductance than that of wild-type plants. Moreover, the transgenic plants also displayed higher drought tolerance than wild-type plants. The data indicate that VIN is a promising target for manipulating stomatal function to increase drought tolerance.

Key words: vacuolar invertase, stomatal behavior, drought tolerance

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油茶冷驯化分子机制的初步研究

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植物的耐寒性是决定植物的地理分布以及作物生产的关键因素。植物在非冻结低温下的冷驯化过程有助于增强植物对冻结低温的耐受性。油茶是山茶属植物中分布最广的物种之一，广泛分布于长江流域及其以南的亚热带山区，是亚热带常绿阔叶林的代表性植物物种之一。与大多数山茶属植物相比，油茶具有较强的耐寒性，常用于培育耐寒的山茶品种，但是关于其耐寒性的分子机制还未见报道。此外，油茶是中国第一大木本油料作物。野生油茶是栽培油茶育种最重要的遗传资源。开发大量适用于野生油茶遗传多样性分析的分子标记，了解不同纬度与海拔野生油茶种群的遗传结构，有助于野生油茶遗传资源的挖掘与利用，促进油茶优良品种的选育。

本研究对庐山和井冈山不同海拔的野生油茶叶片进行了转录组测序。基于转录组数据分析，发现了大量的简单序列重复（SSRs）、单核苷酸多态（SNPs）以及插入缺失（InDels）位点。基于 SNP 位点构建的系统树显示庐山与井冈山的野生油茶间存在明显的遗传分化。庐山和井冈山不同海拔的野生油茶样本分别对应不同的气温条件。研究结果发现，当气温低于 10°C，油茶叶片中的基因表达模式会发生明显变化，大量基因的表达水平会在低温下升高。与 10°C 及以上气温下油茶叶片中的基因表达水平相比，在 2°C 下发生差异表达的基因数量要多于 5°C。此外，重要的调控植物冷驯化响应的 CBF 基因仅发现在 2°C 下差异表达。以上结果提示，随着温度的下降，油茶叶片受到的冷胁迫也逐渐增强，从而更多的与冷驯化相关的基因会发生差异表达。在这些低温下发生差异表达的基因中，跨膜运输蛋白基因是最主要的功能基因类群，而其中又以糖跨膜运输蛋白基因为主。本研究结果为深入了解油茶等常绿阔叶植物耐低温的分子机制奠定了基础。

关键词：油茶，冷驯化，基因表达，转录组，分子标记

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低磷胁迫对玉米叶绿体蛋白质和叶片光系统活性的影响

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对玉米自交系齐 319 和突变体齐 319-96 叶片低磷胁迫下的叶绿体进行了差异蛋白组学研究，结果表明，低磷胁迫下，齐 319 和突变体叶片的叶绿体一部分蛋白表达模式发生了的变化。通过质谱分析鉴定发现齐 319 和突变体叶片叶绿体有 36 个蛋白表达模式存在显著的差异。

在低磷胁迫下，突变体齐 319-96 的 HCF136（蛋白点 A2、A6）和 FtsH（蛋白点 A32）蛋白的表达丰度显著高于野生型 Qi-319， 这表明突变体齐 319-96 的 PS Ⅱ反应中心是 D1 蛋白在低磷胁迫下具有更强的装配周转和修复能力；此外，PS Ⅱ中的捕光叶绿素 a/b 结合蛋白、23 kDa 蛋白 (OEE2) 表达也上调。这些蛋白的上调暗示在低磷下齐 319-96 的 PS Ⅱ反应中心与齐 319 相比保持更高的活性，进而表现为在低磷下与野生型相比具有更高的碳同化效率。

蛋白组数据还显示 PS Ⅰ中的铁氧还蛋白-NADP+氧化还原酶、 联系光系统 Ⅰ和光系统 Ⅱ的 Cytb6-f 复合体蛋白以及光合氧化磷酸化的关键酶 ATP 合酶的 α 亚基和 β 亚基在齐 319-96 的表达丰度显著高于齐 319，这些蛋白表达模式的改变暗示在低磷胁迫下齐 319-96 的光合电子传递与氧化磷酸化能力高于齐 319，表现为在低磷下具有更高的光能捕获、传递和利用能力，从而表现出在低磷下具有更好的碳同化能力。利用快速叶绿素荧光动力学与 820nm 光吸收技术研究了低磷胁迫对玉米叶片 PS Ⅱ、PS Ⅰ的影响，结果表明低磷胁迫下突变体齐 319-96 叶片的 PS Ⅱ、PS Ⅰ性能高于齐 319，受低磷胁迫影响较小，且 PS Ⅱ和 PS Ⅰ具有更好的协调性，在低磷下齐 319 叶片 PS Ⅱ反应中心的电子供体侧性能影响更为严重。因此，在低磷下齐 319-96 叶片的具有更好的光能捕获、传递能力， 从而表现出具有更高 Pn 值和 RuBP 羧化酶活性，具有更高的碳同化速率。

关键词：玉米，突变体，叶绿体，蛋白组学，光系统活性

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Functional characterization of a *Glycine soja* Ca^{2+}ATPase in salt-alkaline stress responses

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It is widely accepted that Ca^{2+}ATPase family proteins play important roles in plant environmental stress responses. However, up to now, most researches are limited in the reference plants Arabidopsis and rice. The function of Ca^{2+}ATPases from non-reference plants was rarely reported, especially its regulatory role in carbonate alkaline stress responses. Hence, in this study, we identified the P-type II Ca^{2+}ATPase family genes in soybean genome, determined their chromosomal location and gene architecture, and analyzed their amino acid sequence and evolutionary relationship. Based on above results, we pointed out the existence of gene duplication for soybean Ca^{2+}ATPases. Then, we investigated the expression profiles of the ACA subfamily genes in wild soybean (*Glycine soja*) under carbonate alkaline stress, and functionally characterized one representative gene *GsACA1* by using transgenic alfalfa. Our results suggested that *GsACA1* overexpression in alfalfa obviously increased plant tolerance to both carbonate alkaline and neutral salt stresses, as evidenced by lower levels of membrane permeability and MDA content, and higher levels of SOD activity, proline concentration and chlorophyll content under stress conditions. Taken together, for the first time, we reported a P-type II Ca^{2+}ATPase from wild soybean, *GsACA1*, which could positively regulate plant tolerance to both carbonate alkaline and neutral salt stresses.

Key words: Carbonate alkaline stress; salt stress; Ca^{2+}ATPase; *Glycine soja*; alfalfa

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A *Glycine soja* methionine sulfoxide reductase B5a interacts with the Ca^{2+}/CAM-binding kinase GsCBRLK and activates ROS signaling under carbonate alkaline stress

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Although researches have extensively illustrated the molecular basis of plant responses to salt and high pH stresses, the knowledge on carbonate alkaline stress is poor and the specific responsive mechanism remains elusive. We have previously characterized a *Glycine soja* Ca^{2+}/CAM-dependent kinase GsCBRLK, which could increase salt tolerance. Here, we characterized a methionine sulfoxide reductase B protein GsMSRB5a as a GsCBRLK interactor by using Y2H and BiFc assays. Further analyses showed that the N-terminal variable domain of GsCBRLK contributed to GsMSRB5a interaction. Y2H assays also revealed the interaction specificity of GsCBRLK with wild soybean MSRB subfamily proteins, and determined that the BoxI/BoxII-containing regions within GsMSRBs were responsible for their interaction. Furthermore, we also illustrated that the N-terminal basic regions in GsMSRBs functioned as transit peptides, which targeted themselves into chloroplasts and thereby prevented their interaction with GsCBRLK. Nevertheless, deletion of these regions allowed them to localize on PM and interact with GsCBRLK. In addition, we also showed that *GsMSRB5a* and *GsCBRLK* displayed overlapping tissue expression specificity and coincident expression patterns under carbonate alkaline stress. Phenotypic experiments demonstrated that *GsMSRB5a* and *GsCBRLK* overexpression in *Arabidopsis* enhanced carbonate alkaline stress tolerance. Further investigations elucidated that *GsMSRB5a* and *GsCBRLK* inhibited ROS accumulation by modifying expression of ROS signaling, biosynthesis and scavenging genes. Summarily, our results demonstrated that GsCBRLK and GsMSRB5a interacted with each other, and activated ROS signaling under carbonate alkaline stress.

Key words: carbonate alkaline stress, methionine sulfoxide reductase, Ca^{2+}/CAM- binding kinase, ROS signaling, *Glycine soja*, Arabidopsis

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Rhizosphere bacteria containing 1-aminocyclopropane-1-carboxylate deaminase increase growth and photosynthesis of pea plants under salt stress by limiting Na^+ accumulation

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Although plant salt tolerance has been improved by soil inoculation with rhizobacteria containing the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase (which metabolises ACC, the immediate precursor of the phytohormone ethylene), it is not always clear whether ion homeostasis and plant water relations are affected. When pea (*Pisum sativum* cv. Alderman) was grown with 70 mM and 130 mM NaCl, the ACC-deaminase containing rhizobacterium *Variovorax paradoxus* 5C-2 increased total biomass by 25% and 54%, respectively. Nutrient flow modelling showed that *V. paradoxus* 5C-2 increased K uptake and root to shoot K flow, while decreasing Na flow and increasing Na deposition in roots. Thus, shoot K^+/Na^+ ratio increased following *V. paradoxus* 5C-2 inoculation. At 70 mM and 130 mM NaCl, rhizobacterial inoculation decreased stomatal resistance by 14% and 31% and decreased xylem balancing pressure by 7% and 21%, respectively. Furthermore, rhizobacterial inoculation improved photosynthetic efficiency (Fv/Fm) by 12% and 19% and increased maximal electron transport rate (ETR) by 18% and 22% at 70 mM and 130 mM NaCl, respectively. Thus *V. paradoxus* 5C-2 mitigates salt stress by improving water relations, ion homeostasis and photosynthesis of pea plants, and may provide an economic means of promoting growth of plants exposed to salt stress.

Key words: water relations, ion homeostasis, photosynthetic efficiency, maximal electron transport rate, nutrient flow modeling

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沙漠植物花花柴幼苗对高温胁迫的耐受性探测

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随着全球气候变化，极端高温对植物发育及作物产量造成的影响越来越受到人们的关注，植物耐高温的分子机制已成为植物抗非生物逆境研究的重要方向。本研究以沙漠植物花花柴为材料，采用不同的温度（40 °C、45 °C 和 50 °C）处理，分别在处理不同时间测定了花花柴幼苗膜透性和膜脂过氧化程度（相对电导率和 MDA 含量）和保护酶系统（SOD 和 CAT）的活性变化。实验结果表明：(1）花花柴幼苗在 40 °C 胁迫时，随着胁迫时间延长，叶片相对电导率和 MDA 含量呈先升高后降低的趋势，分别在 12 h 和 6 h 达到最高；其保护酶系统（SOD 和 CAT）的活性也发生规律性的变化，其中 SOD 和 CAT 的酶活随处理时间的延长逐渐升高，而 POD 活性则在处理 12h 时达到最高，之后随处理时间的延长逐渐降低；(2）在 45 °C 条件处理时，处理 2h 时花花柴幼苗叶片的相对电导率、MDA 含量及 SOD、CAT 活性显著升高，但在随后的 2-8h 时间段，其相对电导率、MDA 含量及 SOD、CAT 活性变化不显著，在处理 12h 以后，相对电导率、MDA 含量显著增加，SOD 和 CAT 活性显著降低；(3）在 50 °C 胁迫时，花花柴幼苗叶片的相对电导率、MDA 含量逐渐升高，且呈显著水平，其 SOD 和 CAT 的酶活在处理 1h 时达到最高，之后显著降低。结论：由于花花柴长期生长与沙漠地区，其具有很强的耐高温性，40 °C 对花花柴的生理胁迫不明显；花花柴对 45 °C 的耐受时间是 8h；50 °C 对花花柴的胁迫时间拐点是 1 h。以上实验结果及结论符合花花柴的生长环境，该结果为以花花柴为材料的耐高温研究提供了参考价值，同时也为花花柴耐高温功能基因的发掘提供了依据。

关键词：花花柴，高温胁迫，高温耐受

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大豆苹果酸转运子在大豆响应低磷胁迫中的功能

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磷缺乏是酸性土壤上限制植物生长的重要因素。根系有机酸，如苹果酸等的分泌，有利于提高植物对土壤中难溶磷的活化和利用。*ALMT* 基因家族编码的苹果酸转运子对于植物根系苹果酸的分泌具有重要的调控作用。本研究对大豆 34 个 *GmALMT* 家族成员进行了表达模式分析，结果发现仅 26 个 *GmALMT* 基因成员的表达受外界磷有效性的影响。进一步选取根系磷饥饿增强表达基因 *GmALMT5* 进行相关的性质和功能分析。亚细胞定位结果表明，GmALMT5 定位于细胞膜上。在大豆毛根和拟南芥超量表达该基因均明显促进了转基因材料苹果酸的分泌。此外，以难溶磷 Ca-P 作为磷源时，超量表达 *GmALMT5* 转基因拟南芥的生物量和磷浓度均显著高于野生型。综上所述，大豆 GmALMT 家族成员在大豆响应低磷胁迫的机制中具有不同的功能。此外，大豆 GmALMT5 编码了膜定位的苹果酸转运子，该苹果酸转运子可能参与了低磷条件下大豆对外部难溶磷的活化和利用。

关键词：大豆，*GmALMT*，苹果酸，低磷胁迫

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ABA accumulation above endogenous levels is necessary for desiccation tolerance in *Physcomitrella patens*

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The moss *Physcomitrella patens*, a model system for basal land plants, highly tolerates several abiotic stresses, including dehydration. We previously reported that *Physcomitrella* survives dehydration to -13 MPa under controlled drying regimes that bring the water potential of the moss to equilibrium with the air in 6 days. Tolerance of more rapid desiccation to water potentials below -100 MPa was only achieved by pre-treatment with exogenous ABA. We now report that gametophores can survive desiccation below -100 MPa under a gradual drying regime that more closely mimics the dehydration dynamics it would experience in its natural habitat. To explain the difference in response to drying rate, we hypothesized that gradual drying allows *P. patens* plants to progressively synthesize to reach levels that are required for the induction of desiccation tolerance. To address this hypothesis, we measured the endogenous ABA levels of protonemata and gametophores under different drying regimes. We also assessed the minimum concentration of exogenous ABA and the length of exposure to ABA required for induction of desiccation tolerance in the moss. Results from this work will provide insight into ongoing studies to uncover the underlying mechanisms of desiccation tolerance in this bryophyte.

