植物、微生物及分子遗传组
论文摘要

杨树抗锈病基因研究

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摘要

森林为人类提供木材、保护人类的生存环境。随着工业的发展，人类对于生物能源的需求增加，杨树人工林具有生长快速的优点能满足人类对于可再生能源的需要。杨树作为林木基因组研究的模式树种在林业生产中也占据着极其重要的位置。杨树锈病是影响杨树栽培与生产的最严重病害之一，近年来杨树锈病在推广美洲黑杨品种中呈爆发趋势，严重影响了杨树的生长。但是危害南方型美洲黑杨的锈菌的研究尚属空白，本研究中对长江中下游地区湖北武汉、湖北石首、江苏南京、江苏泗洪采集的锈菌通过光学显微镜和扫描电镜进行观察，结果表明长江中下游地区的锈菌在形态上和*Melampsora larici-populina*相似。运用不同锈菌的特异引物，进行PCR扩增电泳，在分子水平上证实长江中下游地区的锈菌都属于*Melampsora larici-populina*。本研究中对四个地点种植的88个基因型美洲黑杨锈病病害进行调查，鉴定出9个基因型可能是抗病的。这些结果为培育美洲黑杨抗锈病新品种提供了必要的信息。

Melampsora larici-populina在杨树叶片上夏孢子阶段是侵染杨树导致杨树感染锈病的重要阶段。研究中选取了自然侵染状态下的不同阶段，通过扫描电镜和透射电镜观察目的在于重新构建夏孢子的侵染过程。在侵染的最初阶段，表面具有小刺的夏孢子产生芽孢，相当数量的芽孢直接融合到叶片中，可以观察到叶片角质层的损坏，这表明菌丝的直接侵染。气孔侵染也时常发生，有时一个气孔可以被一个以上的芽管侵染。*M. larici-populina*在侵染杨树叶片的过程中没有形成附着胞，这可能是*M. larici-populina*侵染过程中与其他锈菌侵染过程中的差别。芽管很少有分枝，没有证据显示气孔诱导芽管形成分枝，以及影响分枝的方向。当锈菌夏孢子营养成分耗尽时，夏孢子崩溃，最后变得干瘪。在侵染的最后阶段，锈菌孢子在叶片表面喷发，在叶片表面形成橘黄色锈斑。本研究深入观察了*M. larici-populina*在美洲黑杨叶片上的侵染行为。

前期的研究结果表明抗杨树锈病基因位点*MXC3*位于杨树第四染色体末端。本研究对第四染色体末端*MXC3*所在区域进行分析表明只有一个抗病相关基因。利用RACE技术获得了*MXC3*的全长基因序列，*MXC3*基因cDNA全长2275bp，包括编码653个氨基酸的开放阅读框序列。*MXC3*是在质膜内表达的疏水蛋白。该基因含有两个主要保守域，分别属于奇异果甜蛋白家族和丝氨酸/苏氨酸蛋白激酶。这两个保守域都具有抗病特性。抗病基因型和感病基因型杨树中该基因的序列相似性为94.1%，两个基因的结构也相似，但是在核心区域有区别。氨基酸序列分析表明两个基因在260-280氨基酸位置有较大差别，推定这个区间氨基酸变异是造成两个蛋白质不一样的原因。

水稻叶绿体发育和幼苗生长相关基因*ASL4*的克隆与功能研究

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摘要

叶绿体是光合作用以及多种代谢产物合成和积累的主要场所，叶绿体发育受阻将影响植物体的生长，叶绿体发育由核基因和质体基因共同调控，相关基因突变会造成叶绿体发育受阻，影响光合作用和植株生长。叶绿体核糖体主要负责叶绿体中质体编码蛋白的转录和翻译，已有的研究表明叶绿体核糖体蛋白在核糖体形成、质体蛋白合成、叶绿体分化和逆境响应等过程中行使重要的功能。高等植物中叶绿体核糖体蛋白多数已被鉴定，然而关于叶绿体核糖体蛋白如何调控植株发育等分子机理仍不够清楚。本研究通过MNU化学诱变鉴定一个叶绿体发育受阻突变体*asl4*，该突变体在苗期四叶期之前表现出明显的白化表型，四叶期之后逐渐萎缩死亡；色素含量分析发现突变体中几乎不能合成叶绿体色素；透射电镜观察显示突变体叶片中叶绿体数量极少，且结构异常，不能发育成正常的叶绿体。精细定位将该基因定位于水稻第3染色体短臂50kb区间内，基因组测序发现编码质体核糖体小亚基蛋白S1的基因存在2855bp的序列缺失；转基因互补实验证实*OsASL4*基因为控制突变表型的目的基因。表达分析显示*ASL4*基因在叶绿体发育第二和第三阶段高度积累，暗示该基因参与调控叶绿体发育；叶绿素合成和光合作用相关基因在突变体中表达受到明显抑制。同时定量PCR分析发现NEP依赖的持家基因在突变体中表达明显高于野生型，而PEP依赖的光合基因在突变体中受到严重抑制，说明*ASL4*基因突变可能降低PEP的活性，进一步抑制质体转录。

