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前沿资讯

1．中国农大植保学院在从细胞壁结构层面解析番茄溃疡病菌抵抗逆境机制方面取得新进展

简介：近日，美国微生物学会（ASM）旗下期刊微生物学谱（Microbiology Spectrum）在线发表了植物保护学院李健强、罗来鑫课题组题为《A类青霉素结合蛋白C通过调控肽聚糖的交联程度，进而影响番茄溃疡病菌的肽聚糖组装参与抗逆反应》（“lass A Penicillin-Binding Protein C Is Responsible for Stress Response by Regulation of Peptidoglycan Assembly in Clavibacter michiganensis”）的研究论文。该研究对A类高分子量青霉素结合蛋白PBPC在番茄溃疡病菌抵抗逆境机制中的功能进行了解析，结果表明PBPC可以通过调控细胞壁厚度及肽聚糖的交联程度，进而影响番茄溃疡病菌的抗逆能力，从细菌结构层面解析了低浓度铜离子胁迫下番茄溃疡病菌的抗逆特性。番茄溃疡病菌（Clavibacter michiganensis）是我国重要的检疫性有害生物，引起的番茄细菌性溃疡病是番茄生产中最具毁灭性的病害之一，在多个国家和地区均有该病害的发生报道，严重影响番茄制种业和大田生产的可持续发展。青霉素结合蛋白（penicillin-binding protein, PBP）在细菌细胞壁肽聚糖合成的最后阶段发挥重要作用，主要参与肽聚糖单体的组装和交联过程。VBNC（viable but nonculturable）状态是不产芽胞细菌应对不良环境的一种抗逆反应，但在包括番茄溃疡病菌在内的植物病原菌中，逆境处理条件下病原菌细胞壁结构的变化、青霉素结合蛋白在逆境响应过程中的调控机制仍不明确。作者所在课题组的前期研究结果表明，番茄溃疡病菌可在低浓度铜离子胁迫下进入VBNC状态，失去可培养性。在此基础上，作者在前期发表的研究论文中证实了青霉素结合蛋白在番茄溃疡病菌抗逆过程中发挥了重要作用，并影响其进入和维持VBNC状态。本论文系统研究了VBNC状态番茄溃疡病菌细胞壁肽聚糖结构的变化，及逆境下起关键作用的双功能类PBPC调控的肽聚糖组装机制，结果显示：与对数生长期菌体相比，VBNC状态的野生型番茄溃疡病菌对超声胁迫的抗性显著增强，但PBPC缺失突变体ΔpbpC对超声胁迫的抗性显著降低，VBNC状态ΔpbpC的抗性虽有增强，仍显著低于VBNC状态的野生型菌株。基于此，对WT、ΔpbpC对数生长期和VBNC状态菌体的细胞壁肽聚糖结构进行观察，结果表明WT细胞壁肽聚糖层相对光滑致密，在铜离子胁迫下肽聚糖层厚度和交联程度均稍有增加；而对数生长期的ΔpbpC菌体肽聚糖厚度和交联程度显著低于野生型，“脊-沟（ridge-and-groove）”形貌特征明显，但在铜离子胁迫下肽聚糖交联程度增加，厚度无变化（图2，图3）。有趣的是，PBPC的缺失导致菌体肽聚糖结构的变化，使胞外淀粉酶和胞外多糖的产量显著增加。综上，PBPC的缺失降低了番茄溃疡病菌细胞壁肽聚糖层的厚度和交联程度，改变肽聚糖表观形貌及其对铜离子的响应，导致ΔpbpC不能维持VBNC状态进而细胞死亡。以上结果系统地揭示了PBPC介导的番茄溃疡病菌的抗逆机制，从结构层面解析了VBNC状态细菌的抗逆机制，对以番茄溃疡病菌为代表的革兰氏阳性细菌的生存机制研究及病害防控具有重要意义。植物保护学院已毕业研究生陈星博士为本论文的第一作者，罗来鑫副教授为本论文的通讯作者，安徽农业大学植保学院陈雨教授参与了本研究的部分工作。该研究工作得到北京市自然科学基金（No. 6222025）和国家重点研发计划项目（No. 2017YFD0201601）的资助。论文链接：https://journals.asm.org/doi/10.1128/spectrum.01816-22