Key words: *Physcomitrella patens*; desiccation tolerance; gradual dehydration; equilibrium dehydration; abscisic acid

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A SAL1 loss-of-function *Arabidopsis* mutant exhibits enhanced cadmium tolerance in association with alleviation of endoplasmic reticulum stress

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SAL1, as a negative regulator of stress response signaling, has been studied extensively for its role in plant response to environmental stresses. However, the role of SAL1 in cadmium (Cd) stress response and the underlying mechanism is still unclear. Using *Arabidopsis thaliana* loss-function mutant of SAL1, we assessed Cd resistance and further explored the Cd toxicity mechanism through analysis of ER stress response. The loss of SAL1 function greatly improved Cd tolerance and significantly attenuated ER stress in *Arabidopsis*. Exposure to Cd induced an ER stress response in *Arabidopsis* as evidenced by unconventional splicing of *AtbZIP60* and upregulation of ER stress-responsive genes. Damage caused by Cd was markedly reduced in ER stress response double mutant *bzip28 bzip60* or by application of ER stress alleviating chemical agents, tauroursodeoxycholic acid (TUDCA) and 4-phenyl butyric acid (4-PBA), in wild-type plants. The Cd-induced ER stress in *Arabidopsis* was also alleviated by loss function of SAL1. These results identified SAL1 as a new component mediating Cd toxicity and established the role of ER stress response in Cd toxicity. Additionally, the attenuated ER stress in *sal1* mutant might also shed new light on the mechanism of diverse abiotic stress resistance in the SAL1 loss-of-function mutants.

Key words: *Arabidopsis*, cadmium (Cd) tolerance, endoplasmic reticulum stress, SAL1, stress response

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Jasmonic acid is a crucial signal transducer in heat shock induced sesquiterpene formation in *Aquilaria sinensis*

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Agarwood, a highly valuable resinous and fragrant heartwood of *Aquilaria* plants, is widely used in traditional medicines, incense and perfume. Only when *Aquilaria* trees are wounded by external stimuli do they form agarwood sesquiterpene defensive compounds. Therefore, understanding the signaling pathway of wound-induced agarwood formation is important. Jasmonic acid (JA) is a well-characterized molecule that mediates a plant's defense response and secondary metabolism. However, little is known about the function of endogenous JA in agarwood sesquiterpene biosynthesis. Here, we report that heat shock can up-regulate the expression of genes in JA signaling pathway, induce JA production and the accumulation of agarwood sesquiterpene in *A. sinensis* cell suspension cultures. A specific inhibitor of JA, nordihydroguaiaretic acid (NDGA), could block the JA signaling pathway and reduce the accumulation of sesquiterpene compounds. Additionally, compared to SA and H2O2, exogenously supplied methyl jasmonate has the strongest stimulation effect on the production of sesquiterpene compounds. These results clearly demonstrate the central induction role of JA in heat-shock-induced sesquiterpene production in *A. sinensis*.

Key words: agarwood, sesquiterpene, heat shock, jasmonic acid.

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Mechanism research of flower pigment mutation induced by carbon ions in geranium

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Heavy ion mutation technology as a unique and efficient mutagen has been widely used in germplasm innovation and plant breeding. Flower color as the important qualitative character and economic indicator for ornamental plants become the primary target for ornamental breeding. Here we report one flower color mutant obtained using carbon ion irradiation and attempt to elucidate underlying mutation mechanism. The petal color of the mutant changed from orange-red to light pink, and colors of peduncle, pistil and stamen also displayed significant differences with wild type. Anatomical structure observations showed that the salmon pigments fully filled with the epidermal cells of wild type, while only a small number of pale pink pigments in mutant. HPLC results indicated that the main pigments determined the flower color of wild type were pelargonidin, cyanidin and delphinidin. On the contrary, the unique pigment detected in mutant was cyanidin. The core reason of depigmentation in mutant was the absence of pelargonidin and delphinidin. Quantitative real-time PCR analysis suggested that the significantly down-regulated transcriptional level of early genes CHS, CHI and suppressed expression of gene ANS blocked the biosynthesis of anthocyanin, and that was the crucial reason for the deficiency of anthocyanin which lead to the depigmentation of mutant. These results explained the mechanism of coloration change in mutant induced by carbon ions and provided the research foundation for the follow-up design and color improving in geranium.

Key words: carbon ions irradiation, flower color mutant, depigmentation

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一氧化氮抑制叶绿素合成和诱导单线态氧的生化机制研究

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一氧化氮(nitric oxide，NO)是第一个被发现的参与细胞信号转导的气体信号分子，在植物生理过程中发挥重要作用,它参与调节植物的生长、发育及对外界环境的应激反应。同时一氧化氮对叶绿素的合成的具有一定的抑制作用，尤其是对 5-氨基乙酰丙酸（ALA）的合成抑制强烈。然而 NO 对于大麦和拟南芥的脱植基叶绿素（Chlide）的合成抑制程度不同。NO 处理后明显抑制了大麦黄化幼苗的原脱植基叶绿素（Pchlide）向 Chlide 的转换，而对拟南芥的影响较小。NADPH：Pchlide 氧化还原酶（POR）催化这一步反应。在大麦黄化质体的原片层体中 PORA 和 PORB（比例为 5：1）形成一个大分子的复合物 LHPP。然而，POR 在拟南芥中只能形成同源二聚体。NO 处理造成 POR 蛋白表面半胱氨酸残基的 S-亚硝基化修饰，并使 POR 复合物分解。这种修饰严重抑制了大麦 POR 活性并诱导 Pchlide 的积累以及单线态氧的产生，使大麦叶片在黑暗/高光照交替的环境下产生光漂白和细胞死亡（尤其是在叶尖）。但上述变化在拟南芥中均不显著或变化幅度较小。NO 处理还导致过氧化氢和超氧阴离子积累，并且这种积累在大麦幼苗中更为显著。综合以上数据表明，NO 对叶绿素合成的抑制主要反映在谷氨酰 t-RNA 还原酶和 POR 这两个步骤上。大麦 POR 复合物结构的稳定性对于转绿时避免单线态氧积累和维持细胞氧化还原平衡是至关重要的。一氧化氮主要通过 S-亚硝基化修饰致使 POR 复合物解体并抑制其活性。

关键词：拟南芥、大麦、NADPH：Pchlide氧化还原酶、单线态氧、S-亚硝基化

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Genetic variants associated with salt tolerance of maize (*Zea Mays*)

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Maize is one of the major crops in China. However, land salinization is a major challenge for corn production in a large proportion of corn growth regions. Recently, genome-wide association study based on linkage disequilibrium has been successfully utilized to characterize complex traits of crops, including maize. Here we treated the seedlings of a large maize association population with high concentration of NaCl and investigated the tolerance phenotypes. We scanned about 1.25 million single nucleotide polymorphism (SNP) makers at the whole genome level and detected a couple of SNPs that are significant associated with salt tolerance. Most of the significant SNPs were localized in genes encoding protein kinase, transcription factors, amino acid and sugar metabolism enzymes (name as SAGs). We found that the expression of *SAG31* was strongly associated with salt tolerance level. Sequence analysis to *SAG31* from salt-sensitive and salt-tolerant lines showed that a retrotransposon in the intron was enriched in salt-sensitive lines. The relationships between *SAG31* expression, salt tolerance level and existence of retrotransposon suggest that salt stress may trigger retrotransposon to active some genes for salt tolerance in plant. More genetic and molecular evidences will be presented in future.

Key words: salt stress, maize, GWAS, natural variants

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Highlight stress enhanced the disturbances of the rhythmic expression of Kai genes caused by the knockout of *prx-2cys* in *Synechococcus elongatus* PCC 7942

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The central oscillator of circadian clock in cyanobacterium, *Synechococcus elongatus* PCC 7942, is composed of *kai*A, *kai*B, *kai*C proteins. Many researches confirmed that this self-sustained intracellular circadian clock, so-called Kai clock, have played a pivotal role in the generation and calibration of the time clue for the circadian rhythm in cyanobacterial metabolism. Some proteins, such as SasA、LabA、CikA、RapA, are considered to be the key switches that have operated the output pathways of Kai clock. Peroxiredoxins(Prxs) in short, are families of thiol-specific antioxidant protein, which have be convinced to exert an important function in the whole antioxidant defense system of cyanobacteria, the rhythmic changes redox state of 2Cys-Prx are proved as a conserved non-transcriptional rhythmic marker. To test the relationship between the Kai clock and the PRX-$SO_{2/3}$ rhythmic marker, the expression patterns of clock genes were detected of by qPCR method in Wild-type(WT) and *prx-2cys* gene knockout(Δ*prx-2cys*) trains under different light intensity. The results demonstrated that under the natural light condition(100μ$Em^{-2}s^{-1}$), the rhythmic expression phenotype of the central clock genes were not affected by whether the *prx-2cys* gene were knocked out in *S.elongatus* PCC 7942 cells, while the expression patterns of the genes operating the output pathway were slightly affected. Interestingly, under a high light stressed condition(400μ$Em^{-2}s^{-1}$), the expression patterns of the output pathway genes were severely affected both in WT and Δ*prx-2cys* strains. The circadian rhythmic expression pattern of the central clock genes nearly disappeared in WT strains, while more robust rhythmic expression profiles of *sasA*、*labA*、*cikA*、*rapA* were restored inΔ*prx-2cys* cells by an unknown method.

Key words: cyanobacterium, peroxiredoxin, gene knockout, antioxidant, circadian rhythm

P104

The research of photosynthesis and chlorophyll fluorescent parameters variation between two species of Sedum under cadmium treatment

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The hyperaccumulating plant is a promising phytoremediation candidate accumulating substantial heavy metal ions without obvious signs of poisoning. We used the hyperaccumulating ecotype of Sedum alfredii Hance(HE) and non- hyperaccumulating ecotype of Sedum alfredii Hance (NHE) for physiology experiment. Hydroponic experiments were conducted using a modified culture solution. We determined the chlorophyll content, photosynthetic parameter, chlorophyll fluorescence characteristics at different time points. The results showed that along with the prolonging of stress time, the chlorophyll content a (Chla) and the chlorophyll content b (Chlb) finally reached a steady after a big drop. The Pn, Gs, Tr, Ci reduced at the beginning of stress, and then showed a rising trend, which illustrated the cadmium tolerance of two species of Sedum. The difference of Pn and Tr between two species of Sedum show that the cadmium tolerance of HE was stronger than the NHE. Compared these chlorophyll fluorescence characteristics(F0, Fm, ΦPSII、NPQ, qP, ETR), we found that cadmium tolerance of two species of Sedum was completely different. Verifing the cadmium tolerance of two species of Sedum would have a great help for the control of environmental pollution.

Key words: hyperaccumulating ecotype, Sedum alfredii, non-hyperaccumulating ecotype Sedum alfredii; cadmium stresses, chlorophyll, photosynthetic characters, fluorescence parameter

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A senescence-associated *Hevea brasiliensis LEA3* gene is regulated by multiplicate hormones and stresses

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Late embryogenesis abundant (LEA) proteins are a class of small proteins closely associated with the desiccation tolerance in organisms. In this study, an LEA gene (*HbLEA3-1*) was isolated from the rubber tree senescent leaves. The 972-bp full-length cDNA contains an ORF putatively encoding a polypeptide of 100 amino acids with a calculated molecular weight of 11.61 kDa and an isolectric point of 9.07. Composed of a hydrophobic N-terminal and a hydrophilic C-terminal and with a grand average of hydrophilicity of -0.498, the protein is predicted to contain an LEA_3 domain and a transit peptide for chloroplast localization. The search of the rubber tree genome suggested the presence of another copy, but no evidence supported its expression. qRT-PCR analysis indicated that *HbLEA3-1* is mainly expressed in leaves with a steadily increasing pattern during leaf development except for a transient decline in early-senescent leaves. Furthermore, the gene expression is regulated by several senescence-inducers such as cold, PEG, NaCl, H_2O_2, ABA, ethylene, MeJA and SA. Our results suggest a role of *HbLEA3-1* in hormone and abiotic stress responses as well as leaf development.