关键词

水稻，生长发育，基因克隆，叶绿体核糖体，分子机制

The Regulatory Network of Secondary Cell Wall Cellulose Bisynthesis in Rice

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Abstract

As the one of the most important staple food crops, rice produces huge agronomic biomass residues, which contain lots of secondary cell walls (SCWs) consisting of cellulose, hemicelluloses and lignin. The underlying transcriptional regulation mechanism of SCWs biosynthesis remains elusive. In this study, we isolated a novel NAC family transcription factor (TF), OsSND2 using yeast one-hybrid screening using the secondary wall NAC-binding element (SNBE) on the promoter region of *OsMYB61* as bait. We used an electrophoretic mobility shift assay (EMSA) and chromatin immunoprecipitation analysis (ChIP) to further confirm that OsSND2 can directly bind to the promoter of *OsMYB61* both *in vitro* and *in vivo*. OsSND2, a close homolog of AtSND2, was localized in the nucleus with transcriptional activation activity. Expression pattern analysis indicates that *OsSND2* mainly expressed in internodes and panicles. Overexpression of OsSND2 resulted in rolled leaf, increased cellulose content and up-regulated the expression of SCWs related genes. The knockout of OsSND2 using CRISPR/Cas9 system decreased cellulose content and down-regulated the expression of SCWs related genes. Furthermore, OsSND2 can also directly bind to the promoters of other MYB family TFs by transactivation analysis in yeast cells and rice protoplasts. Altogether, our findings suggested that OsSND2 may function as a master regulator to mediate SCWs biosynthesis. Therefore, study the function of OsSND2 can provide a strategy for manipulating plant biomass production.

Keywords

Secondary Cell Wall (SCW), Rice, Cellulose Synthesis, Transcription Factor (TF), NAC, MYB

Identification and Analysis of Adenine N6-Methylation Sites in the Rice Genome

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Abstract

DNA N6-methyladenine (6mA) is a noncanonical DNA modification present at low levels in different eukaryotes1-8, but its prevalence and genomic function in higher plants is unclear. Using mass spectrometry and immunoprecipitation and validation with analysis of single-molecule real-time sequencing, we observed that about 0.2% of all adenines are 6mA-methylated in the rice genome. 6mA occurs most frequently at GAGG motifs and is mapped to about 20% of genes and 14% of transposable elements (TEs). In promoters, 6mA marks silent genes, but in bodies correlates with gene activity. 6mA overlaps with 5-methylated cytosine (5mC) at CG sites in gene bodies and is complementary to 5mC at CHH sites in TEs. We show that OsALKBH1 may be potentially involved in 6mA demethylation in rice. The results suggest that 6mA is complementary to 5mC as an epigenomic mark in rice and reinforces a distinct role for 6mA as a gene-expression associated epigenomic mark in eukaryotes.

Keywords

N6-methyladenine, Epigenome, Rice

莲(*N. nucifera* Gaertn.)基因组研究进展

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摘要

莲(*Nelumbo nucifera* Gaertn.)是我国最重要的一种水生蔬菜作物，有食用、药用和观赏价值，也具园林造景和水体净化等环境生态价值；还是幸存的活化石、基部真双子叶植物。迄今，缺乏莲高密度遗传图谱、也缺乏物理图谱。为此，本研究完成了“中国莲基因组全序列测序”并对中国莲品系泰国“清迈莲”进行重测序，快速开发出大量的SSR、SNP等分子标记。在此基础上，进行了以下工作：

(1) 以“中国太子莲”和“泰国清迈野莲”为亲本，构建了F_2代分离群体，通过对181株F_2代植株进行RAD-Seq简化基因组测序，共计鉴定出217,577个高质量的多态性SNP。根据SNP的分型结果，将其合并得到了2,371个bin标记，并最终构建得到了包含217,577个SNP标记，分为8个连锁群，总图距为789.54cM的高密度遗传连锁图。通过改变作图算法、加入198个多态性SSR标记并与其物理位置比较、以及与已有莲连锁图进行比较等方式对遗传图谱的准确性进行了验证，其结果都与原连锁图的标记顺序高度一致，表明所构建的连锁图具有较高的稳定性和准确性。

(2) 利用BioNano光学图谱技术对“中国太子莲”基因组中的酶切位点进行检测组装，最终得到了总长为645.12Mb的莲的第一张光学图谱，并将最初的“中国太子莲”基因组草图的ScaffoldN50提升了1.5倍。通过整合多个图谱信息将“中国太子莲”中97.9%的序列以及“中国古莲”中97.6%的序列锚定到8个连锁群（染色体）上，将莲基因组组装到染色体水平上，还鉴定了假染色体上的着丝粒区。