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2．中国农大林建涵教授团队在生物传感器方面研究取得新进展

简介：近日，中国农大信息与电气工程学院林建涵教授团队在传感器领域著名期刊《生物传感器与生物电子学》（Biosensors and Bioelectronics）发表最新研究成果，题为“利用一次性离心管模仿沙漏实现大体系样本中食源性致病菌的生物传感”(Hourglass-mimicking Biosensor Based on Disposable Centrifugal Tube for Bacterial Detection in Large-volume Sample)，在生物传感器方面研究取得了新进展。沙门氏菌污染可能发生在食品供应链的任何一个环节，快速准确筛查沙门氏菌是预防和控制食源性疾病暴发的关键。日常筛查时，致病菌检测仍然面临一些瓶颈问题，主要包括：食品基质复杂，容易造成严重干扰；细菌浓度很低，检测信号通常很弱；仪器试剂成本较高，实际应用难度较大；实验操作冗长，基层人员难于实施。该研究基于免疫学方法，融合工程技术和纳米材料，开发了一种简单、快速、灵敏的细菌检测新技术。该研究以常见实验室耗材-离心管作为实验平台，以常见食源性致病菌-沙门氏菌为研究模型，利用哈尔巴赫环形磁铁结合铁球框架，并利用Arduino Uno控制步进电机，将离心管像沙漏一样反复自下而上混合，从大体积食品样本中高效分离沙门氏菌，并利用金核铂纳米酶高效放大生物信号，研制了一种用于沙门氏菌超灵敏检测的新型比色生物传感器。该生物传感器对10mL样品中目标细菌的分离效率可达到95%，可在1小时内实现沙门氏菌的定量检测，检测下限达到了16 CFU/mL。中国农业大学是该研究工作的第一完成单位，美国阿肯色大学是合作完成单位，博士后王蕾为第一作者，林建涵教授为通讯作者，该研究工作得到了Walmart基金会的资助和Walmart食品安全协作中心的支持。林建涵教授团队一直致力于融合工程技术、生物技术和纳米材料，研究开发新型的生物传感器技术与装备，为保障食品安全和动物防疫贡献系统解决方案，近5年已在《生物传感器与生物电子学》（Biosensors and Bioelectronics）、《ACS传感器》（ACS Sensors）、《传感器与执行器B：化学》（Sensors and Actuators B: Chemical）、《治疗诊断学》（Theranostics）、《芯片实验室》（Lab on a Chip）、《食品化学》（Food Chemistry）、《分析化学》（Analytical Chemistry）等多个学科顶级期刊发表六十余篇研究论文。
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3．万建民院士团队揭示生长素调控水稻光合产物源库分配的新机制

简介：8月30日，南京农业大学万建民院士团队关于生长素调控水稻光合产物源库分配和生长发育机制的研究成果“Auxin regulates source-sink carbohydrate partitioning and reproductive organ development in rice”在PNAS上发表。光合作用产物在源（叶片）和库（果实和种子）器官间分配与运输将直接影响农作物的产量。作物利用蔗糖转运子（SUTs/SUCs）将光合产物（主要是蔗糖）从源（叶片）经过韧皮部的筛管组织长距离输送到库器官，然后在库中卸载；一方面库中的蔗糖产生膨压将为