Key words: Rubber tree (*Hevea brasiliensis*), late embryogenesis abundant, *LEA3*, leaf development, leaf senescence, abiotic stress, hormone

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水杨酸调控基因 *AtSAJ1* 在植物对逆境胁迫应答中的作用机制

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水杨酸（Salicylic Acid, SA）是植物体内普遍存在的内源信号分子，在植物的生物和非生物胁迫应答方面具有重要的生理功能，然而对其如何调控植物相关反应的分子机制仍知之甚少。我们前期克隆了一个拟南芥未知功能蛋白——AtSAJ1（DNAJ类锌指蛋白），该基因在mRNA水平受SA和病原菌诱导显著上调表达；有趣的是，在蛋白水平上AtSAJ1受SA诱导降解。遗传分析表明AtSAJ1的突变体产生了对病原菌耐受性提高的表型；经过深入研究发现AtSAJ1定位在叶肉细胞的叶绿体和根中的质体上，利用SA途径相关突变体和转基因植株与atsaj1构建双突变体，分析了AtSAJ1在SA途径的功能和遗传位置；继而通过蛋白互作和遗传手段筛选到AtSAJ1在SA途径中的互作因子D。我们的研究将有助于揭示AtSAJ1在SA途径中的生理功能及其介导SA参与逆境胁迫应答的分子机理，为进一步了解SA信号参与逆境胁迫应答的机制和作物抗性分子育种提供理论基础。

关键词：拟南芥，水杨酸，AtSAJ1，逆境胁迫，蛋白互作

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Identification of the differentially expressed genes in rice in response to the pathogen *Rhizoctonia solani*

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Rice sheath blight is an important disease caused by the necrotrophic pathogen *Rhizoctonia solani*. However, the details of the rice defense response against *R. solani* remains to be elucidated. Here, a high-throughput sequencing technology was used to analyze the defense response of leaf sheath rice seedlings to rice sheath blight. We identified 250 differentially expressed transcripts by comparing the 24 h and 48 h post-inoculation (hpi) samples with their controls. With the increasing damage, two-thirds of the defense related genes were up-regulated and peaked at 48 hpi. These genes included a series of pathogenesis-related genes that possess antifungal activities. In addition, a functional analysis found that some differentially expressed genes, including five transcription factors families, were associated with Jasmonic acid (JA) signaling and secondary metabolism, implying that the plant might prevent the growth of rice sheath blight fungus by JA signaling to regulate metabolic reproduction. We had also found a microRNA (miRNA) precursor was up-regulated at 48h, suggesting that miRNA post-regulation might be play a role in resistance to rice sheath blight. These results have improved our understanding of the defense mechanisms of rice resistance to *R. solani* and would provide a molecular breeding resource.

Key words: rice sheath blight, *Rhizoctonia solani*, defense response, pathogenesis -related, jasmonic acid signaling

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褐飞虱唾液对水稻防卫反应的影响

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褐飞虱(*Nilaparvatalugens*(Stal))是水稻的一种重要害虫，它通过刺穿水稻的韧皮部筛管后直接吸取水稻汁液，引起水稻减产甚至成片死亡。已有的研究表明，蚜虫的唾液中存在能抑制植物防卫反应的效应子。褐飞虱同为刺吸式昆虫，因此推测，在褐飞虱的唾液中也存在抑制水稻防卫反应的效应子。为此，本研究选取已知的水稻防卫蛋白 POD（过氧化物酶）和过氧化氢酶作为研究对象，通过比较褐飞虱 (*Nilaparvata lugens*(Stal))直接取食水稻(*Oryza sativa* L.)及提取褐飞虱唾液人工模拟褐飞虱取食，分别设置直接针刺、褐飞虱唾液+50mg/mL Amp 注射水稻、注射蒸馏水等对照，运用单因素方差分析方法对不同方法处理的水稻防卫蛋白相关酶的酶活进行分析。结果表明：1）注入褐飞虱唾液或褐飞虱唾液加 50mg/mL 抗生素的混合物与褐飞虱刺吸效果一样，均能使水稻 POD 活性升高，且变化幅度较为接近。此结果证明，褐飞虱唾液中的某种成分充当影响水稻抗性反应的效应子，而非褐飞虱唾液中的共生菌。2）人工模拟褐飞虱刺吸过氧化氢酶的酶活力变化与褐飞虱取食一致，呈先上升再下降的趋势，在刺吸第 6 小时，过氧化氢酶酶活性值达到最高。但是，在注射含抗生素的褐飞虱唾液后，过氧化氢酶的酶活力发生明显的变化，呈现先下降在上升的趋势。结果表明褐飞虱唾液中的内生菌是影响其酶活性改变的重要因素。为进一步确定可能引起水稻防卫反应的这些内生菌种类，本研究进一步利用马铃薯葡萄糖培养基、高氏一号培养基、营养琼脂、酵母膏胨葡萄糖培养基四种培养基对褐飞虱唾液内生菌进行了的分离纯化，并从微生物形态学角度、细菌的生理生化特性对分离纯化的内生菌进行了鉴定，结合 16SrRNA 测序，发现能引起植物防卫反应的可能的内生菌源于肠杆菌属、洋葱假单胞菌属或丁香假单胞菌属。

关键词：防卫蛋白，唾液，内生菌，酶活

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Comparative proteomics studies on brown planthopper responding to host rice resistance

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The brown planthopper (BPH, *Nilaparvata lugens*) is one of the most destructive pests of rice. Comparative proteomics analyses of BPH will help to find new measures to control BPH. In this work, comparative proteomics analyses of the fourth instar nymphs of biotype I and II which infested the susceptible rice TN1 and the resistant rice Mudgo, were carried out by isobaric tag-based methodology for relative peptide quantification (iTRAQ) technique. The results revealed that 7385 protein fragments were detected, and 696 fragments were quantified. Total 201 proteins showed obviously different expression levels. These proteins were expressed differently between the two types of BPH, or the same type BPH feeding on the two rice varieties with different resistant levels. 27 proteins were selected to investigate the expression level of the corresponding genes by fluorescent quantitative real-time PCR. The results revealed that most of the genes have the same expression trends with their coding proteins. It is noteworthy that three genes (*vitellogenin*, *fatty acid synthase*, *protein kinase*) were expressed at an extremely different pattern, and probably *involved in* regulating biotype formation and/or the acute stress reaction. This study will help in intensifying the knowledge of the interaction mechanism of BPH and rice, including: ① during the acute stress reaction of BPH to host rice resistance, the variation laws of the midgut proteomics, which will indirectly provide some important information about the resistance mechanism of rice; ② the discrepancy between different biotype BPH midgut proteomics, which will provide some important information for division of biotype and elucidating the molecular mechanism of the formation of a special biotype.

Key words: brown planthopper (*Nilaparvata lugens* Hemiptera: Delphacidae), biotype, host rice resistance, isobaric tag-based methodology for relative peptide quantification (iTRAQ), comparative proteomics

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烯效唑对淹水胁迫下大豆叶片光合特性及产量的影响

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研究烯效唑对淹水胁迫下大豆叶片光合特性及产量的影响，以其明确烯效唑减轻大豆淹水胁迫伤害的生理作用。本研究以耐涝品种垦丰 14 和涝渍敏感品种垦丰 16 为试验材料，采用盆栽方式，在三节期（V3）叶面喷施 50mg/L 烯效唑，之后进行淹水胁迫处理，于三节期（V3）、淹水 3d（V3-3d）、淹水 5d（V3-5d）以及恢复正常水分条件后（始花期 R1、始荚期 R3、始粒期 R5 期）测定叶片光合参数，分析烯效唑对淹水胁迫下大豆叶片叶绿素相对含量、光合特性及产量的调控效应。结果表明：淹水胁迫期间（V3-3d~V3-5d），垦丰 16 叶片光合特性各指标的降低幅度高于垦丰 14；恢复正常水分处理后，垦丰 14 较垦丰 16 具有较强的补偿效应，表现为叶片净光合速率显著提高。与淹水胁迫下喷施清水进行对比，喷施烯效唑能提高淹水胁迫期间大豆叶片净光合速率、蒸腾速率、气孔导度和胞间 CO_2 浓度，并且在恢复正常水分管理后喷施烯效唑处理下的大豆叶片 SPAD 值、光合特性较正常水分处理下的高。淹水胁迫条件下喷施烯效唑处理使垦丰 14 的单株产量较正常水分、喷施清水处理下的分别高 105.81%、28.17%，使垦丰 16 的单株产量分别提高 10.16%、23.93%。说明喷施烯效唑可有效改善淹水胁迫下大豆叶片光合特性，从而增加其单株产量，且不同品种对烯效唑的调控效应存在差异。

关键词：大豆，烯效唑，淹水胁迫，光合特性，产量

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Plastid-mediated crop protection by RNA interference

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Plastid (chloroplast) genetic engineering has a great promise in plant biotechnology. It has several attractive advantages such as high-level of transgene expression owing to the polyploidy of the plastid genetic system, transgene containment via maternal inheritance and absence of gene silencing and pleiotropic effects. It has been demonstrated that double-stranded RNAs (dsRNAs) targeted against essential genes can trigger a lethal RNAi response in insect pests. Previously, we found chloroplasts can be capable of stably accumulating long dsRNAs, in which case dsRNA expression from the plastid genome provide better protection against Colorado potato beetle (CPB), a notorious agricultural pest, than dsRNA expression from the nuclear genome (Zhang et al., Science, 2015). Encouraged by this primary success, we aimed to employ this strategy to target another important insect pest, cotton bollworm (CBW). However, feeding transplastomic plants expressing dsRNA against essential genes of CBW did not cause the gene silencing and suppress the growth of CBW larvae. While no effective RNAi responses were observed in CBW, we found that a secreted RNA nuclease from midgut cell of CBW larvae can quickly degrade the ingested dsRNA, which might impede the effect of RNAi. From this findings, we proposed an approach how to escape the activity of this RNA nuclease and control the insects that showed no or less sensitivity to RNAi. In the end, I will summarize an updated working model of plastid-mediated crop protection and the potential application of this technology.

Key words: crop protection, plastid genetic engineering, RNAi, RNA nuclease

P112

N-3-oxo-hexanoyl-homoserine lactone, a bacterial quorum sensing molecule, plays an important role in plant salt tolerance

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N-acyl-homoserine lactones (AHLs) are the quorum sensing (QS) signal molecules to coordinate the collective behavior in a population in Gram-negative bacteria. Recent evidences demonstrate their roles in plant root growth and defense responses. In this present, we show that the treatment of plant roots with *N*-3-oxo-hexanoyl-homoserine lactone (3OC6-HSL), one molecule of AHLs family, resulted in enhanced salt tolerance in Arabidopsis, wheat and rice. The genetic analysis revealed that the growth inhibition phenotype in the length of primary roots, the fresh weight and the height of seedlings under salt stress condition were significantly improved by 3OC6-HSL. The contents of chlorophyll were increased and the Na^+/K^+ ratios were decreased by 3OC6-HSL in Arabidopsis, wheat and rice under salt stress condition. The level of proline were raised and the expression of proline biosynthesis related genes *AtP5CS, TaP5CS* and the proline transport gene *TaPT* were upregulated by 3OC6-HSL under salt stress condition, which suggested that the proline biosynthesis and transportation may be involved in 3OC6-HSL-mediated plant salt tolerance. qRT-PCR analysis showed that the expression of some salt-responsive genes including ABA-dependent genes *RD22* and *COR15a,* osmotic responsive gene *ADH,* ion-homeostasis related genes *SOS1* and *SOS2* were induced by 3OC6-HSL in Arabidopsis. But only the expression of *COR15a* was upregulated with a stronger induction by 3OC6-HSL than by NaCl under salt stress condition. 3OC6-HSL had no effect on the expression of ABA-independent gene *ERD1* under salt stress condition. These results indicated that ABA-dependent salt resistant pathway might involve in AHL-induced salt tolerance. In summary, genetic, physiological and molecular evidences showed that 3OC6-HSL plays an important role in plant salt tolerance. It provides a new insight into the plant-microbe inter-communication.

Key words: AHL, quorum sensing, salt tolerance, inter-communication, plant

P113

Increasing CK content enhances rice resistance to sheath blight caused by necrotrophic pathogen *Rhizoctonia solani*

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Sheath blight (SB), caused by necrotrophic fungus *Rhizoctonia solani Kühn* (*R. Solani*), is one of the most destructive diseases in rice worldwide. Increasing studies have showed that plant hormone cytokinins (CKs) play an important role in regulation of plant disease resistance. However, its role in enhancing rice resistance to SB remains unknown. Here, we found that exogenous application of synthetic CK kinetin (KT) reduced the severity of *R. Solani*-induced symptoms. Transgenic rice lines with increased endogenous CK levels by engineering CK-biosynthesis gene *IPT* delayed senescence or a stay-green phenotype and significantly enhanced SB resistance in both field tests and detached leaf inoculation assays. In contrast, transgenic rice lines overexpressing *CKX4*, encoding a CK-degradation enzyme, contain significantly lower CKs content compared to wild type and show significantly enhanced susceptibility to SB. We also found a 'stay-green' rice mutant, which maintains high CK content due to loss of function of CK degradation, displays higher resistance to SB at the later developmental stage. We conclude from our microscopic examination that the mechanism leading to enhanced resistance by increasing CK content is due to stronger ability of the transgenic lines or the mutant plant to inhibit cell death caused by *R. solani*, which ultimately results in poor pathogen infection. Importantly, we found that the transgenic lines and the 'stay-green' mutant with increased CK levels are almost the same as WT in their morphological and yield traits. Taken together, our results demonstrate that modulating CK levels is a feasible approach to the development of new rice varieties with excellent SB disease resistance, which is of great importance in rice breeding toward SB resistance.

