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前沿资讯

1．华中农业大学油菜团队揭示代谢物转运蛋白BnaPPT1和BnaBASS2影响油菜籽粒油脂积累

简介：近日，我校油菜团队在Journal of Advanced Research和Plant Biotechnology Journal分别发表题为“BnaPPT1 is essential for chloroplast development and seed oil accumulation in Brassica napus”和“Pyruvate transporter BnaBASS2 impacts seed oil accumulation in Brassica napus”的研究论文。研究结果表明磷酸烯醇式丙酮酸转运蛋白BnaPPT1和丙酮酸转运蛋白BnaBASS2在油菜籽粒油脂积累方面起着重要作用。植物细胞内，磷酸烯醇式丙酮酸盐/磷酸盐转运蛋白（PPT）和胆汁酸-钠转运蛋白（BASS2）分别将糖酵解途径代谢物磷酸烯醇式丙酮酸和丙酮酸从细胞质转运到质体，从而满足质体中的脂肪酸合成和其他代谢活动。植物中，PPT和BASS2对质体中脂肪酸合成以及种子中油脂积累的影响尚不清楚。发表在Journal of Advanced Research的研究以甘蓝型油菜为研究对象，发现了叶绿体膜定位的BnaPPT1在叶片膜脂合成和叶绿体发育中发挥着重要作用，并影响叶片光合作用。研究结果表明，将BnaPPT1敲除后，油菜生长缓慢，叶片呈淡黄色。叶片色素测定和叶绿体超微结构观察显示，突变体中叶绿素及叶绿素前体含量均显著降低，叶绿体的基粒片层变薄，形成更多更小的叶绿体和淀粉粒。基于液相色谱串联质谱法（LC-MS/MS）的脂质代谢物分析显示，突变体中叶绿体膜脂主要成分MGDG、DGDG和PG含量分别降低67.8%、68.5%和16.4%。此外，BnaPPT1影响了油菜种子的糖酵解途径和脂质代谢途径，在种子油脂合成中发挥着重要作用。BnaPPT1突变体种子含油量降低了2.2 ~ 9.1%，而过表达BnaPPT1显著提高了种子含油量2.1 ~ 3.3%。发表在Plant Biotechnology Journal的研究创建了BnaA05.BASS2的过表达油菜材料，通过基因编辑创建了BnaA05.BASS2 and BnaC04.BASS2-1的油菜双突变体。油菜BASS2双突变体的株高、单株角果数、粒重和单株产量等均显著下降，而过表达植株的主要农艺性状基本没有受到影响。连续3年对含油量表型分析表明，突变体的籽粒含油量降低了2.8 ~ 5.0%，而过表达BnaA05.BASS2显著提高了籽粒含油量1.4 ~ 3.4%。BnaBASS2过表达显著提高了参与油脂合成和油体形成的多个基因的表达水平，促进了籽粒中的油脂积累。基于液相色谱串联质谱法的代谢组分析表明敲除BnaA05.BASS2 and BnaC04.BASS2-1可以显著降低叶绿体中丙酮酸盐的含量，证明了BnaBASS2负责向质体转运丙酮酸盐。同时，BnaBASS2可以显著影响油菜种子叶绿体中糖酵解、脂肪酸合成和能量相关代谢产物的含量。综上所述，BnaPPT1和BnaBASS2在质体代谢物转运方面发挥着重要的作用，可以显著影响油菜籽粒中的油脂积累。我校油菜遗传改良团队的唐珊博士为两篇论文的第一作者，郭亮教授为通讯作者，鲁少平副研究员和姚璇副教授也参与了该研究的指导。两项研究得到了国家自然科学基金、湖北洪山实验室重大项目、华中农业大学-中国农业科学院深圳农业基因组研究所联合项目、中国博士后科学基金和湖北省博士后创新岗位资助。

