

果树代谢组学研究进展

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摘 要: 果树代谢组学是继基因组学、蛋白质组学之后又一新兴的组学技术, 主要是从代谢水平研究果树整体或局部代谢物变化差异, 帮助发现新功能基因和了解代谢网络。目前果树代谢组学研究刚刚起步, 相关研究相对较少, 该文介绍了果树代谢组学的主要研究内容与方法以及在果树上的相关应用。

关键词: 代谢组; 代谢组学; 果树; 核磁共振; 质谱

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Advance in Research of Fruit Tree Metabolomics

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Abstract: Fruit metabolomics is the second in genomics, proteomics after another emerging genomics technologies. Fruit metabolomics mainly study metabolite changes of the whole fruit or partial, helping to discover new functional genes and understanding of metabolic networks. Currently fruit metabolomics study has just started, relatively few studies, this paper describes the main fruit metabolomics research direction, technical processes and related applications.

Key words: metabolome; metabolomics; fruit tree; NMR; MS

果树含有超过 20 万种的小分子代谢物, 它们参与细胞形态建成、细胞间信号传导、细胞防御以及果树应激等生理反应, 其中部分代谢物水平还影响代谢通量、基因表达和蛋白质稳定^[1-6]。但是大部分代谢物化学结构、功能及其代谢途径未明, 限制了人们对果树代谢网络的了解。继基因组学、蛋白质组学之后, 代谢组学和转录组学的出现标志着果树研究已进入了后基因组时代。果树代谢组学技术不再局限于传统的有针对性代谢物化学成分分析, 而是从系统生物学的角度对代谢物进行定量定性, 已成为能检测复杂生物体变化的技术手段^[7-8]。近年来, 植物代谢组学的技术已被广泛地应用于番茄^[2,9-11]、大豆^[12-14]、马铃薯^[15-16]、拟南芥^[17-20]、烟草^[21]、水

稻^[22-23]、咖啡^[24-27]及水仙等植物^[28-30]。目前, 果树代谢组学研究相对较少, 但果树代谢组代表着现有不同层次细胞、组织的最终调控结果, 相较于基因组学和蛋白质组学的影响更直接。因此, 本文主要介绍近年来果树代谢组学研究进展, 以期对果树代谢组学的研究发展提供参考。

1 果树代谢组学

1998 年 Stephen Oliver 首次提出代谢组 (metabolome) 是指生物体内所有低分子量化合物的集合^[31]。2002 年由 Oliver Fiehn 引入“代谢组学”一词, 指出所有代谢物应被定量定性分析。近年来代谢组学 (metabolomics) 以效仿蛋白质组学和基

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因组学的研究思想,通过观察生物体系(细胞、组织、生物体)受到刺激或干扰后代谢产物种类和数量的变化规律,来研究生物体系的代谢途径和代谢网络^[32-33]。目前代谢组学技术已经被广泛地应用于不同科学领域,包括医学^[34-35]、中药^[36-39]、食品科学^[40-42]、微生物^[43]和动物^[7]等。

果树代谢组学是对果树的特定器官、组织或细胞中所有小分子代谢产物进行定量或定性分析^[44]。传统的果树代谢研究主要集中于以分离鉴定某一单一成分、功能作为主要目的,而果树代谢组学研究是利用代谢组学技术高通量、低成本的特点,系统、全面地研究果树(或某一组织、器官)中所有小分子物质及其随着时间的变化关系。得益于其高通量和低成本的特点,果树代谢组学的应用也从最初单纯的代谢分析、单一代谢途径描述走向与其他组学技术结合、围绕特定的生物学问题,来共同揭示植物生命活动的奥秘^[6,45-46]。

2 果树代谢组学研究内容与方法

2.1 研究内容

Oliver Fiehn^[47]根据研究目的和对象的不同,将生物体系的代谢组学研究分为4个层次:代谢靶标分析、代谢轮廓分析、代谢组学和代谢指纹分析。不同层次的代谢组学技术已成为后基因组研究过程中所不可或缺的研究手段,并揭示了基因序列与代谢网络功能间的直接联系^[48-49]。相较于传统代谢研究手段,果树代谢组学研究具有潜在的巨大发展空间:研究果树代谢物水平,了解其遗传因素,进而对果实质量做出评估,培育新品种^[50-51];研究不同基因型果树的代谢物图谱,发现新的功能基因以及对转基因水果风险性的评估^[52-55];研究不同生态条件下果树代谢产物的差异,确定不同地区果树指纹图谱,有利于判别果实的地理起源^[56-58];研究果树对生物性或非生物性刺激的应激反应以及宿主与病毒间的相互作用时代谢物变化,揭示其变化原因^[56,59-66]。

