

基于全长转录组和代谢组联合分析 揭示湿地松胚性愈伤褐变的发生机制

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摘要:【目的】胚性愈伤团的褐变与基因型密切相关, 利用抗/易褐变无性系材料解析胚性愈伤褐变的分子机制, 为松树抗褐变资源选择及体细胞胚胎发生体系优化提供理论依据。【方法】以来自同一湿地松半同胞家系的抗/易褐变胚性无性系为研究对象, 将二者的胚性愈伤团于继代后第 0 天, 第 14 天和第 28 天取样, 进行全长转录组、二代转录组测序分析, 并利用液相色谱串联质谱 (LC-MS/MS) 开展黄酮/酚类化合物靶向代谢组测定及分析, 通过多组学关联分析、共表达分析等方法, 挖掘与胚性愈伤团褐变相关的重要基因及调控网络。【结果】基于三代测序获得的 63334 条全长转录本序列 (注释率 97.27%) 进行表达谱分析, 发现抗褐变无性系虽在迅速生长, 但继代后 0 到 14 天的差异表达基因仅有 17 个, 两个无性系间的差异表达基因则非常多, 超过 13710 个。两个无性系的差异表达基因呈现相异的表达趋势, 差异关键时间节点为继代后 14d, 易褐变胚性愈伤团中在 14d 提前发生上调的基因与抗褐变胚性愈伤团中推迟到 28d 才上调的基因相似, 与类黄酮生物合成过程、应激反应、苯丙素类生物合成等相关 GO (Gene Ontology) 条目和 KEGG (Kyoto Encyclopedia of Genes and Genomes) 通路有关。超过 14d 继代周期后, 两个无性系的胚性愈伤中都发现较多基因的表达下调, 与甲基化、染色质修饰、RNA 修饰等相关的 GO 条目和 KEGG 通路有关。通过 WGCNA (Weighted correlation network analysis) 分析进一步挖掘具有特定表达模式的共表达基因, 共获得了 14 个共表达模块 (Module), 其中 lightcyan 模块的基因显著富集到与苯丙素类和类黄酮类生物合成过程相关的 GO 条目和 KEGG 通路中, 以该模块构建褐变转录调控网络, 获得包括 WRKY6、ERF4 等转录因子在内的 9 个转录因子及 31 个关键基因。靶向代谢组分析获得了 61 个黄酮/酚类化合物, 易褐变的组织中大部分这些化合物的含量更高。多组学关联分析表明 4-羟基肉桂酸 (4-Hydroxycinnamic acid)、柚皮素 (Naringenin) 在易褐变胚性愈伤继代后 14d 明显积累, 与相关基因的表达模式相吻合, PePAL、PeCYP73A4、PeCHS 和 PeCHI 的高表达促进了这些物质积累, 而 PeWRKY6、PeERF4、PeNAC098 等转录因子可能上调了这些基因, 共同引起了褐变的发生。【结论】抗褐变无性系能够在 14 天的继代周期内维持内部基因表达的相对稳定, 仅在超过继代周期后才发生明显变化。而易褐变无性系则存在很多基因“不合时宜”地提前发生表达变化, 导致酚类化合物的提前积累。PeWRKY6 和 PeCYP73A4 等关键基因与 4-羟基肉桂酸和柚皮素等在胚性愈伤的过度积累相关而引发褐变。

关键词: 湿地松; 胚性愈伤团; 褐变; 全长转录组; 黄酮/酚类靶向代谢组

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The Mechanism of Embryogenic Callus Browning in Slash Pine Revealed by Integrative Analysis of Full-Length Transcriptome and Metabolome

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Abstract: **【Objective】** The browning of embryogenic callus is closely related to genotype. By utilizing anti-browning and easy-browning embryonic clones, this study aims to elucidate the molecular mechanisms underlying the browning of embryogenic callus, providing a theoretical basis for selecting anti-browning resources in pine trees and optimizing somatic embryogenesis protocols. **【Methods】** The study focused on anti-browning and easy-browning embryogenic clones derived from the same half-sibling family of slash pine (*Pinus elliottii*). Embryogenic callus from both clones were sampled on 0, 14, and 28 days after subculture (DAS). Full-length transcriptome and second-generation transcriptome sequencing analyses were conducted, and liquid chromatography-tandem mass spectrometry (LC-MS/MS) was employed for targeted metabolomic analysis of flavonoids and/or phenolic compounds. Through multi-omics association analysis and co-expression analysis, important genes and regulatory networks related to the embryogenic callus browning were identified. **【Results】** Expression profile analysis based on 63,334 full-length transcripts obtained from three generations of sequencing (with an annotation rate of 97.27%) indicated that while the anti-browning clone exhibited rapid growth, only 17 differentially expressed genes (DEGs) were found between 0 and 14 DAS. In contrast, there were over 13,710 DEGs between the two clones. The DEGs between the two clones exhibited divergent expression trends, with a key differential time point at 14 DAS. Genes that were upregulated earlier on 14 DAS in the easy-browning embryogenic callus were similar to those that did not upregulate until 28 DAS in the anti-browning callus. These genes were associated with Gene Ontology (GO) terms and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways related to flavonoid biosynthesis, stress responses, and phenylpropanoid biosynthesis. Exceeding the subculture period of 14 days, a significant number of genes showed downregulation in the embryogenic callus of both clones, associated with GO terms and KEGG pathways related to methylation, chromatin modification, and RNA modification. Further exploration through Weighted Correlation Network Analysis (WGCNA) revealed 14 co-expression modules, with genes in the lightcyan module significantly enriched in GO terms and KEGG pathways related to phenylpropanoid and flavonoid biosynthesis. A transcriptional regulatory network for browning was constructed based on this module, identifying 9 transcription factors, including WRKY6 and ERF4, along with 31 key genes. Targeted metabolomic analysis identified 61 flavonoid/phenolic compounds, most of which were found at higher levels in the easy-browning callus. Multi-omics association analysis indicated a significant accumulation of 4-hydroxycinnamic acid and naringenin in the easy-browning callus on 14 DAS, consistent with the expression patterns of related genes. High expressions of PePAL, PeCYP73A4, PeCHS, and PeCHI promoted the accumulation of these substances, while transcription factors PeWRKY6, PeERF4, and PeNAC098 may have upregulated these genes, collectively contributing to

browning.【Conclusion】The anti-browning clones were able to maintain relative stability in internal gene expression during the 14-day subculture period, with significant changes occurring only after exceeding the subculture period. Conversely, the easy-browning clones exhibited many genes that underwent premature expression changes, leading to an early accumulation of phenolic compounds. Key genes such as PeWRKY6 and PeCYP73A4 were associated with the excessive accumulation of 4-hydroxycinnamic acid and naringenin in embryogenic callus, triggering browning.

Keywords: slash pine; embryogenic callus; browning; full-length transcriptome; targeted metabolomics of flavonoids/phenolic compounds