其他物质（包括营养物质、水和信号分子）长距离运输创造了驱动力，另一方面，运输到库中的蔗糖也是作物籽粒合成淀粉的主要原料。众所周知，植物激素生长素调控作物生长发育的各个方面，然而，目前关于生长素和蔗糖这两大信号分子如何协同调控作物生长发育的分子机制依然不清楚。在前期研究中，该团队筛选获得了一个水稻生长素氧化双加氧酶（Dioxygenase for Auxin Oxidation, DAO）失活的dao突变体（Dev Cell, 2013），该突变体在抽穗开花时期不能将有活性的生长素（IAA）氧化成无活性的OxIAA，破坏了体内IAA的动态平衡，出现颖壳不能正常张开、花药不能正常开裂、籽粒不能正常灌浆和单性结实的表型。本研究发现dao突变体由于生长素水平升高导致植株叶片中蔗糖含量增加，但花器官（主要是浆片、花药和子房）中的蔗糖含量却显著降低。对野生型和突变体叶片进行[14C]-蔗糖喂养实验，结果显示在dao突变体叶片中显著积累放射性蔗糖，但在花药和子房中放射性蔗糖却显著减少。RNA-Seq分析显示，在dao突变体浆片中生长素转录因子OsARF18表达上调，而OsARF2表达下调。OsARF18的过表达系或OsARF2敲除系能够再现dao突变体的表型。EMSA实验证明OsARF2 通过直接结合OsSUT1启动子中的糖响应元件(SuRE) 调节OsSUT1的表达，而OsARF18 通过直接结合生长素反应元件AuxRE 或SuRE 抑制OsARF2和OsSUT1 的表达，从而调控蔗糖从源到库的运输。而在dao和Osarf2 突变体中过表达OsSUT1基因，能显著提高突变体颖花张开率和小穗结实率。这些研究结果揭示了IAA-OsARF18-OsARF2生长素信号级联反应调控蔗糖转运子OsSUT1表达，调节蔗糖从源（叶片）到库（浆片、花药和子房）的分配，进而影响水稻颖壳张开、花药开裂和籽粒灌浆结实的分子机制，将为如何协调源-库-流提高农作物产量提供理论依据。南京农业大学赵志刚教授、王超龙讲师、余晓文教授为论文的共同第一作者，万建民院士和赵志刚教授为本文的通讯作者，中国农科院作物科学研究所王海洋研究员和江苏省农业科学院生物技术所张云辉研究员也参与了此项研究。感谢Yunde Zhao 教授（University of California, San Diego）和W. Terzaghi教授（Wilkes University）对本研究提出非常好的建议，该研究得到国家自然科学基金重大项目、国家重点研发计划、江苏省重点研发计划等项目的资助。万建民院士团队长期从事水稻籼粳亚种间杂种育性的基础研究，十三五以来在水稻籼粳交杂种优势利用和生长发育领域发表研究论文多篇，包括Genetics（2016），Science（2018），Plant Physiol.（2019），PNAS（2022）等。这些研究结果将为水稻籼粳交杂种优势利用提供重要基因资源，为水稻超高产育种提供理论支撑。论文链接：https://www.pnas.org/doi/10.1073/pnas.2121671119

来源：南京农业大学农学院

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http://news.njau.edu.cn/2022/0830/c18a119987/page.htm

学术文献

1. Highly efficient Agrobacterium-mediated transformation and plant regeneration system for genome engineering in tomato(用于番茄基因组工程的高效农杆菌介导转化和植株再生系统)

简介：Tomato (Solanum lycopersicum L.) is an important vegetable and nutritious crop plant worldwide. They are rich sources of several indispensable compounds such as lycopene,

minerals, vitamins, carotenoids, essential amino acids, and bioactive polyphenols. Plant regeneration and Agrobacterium-mediated genetic transformation system from different explants in various genotypes of tomato are necessary for genetic improvement. Among diverse plant growth regulator (PGR) combinations and concentrations tested, Zeatin (ZEA) at 2.0 mg l?1 in combination with 0.1 mg l?1 indole-3-acetic acid (IAA) generated the most shoots/explant from the cotyledon of Arka Vikas (36.48 shoots/explant) and PED (24.68 shoots/explant), respectively. The hypocotyl explant produced 28.76 shoots/explant in Arka Vikas and 19.44 shoots/explant in PED. In contrast, leaf explant induced 23.54 shoots/explant in Arka Vikas and 17.64 shoots/explant in PED. The obtained multiple shoot buds from three explant types were elongated on a medium fortified with Gibberellic acid (GA3) (1.0 mg l?1), IAA (0.5 mg l?1), and ZEA (0.5 mg l?1) in both the cultivars. The rooting was observed on a medium amended with 0.5 mg l?1 indole 3-butyric acid (IBA). The transformation efficiency was significantly improved by optimizing the pre-culture of explants, co-cultivation duration, bacterial density and infection time, and acetosyringone concentration. The presence of transgenes in the plant genome was validated using different methods like histochemical GUS assay, Polymerase Chain Reaction (PCR), and Southern blotting. The transformation efficiency was 42.8% in PED and 64.6% in Arka Vikas. A highly repeatable plant regeneration protocol was established by manipulating various plant growth regulators (PGRs) in two tomato cultivars (Arka Vikas and PED). The Agrobacterium-mediated transformation method was optimized using different explants like cotyledon, hypocotyl, and leaf of two tomato genotypes. The present study could be favourable to transferring desirable traits and precise genome editing techniques to develop superior tomato genotypes.