2.2 研究技术

果树代谢组学研究一般需要检测大量数据作为研究分析材料,因此要求果树代谢组学技术必须具有高通量、高灵敏度、低成本的特点。目前,在代谢组学研究中主要研究技术有:气相色谱-质谱联用技术(GC-MS),毛细管电泳-质谱联用技术(CE-MS),液相色谱-质谱联用技术(LC-MS),液相色谱-电化学-质谱联用技术(LC-EC-MS),核磁共振联用技术(NMR),液相色谱-核磁共振联用技术 LC-NMR,傅

立叶红外光谱技术(FT-IR)^[47,67-69]。而在果树代谢组学的研究中以色谱-质谱联用技术(GC-MS和LC-MS)与核磁共振技术(NMR)的应用最广泛^[68,70]。其中核磁共振技术能够实现对果实样品的非选择性、非破坏性分析,极大地满足了果树代谢组学对尽可能多的化合物进行检测的目标^[71-72],且具有客观性强,检测范围广的特点,但灵敏度较低。而色谱-质谱联用技术兼备了色谱高通量、高分离度的特点,且质谱具有高灵敏度和特异性,但检测范围却不如核磁共振。因此,两种方法的联用互补将会使果树代谢组学技术获取更全面的代谢信息^[73-74]。

2.3 数据分析

果树代谢组学技术高通量的特点必然会产生大量的数据信息,因此数据处理成为信息挖掘的关键环节。果树代谢组学的数据处理手段为模式识别技术,可分为非监督(unsupervised)学习方法和有监督(supervised)学习方法。非监督学习方法对采集的数据直接进行归类,不需样品的任何背景信息。通过建立代谢产物与这些原始信息的联系,筛选标志性物质。非监督学习方法主要有聚类分析(hierarchical clustering analysis, HCA)、非线性映射(nonlinear mapping)、主成分分析(principal components analysis, PCA)等,其中以PCA应用较为广泛。PCA是以互相垂直的主成分作为多维数据的代表来描述大数据的内部结构^[75]。由于没有背景信息的参考,此方法能更为客观地快速提供分析对象的整体信息概况,但容易忽视部分相关性较小的变量。因此,PCA模型的代表性具有一定的偏差,往往需要同有监督学习方法相结合。

有监督学习方法按照样品背景信息进行归类建立类别间的数学模型,对未知样本进行预测,侧重类别间的比较。主要方法有人工神经网络(neural network, ANN)、偏最小二乘法(partial least squares, PLS),正交偏最小二乘判别分析(orthogonal partial least-squares discriminant analysis, OPLS-DA)、偏最小二乘法-判别分析(partial least squares-discriminant analysis, PLS-DA)及软独立建模分类法(soft Independent modeling classification, SIMCA)。其中以PLS-DA应用较为普遍。PLS-DA是首先采用偏最小二乘法对高维数据进行降维,再通过Fisher进行线性判别。此方法预测组别能力较强于PCA,因此在代谢组学中常采用PLS-DA和PCA对数据进行共同分析以获取更为客观的数据信息。

3 果树代谢组学的应用

3.1 果树生长发育代谢水平变化

果树代谢组学的应用为果树科学管理,果实品质分析提供了更加全面的理论依据。Martinelli^[76]采用气相质谱技术研究灌溉量对油橄榄生长发育中代谢物变化的影响,为油橄榄园水分科学管理提供理论依据。潘志勇等^[77]采用实时直接分析(DART)质谱技术对红暗柳橙和野生型暗柳橙果实进行代谢物检测,发现了红暗柳橙低酸性的主要成因。Cho等^[78]通过核磁共振技术检测柑橘果实不同成熟阶段代谢物变化规律,以此来预测果实成熟度。

¹H-NMR 技术常被应用于系统研究植物在不同条件下的整体代谢物变化、代谢规律及其调控机制,如金丝桃属^[79]、冬青属^[80]、大麻属^[81]植物的分类及品种差异。在葡萄上,Alì^[82]采用¹H-NMR 技术检测 4 个不同品种的葡萄在果实发育过程中代谢组的变化情况,发现在果实发育早期含有较高的有机酸和酚,而到后期果糖和葡萄糖含量迅速增加,再次证明了转色期是葡萄品质的关键时期。Son^[83-84]通过核磁共振测定葡萄、葡萄酒代谢物组成。Mattivi^[85]也通过定向代谢组学方法分析不同葡萄品种花青素和类黄酮的组成成分。