来源：植物科学技术学院

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全文链接:

http://news.hzau.edu.cn/2022/0906/64396.shtml

2．基因组所研究揭示水稻AGO1d调控温敏雄性不育的作用机制

简介：近日，基因组所费启立课题组联合上海交通大学生命科学技术学院梁婉琪课题组和美国唐纳德·丹弗斯植物科学中心的Blake Meyers团队在《新植物学家（New Phytologist）》在线发表了题为"Temperature-sensitive male sterility in rice determined by the roles of AGO1d in reproductive phasiRNA biogenesis and function "的研究论文，报道了水稻AGO1d通过介导生殖特异的21-和24-nt phasiRNAs的生成和功能调控温敏雄性不育的作用机制。水稻是我国重要粮食作物，解析其雄性不育机制促进两系和三系法杂交育种，对于保障粮食产量具有重大意义。phasiRNAs是植物中一类特殊的siRNA，它的生成依赖于22-nt miRNA/AGO蛋白沉默复合体（RISC）切割长非编码转录本（如 TAS 和 PHAS ）或者NB-LRR等编码基因转录本所触发，随后RNA依赖的RNA聚合酶6（RDR6）将切割靶点下游的片段合成双链RNA，再分别由DCL4和DCL5连续相位切割产生21-和24-nt的次级siRNAs（phased secondary siRNAs, phasiRNAs）。在禾本科植物花药发育过程中， PHAS 位点大量时空特异性转录非编码RNAs，并由22-nt miR2118和miR2275分别触发21-和24-nt phasiRNAs的生成。近些年的研究发现这两类生殖特异的phasiRNAs在雄性生殖发育中发挥重要作用，其功能缺失会导致水稻和玉米雄性不育。然而，在phasiRNAs生成过程中，哪一类特定AGO蛋白加载了miR2118和miR2275以介导phasiRNAs生成，以及21-nt phasiRNAs是否与MEL1（AGO5c）以外的AGO蛋白结合，尚不清楚。 该研究发现 AGO1d 在小孢子母细胞减数分裂前和减数分裂期间花药四层壁细胞中特异性积累。通过CRISPR/Cas9系统编辑 AGO1d 基因，发现 AGO1d 敲除导致低温环境下花药发育异常、花粉不育，而在常温环境下花药发育正常、花粉可育，即表现出温敏雄性不育表型。进一步通过花药半薄切片发现低温环境下ago1d 敲除株系花药在小孢子发育后期（S10）绒毡层细胞程序性死亡异常，花粉形成过程中淀粉积累减少，最终导致花粉不育，水稻结实率降低。为探究其作用机制，作者通过水稻花药的AGO1d RIP-seq、小RNA-seq和转录组测序，发现AGO1d能够直接与miR2118和miR2275结合，且 ago1d 敲除花药中21-和24-nt phasiRNAs含量显著下降。同时，RIP-seq结果显示AGO1d可与5‘-末端尿苷（5‘-U）的21-nt phasiRNAs结合，而先前研究发现MEL1可与5‘-末端胞苷（5‘-C）的21-nt phasiRNAs结合，这表明AGO1d不同于MEL1，可以在phasiRNAs的生成和功能中具有双重作用。对转录组数据的分析表明，水稻花药发育时期关键调控通路的失调，如转录调控、糖代谢以及脂代谢的失调，可能导致了低温下 ago1d 敲除株系的不育性。通过多组学和代谢分析，发现AGO1d可通过介导21-nt phasiRNAs生成和功能在糖酵解途径中负调控D-果糖1,6-二磷酸的合成，证实了phasiRNAs在调节糖代谢中的功能。该研究对于水稻等作物两系杂交育种具有重要指导意义。基因组所费启立研究员、上海交通大学生命科学技术学院梁婉琪研究员和美国唐纳德·丹弗斯植物科学中心Blake Meyers教授为本论文通讯作者，费启立课题组博士后施传琳和梁婉琪课题组博士后张杰为本论文共同第一作者，费启立课题组博士后武炳瑾和助理余昌秀以及Blake Meyers课题组博士Rachel Jouni也参与了此项研究。本研究得到了国家自然科学基金、广东省基础与应用基础研究基金和深圳市大鹏新区科技创新和产业发展专项资金的支持。 原文链接：https://doi.org/10.1111/nph.18446