Keywords: rice, cytokinins, sheath blight, stay-green, disease resistance

P114

A bZIP transcription factor, CaLMF, mediates light regulated camptothecin biosynthesis in *Camptotheca acuminata*

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Camptothecin (CPT) has powerful biological activities and its analogs, irinothecan and topothecan, are effective anti-cancer drugs for clinical therapy. CPT was firstly isolated from *Camptotheca acuminata* and its low accumulation in *planta* limited drug supply in the market. Among environmental factors and plant hormones/elicitors, which could regulate the biosynthesis of CPT, light intensity is a very important negative regulator for CPT biosynthesis in *C. acuminata*. The present work describes the molecular cloning and functional identification of a bZIP transcription factor, *CaLMF*, which is activated by light and predominately expressed in old leaves in *C. acuminata*. Over-expression of *CaLMF* down-regulates the expression of CPT biosynthesis genes and decreases the accumulation of CPT, while light-regulated expression of CPT biosynthesis genes and CPT production are abolished in *CaLMF* silenced plants. Our results show that CaLMF is a significant light signaling component, where it mediates light-regulated CPT biosynthesis in *C. acuminata*.

Key words: *Camptotheca acuminata*, camptothecin biosynthesis, bZIP transcription factor, virus-induced gene silencing, light intensity

P115

TOPP4 regulates the stability of PHYTOCHROME INTERACTING FACTOR5 during photomorphogenesis in *Arabidopsis*

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In plants, photoreceptors transfer light signals to phytochrome-interacting factors (PIFs), inducing the rapid phosphorylation and degradation of PIFs to promote photomorphogenesis. However, the phosphatase responsible for PIF dephosphorylation remains unknown. In this study, we identified a type 1 protein phosphatase, TOPP4, that is essential for PIF5 protein stability in Arabidopsis (Arabidopsis thaliana). Compared with the wild type, the dominant-negative mutant, topp4-1, displayed reduced hypocotyl length and larger apical hook and cotyledon opening angle under red light. Overexpression of topp4-1 in the wild type led to defects that were similar to those in the topp4-1 mutant. Red light induced phytochrome B (phyB)-dependent TOPP4 expression in hypocotyls. The topp4-1 mutation weakened the closed cotyledon angle of phyB-9 and phyA-211 phyB-9, while overexpression of TOPP4 significantly repressed the short hypocotyls of phyB-green fluorescent protein seedlings, indicating that TOPP4 and phyB function in an antagonistic way during photomorphogenesis. Protein interaction assays and phosphorylation studies demonstrate that TOPP4 interacts directly with PIF5 and dephosphorylates it. Furthermore, TOPP4 inhibits the red light-induced ubiquitination and degradation of PIF5. These findings demonstrate that dephosphorylation of PIF5 by TOPP4 inhibits its ubiquitin-mediated degradation during photomorphogenesis. These data outline a novel phytochrome signaling mechanism by which TOPP4mediated dephosphorylation of PIF5 attenuates phytochrome-dependent light responses.

Key words： photomorphogenesis, PIF5, phytochrome ,TOPP4, ubiquitin-mediated degradation

P116

A small heat shock protein involved in regulation of cyclic electron transport around photosystem I in *Arabidopsis*

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The light reactions in photosynthesis drive the occurrence of both linear and cyclic electron transport around photosystem I (PSI). Linear electron transport generates both ATP and NADPH, whereas PSI cyclic electron transport produces ATP without producing NADPH. There is clear genetic and molecular evidence for the existence of at least two distinct pathways for PSI cyclic electron transport in angiosperms: a main pathway that depends on PGR5 and PGRL1 proteins, and a minor pathway that is mediated by a chloroplast NADH dehydrogenase-like complex-dependent pathway. Although great progress has been made in understanding the physiological significance of photosystem I cyclic electron transport, our knowledge of the machineries involved remains very limited. Here, we report the isolation and identification of an Arabidopsis small heat shock protein which interacted with PGR5 and PGRL by yeast two-hybrid system analysis. The small Hsp was further proved the chloroplast localization by the GFP fusion protein. We got the mutant plant by CRISPR/Cas9 system and found the decrease of PGRL1 in this mutant. We propose that this small heat shock protein may regulate PGR5–PGRL1 protein-dependent pathway by interacting with PGR5 and PGRL especially under stressed conditions.

Key words: cyclic electron transport, linear electron transport, small heat shock

P117

高光胁迫下 SA 对拟南芥 PSII 保护作用研究

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在自然条件下，许多植物都会受到不同程度的生物与非生物胁迫。光照是光合生物生长发育所需要的重要环境因子。然而，高光强会明显抑制植物的生长发育，从而影响作物以及果实的产量。水杨酸（salicylic acid，SA）作为一种自然产生的植物激素，能调控植物的多种生理和生化功能。作为一种信号分子，SA 在植物应答环境胁迫时也起着非常重要的作用。前人对 SA 在植物中的抗逆作用已有大量的研究，发现外源 SA 可提高植物抗病性、抗旱性、抗盐性等，并对其机理有深入阐述，但很少研究关注 SA 在逆境下对光合结构的具体保护机制。最近，我们的研究发现，外施 SA 能通过不同的方式来调控小麦的叶绿素荧光以及光系统 II（PSII）的 CP29 等蛋白的含量，这加深了我们对 SA 保护植物 PSII 机制的理解，但具体的调控机理仍不清楚。目前，我们以双子叶模式植物拟南芥为对象，对高光条件下 SA 处理前后生理参数、叶绿素荧光参数、PSII 主要功能蛋白表达水平、类囊体膜蛋白的迁移、PSII 蛋白磷酸化水平进行了研究，并重点研究类囊体膜复合物以及类囊体超微结构的变化。期望阐明 SA 在逆境下如何通过调控 PSII 蛋白表达、类囊体蛋白磷酸化以及 PSII 复合物来对植物 PSII 进行保护的具体机制。这不仅有重要的学术意义，而且在农业生产和果实的产量中也具有潜在的应用价值。

关键词：拟南芥，水杨酸，高光胁迫，PSII

P118

光敏色素相互作用因子 *OsPIL11* 在水稻生长发育中的作用

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植物光敏色素（phytochrome）是一种可溶性色素蛋白质，主要感受红光和远红光，通过与光敏色素相互作用因子（phytochrome-interacting factors, PIFs）调控光形态建成。在水稻基因组中预测到 6 个 PIF 转录因子的编码基因（*OsPIL11* ~ *OsPIL16*）。我们分析了水稻 PIF 转录因子 *OsPIL11* 在水稻生长发育中的作用。在暗培养条件下，*OsPIL11* 过表达株系（*OsPIL11*-OX）幼苗的中胚轴显著长于野生型，而胚芽鞘生长受到抑制。有报道表明，独脚金内酯负调控中胚轴延伸，因为我们比较了野生型和 *OsPIL11*-OX 株系中独脚金内酯（SLs）途径基因的表达，结果显示，SLs 合成途径和信号途径基因在过表达株系中的表达水平均显著降低，推测 *OsPIL11* 是 SLs 途径的负调控因子，这可能决定着 *OsPIL11*-OX 具有较长的中胚轴。在光照条件下，*OsPIL11*-OX 株系的成熟期株高显著增高，分蘖数、枝梗数及穗粒数减少，结实率降低。GUS 染色结果显示，*OsPIL11* 基因在水稻根、节、叶鞘、幼穗、颖壳中均有表达。这些结果表明，*OsPIL11* 影响水稻生长发育过程中多个生物学过程。

关键词：水稻，光敏色素相互作用因子，中胚轴，独脚金内酯，生长发育。

P119

The genetic diversity of *Brassica* cytoplasmic DNA and beyond

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Brassica napus (rapeseed) is a recent allotetraploid plant that is cultivated as the second most important oilseed crop worldwide. Compared with its diploid ancestor species (*B. rapa* and *B. oleracea*), *B. napus* performs much better on many agronomic characters, e.g. colony photosynthetic capacity and economic coefficient. The origin of *B. napus* and its genetic relationships with its relatives still remain largely unresolved. Here, The cpDNA from a total of 488 worldwide *B. napus* accessions, 139 *B. rapa* accessions and 49 *B. oleracea* accessions were populationally re-sequenced using Illumina Solexa sequencing technologies. Their intra-specific cpDNA variants and their allelic frequencies were called genome-widely and further validated by genotyping analyses. The cpDNA of the current worldwide *B. napus* population comprises more than 400 variants (SNPs and short InDels) and maintains one predominant haplotype (Bncp1). Sequencing determination of the Bncp1 cpDNA haplotype eliminated its direct inheritance from any of the investigated *B. rapa* or *B. oleracea* accessions. The variant-based polymorphism information content (PIC) analysis demonstrated that *B. napus* has a much lower cpDNA diversity than *B. rapa*. However, a vast majority of the wild and cultivated *B. oleracea* appeared to share one same distinct cpDNA haplotype. This finding suggests that the cpDNA of the three *Brassica* species are well differentiated. Presumably, Bncp1 haplotype may originate from other *Brassica* relatives. There is also a big possibility that it may result from the interactions between cpDNA mutations and the natural/artificial selection. A series of experiments have been designed and being performed to clarify the evolutionary mechanism, and also to dissect the functional mechanism of the major *B. napus* cytoplasmic DNA haplotypes. These obtained variation data will provide primary information for the chloroplast/mitochondrion related researches, and germplasm enhancement for *B. napus*.

Key words: *Brassica napus*, cpDNA, genetic diversity, haplotype, evolution.

P120

Nuclear accumulation of the photoreceptor UVR8 is regulated by COP1

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Light plays critical roles throughout the lifecycle of plants. UV-B is an intrinsic component of solar radiation. Plants respond to UV-B through the UVR8 photoreceptor signaling pathway. Homodimeric UVR8 perceives UV-B via specific intrinsic aromatic amino acids as chromophores. This leads to rapid dimer dissociation. UVR8 monomers interact with CONSTITUTIVE PHOTOMORPHOGENIC 1 (COP1) to relay the captured UV-B signal for UV-B photomorphogenesis. While it was described quite some years earlier that UV-B triggers UVR8 protein translocation from cytosol into nucleus, the molecular mechanism regulating this process has remained unknown. The E3 ligase COP1 is a central negative regulator for photomorphogenesis under visible light and far-red light. Intriguingly, COP1 plays positive roles in UVR8-mediated UV-B photomorphogenesis. UV-B signaling is essentially abolished in *cop1* mutant alleles with unknown mechanisms. Through GR-UVR8 transgenic lines, we demonstrated that UVR8 is active in nucleus for UV-B photomorphogenesis. Moreover, the UV-B-triggered UVR8 nuclear accumulation is absent in *cop1* mutants. Our results emphasize the importance of nuclear-localized UVR8 and highlight a previously unknown activity of COP1 in mediating UVR8 nuclear accumulation in response to UV-B.

Key words: UV-B, photomorphogenesis, UVR8, COP1, nuclear accumulation

P121

Agronomic nitrogen-use efficiency of rice can be increased by an artificial decrease in the expression ratio of the *OsNRT2.1* and *OsNAR2.1* genes in culms

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The importance of the nitrate (NO_3^-) transporter for yield and nitrogen-use efficiency (NUE) in rice was previously demonstrated using map-based cloning. In this study we enhanced the expression of the *OsNRT2.1* gene, which encodes a high-affinity NO_3^- transporter, using a ubiquitin (*Ubi*) promoter and the NO_3^--inducible promoter of the *OsNAR2.1* gene to drive *OsNRT2.1* expression in transgenic rice plants. Transgenic lines expressing *pUbi:OsNRT2.1* or *pOsNAR2.1:OsNRT2.1* constructs exhibited increased total biomass including yields of approximately 21% and 38% compared with wild-type (WT) plants. The agricultural NUE (ANUE) of the *pUbi:OsNRT2.1* lines decreased to 83% of that of WT plants, while the ANUE of the *pOsNAR2.1:OsNRT2.1* lines increased to 128% of that of WT plants. The dry matter transfer (DMT) into grain decreased by 68% in the *pUbi:OsNRT2.1* lines and increased by 46% in the *pOsNAR2.1:OsNRT2.1* lines relative to the WT. The expression of *OsNRT2.1* in shoot culms showed that *Ubi* enhanced *OsNRT2.1* expression by 3 to 20-fold and *OsNAR2.1* promoters increased by 33% to 45% higher than the WT. The ratio of *OsNRT2.1* to *OsNAR2.1* expression was altered in the transgenic lines with ratios of approximately 11.3:1 and 4.7:1 in the *pUbi:OsNRT2.1* and *pOsNAR2.1:OsNRT2.1* lines, compared with a ratio of 7.2:1 in WT plants. We show that increased expression of *OsNRT2.1*, especially in combination with a relative lower expression ratio with its partner gene *OsNAR2.1*, can improve yield and NUE in rice.