3.2 果实贮藏与保鲜

延长果实贮藏保鲜时间有利于增加果树经济价值、提高果实品质,代谢组学技术的应用为果实贮藏环境的调控、化学保鲜剂安全性的评估提供了可靠的科学参考。Rudell 等^[86-87]采用 GC-MS 和 LC-MS 技术发现紫外-白光照射下苹果果皮和果实质地的代谢途径均受到影响,而用加热模拟贮藏过程中温度变化表明了苹果代谢物谱轮廓显著改变,发病机率增大。在柑橘上也有同样的发现,研究采用 GC-MS 和高效液相色谱-四级杆-飞行时间串联质谱仪分析热处理与对照样品间初生代谢物和次生代谢物的差异,发现 52℃ 温水浸泡过的柑橘在贮藏过程中醇、脂肪酸类和黄酮类物质增加,提高了果实抗性^[88]。Romina 等^[89]采用 GC-MS 和 LC-MS 技术分析鳄梨成熟期代谢产物的变化,进而预测鳄梨达到即食成熟度的时间。

3.3 果树与病害的互作关系

为研究柑橘黄龙病发病过程中病原菌与宿主间的互作关系,Hong 等^[90]采用气相色谱-质谱技术研究黄龙病潜育期和显症期叶片组织中小分子代谢物的含量差异,发现黄龙病病菌侵染过程中氨基酸和

抗病性相关代谢水平上调,而糖类代谢水平在潜育期和显症期中差别显著。为进一步快速判别黄龙病与缺锌症状,Cevallos-Cevallos 等^[91]利用气相质谱联用仪分析了黄龙病与缺锌对夏橙叶片代谢组变化,发现 6 种代谢物可作为黄龙病侵染的生物指标。Cevallos-Cevallos 等^[92]采用 GC-MS 技术研究不同品种柑橘对黄龙病的敏感值。在研究苹果贮藏期果肉内部褐变的病变机理方面,Jinwook^[93]采用代谢组学技术研究 DPA 处理后苹果代谢物水平变化,发现果肉褐变与氨基酸、乙醛、乙醇和乙酸乙酯挥发性化合物的积累有关。Luca 等^[94]分别采用 GC-MS 和 PTR-MS 两种代谢组学技术研究不同树莓品种对灰霉病的敏感度,发现萜类等几种挥发性有机化合物为主要影响因子。

3.4 果树逆境的代谢变化

有关逆境对果树的影响,过去研究常停留在逆境是否对果树造成一定的生理伤害,但却无法对逆境危害的影响做出准确的评估,而果树代谢组学技术的应用正好解决了这一问题。先前的研究发现油橄榄水分亏缺会影响橄榄油的品质,但却无法对水分的逆境影响做出准确的评估^[95-97]。Martinelli 等^[98]利用气相质谱联用仪分析不同水分胁迫下油橄榄代谢物谱的变化差异,发现葡萄糖酸可能是作为水分亏缺对品质影响的主要因子。在葡萄上,Uri 等^[99]利用代谢组学技术分析水分亏缺对葡萄代谢水平的影响。Choi 等^[100]采用核磁共振技术分析硝酸银对葡萄细胞悬浮培养过程中代谢产物的影响。

此外,代谢组学技术在判别果树地理起源上应用也十分广泛。Longobardi^[57]为了能区分意大利樱桃的原产地,通过采用非针对性¹H-NMR 代谢指纹分析和监督模式识别(PLS-DA 和 LDA)技术相结合成功地建立 LDA 模型,并将苹果酸、葡萄糖、果糖、谷氨酰胺和琥珀酸盐作为区分樱桃地理起源的具有分类性能的代谢物。Longobardi 等^[101]也采用指纹图谱技术鉴定橄榄油的原产地。

4 展望

虽然基因组学与蛋白质组学技术已经日益成熟,但由于研究技术的限制,果树基因转录和代谢物之间的调控机制仍无法解决。因此,代谢组学技术在了解果树代谢网络以及转录基因的功能上起到了关键的作用^[102]。随着质谱和核磁共振等相关联用技术的快速发展,利用代谢组学与其他组学技术相结合,从多维化的角度研究和预测果树复杂的代谢

系统已成为未来果树研究的主要方向。据 Scopus^[31]对近年来出版物的统计分析,代谢组学研究正处迅速发展阶段,其内在的潜力已获得越来越多

科研人员的重视,将会为果树生物学带来一场新的革命!

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