(3) 同时，完成了莲叶绿体基因组和莲线粒体基因组物理图的构建。

以上工作不仅对莲的遗传育种、生产、药物资源开发意义重大，而且有助于了解单子叶植物与双子叶植物的进化关系以及莲的起源和分类地位，以巩固我国莲研究领域在世界上的优势地位。

关键词

莲(*Nelumbo nucifera* Gaertn.)，遗传图谱，BioNano光学图谱，叶绿体基因组，线粒体基因组

Broadening the Targeting Range of CRISPR-Cpf1 Genome Editing in Rice by Modifying PAM Recognition

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Abstract

Clustered regularly interspaced short palindromic repeats (CRISPR)-Cpf1 is a newly developed genome editing tool for eukaryotic cells, including plant cells. During DNA cleavage, the commonly used *Acidaminococcus* sp. *BV3L6* Cpf1 (*As*Cpf1) and *Lachnospiraceae bacterium ND2006* Cpf1 (*Lb*Cpf1) require a TTTV protospacer adjacent motif (PAM) at their target sites, limiting the targeting range of these proteins in the genome. Engineering *As*Cpf1 by introducing PAM-interaction mutations can relax PAM recognition specificity in human cells. However, the limitations of *As*Cpf1 remain unresolved in plants. In addition, unlike in animal systems, plant genome editing may prefer variants of *Lb*Cpf1, as *Lb*Cpf1 exhibits much higher editing efficiency than does *As*Cpf1 *in planta*. In this study, we generated variants of rice codon-optimized *As*Cpf1 and *Lb*Cpf1. The *Lb*Cpf1 variants that contain the mutations G532R/K595R and G532R/K538V/Y542R efficiently induced targeted mutagenesis at the sites with non-canonical TYCV/CCCC and TATV PAMs, respectively. These variants increased the *Lb*Cpf1 targeting range in the rice genome by nearly twofold, which greatly enhances the application of the CRISPR-Cpf1 system in plant research and crop breeding.

Keywords

Cpf1, PAM, Variant, Genome Editing, Rice

Identification and Functional Analyses of the Relative Genes of Proanthocyanidin Precursors Transport in Brown Cotton (Gossypium Hirsutum)

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Abstract

Proanthocyanidins belong to the flavonoids, and are the main components of the fiber pigments in brown cotton. Proanthocyanidins of brown cotton fibers are mainly synthesized in the cytoplasm by phenylpropane metabolism pathway, and are transported to the vacuoles through transporters or vesicles trafficking and accumulated in the vacuoles, and are finally polymerized and oxidized. Although it is relatively clear about the biosynthesis and regulation of proanthocyanidins in brown cotton, the molecular mechanism of proanthocyanidins precursors transport is still poorly understood. Identifying the transporter genes of proanthocyanidins and analyzing theirs functions are the key questions for elucidating the molecular mechanism of proanthocyanidins transmembrane transport. The project groups have early identified the related transporter GhTT19, GhTT12 and GhTT13 from brown cotton by homologous gene cloning technology. In view of this, the genetic transformation systems of Arabidopsis and cotton were utilized to verify the transport functions; Enzyme kinetic analyses and yeast in vitro experiments were used to determine the transport substrates and modes; The genes specific expressions, subcellular localizations and accumulations of proanthocyanidins in transgenic plants were analysed to explain the mechanism that the proanthocyanidins precursors are transported to the vacuoles by the transporters, and to reveal biological essence from the molecular level that the fiber color is unstable and the pigment distribution is uneven in brown cotton. These experimental results provide a theoretical basis for the improvement of the color and quality of brown cotton fibers.

Keywords

Brown Cotton, Proanthocyanidin, Transport, Bioinformatics, Genetics Transformation

不同棉花原花青素含量及合成相关基因表达分析

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摘要

原花青素（Proanthocyanidins，PAs）是一种由不同结构的儿茶素（Catechin）或表儿茶素(Epicatechin)聚合而成的类黄酮物质，广泛存在于植物中，在抗氧化和抗衰老等过程中具有重要的生物学功能。本文主要采用高效液相色谱（HPLC）测定不同棉花品种的不同组织部位以及不同发育时期纤维中原花青素含量的变化。不同棉花品种根、茎、叶、花瓣、花萼和纤维中原花青素含量测定的结果表明，叶和花萼中含量最高，其次是茎，而花瓣和根中含量较少，而不同品棉花种在相同组织部位中原花青素的含量存在显著差异，其中四倍体陆地棉TM-1含量最高，最低为亚洲棉。C18色谱柱（4.6mm×150mm，5μm），甲醇(A)-乙腈(B)为流动相进行梯度洗脱，电喷雾离子源为质谱离子源,获得不同化合物的质谱数据。结果根据高分辨质谱结果和MS/MS碎片信息,结合对照品质谱信息及相关文献,共鉴定推断原花青素前体物质的结构。选取16个类黄酮合成关键基因进行qRT-PCR表达分析，结果显示这些基因在棉花各个组织部位中均有不同程度表达，其中GHAHA5、GHAHA7、GHAHA9、GHAHA10、GHAHA11表达水平与整个棉花生长周期的原花青素含量基本保持一致，推测这些基因参与原花青素的生物合成。另外，GHTT3、GHTT18、GHBAN和GHTT12也在原花青素的合成途径中起着重要作用，其表达能够促进PA和其他类黄酮物质含量的增加。这些研究结果为进一步解析棉花原花青素合成与积累的分子机制提供理论依据。