Key words: OsNAR2.1 promoter, *Oryza sativa*, OsNRT2.1, agronomic nitrogen-use efficiency

P122

磷铁平衡调控磷吸收与利用的机制初探

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磷是植物生长发育所必须的大量营养元素。现代农业生产，往往需要大量磷肥，以促进和保持作物产量，不仅消耗世界范围内有限的磷矿，同时增加农业生产成本，造成水体富营养化和环境破坏。如何减少磷肥使用量，提高已有磷利用效率，一直是磷研究过程中重要的研究方向。缺磷胁迫引起拟南芥植物根系铁含量增加，形成铁毒害，抑制主根伸长，阻断了植物通过根系可塑性改变增加磷吸收的可能性，适合的磷铁平衡，在植物磷高效吸收与利用方面具有重要作用，但报道甚少，分子机制模糊。本研究通过模式植物拟南芥，由转录因子组成的转录调控，在植物生长、发育和各种逆境应答过程中的重要作用为出发点，筛选了 55 个转录因子家族描述信息里面应答生物与非生物逆境大部分转录因子，通过三引物鉴定获得纯系材料，采用严格低磷胁迫筛选，获得了参与拟南芥调控低磷未被报到的转录因子 *attflpg2*，*attflpt1* 和 *attfp1* 突变体材料，同时植物缺铁应答未报到 *attfo3* 突变体材料。与野生型相比，突变体 *attflpg2-2*，含有蔗糖 10g 的 1/2MS 盐正常与缺磷培养基上根毛极度缺失，同时低磷胁迫下主根增长，地上部分没有任何差异，磷诱导根毛大量产生下，通过根可塑性改变增加磷吸收，但低磷胁迫下 *attflpg2-2* 根毛极度缺失缺，却表现出对低磷胁迫不敏感。大量正交梯度实验摸索 *attflpg2-2* 突变体材料对低磷胁迫不敏感原因，同时包括 *attflpt1*，*attfp1*，*attfo3*，*attfg2-1* 和 *attfp2-2*。在适合的磷铁供应比例范围内，低磷胁迫敏感突变体 *attflpt1* 和 *attfp1*，转变为对低磷胁迫拥有较强的抗性。而铁应答 *attfo3* 突变体无论在任何磷铁供应比例下，均未表现出任何差异，同时还有铁敏感突变体 *attfg2-1* 和 *attfp2-2*。因此，磷铁平衡在很大程度上影响植物对磷的吸收与利用效率。

关键词：离子平衡，转录因子，磷高效吸收，根毛缺陷

P123

The dissection of the genetic architecture of amino acid composition in maize kernels by combing genome-wide association study and linkage mapping

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Maize (Zea mays) is one of the most widely grown crops worldwide. It is not only a staple food for people and animals, but also an important industrial material for fuel and other applications. The amino acid composition and quantity of seed storage proteins is related to the nutritional quality of seeds. Amino acids are both constituents of proteins, providing the essential nutrition for humans and animals, and signaling molecules regulating the growth and development of plants. However, the maize cultivars widely planted usually have insufficient levels of essential amino acids, such as lysine and tryptophan. In present study, we measured the levels of 17 different total amino acids in mature kernels from a maize diversity inbred collection and three recombinant inbred line (RIL) populations. In total, 586 and 778 significant loci (QTLs) with 6.83% and 7.95% average phenotypic variation were identified by GWAS and linkage mapping, respectively. About 44.3% of the loci identified by GWAS were verified by expression QTL, and only 5.5% overlapped with mapped QTLs in the three RIL populations. For each trait, 2.9 and 4.2 loci (QTLs) were identified on average, which implies that the genetic architecture of amino acids is relative simple: only controlled by limited loci. GRMZM2G015534, GRMZM2G143008 and one QTL were further validated by using molecular approaches. The amino acid biosynthetic and catabolic pathways were reconstructed on the basis of candidate genes proposed in this study. Our results provide insights into the genetic basis of amino acid biosynthesis in maize kernels and may facilitate marker-based breeding for quality protein maize.

Key words: amino acid, Quality Protein Maize (QPM), network, Genome-Wide Association Study (GWAS), linkage mapping, metabolism.

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Overexpression of a pH-sensing nitrate transporter in the membrane to increase rice crop yields

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Cellular pH homeostasis is fundamental for life and all cells adapt to maintain this balance. In plants the chemical form of nitrogen supply, nitrate and ammonium, is one of the cellular pH dominators. We report that the rice nitrate transporter OsNRT2.3 is transcribed into two spliced isoforms with natural variation in their expression ratio. One splice form, OsNRT2.3b is plasma membrane located mainly expressed in the phloem, and which has a regulatory motif on the cytosolic side that acts to switch nitrate transport activity on or off by a pH sensing mechanism. High OsNRT2.3b expression in rice enhances the pH buffering capacity of the plant, increasing N, Fe and P uptake. In field trials, increased expression of OsNRT2.3b improved grain yield and nitrogen use efficiency (NUE) by 40%. These results indicate that pH sensing by the rice nitrate transporter OsNRT2.3b is important for plant adaption to varied N supply forms and can provide a target of improving NUE.

Key words: nitrate transporter, pH-sensing, nitrogen use efficiency, yield, rice

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硝酸根转运体 *OsNPF7.2* 基因影响水稻在高浓度硝酸根为氮肥下的生长

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目前已报道众多的植物 *NPF*(*Nitrate Transporter1/Peptide Transporter Family*)基因家族编码的转运体蛋白，它们分别能转运硝酸根、小肽、氨基酸、植物激素和苷子苷。其中的多个硝酸根转运体广泛地参与到植物对硝酸根的吸收、分配和再利用。为了进一步了解 *NPF* 编码的硝酸根转运体在水稻硝酸根营养生理中的作用，我们分析了水稻 *OsNPF7.2* 基因的表达模式，及其编码蛋白的硝酸根转运活性和亚细胞定位，并进一步探究其表达干扰株系在高浓度（1-10mM）硝酸根为氮肥下的生长特性。结果表明，水稻 *OsNPF7.2* 基因主要表达于根的厚壁组织、皮层和中柱，在叶脉中也有表达，其在根中的表达受高浓度硝酸根的诱导。水稻 OsNPF7.2 蛋白是低亲和的硝酸根转运体，主要定位于液泡膜上。T-DNA 插入和 RNA 干扰能下调水稻 *OsNPF7.2* 基因的表达。相较于其野生型，这些表达干扰的水稻株系在高浓度硝酸根为氮肥下的生长均明显受阻。这表明，水稻 *OsNPF7.2* 基因参与硝酸根的跨液泡膜转运过程，并在高浓度硝酸根为氮肥下的植株生长中发挥作用。

关键词：水稻，硝酸根，氮，液泡膜，生长，NPF

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高铵营养下谷氨酸过量积累对小麦苗期根系生长的胁迫作用与机理

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目前大多数对于高铵胁迫相关的机理研究大多集中在铵本身对于植株生长的作用上面，往往忽略了铵代谢过程中 N 同化产物与耐铵性的关系。本研究目的在于阐明高铵条件下铵同化产物-谷氨酸对小麦根系生长的影响及机理。本研究采用水培试验，以 5mM NH_4^+为单一氮源，为了研究铵同化产物的影响在溶液中并加入 1 μM 甲硫氨酸亚砜（MSO，谷氨酰胺合成酶的专一性抑制剂）及 1 mM 谷氨酸（初级 N 同化产物）处理，以 5mM NO_3^-硝态氮为对照。铵态氮处理显著降低了两个小麦品种的植株干重、总根长、根表面积和根体积，但是徐麦 25 受到的影响较少，但是当加入 MSO 后，抑制作用减弱，加入谷氨酸后抑制作用进一步加强。铵态氮条件下，两个品种中谷氨酰胺合成酶（GS）和谷氨酸脱氢酶（GDH）的活性显著增加，GS1 和 GDH 基因表达显著提高，但是 GS2 的基因没有显著影响，因此导致铵同化产物谷氨酸的增加，但是加入 MSO 后降低谷氨酸的含量。与矮抗 58 相比，徐麦 25 因为具有较高的谷草转氨酶（GOT）和谷丙转氨酶（GPT）活性，因此谷氨酸含量较低。铵态氮营养降低了根系中生长素（IAA）的含量，同时使生长素在地上部分和根的比值升高，但是徐麦 25 中的比值低于矮抗 58。而加入谷氨酸后比值进一步升高，但是加入 MSO 后比值降低。生长素转运载体 PIN 基因的表达在铵态氮条件下受到显著抑制，加入谷氨酸后进一步抑制了 PIN 的表达，但是加入 MSO 后 PIN 的抑制程度得到缓解。同时铵态氮条件下，可溶性糖含量以及其在根中和地上部分的比值显著降低。结果表明，高铵耐性品种具有较高的氨基转化能力以减少谷氨酸的过量积累，从而保持较高的生长素运输能力，不仅可以维持根中生长素浓度，还可促进可溶性糖从地上部分向根系的运输，维持较高的根系生长。

关键词：小麦，高铵胁迫，谷氨酸，氨基转化，生长素运输

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Metabolic characteristics in meal of black rapeseed and yellow-seeded progeny of *Brassica napus–Sinapis alba* hybrids

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Breeding of yellow-seeded rapeseed (*Brassica napus*) is preferred over black-seeded rapeseed for the desirable properties of the former. This study evaluated the metabolites and nutritive values of black-seeded rapeseed meal and yellow-seeded meal from the progeny of a *B. napus–Sinapis alba* hybrid. Yellow-seed meal presented higher protein (35.46% *vs.* 30.29%), higher sucrose (7.85% *vs.* 7.29%), less dietary fiber (26.19% *vs.* 34.63%) and crude fiber (4.56% *vs.* 8.86%), and less glucosinolates (22.18 *vs.* 28.19 μmol/g) than black-seeded one. Amounts of ash (3.65% *vs.* 4.55%), phytic acid (4.98% *vs.* 5.60%), and total polyphenols (2.67% *vs.* 2.82%) were decreased slightly in yellow-seeded meal compared with black-seeded meal. Yellow-seeded meal contained more essential amino acids than black-seeded meal. Levels of the mineral elements Fe, Mn, and Zn in yellow-seeded meal were higher than black-seeded meal. By contrast, levels of P, Ca, and Mg were lower in yellow-seeded meal. Moreover, yellow-seeded meal showed lower flavonol (kaempferol, quercetin, isorhamnetin, and their derivatives) content than black-seeded meal. Comparison of metabolites between yellow and black rapeseed confirmed the improved nutritional value of meal from yellow-seeded *B. napus*, and this would be helpful to the breeding and improvement of rapeseed for animal feeding.

Key words: *Brassica napus*, yellow-seeded rapeseed, black-seeded rapeseed, seed meal, metabolites

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Application of virus-induced gene silencing approach in *Camptotheca acuminata*

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Camptotheca acuminata is the major plant for the production of camptothecin (CPT), an important anti-cancer drug used for the treatment of various cancers throughout the world. The low accumulation of CPT in plants limited its supply in the market and led to urgent need of promoting its accumulation by means of plant metabolic engineering, which relied on deep understanding of CPT biosynthesis pathway. However, missing of most of pathway genes restricted the attempts for regulating CPT biosynthesis. To unveil the CPT biosynthesis pathway, many efforts have been done for pathway gene identification and the application of large-scale RNA-sequencing technique accelerated screening of candidate genes which could be involved in CPT biosynthesis. To identify the function of these candidate genes in *planta*, it needs to develop an effective approach, such as virus-induced gene silencing (VIGS) in *C. acuminata*. In this work, a Tobacco Rattle Virus-based VIGS method was developed and the application of this method successfully silenced the expression of four known genes involved in the early steps of CPT biosynthesis and resulted in clear decrease of CPT and 10-hydroxycamptothecin (10-HCPT) accumulation. This VIGS approach could be further applied to functional identification of candidate genes to elucidate CPT biosynthesis in *C. acuminata*.

Key words: *Camptotheca acuminata*, virus-induced gene silencing, camptothecin biosynthesis pathway, alkaloids

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Rice gene *OsAMT1.1* functions in ammonium uptake and ammonium–potassium homeostasis over their low and high concentration ranges

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Rice (*Oryza sativa*) grown in paddy field is the ammonium (NH_4^+) preferring crop; however, its AMT-type NH_4^+ transporters in mediating root N acquisition have not been well-characterized yet. Here, we analyzed the expression patterns and physiological functions of *OsAMT1.1* gene belonging to AMT1 subfamily in rice. OsAMT1.1 is located in the plasma membrane and mainly expressed in root epidermis, stele and mesophyll cells. Disruption of *OsAMT1.1* gene decreased NH_4^+ uptake, root and shoot growth at both low- and high-NH_4^+ supply levels. OsAMT1.1 contributed to short-term (5 min) $^{15}NH_4^+$ influx rate by about one quarter, irrespective of varied NH_4^+ concentrations. Knockout of *OsAMT1.1* significantly decreased total N transport from root to shoot at low NH_4^+ level. Moreover, the *osamt1.1* mutants in comparison to WT showed increase of potassium (K) absorption rate under high NH_4^+ condition but decrease under low NH_4^+ condition. The mutants contained significant higher K in both roots and shoots at limited K (0.1 mM) supply when NH_4^+ was replete. Taken together, the results indicated that OsAMT1.1 contributes significantly to the NH_4^+ uptake from both low- and high-NH_4^+ environments and plays an important role in N-K homeostasis in rice.