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http://agri.ckcest.cn/file1/M00/03/3E/CsgkOYeBdI6ANeJCACjNtEv_CFY115.pdf

2. AGROBACTERIUM-MEDIATED TRANSFORMATION OF TWO TOMATO CULTIVARS (LYCOPERSICON ESCULENTUM MILL.) CV. SANDRA AND ROCKY(农杆菌介导的两个番茄品种（LYCOPERSICON ESCULENTUM MILL.）CV。桑德拉和洛基)

简介：An efficient protocol for Agrobacterium-mediated transformation of tomato cultivars Sandra and Rocky was conducted to examine the possibility of producing transgenic tomato plants cultivars harbouring the nptII gene, conferring kanamycin resistance. To achieve this aim, tomato cotyledon explants were transformed using EHA105 Agrobacterium tumefaciens strain harboring the binary vectors pBI121 which contains Gus gene, and neomycin phosphotransferase II (nptII) as selectable marker gene under the control of a CaMV35S promoter and nopaline synthase (nos) Terminator. Transformant detection was carried out in three distinct ways. First antibiotic selection, Kanamycin at a concentration of100 mgl-1 found to be efficient for this purpose. Second histochemical GUS assay revealed the presence

of blue colored zones in a number of shoots and leaves for both in vitro and the greenhouse-grown transgenic plants. Third PCR analysis indicated positive result by showing the fragment for nptII gene in tested transformants, while was absent in non-transgenic control (wild type). On the other hand, the results showed that Sandra cultivar was more efficient for regeneration and subsequently transformation frequency than Rocky cultivar, which record 26.66% of transformation frequency compared with 11.57% in Rocky cultivar.
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3. Rapid delivery of Cas9 gene into the tomato cv. 'Heinz 1706' through an optimized Agrobacterium-mediated transformation procedure(将Cas9基因快速导入番茄品种Heinz 1706'通过优化的农杆菌介导转化程序)

简介：Solanum lycopersicum 'Heinz 1706' is a pioneer model cultivar for tomato research, whose whole genome sequence valuable for genomics studies is available. Nevertheless, a genetic transformation procedure for this cultivar has not yet been reported. Meanwhile, various genome editing technologies such as transfection of clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated (Cas) ribonucleoprotein complexes into cells are in the limelight. Utilizing the Cas9-expressing genotype possessing a reference genome can simplify the verification of an off-target effect, resolve the economic cost of Cas9 endonuclease preparation, and avoid the complex assembly process together with single-guide RNA (sgRNA) in the transfection approach. Thus, this study was designed to generate Cas9-expressing 'Heinz 1706' lines by establishing an Agrobacterium tumefaciens-mediated transformation (ATMT) procedure. Here, we report a rapid and reproducible transformation procedure for 'Heinz 1706' by finetuning various factors: A. tumefaciens strain, pre-culture and co-culture durations, a proper combination of phytohormones at each step, supplementation of acetosyringone, and shooting/rooting method. Particularly, through eluding subculture and simultaneously inducing shoot elongation and rooting from leaf cluster, we achieved a short duration of three months for recovering the transgenic plants expressing Cas9. The presence of the Cas9 gene and its stable expression were confirmed by PCR and qRT-PCR analyses, and the Cas9 gene integrated into the T0 plant genome was stably transmitted to T1 progeny. Therefore, we anticipate that our procedure appears to ease the conventional ATMT in 'Heinz 1706', and the created Cas9-expressing 'Heinz 1706' lines are ultimately useful in gene editing via unilateral transfection of sgRNA into the protoplasts.
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4．Overexpression of Pti4, Pti5, and Pti6 in tomato promote plant defense and fruit ripening(番茄中Pti4、Pti5和Pti6的过度表达促进植物防御和果实成熟)