来源：基因组所

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全文链接:

http://www.agis.org.cn/xwzx/kyjz/fc903f2d9a9b40229dcf3b31160e41d3.htm

3. Plant Biotechnology Journal | 单细胞转录组测序揭示了大白菜腹面和背面叶肉细胞的分化

简介：近日，Plant Biotechnology Journal在线发表了中国农业科学院蔬菜花卉研究所蔬菜分子设计育种团队完成的题为“Single-cell transcriptome reveals differentiation between adaxial and abaxial mesophyll cells in Brassica rapa”（单细胞转录组测序揭示了大白菜背腹面叶肉细胞的分化）的论文。该研究系统揭示了大白菜叶片单细胞的异质性，并在单细胞水平区分了叶片腹面的栅栏组织细胞和背面的海绵组织细胞，同时揭示了这两类细胞的分化，是大白菜叶球形成机制研究的一个重要进展。白菜类蔬菜属于十字花科芸薹属，是我国重要的叶菜类蔬菜作物，包含了许多叶片形态多样的亚种，如结球的大白菜和非结球的小白菜、菜心、水菜和乌塌菜等。叶肉细胞既是叶片中最大的细胞群，也是光合作用的主要场所，包括了叶片腹面的栅栏组织和背面的海绵组织。栅栏组织和海绵组织细胞的差异和分化对于维持叶片形态具有重要意义。近年来随着单细胞RNA测序技术的应用，在单细胞水平探索叶片栅栏组织和海绵组织细胞之间的分化，并确定其关键的调控基因，对于揭示白菜类蔬菜作物叶片发育和叶球形成的机制非常重要。然而，人们对其知之甚少。　　该团队通过单细胞转录组测序构建了大白菜叶片的单细胞转录组图谱，其中包含16,055个高质量细胞，涵盖8种细胞类型即叶肉细胞、表皮细胞、维管细胞、维管束鞘、保卫细胞、增殖细胞、韧皮部和木质部（图1）。该团队进一步采用改良的tape-sandwich方法分离了叶片栅栏组织和海绵组织，通过转录组分析和原位杂交，发现并验证了一批新的标记基因，从而将腹面的栅栏细胞和背面的海绵细胞在单细胞水平上区分开来。单细胞转录组分析发现了二者之间存在明显的功能差异，即栅栏细胞主要行使光合作用，而海绵细胞主要响应外部环境刺激。而且还发现，栅栏细胞富含大量核糖体蛋白编码基因，其中部分同源基因在拟南芥中已经被证明参与了叶片背腹极性的建成。有趣的是，大多数已知的背腹极性基因在成熟叶片中几乎不表达，而是在叶片发育的起始或早期阶段特异性表达。 该研究为进一步了解白菜类和其他芸薹属作物的叶片发育和形态发生的复杂过程提供了新的见解。此外，本研究还提供了许多细胞类型特异性标记基因，这将促进单细胞转录组测序技术在白菜中的应用。研究结果对解析大白菜叶球形成机制具有重要意义。　　蔬菜分子设计育种团队已毕业博士生郭新磊和梁建丽副研究员为共同第一作者，武剑研究员、王晓武研究员为论文通讯作者。本研究得到了国家重点研发计划(2021YFF1000101)、基本科研业务费（Y2020PT21）和创新工程项目的支持。　　文章链接：https://doi.org/10.1111/pbi.13919

来源：分子遗传-梁建丽

发布日期:2022-09-02

全文链接:

https://ivf.caas.cn/xwdt/zhxw/19ec4c3fee654ab9855a693329924e8a.htm

学术文献

1. QTL mapping of fruit aroma compounds in cucumber (Cucumber sativus L.) based on the recombinant inbred line (RIL) population(基于重组自交系群体的黄瓜果实香气成分QTL定位)