关键词

原花青素，前体，组织器官，含量，RT-PCR

WOX11 Recruits a Histone H3K27me3 Demethylase to Promote Gene Expression During Shoot Development in Rice

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Abstract

WUSCHEL-related homeobox (WOX) genes are key regulators of meristem activity and plant development, the chromatin mechanism of which to reprogram gene expression remains unclear. H3K27me3 is a chromatin mark of developmentally repressed genes. How the repressive mark is removed from specific genes during plant development is largely unknown. Here we show that WOX11 interacts with the H3K27me3 demethylase JMJ705 to activate gene expression during shoot development in rice. Genetic analysis indicates that WOX11 and JMJ705 cooperatively control shoot growth and commonly regulate the expression of a set of genes involved in SAM identity, chloroplast biogenesis, and energy metabolism in the shoot apex. Loss of WOX11 led to increased levels of H3K27me3 and overexpression of JMJ705 decreased the levels at a subset of common targets. JMJ705 is associated with most of the WOX11-binding sites found in the tested common targets in vivo, regardless of presence or absence of the JMJ705-binding motif. Furthermore, wox11 mutation reduced JMJ705-binding to the tested common targets. The results suggest that recruitment of JMJ705 to genes of specific developmental pathways is promoted by DNA-binding transcription factors and that WOX11 functions to stimulate rice shoot growth through epigenetic reprogramming of genes involved in meristem development and energy generating pathways.

Keywords

WOX11, Histone Demethylase, H3K27me3, Shoot Development, Gene Expression

浓香型白酒窖泥功能微生物挖掘

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摘要

浓香型白酒是中国白酒的一个重要品种。它由谷物在窖池中经过复杂发酵而成。窖泥中含有大量微生物，对白酒产量和品质具有非常重要的影响。然而，窖泥中微生物群落结构极其复杂，人们对窖泥功能微生物的认识尚不全面深入。对它们的研究有利于及时监测、维护和改善窖泥品质。

本课题通过对优质和劣质窖泥进行HiSeq测序，比较分析了它们的微生物组成，发现两种与窖泥品质相关的微生物*C. kluyveri*和*S. wolfei*，对它们的检测可以快速判断酿酒窖泥品质。

在此基础上，从窖泥中分离到3株高产己酸菌，鉴定其均为*C.kluyveri*。将其中一株产己酸量达到4.36g/L的菌株命名为*C.kluyveri* JZZ。

培养己酸菌的常用培养基EAM (Ethanol Acetic acid-Na Medium) 含有$CaCO_3$，不利于科学研究且易导致窖泥板结退化。本课题在EAM培养配方基础上，不加$CaCO_3$，调整pH、乙醇添加量、培养温度，得到培养配方NCEAM (None-$CaCO_3$ EAM)。与优化的EAM培养配方相比，该配方在不显著影响己酸菌生长的情况下，使己酸产量提高16.24%，达5.08 g/L。

鉴于分离自窖泥的*C. kluyveri*基因组序列未见报道，本项目首次对窖泥来源的*C.kluyveri* JZZ进行基因组测序。JZZ基因组包含一条4454353 bp的环形染色体和一条58581 bp的环形质粒；共有4158个编码蛋白；具有完整的利用乙醇合成己酸的代谢通路。与另外2株*C. kluyveri*相比，JZZ基因组较长；有大量基因重排；具有更多的防御机制相关基因和DNA复制、重组、修复相关基因，较少的次级代谢产物合成、转录和代谢相关基因；另外，JZZ具有960个独有基因，且独有基因中防御机制和转录相关基因明显聚集。JZZ基因组成对其在窖泥环境中生存和发挥生物学功能可能具有重要意义。

将*C. kluyveri* JZZ应用于人工窖泥制作和酿酒生产，出酒率、优质率分别为40.95%、22.61%，实现了人工窖泥快速成熟及当年用于生产。

关键词

浓香型白酒，窖泥，*Clostridium kluyveri*，培养条件，基因组，人工窖泥