简介：Pseudomonas syringae pv. tomato (Pst) is a pathogenic microorganism that causes bacterial speck disease and affects tomato yield and quality. Pto is a disease resistant gene for plant to recognize and defense against Pst. Pto interacts with Pti (Pto interacting) proteins, which include three transcription factors, Pti4, Pti5, Pti6, and they were thought to be downstream of Pto-mediated pathway to promote the expression of disease-related genes. In the present work, the overexpression plants of Pti4, Pti5 or Pti6 were obtained by Agrobacterium-mediated transformation in tomato. The Pti4/5/6-overexpressed lines indicated enhanced expression of pathogenesis-related genes and resistance to pathogenic bacteria Pst DC3000. Meanwhile, the transgenic plants showed that Pti4/5/6 function in ripening but performed no obvious adverse influence on flowering time, seed-setting rate, weight and soluble solids content of fruits. Furthermore, Pti-overexpressed fruits exhibited increased enzymatic activities of phenylalnine ammonialyase, catalase, peroxidase and decreased content of malondialdehyde. Additionally, cell-free and in vivo ubiquitination assay indicated that Pti4, Pti5 and Pti6 degraded by 26S proteasome which suggested that these Pti transcription regulators' functions could be regulated by ubiquitin-mediated post translational regulation in tomato.

来源：Plant Science

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全文链接:

http://agri.ckcest.cn/file1/M00/10/10/CsgkOGMqyEOAVdUEACtKHEh-ado564.pdf

相关专利

1．番茄褐色皱纹果病毒的侵染性克隆载体及其构建方法和应用

简介：本发明公开了一种番茄褐色皱纹果病毒的侵染性克隆载体及其构建方法和应用，在获得番茄褐色皱纹果病毒基因组全序列的基础上，构建了该病毒的侵染性克隆，将包含35S启动子的番茄褐色皱纹果病毒cDNA序列构建入双元载体pCB301中，通过农杆菌介导转化后在寄主体内转录、复制、侵染，以用于病毒的功能基因组研究。本发明方法为研究该病毒基因组结构和功能及病毒与寄主之间的相互作用研究提供了成熟的体系。

来源：佰腾网

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全文链接:

https://www.baiten.cn/patent/detail/5a8dc4941305110d0ff9256c03560bd8faf098982bb2844e?sc=&fq=&type=&sort=&sortField=&q=%E7%95%AA%E8%8C%84%E8%A4%90%E8%89%B2%E7%9A%B1%E7%BA%B9%E6%9E%9C%E7%97%85%E6%AF%92%E7%9A%84%E4%BE%B5%E6%9F%93%E6%80%A7%E5%85%8B%E9%9A%86%E8%BD%BD%E4%BD%93%E5%8F%8A%E5%85%B6%E6%9E%84%E5%BB%BA%E6%96%B9%E6%B3%95%E5%92%8C%E5%BA%94%E7%94%A8&rows=10#1/CN202110544185.2/sqdetail/abst

2．一种番茄侧枝发生的调控方法

简介：本发明适用于植物基因工程技术领域，提供了一种番茄侧枝发生的调控方法，包括一种番茄SlERF025基因，其核苷酸序列如Seq No.1所示，还包括以下步骤：步骤（1）：番茄SlERF025基因的克隆；步骤（2）：目的基因片段的回收；步骤（3）：将目的基因连接到表达载体；步骤（4）：重组质粒转化大肠杆菌；步骤（5）：农杆菌介导番茄遗传转化。本发明所提供的一种番茄侧枝发生的调控方法为调控植物分枝方面的应用，是将SlERF025基因利用根癌农杆菌介导的方式转入番茄植株，观察转基因植株与野生型植株的表型差异并进行对比和生理指标的测定，同时研究该基因对植物分枝的控制，探索关于调控植物分枝的新方法。

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http://agri.ckcest.cn/file1/M00/03/3E/CsgkOYeBfA-AUsECAAzXlgvP9yE244.pdf