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一个大豆氨基酸透性酶基因功能的研究

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氨基酸透性酶（amino acid permeases, AAPs）是一类在植物不同器官的氨基酸转运过程中均发挥着重要作用的氨基酸转运蛋白。无论是在植物的营养生长还是种子发育过程中，氨基酸的吸收和由“源”向“库”的转运都离不开氨基酸转运蛋白。目前对该蛋白家族的研究多集中在模式植物拟南芥中，而在重要的经济作物大豆中几乎为空白。本文主要报告对大豆氨基酸透性酶第 6 亚家族一个成员的功能研究。我们发现该基因具有低氮响应特点，并利用转基因拟南芥证明其编码产物具有吸收和运输广谱氨基酸的功能。我们进一步以天隆一号大豆为受体材料，利用农杆菌介导的大豆子叶节转化法获得过表达该基因的转基因大豆材料。对多个单拷贝纯合株系的研究发现：该基因过表达提高了转基因大豆幼苗对多种氨基酸的吸收和运输，最终影响种子中游离氨基酸的种类和含量，转基因大豆种子中总氮含量增加，碳氮比下降；转基因大豆对低氮抗性增强。相关研究成果的取得，为深入解析氨基酸透性酶第 6 亚家族的功能及高产高效大豆新品种的培育奠定了重要基础。

关键词：大豆，氨基酸透性酶，游离氨基酸，氨基酸运输

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Prequel to synthetic biology for pathway elucidation: from candidate genes screening to intermediates identification in plants

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Natural products extracted from plants have wide variety of biological activities that are used as medicines, pesticides, industrial raw materials, and so on. As increased demand of these compounds, its supply is potentially challenged by limitations in biological sourcing. The recent progress of synthetic biology provides an alternative way for producing these molecules in heterologous species, such as yeasts, bacteria and other model plants by transferring whole biosynthesis pathway. To develop such bioengineering approach, the essential prerequisite is the availability of whole pathway genes. Recently, many transcriptomic and genomic investigations were carried out, which supplied tons of gene sequences information and dramatically speeded up the discovery of pathway biosynthesis genes. Using monoterpenoid indole alkaloids (MIAs) synthesized in *Catharanthus roseus* as an example, we describe potent methods for pathway elucidation, including candidate genes screening, genes function validation, and pathway intermediates identification. These methods could be further applied to other valuable plant species for supplying essential gene elements to establish synthetic biology platform.

Key words: plant secondary metabolism, synthetic biology, pathway elucidation, pathway genes screening, pathway intermediates identification

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OsYSL15 在铁由根向地上部转运过程中的功能研究

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铁是植物必需的微量元素，在植物的生长发育过程中起到重要作用，如果植株缺铁则会导致植株矮小、新叶变黄。铁从根部吸收以后，将会以共质体途径和质外体途径运输，后者被内皮层中的凯氏带阻拦，在那里完成选择性的吸收，再装载进入木质部进而完成地下向地上的长距离运输。然而，在整个转运过程中，却没有功能明确的转运蛋白被报导出现。*OsYSL15* 长期以来被认为在吸收三价铁复合物这一过程中发挥重要作用，而其在内皮层和中柱间也存在较为强烈的表达，暗示其在根部向地上部转运的过程中同样会发挥重要作用。本研究以 *OsYSL15* 突变体和野生型为实验材料，使用不同浓度二价铁对植株进行为期三周的处理，发现突变体植株长势明显弱于野生型。对根部和地上部铁含量进行测量分析，发现突变体地上部铁含量下降，且转运系数显著性小于野生型。进一步测量木质部伤流液中铁含量，发现突变体中的铁含量均显著性小于野生型。根部普鲁士蓝加强剂染色横切观察表明突变体中的铁在内皮层大量截留。最后比较突变体和野生型中铁稳态相关部分基因的表达情况，结果发现铁长距离运输转运蛋白 *OsYSL2* 的表达显著增强，可能是突变体植株为提高地上部铁转运而采取的策略。综上所述，我们可以得出 *OsYSL15* 在铁跨内皮层转运的过程中发挥重要作用。

关键词： *OsYSL15*，铁，地下向地上部转运，内皮层

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Agrobacterium-mediated Transformation of *Dendrobium officinale*

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Dendrobium officinale, belonging to *Dendrobium* genera in *Orchidaceae*, have both ornamental and medicinal values. To date, a few studies on gene transfer techniques in orchids have been reported. However, based on the specific physiological characters of *D. officinale,* to establish a systematically optimized transformation procedure is requisite. Here, we report a routine gene transformation protocol via *Agrobacterium tumefaciens* in *D. officinale. A. tumefaciens* harbouring pCambia1305 plasmid carrying β-glucuronidase as the reporter gene and hygromycin phosphotransferase as the plant selectable marker gene was used for genetic transformation of *D. officinale*. Protocorm-like bodies (PLBs) obtained from *D. officinale* seeds were used as the target explants for transformation. Different parameters were optimized to improve the transformation efficiency, such as strain type of *A. tumefaciens*, cell density during Agrobacterium-infiltration, co-cultivation period, surfactants concentration and sonication duration. Putatively transformed tissues were obtained by selection on solid MS media supplemented with 6-benzylaminopurine (6-BA), α-naphthalene acetic acid (NAA) and 20 mg/L hygromycin. The results showed while GV3101 cell density of OD_{600} = 0.6, surfactant concentration = 0.001%, acetosyringone concentration = 100 μmol/L, with addition of 3-min sonication and incubation of 28°C 12 hours followed by 20°C 5 days, the highest transformation efficiency was obtained. The transformed PLBs were found GUS-stain positive via histochemical analysis, indicating that the foreign DNA had been successfully integrated into the *D. officinale* genome. Our optimized transformation method is applicable in practice of genetic and physiology research and genetic engineering-based breeding in *D. officinale*.

Key words: *Dendrobium officinale*; genetic transformation; transgenic technology

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Fine mapping a major QTL qFCC7L for chlorophyll content in rice (*Oryza sativa* L.) cv. PA64s

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Chlorophyll (Chl) content is an important agronomic trait directly affecting the photosynthetic rate. Using a high-density genetic map of 132 recombinant inbred lines (RILs) derived from the cross between 93-11 and PA64s, we detected the quantitative trait loci (QTLs) for Chl content of the top three leaves under two nitrogen (N) conditions at two developmental stages. A total of 32 main-effect QTLs located on chromosomes 1, 4, 5, 6, 7, 8, and 12 were identified, and these QTLs individually accounted for 6.0–20.8 % of the total phenotypic variation. A major QTL *qFCC7L* affecting the Chl content under low N condition was identified, and its positive allele came from PA64s. This QTL might be associated with the ability to tolerate low-N stress in rice. The chromosomal segment substitution line (CSSL) with the corresponding segment from PA64s had a higher SPAD value and photosynthetic rate than 93-11 and showed a lower specific leaf area (SLA). We performed a fine-mapping using a BC4F2 population via marker-assisted backcross and finally mapped this QTL to a 124.5 kb interval on the long arm of chromosome 7.Candidate gene analysis showed that there were sequence variations and expression differences in the predicted candidate gene between the two parents. These results suggest that the QTL *qFCC7L* may be useful for breeding the rice varieties with higher photosynthetic rate and grain yield.

Keywords: chlorophyll content, photosynthetic rate, QTL analysis, low nitrogen condition, rice

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Genomic characteristics and QTL identifications of chromosomal single-segment substitution lines of *O. meridionalis* in the genetic background of *O. sativa*

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O. meridionalis indigenous to Australia is an AA genome wild species in *Oryza*, it has been considered an important germplasm resource for improvement of cultivated rice. In this study, a total of 99 chromosomal single-segment substitution lines (SSSLs) were developed by using *O. meridionalis* as a donor and an elite indica cultivar, Huajingxian 74 as recipient, through successive backcrossing and SSR marker-based genotyping. The 99 substituted segments were distributed on 12 chromosomes with the estimated total lengths of 1580.16 cM and 15.11 cM mean length of every segment. The 837.94 cM coverage length of substituted segments covered 54.98 percent of rice genome. Fourteen quantitative traits were evaluated in SSSLs for two seasons, including plant vegetative growth traits, i.e., heading date, plant height, flag leaf length, flag leaf width; Grain yield related traits, i.e., panicle numbers per plant, panicle length, spikelet numbers per panicle, grain density, number of primary branches per panicle, seed-setting rate, 100-grain weight, grain length, grain width, and ratio of grain length to width. A total of 161 putative QTLs detected for thirteen traits except panicle numbers per plant. Among them, 32 QTLs related to nine traits were identified both in the late season of 2014 and the early season of 2015, in which 10 QTLs had the positive additive effects. The additive effect of the QTLs ranged from 0.10 to 33.55, and the contribution of the additive effect to traits were from 0.06-20.92 percent. The present study demonstrated that the SSSLs offer both opportunities and a good germplasm platform for identification and transformation beneficial genes of *O. meridionalis*, as well as a precious long-time *in-vivo* conservation reservoir for *O. meridionalis*.

Key words: *O. meridionalis*, chromosomal single-segment substitution lines (SSSLs), QTL identification, substitution mapping

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Lipid metabolism is involved in the development of functional stomata in *Arabidopsis*

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Stomata that are bordered by pairs of guard cells are specialized for regulating gas exchange and transpiration in plants. During the past decade immense progress has been made in understanding the early stages of guard cell differentiation, but the downstream development for functional stomatal guard cells remains poorly understood. Here we reported two *Arabidopsis* mutants, *wizened stomata 1* (*wiz1-1*) and *wizened stomata 2* (*wiz2-1*), which have 31% and 16% deformed stomata, respectively. These abnormal stomata bear a general resemblance to the flat tires of cars, and the sizes of wizened guard cells in *wiz1-1* and *wiz2-1* mutants are 85% and 75% of the wild type (WT) ones. Except for the stomatal shape and size, mutations in *WIZ1* and *WIZ2* did not affect the stomatal density and stomatal indices. In addition, stomatal conductance (g_s) induced by low [CO_2] and light illumination was significantly reduced in mutants. When tested with the light- and fusicoccin-induced stomatal opening bioassays, the deformed stomata of *wiz1-1* and *wiz2-1* showed nearly no aperture changes while the normal shaped stomata behaved comparably to those of WT in terms of stomatal movement. Genetic analysis showed that these two mutants are not allelic. By tracing developmental process of deformed stomata in *wiz1-1* and *wiz2-1*, we found that the morphological defects occurred after guard mother cells were divided to form young stomata, suggesting that both WIZ1 and WIZ2 regulate guard cell differentiation at the late stage. The development of the ventral wall of the wizened guard cells was disturbed, and large lipid drops (LD) accumulated in the wizened guard cells. These results imply that WIZs may play important roles in the development of fully functional stomata through modulating LD biogenesis in *Arabidopsis thaliana*. We are currently working on the fine gene mapping and dissecting the molecular mechanisms regulating the development of functional stomata by the *WIZ* genes.

Key words: environmental stress, stomatal function, guard cell, lipid metabolism, plant development

P137

Ecological adaptation of heading date and photoperiod sensitivity in Chinese *japonica* rice

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Heading date (HD) and photoperiod sensitivity (PS) are important traits for the adaptation of rice to different cultivation areas and cropping seasons, and are significantly affected by environmental factors. Identification of the HD and PS adaptation among rice varieties could prove invaluable information during rice germplasm management and help researchers develop strategies to improve cultivars. In our study, HDs of 583 major inbred *japonica* varieties native to different regions in China were analyzed under organic farming conditions in Hangzhou and Sanya for four years. Basic vegetative phase (BVP), photoperiod sensitivity index (PSI), the original climatic and geographical information (temperature, latitude and longitude) were analyzed. Phenotypic variation analysis showed BVP, HD and PSI exhibited distinct ecological and geographical distributions in China. Correlation and regression analysis showed that BVP, latitude, longitude, PSI, and temperature could affect HD differently in the five main ecologically distinct rice growing regions. Five regression equations were obtained for predicting the adaptation of *japonica* rice in the five ecological regions. The Shannon-Weaver diversity index on the phenotype of BVP, HD and PSI showed an overall mean of 0.47 in the germplasm collection, and exhibited wide ecologically and geographically distribution. Cluster analysis demonstrated that HD plays an important role in the regional adaptation of rice and that there had been some changes in the regionalization of Chinese *japonica* varieties during the past 50 years. Cluster analysis also showed that there were sub-regions between and in the five main regions, These results would be useful for rice introduction and commercial extension of rice cultivars to different cropping regions in China, and also be important to analyze the effects of global warming on rice regionalization. Meanwhile, methods used in the study have reference values to the rice regionalization analysis in other countries owning a number of rice ecological regions.

Key words: ecological adaptation, heading date, *japonica* rice, photoperiod sensitivity

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Development and application of marker-assisted reverse breeding using hybrid maize germplasm

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Humankind has been through different periods of agricultural improvement aimed at enhancing our food supply and the performance of food crops. In recent years, whole genome sequencing and deep understanding of genetic and epigenetic mechanisms have facilitated new plant breeding approaches to meet the challenge of growing population, dwindling resources, and changing climate. Here we proposed a simple and fast molecular breeding method, marker-assisted reverse breeding (MARB), which will revert any maize hybrid into inbred lines with any level of required similarity to its original parent lines. This method has the advantages of fast speed, fixed heterotic mode, and quick recovery of beneficial parental genotypes compared to traditional pedigree breeding using elite hybrids. Meanwhile, MARB has the advantage of not requiring sophisticated transformation and DH technologies over RNAi-mediated reverse breeding. In addition, MARB can also be used with feed corn harvested from big farms, which is often similar to F_2 populations, and the relevant transgenes in the population can be eliminated by marker-assisted selection. As a result, the whole global commercial maize hybrids can be utilized as germplasm for breeding with MARB technology. Starting with an F_2 population derived from an elite hybrid, our experiment indicates that with three cycles of marker-assisted selection, selected lines could recover over 80% of the parental genotypes and associated beneficial genes in a fixed heterotic mode. The success application of MARB in maize suggests that this technology is applicable to any hybrid crop with enough available SNP markers. Several issues associated with MARB were discussed, including its rationale, efficiency and advantages, along with food/feed and environmental safety issues and applications of MARB in variety protection and marker-assisted plant breeding.