简介：The fresh and unique flavor of cucumber fruits, mainly composed of aldehydes and

alcohols, is one of the most important fruit quality. However, little is known about the genetic basis of aroma compounds in cucumber fruit and the relative quantitative trait locus (QTLs). In this study, a genomic screening of QTLs underlying aroma compounds was performed based on the genetic linkage map constructed by 1301 single nucleotide polymorphism (SNP) markers from genotyping-by-sequencing of a recombinant inbred lines (RILs) population developed from Q16×Q24. Significant genetic variations of aroma compounds in the RILs population were observed, and a total of 28 QTLs were screened. A major QTL (qol8-2.1) related to (E, Z)-2, 6-nonadien-1-ol was detected with a markedly high LOD score (10.97 in 2020 and 3.56 in 2019) between mk190 to mk204 on chromosome 2. Genome scans found that a cluster of 9 lipoxygenase genes was identified in this region. A significant positive correlation was detected between CsaV3_2G005360 (CsLOX08) and (E, Z)-2, 6-nonadien-1-ol, and five amino acid variations were detected between the CsLOX08 protein sequences of two parental lines. Based on the genome variation of CsLOX08, we developed an InDel marker. Genotyping of InDel markers was consistent with the content of (E, Z)-2, 6-nonadien-1-ol in RILs, which were also verified in 9 cucumber inbred lines. The result will give breeders guidance for better flavor in cucumber.

来源：Horticulture Research

发布日期:2022-07-06

全文链接:

http://agri.ckcest.cn/file1/M00/10/0F/CsgkOGMa1PSAPTs3ACoMVYhqtSA901.pdf

2. Integrated Metabolome and Transcriptome Analysis Provide Insights into the Effects of Grafting on Fruit Flavor of Cucumber with Different Rootstocks（综合代谢组学和转录组学分析为嫁接对不同砧木黄瓜果实风味的影响提供了见解）

简介：Rootstocks frequently exert detrimental effects on the fruit quality of grafted cucumber (Cucumis sativus L.) plants. To understand and ultimately correct this deficiency, a transcriptomic and metabolomic comparative analysis was performed among cucumber fruits from non-grafted plants (NG), and fruits from plants grafted onto different rootstocks of No.96 and No.45 (Cucurbita moschata. Duch), known to confer a different aroma and taste. We found remarkable changes in the primary metabolites of sugars, organic acids, amino acids, and alcohols in the fruit of the grafted cucumber plants with different rootstocks, compared to the non-grafted ones, especially No.45. We identified 140, 131, and 244 differentially expressed genes (DEGs) in the comparisons of GNo.96 vs. NG, GNo.45 vs. NG, and GNo.45 vs. GNo.96. The identified DEGs have functions involved in many metabolic processes, such as starch and sucrose metabolism; the biosynthesis of diterpenoid, carotenoid, and zeatin compounds; and plant hormone signal transduction. Members of the HSF, AP2/ERF-ERF, HB-HD-ZIP, and MYB transcription factor families were triggered in the grafted cucumbers, especially in the cucumber grafted on No.96. Based on a correlation analysis of the relationships between the metabolites and genes, we screened 10 candidate genes likely to be involved in sugar metabolism (Fructose-6-phosphate and trehalose), linoleic acid, and amino-acid (isoleucine, proline, and valine) biosynthesis in grafted

cucumbers, and then confirmed the gene expression patterns of these genes by qRT-PCR. The levels of TPS15 (Csa3G040850) were remarkably increased in cucumber fruit with No.96 rootstock compared with No.45, suggesting changes in the volatile chemical production. Together, the results of this study improve our understanding of flavor changes in grafted cucumbers, and identify the candidate genes involved in this process.