Key words: maize, marker-assisted reverse breeding; SNP

P139

Possible mechanism of reduced tiller number in the ascorbic acid-deficient rice suppressed for L-galactono-1, 4-Lactone dehydrogenase

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The tiller of rice (*Oryza sativa* L.), which determines the panicle number per plant, is an important agronomic trait for grain production. Ascorbic acid (Asc) is a major plant antioxidant that serves many functions in plants. L-galactono-1,4-lactone dehydrogenase (GLDH, EC 1.3.2.3) is an enzyme that catalyzes the last step of Asc biosynthesis in plants. Here we show that the GLDH-suppressed transgenic rices, GI-1 and GI-2, which have constitutively low (between 30% and 50%) leaf Asc content compared with the wild-type plants, exhibit significantly reduced tiller number. Moreover, lower growth rate and plant height were observed in the Asc-deficient plants relative to the trait values of the wild-type plants at different tillering stages. Further examination showed that the deficiency of Asc resulted in a higher lipid peroxidation, a loss of chlorophyll, a loss of carotenoids and a lower rate of CO_2 assimilation. In addition, the level of abscisic acid was higher in GI-1 plants, while the level of jasmonic acid was higher in GI-1 and GI-2 plants at different tillering stages. The results we presented here indicated that Asc deficiency was likely responsible for the promotion of premature senescence, which was accompanied by a marked decrease in photosynthesis. These observations support the conclusion that deficiency of Asc alters tiller number in the GLDH-suppressed transgenics through promoting premature senescence and changing phytohormones related to senescence.

Key words: ascorbic acid, L-galactono-1,4-lactone dehydrogenase, rice, tiller number

P140

水稻产量一般配合力及相关基因功能研究

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水稻是世界上主要的粮食作物之一。杂交水稻的大面积推广和应用，大幅度地提高了水稻产量。在杂种优势利用中，育种亲本的选择是其成败的重要因素之一。深入分析亲本配合力，合理选配亲本，是筛选强优势杂交组合的一个重要手段。因此，发掘和深入研究水稻配合力相关基因具有重要的理论意义和应用价值。本研究利用以优良恢复系蜀恢527为轮回亲本、籼稻ZDZ057为供体构建的导入系，发现了显著影响水稻产量及相关性状的一般配合力和杂种优势的重要QTL。QTL的产量性状一般配合力和杂种优势效益较大，而且在不同环境条件下表现稳定。利用继续回交获得的近等基因系，将一般配合力基因精细定位于230.5kb的区间。对候选区间进行基因预测发现，该区间共有36个预测基因，其中7个基因已被克隆，14个基因均为假定蛋白，其他15个基因已有报道，接下来，对上述36个基因分别进行功能分析和验证，以找到影响水稻产量一般配合力的关键基因。

关键词：水稻，一般配合力，QTL定位;基因

P141

A tandem array of *ent*-kaurene synthases in maize with roles in gibberellin and more specialized metabolism

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While most commonly associated with its role in gibberellin (GA) phytohormone biosynthesis, *ent*-kaurene also serves as an intermediate in more specialized diterpenoid metabolism, as exemplified by the more than 800 known derived natural products. Among these are the maize kauralexins. However, no *ent*-kaurene synthases (KSs) have been identified from maize. The maize GA-deficient *dwarf-5* (*d5*) mutant has been associated with a loss of KS activity. The relevant genetic lesion has been previously mapped, and was found here to correlate with the location of the KS-like gene *ZmKSL3*. Intriguingly, this forms part of a tandem array with two other terpene synthases (TPSs). Although one of these, *ZmTPS1*, has been previously reported to encode a sesquiterpene synthase, and both ZmTPS1 and that encoded by the third gene, *ZmKSL5*, have lost the N-terminal γ-domain prototypically associated with KS(L)s, all three genes fall within the KS(L) or TPS-e sub-family. Here it is reported that all three genes encode enzymes that are targeted to the plastid in planta, where diterpenoid biosynthesis is initiated, and which all readily catalyze the production of *ent*-kaurene. Consistent with the closer phylogenetic relationship of ZmKSL3 with previously identified KSs from cereals, only transcription of this gene is affected in *d5* plants. On the other hand, the expression of all three of these genes is inducible, suggesting a role in more specialized metabolism, such as that of the kauralexins. Thus, these results clarify not only gibberellin phytohormone, but also diterpenoid phytoalexin biosynthesis in this important cereal crop plant.

Key words: gibberellin, phytoalexin, kaurene, terpene synthase, gene duplication

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The rice *Yellow-Green Leaf 18* locus is essential for chlorophyll synthesis, plant growth and retrograde plastid-nuclear signaling

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Chlorophyll synthesis from glutamyl-tRNA to chlorophyll b needs 15 steps of enzymatic reaction, and all 27 genes encoding these 15 enzymes have been identified in higher plants represented by Arabidopsis (*Arabidopsis thaliana*). However, only nine genes encoding six enzymes in chlorophyll synthesis pathway have been identified in rice (*Oryza sativa*). In this study, a spontaneous mutant, *yellow-green leaf 18* (*ygl18*), was isolated in rice (*Oryza sativa*). This mutant showed yellow-green leaf, decreased chlorophyll level, and clime-dependent growth difference. Map-based cloning of this mutant identified the *YGL18* gene, and this gene has not yet been studied in rice. *YGL18* is expressed in green tissues, especially in leaf, and it functions in chloroplast. Bioinformatic analysis showed that *YGL18* encodes one of the key enzymes of the chlorophyll synthesis pathway. Multiple sequence alignment revealed that the amino-acid substitution of leucine (Leu) to phenylalanine (Phe) in ygl18 is originally conserved in different photosynthesis organisms. In-vitro enzymatic assays identified the YGL18 enzymatic activity, but ygl18 almost lost the enzymatic function. *YGL18* plays important roles in light-dependent and photoperiod-regulated chlorophyll synthesis. Plastid-related nuclear genes were regulated in *ygl18* leaves, and the majority of genes in the tetrapyrrole synthesis pathway were up-regulated, implying that there is a new model on retrograde plastid-nuclear signaling. Based on these findings, it is suggested that *YGL18* plays essential roles in chlorophyll synthesis, plant growth and regulating plastid-nuclear signaling.

Keywords: chlorophyll synthesis, *yellow-green leaf 18*, plant growth, plastid-nuclear signaling

P143

Study on the genetic mechanism for abnormal heading in hybrid rice

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Heading stage is the transitional stage from vegetative growth to reproductive growth stage. The length of heading date is mainly determined by photoperiod sensitivity (PS), temperature sensitivity (TS) and basic vegetative growth (BVG) in rice. We developed a BC_2F_3 population by using the recurrent parent Shuhui 527 and the recipient parent Fuhui 838 in the backcross breeding process, then the BC_2F_3 introgression lines were crossed with four male sterile lines (XieqingzaoA,Gang46A, II -32A and Jin23A), a part of hybrid combinations of which can't flower in some long day areas, i.e Hefei, but obviously late heading in some short day areas i.e Sanya. This phenomenon of abnormal heading was considered as ″big green rice″. A total of 12 QTL for Photoperiod sensitivity was found in more than two environments or populations by using the one-way ANOVA. By two-way ANOVA, a total of 31 pairs of QTL were detected. The results of RiceSNP50 chip in Peking University showed that *se*5, *phyA* and *Osf*l may be upstream photosensitive genes, which causing abnormal heading of those hybrid combinations derived from the crosses between introgression lines and Gang46A. These genes delayed the flowering time by repressing other genes in the flowering pathway such as *Ehd1* and *Hd1*. These results lay a foundation for RNA-seq analysis and SNP genotype analysis to identify the differentially expressed flowering genes and the genetic mechanism of hybrid rice ″big green rice″.

Key words: hybrid rice, heading date, day length, photoperiod sensitive genes

P144

Intragenic recombination between two non-functional *semi-dwarf 1* alleles produced a functional *SD1* allele in a tall recombinant inbred line in rice

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Intragenic recombination is one of the most important source of genetic variability. In our previous study, a tall line RI92 with plant height of about160 cm was observed in the progeny of the cross between two semi-dwarf *indica* cultivars Zhenshan 97 and Minghui 63. Genome-wide genotyping and sequencing indicated that the genome constitution of RI92 was completely from both parents. In order to uncover this tall plant height, a near isogenic F_2 population (BC_3F_2) was developed by three continuous backcrosses between RI92 and Zhenshan 97 as recurrent. Bulk segregant analysis in the BC_3F_2 population revealed that marker RM3523 was linked to the plant height gene, which was in the adjacent region of ˝green revolution gene˝ *semi-dwarf 1* (*sd1*). Sequencing analysis of *sd1* among Zhenshan 97, Minhui 63 and its offspring RI92 revealed that an intragenic recombination was occurred at *sd1* and resulted a functional *SD1* in RI92. The recombination breakpoint was in the region between +1721 and +2575. Paclobutrazol (PBZ) treatment of NILs between *SD1/SD1* and *sd1/sd1* further confirmed *SD1* is the gene contributing to the tall plant height. 4-fold high recombination rate as compared to the genome-wide average was observed in *SD1* located bins in two RIL populations, which indicated that the intragenic recombination at *SD1* was occurred due to recombination hotspot. Genome re-sequencing identifed only 34 recombination bins in RI92, which suggested limited recombinaitons in the process of developing recombinant inbred lines. The intragenic recombination between two parental non-functional *sd1* alleles produced a functional *SD1* in RI92, which led to a tall plant height. These results indicated that intragenic recombination could create new alleles in the progeny distinct from parental alleles and diversify natural variation. Limited recombination bins in RI92 suggested that increasing population size or changing the crossing style (intercross) would be alternatives to enhancing mapping resolution.

Key words: plant height, bulk segregant analysis, *semi-dwarf 1*, functional and non-functional alleles, intragenic recombination

P145

Improving eating and cooking qualities of rice grain by molecular marker-assisted breeding

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Rice (*Oryza sativa* L.), one of the important cereal crops, is the major stable food for more than 50% population in the world. Increasing rice grain yield is a crucial challenge, and improving its eating and cooking quality (ECQ) is also an important breeding goal. Starch is the major storage material in rice seeds, which comprises two main types of glucan homopolymer: amylose and amylopectin. The eating and cooking qualities of rice grain are closely related to the ratio of amylose to amylopectin and the architectural features of amylopectin. For improving the ECQ of cultivated species in Northeast of China, the apparent amylose content (AAC) and Rapid Visco Analyser (RVA) profile of 130 rice varieties were measured; nine rice varieties showed highly starch quality were used as donor candidates, and hybridized with the cultivated specie Longjing26 in Northeast of China expecting ultimately to obtain the superior rice variety. Meanwhile, we hope to find some quantitative trait loci (QTLs) related to the starch quality by constructing Recombinant Inbred Line (RIL). Now, some AAC related QTLs have been found by mapping the Recombinant Inbred Line of RCC147xLongjing26; next, we hope to use those QTLs to improve ECQ of Longjing26

Key words: ECQ, AAC, RIL, QTL

P146

Genome-wide association study dissects the genetic architecture of oil biosynthesis and plant architecture in rapeseed (*Brassica napus* L.)

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Genome-wide association study (GWAS) is a powerful tool for dissect complex agronomic traits in crops species. Rapeseed is one of the most economically important polypoid oil crops in the world. In this study, we re-sequenced 406 diverse rapeseed accessions to construct a haplotype map of the rapeseed genome. Using an Illumina HiSeq 4000, a total of 55.6 billion paired-end reads of 150bp in length were generated, with an average coverage depth of more than 10×for each accession. After mapping against the reference sequences of oilseed cultivar 'Darmor-*bzh*', 28,006,956 single nucleotide polymorphisms (SNPs) were identified. Using these SNPs, genome-wide association studies were performed for mapping traits. We investigate 36 agronomic traits in four environments and examined the major loci of genes related to oil content, fatty acid biosynthesis, seed weight, plant height and primary branch. Many of the candidate genes for oil content encode enzymes involved in oil metabolism, including two significant associations on A8 and C3 of *Brassica napus* which were close to the key gene *Bna. FAE1* for erucic acid content. Some other QTLs were also identified for plant architecture (plant height and primary branch). Our results provide insights into the genetic basis of oil biosynthesis in oilseed and may facilitate marker-based breeding for oil production and yield.

Keywords: *Brassica napus*, GWAS, oil content, plant architecture, yield

P147

A haplotype-based GWAS method gain novel insights of agriculturally complex traits using a maize multi-parent synthetic population

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Maize (*Zea Mays* L.) is one of the most important crops globally for food, feed and fuel. Aiming to efficiently design breeding schemes, the global molecular breeders are struggling to clarify the determinants underlying agriculturally important traits, almost inherited as the 'quantitative traits', whose genetic basis are attributed to many modest-effect loci, epistasis and interaction with environments. To tradeoff between diversity and confounding influence, we here propose a maize synthetic population, tailor-made for genetics and breeding, with firstly thoroughly intercross between 24 maize elite inbred lines (centered by 'HZS' pedigree but genetically diverse) and subsequently sufficiently inbreeding (single-seed decent for over six generations). Up to present, we have collected an unprecedently huge dataset in this maize synthetic population: 1) NGS-based genetic variants (50M SNP, 2.8M InDel and 0.66M SV); 2) multi-environmental phenotypes (from agronomic to yield related traits). The large-scale data for synthetic population endows the popular GWAS, single-marker based linear mixed model, sufficient power for identifying specific loci or known genes. However, frustratingly, only one-half or less heritability allow to be accounted for jointly by all identified loci for any a given trait, the genetic base of complex traits remains ambiguous, also named as 'missing heritability'. Here, we provide a probabilistic estimate of mosaic structure for any a progeny in synthetic population that presumed as a reshuffle across 24 parental genomes, using a combined method of the hidden markov model (HMM) and linkage disequilibrium (LD). We developed a novel statistical methodology, 'haplotype-based GWAS', that tested allelic effect attributed to each parent state rather than the substitute effect between states in each marker, with an assumption that multiple- and independent-causal variants jointly influence trait variations in a given inherited genomic region. The haplotype-based method provides opportunities to further dissect complex traits, and potentially settle the missed heritability that never solved before solely using traditional method.

Key words: *Zea Mays*, multi-parent population, haplotype method, missing heritability

P148

Fingerprinting construction and genetic relationship analysis of blueberry based on EST-SSR markers

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In this study, for the purpose of cultivar identification and apolegamy of excellent hybridization combination to provide basis at the molecular level, EST-SSR markers were employed to analysis on fingerprinting and genetic diversity of 30 blueberry cultivars. Ten of 40 pairs of EST-SSR primers were screened out based on 5 cultivars which was selected randomly. The 10 primer pairs amplified a total of 206 alleles (including 203 polymorphic alleles) among the 30 cultivars, and the ratio of polymorphism was as high as 98.12%. Alleles amplified by each pair of primers ranged from 9 to 48, with a mean of 20.6, and the mean of polymorphism alleles for each pair was 20.3. 30 cultivars were univocally identified using only one EST-SSR primer pair(CA231), which one's alleles was the most and the ratio of polymorphism was 100%. UPGMA cluster analysis of genetic relationship between cultivars showed that all the materials were clustered into one group at the genetic similarity coefficient of 0.52, and 77.7% of the cultivars were still clustered together at the genetic similarity coefficient of 0.63. The results indicated that the genetic basis among blueberry cultivars was narrow. It is necessary to strengthen utilization of wild resources and interspecific resources in the breeding.

Key words: blueberry, EST-SSR, fingerprinting, genetic diversity

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水稻多分蘖矮杆基因 *MTD1* 的分离与克隆

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植株高度和分蘖数是决定水稻农艺性状和影响谷粒产量的关键因素。该研究通过 EMS 诱变处理水稻粳稻品种 Wuyunjing 7 (W7) 获得一个在株高和分蘖数上有显著特点的多分蘖矮杆突变体(*mtd1*)。与野生型相比，*mtd1* 表现出多效性缺陷，包括植株高度变矮、分蘖数目增多、脆性茎节及生育期延迟等，除此之外，每穗粒数、一次支梗和二次支梗数比野生型显著减少。遗传学分析表明 *mtd1* 受一对单隐性核基因控制，采用图位克隆策略，*MTD1* 基因最后被定位在第 9 号染色体短臂上 M6 和 M7 这两个标记之间一段 66kb 的物理区间内，该区段包含 5 个开放阅读框，其中包括已克隆的脆秆基因 *(BC12)*，测序结果显示 *MTD1* 基因与 *BC12* 等位，只是突变位点不同，*mtd1* 突变体中仅仅只有一个基因发生单碱基 G 到 A 的突变，从而导致翻译的提前终止。过量表达 *MTD1/BC12* 能恢复野生型的表型，说明 *mtd1* 的突变是由于 BC12 基因突变导致的。在表型上，分蘖数增多是本研究的一个新颖之处，水稻中与分蘖相关基因的 qPCR 分析结果同样也支持 *mtd1* 突变体的多分蘖表型；GA 处理实验表明该突变体是一个与 GA 合成有关的敏感型突变体，α-淀粉酶活性测定结果进一步证明并非 GA 信号传导途径导致植株矮化。这些结果都有利于大家深入理解控制水稻矮秆、分蘖性状的分子机制。

关键词：水稻，矮秆，多分蘖矮秆突变体*mtd1*，基因克隆，遗传分析

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姜花香气物质生物合成关键基因鉴定和功能分析

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花香是观赏植物重要的审美和商品性状之一，对植物本身而言，花香可作为信号物质来吸引授粉者以及趋避病原菌和食草昆虫对花器官的危害，从而提高作物的产量和品质。姜花是姜科姜花属多年生草本植物，具有洁白的颜色、美丽的花型和迷人的芳香，是备受人们喜爱的香型切花和园林应用花卉。姜花盛开时释放大量的挥发性香气物质，主要为沉香醇、(*E*)-β-罗勒烯、(*E*,*E*)-α-金合欢烯等萜类物质和苯甲酸甲酯等苯基/苯丙烷类物质。然而，上述物质的生物合成和代谢调控机理仍不明确。本研究利用 Illumina 测序平台，首次获得了姜花的转录组信息，包含 65,591 条 unigene 序列，通过生物信息学分析，我们分别挖掘出 35 和 33 个萜类和苯丙烷类香气物质生物合成的候选基因。利用 RNA-Seq 技术分析了姜花花发育过程中的基因表达规律，结合姜花香气物质的变化规律，初步筛选出香气物质合成和代谢调控的关键基因，包括参与萜类香气物质合成的 *HcTPS3/5/8/10* 以及参与苯甲酸甲酯生物合成的 *HcBSMT1/2*。体外功能分析和亚细胞定位试验表明，HcTPS3/10 分别为(*E*)-β-罗勒烯合成酶和(*E*,*E*)-α-金合欢烯合成酶，HcTPS5/8 为沉香醇合成酶。体外和体内功能分析表明，HcBSMT1/2 均可催化苯甲酸生成苯甲酸甲酯，催化水杨酸生成水杨酸甲酯，而 HcBSMT1 催化苯甲酸的 *K*m 值是 HcBSMT2 的 16.6 倍，说明 HcBSMT2 在苯甲酸甲酯合成中起主要作用。荧光定量 PCR 分析表明，以上基因均为花部位特异表达基因，基因表达均受到花发育的调控，且表达规律与相应香气物质的释放规律呈显著正相关。本研究为姜花属植物花香性状改良以及利用基因工程手段培育优质香型花卉新品种奠定了基础。

关键词：花香，姜花，RNA-Seq，生物合成，代谢调控

P151

Decipherment of major loci controlling 20 agronomic traits by a genome-wide association study in tomato recombinant inbred lines

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During the domestication and improvement in the past centuries, tomato morphology has greatly changed along with a dramatic increase in fruit weight. To understand the genetic basis that directed the breeding of elite varieties in the past and guides for future practice of tomato improvement, we carried out a genome-wide association study of 20 agronomic traits using a recombinant inbred lines derived from the wild and cultivated tomatoes. In the total of 338 significant loci identified, ~70% are newly reported, which explain ~49% of the phenotypic variances in average, and a high quality local *de novo* assembly further pinpointed responsible mutations at single gene resolution. Using near isogenic lines for two loci, we proved our association analysis reliable and accurate. These findings allow us to construct a trait-locus network that largely explains the correlation among different traits, which will facilitate the identification and isolation of key genes governing these important agronomic traits and provide a powerful tool for genetic improvement of high-yield and superior-quality of new elite tomatoes.

Key words: tomato, GWAS, agronomic traits, recombinant inbred lines (RIL), trait-locus network

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滇龙胆环烯醚萜氧化酶基因和香叶醇 10-羟化酶基因启动子的克隆和功能预测

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滇龙胆（*Gentiana rigescens*）为云南道地药材，其主要药效成分为龙胆苦苷等环烯醚萜类化合物，其在叶和茎等绿色器官中合成，在根中累积。但目前存在的问题，一是滇龙胆野生资源由于人为连年采挖，导致野生资源濒危；二是现有的滇龙胆品种产量低、品质不高、抗病性较差、适宜种植范围较小。2013年6月，云南省启动了龙胆草航天育种工程，目标是选育高产、优质、高抗、适宜机械化生产和不同海拔种植的龙胆草品种，且使龙胆草干燥根龙胆苦苷含量达10%以上。现代分子生物学手段为植物育种提供了新的思路和手段。通过研究关键酶基因及其启动子，能够为新品种选育及通过生物技术手段生产主要药效成分奠定基础。

环烯醚萜氧化酶（GrIDO）和香叶醇10-羟化酶（GrG10H），是龙胆苦苷生物合成途径中的关键酶，对环烯醚萜类的生物合成其重要作用。本课题组通过RT-PCR技术分别克隆到*GrIDO*基因和*GrG10H*基因，通过genome walking技术克隆到它们的启动子。*GrIDO*基因启动子长720 bp，通过PlantCARE网站对该序列进行分析，结果表明，该启动子主要包含4个与光响应有关的元件（2个I-box、1个CATT-motif和1个chs-CMA2a）、2个MeJA响应元件（CGTCA-motif和TGACG-motif）、1个ABA响应元件（ABRE）、1个热胁迫响应元件（HSE）、1个MYB结合位点（MYB Binding Site）和一个防御胁迫响应元件（TC-rich repeat），这表明滇龙胆*GrIDO*基因的表达，不仅受光、MeJA、ABA、热刺激的诱导调控，同时还与胁迫反应及MYB转录因子有关。*GrG10H*基因启动子长820 bp，包含3个MeJA响应元件（TGACG-motif CGTCA-motif CGTCA-motif）、1个生长素响应元件（TGA-element）、水杨酸响应元件（TCA-element）、1个防御胁迫元件（LTR）、MYB结合光响应位点（MRE）、4个光响应元件（I-BOX，G-BOX、ATCT-motif、Box 4），表明该基因不仅受MeJA、水杨酸、光、生长素的调控，而且还与防御胁迫相关。下一步，将进行通过报告基因验证启动子功能，通过酶活分析基因和基因表达分析验证基因功能。

关键词：滇龙胆，环烯醚萜氧化酶基因，香叶醇10-羟化酶基因，启动子，序列分析

P153

Combining the high-throughput maize phenotyping and quantitative trait loci analysis to reveal the dynamic genetic architecture of maize plant growth

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With the increasing demands in crop breeding for novel traits, plant research community faces to quantitatively analyze the structure and function of large numbers of plants. A clear goal of high-throughput phenotyping is to bridge the gap between genomics and phenomics. In this study, we obtained 106 traits from a maize recombinant inbred line population across 16 development stages using the automatic phenotyping platform.The results showed that the exponential model had better predication ability for biomass accumulation, even in the early growth stage. QTL mapping with high density genetic linkage map was used to uncover the genetic basis of these complex agronomic traits, and have identified 2265 QTLs for all investigated traits. We also conduct the traits-loci network analysis and 3 house-keeping loci were detected in the hub points for 2 traits. These results reveal the dynamic genetic architecture of maize plant growth thus enhance maize ideotype breeding in the near future.

Key words: maize, high-throughput phenotyping, dynamic genetic architecture, QTL mapping

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Genetic analysis of rice grain shape mutants and molecular cloning of rice *SLENDER GRAIN 1* gene

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Rice grain shape, which is closely related to seed development and is determined by multiple factors, is an important trait affects both production and its economic value. In order to investigate the molecular mechanism controlling grain shape, a mutant population with *japonica* rice Zhonghua11 background was generated by EMS (ethyl methane sulfonate) mutagenesis. Various mutants with alternation of grain shape were identified and *slender grain 1* (*slgr1*), a mutant with significant increase of grain length was selected for further analysis. The grain length of *slgr1* is ~18% longer than that of wild-type, but significant alternation was found in neither width nor thickness of grain. Genetic analysis revealed that the increased grain length was controlled by a single recessive gene, which was further mapped by MutMap method. Five candidate genes with amino acids change of SLGR1 were identified and will be further analyzed. Detailed results will be presented.

Key words: rice, grain length, EMS mutation, MutMap

P155

Target design breeding of improved KY131 with introgression of rice blast resistance genes through genome editing

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Rice blast caused by the fungus *Magnaporthe grisea*, one of the main diseases in rice, has serious influence on rice production and food security. Maker-assisted selection (MAS) approach is a quite effective strategy for genetic improvement of resistance in rice. In this study, we obtain two improved lines of Kongyu 131, a leading japonica cultivar, introgressed with rice blast resistance genes, *Pi1* and *Pi2*, respectively (from Yuqing He's lab). In previous work, Kongyu131 with *Pi1* and *Pi2* showed greatly improvement of rice blast resistance. However, the improved lines of Kongyu 131 headed later than the wild-type Kongyu 131. Because *Pi2* is closely linked to the heading date gene *Hd1*, a large segregation population is of great necessity in order to restore the natural heading date of the improved version of Kongyu 131 by recombination mediated elimination of *Hd1*. Background analysis with the Rice60K SNP chip indicated that Kongyu 131 with *Pi2*, from the large segregation population, which had the same flowering time with Kongyu 131 is without *Hd1*. Meanwhile, Kongyu 131 with *Pi1* still had several donor chromosome segments, which may contain the unknown heading date loci resulting in the later heading phenotype. Heading date is a critical factor in rice adaption, and it can also affect the rice yield. So, in this study, we conduct target design breeding of Kongyu 131 with *Pi1* by gene editing. We re-sequenced the major flowering genes in the improved line of Kongyu 131 with *Pi1*. The results showed that all the major flowering inhibitor genes re-sequenced are without function but for gene *X*. Therefore, we designed CRISPR/Cas9 vector based on gene *X* to induce gene *x* knock out mutants. In the generation T0, we obtained more than 30 individual plants, of which 8 random plants were used for mutants screen. Six out of 8 transgenic positive plants were identified via PCR method based on *Cas9* sequence, and all were mutants including homozygous, biallelic, and chimeric mutants through TA clone sequencing. In 2016, field survey is ongoing in Wuhan (Hubei Prov.) and Harbin (Heilongjiang Prov.). To sum up, we aim at targeting design breeding of Kongyu 131 via MAS and gene editing.

Key words: rice blast, heading date, target design breeding, gene editing