来源： International Journal of Molecular Sciences

发布日期:2019-07-23

全文链接:

http://agri.ckcest.cn/file1/M00/03/3D/CsgkOYdxhm6AFHAsADMxwLwOEos242.pdf

3. A Protocol for Agrobacterium-mediated Transformation of Cucumber (Cucumis sativus L.)from cotyledon explants（农杆菌介导的黄瓜（Cucumis sativus L.）子叶外植体转化方案）

简介： Genetic transformation is central to the discovery, delivery, and functional analysis of genes, and for associated crop improvement, and yet is technically challenging in many plant species. As an example, cucumber (Cucumis sativus L.) is a model species for the study of phloem characteristics, raffinose family oligosaccharide (RFO) metabolism and sex determination, but methods for its genetic transformation have generally had low efficiency, thereby limiting basic and applied studies. Here, we describe a rapid and efficient protocol for Agrobacterium-mediated transformation of cucumber cotyledon explants, using vacuum infiltration. The transformation efficiency of the regenerated plants was as high as 54%, and the final positive plantlet transformation efficiency was approximately 26% of the total number of infected explants and this is obviously higher than previously published protocols. Transgenic plantlets can be obtained in 3 to 4 months and the transgenic T1 seeds generated in the subsequent 3 to 4 months after self-pollination. Using this protocol, we have obtained more than 600 transgenic cucumber lines. This protocol is of great importance for studies of cucumber and of other Cucurbitaceae species, since it enables gene functional analysis, and so opens a pipeline for rapid cucumber variety improvement.

来源： research square

发布日期:2017-09-19

全文链接:

http://agri.ckcest.cn/file1/M00/03/3D/CsgkOYdxhSCAZdTcAAwZ1Jaeqac917.pdf

4. Improvement of Agrobacterium-mediated transformation of cucumber (Cucumis sativus L.) by combination of vacuum infiltration and co-cultivation on filter paper wicks（真空渗透与滤纸芯共培养相结合提高农杆菌介导黄瓜转化率）

简介： An improved method for genetic transformation of cucumber (Cucumis sativus L. cv. Shinhokusei No. 1) was developed. Vacuum infiltration of cotyledonary explants with Agrobacterium suspension enhanced the efficiency of Agrobacterium infection in the proximal regions of explants. Co-cultivation on filter paper wicks suppressed necrosis of explants, leading to increased regeneration efficiency. Putative transgenic plants were

screened by kanamycin resistance and green fluorescent protein (GFP) fluorescence, and integration of the transgene into the cucumber genome was confirmed by genomic polymerase chain reaction (PCR) and Southern blotting. These transgenic plants grew normally and T1 seeds were obtained from 7 lines. Finally, stable integration and transmission of the transgene in T1 generations were confirmed by GFP fluorescence and genomic PCR. The average transgenic efficiency for producing cucumbers with our method was 11.9 ± 3.5 %, which is among the highest values reported until date using kanamycin as a selective agent.

来源： Plant Biotechnology Reports

发布日期:2012-09-18

全文链接:

http://agri.ckcest.cn/file1/M00/10/0F/CsgkOGMa04iAF1sQAAkhV6G3Sm8528.pdf

相关专利

1．番茄周期素依赖性激酶S1CDK8基因及应用

简介：本发明公开了中介体成员番茄周期素依赖性激酶S1CDK8基因及应用，本发明首次从番茄中获得番茄周期素依赖性激酶S1CDK8基因，并且通过利用农杆菌介导的方法将S1CDK8在番茄MT(micro-Tom)中敲除进行功能验证，得出S1CDK8基因缺失可同时诱导整株番茄各器官的畸形发育，出现株高降低、雄性不育、花器官异常等症状，这也体现了S1CDK8在植物发育的进程中扮演的关键作用。

来源： 佰腾网

发布日期:2021-11-19

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http://agri.ckcest.cn/file1/M00/03/3D/CsgkOYdxinCAF2zLAA34jKcKp4w116.pdf

2．创制番茄果实色泽材料的多基因编辑载体及方法

简介：本发明涉及一种创制番茄果实色泽材料的多基因编辑载体及方法，该多基因编辑载体采用CRISPR/Cas9介导的基因编辑体系，能够实现同时对番茄PSY1基因、SGR基因和S1MYB12基因进行精确编辑，利用农杆菌介导的转化技术快速得到相关变异材料，丰富番茄育种资源，为番茄品种选育提供新思路。

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