尾叶桉组培苗根系发育不同时期转录组分析

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摘要：研究尾叶桉 Eucalyptus urophylla 组培苗根系发育过程中不同时期基因的差异表达情况，为挖掘尾叶桉生根相关功能基因提供数据基础。以 1 个尾叶桉组培无性系（E）为材料，根据根系发育过程中基部形态变化选取 0d（E0），1d（E1），4d（E4）和 6d（E6）基部 2 mm 材料进行转录组测序分析，运用基因差异表达分析、GO 分析和 KEGG 分析，获得促进尾叶桉根系发育的候选功能基因。共获得 7944 个差异表达基因（DEGs），其中，E1（根系发育 1d）与对照（E0）相比 DEGs 数量最多，共 5902 个；其次为 E4 vs E1，共 2840 个 DEGs；E4 vs E1 差异表达基因最少，仅为 1094 个。GO 分析显示，转移至 IBA 培养基上 1d 后，DEGs 显著富集到代谢过程、细胞过程及生物学调控过程中。KEGG 分析显示，E1 vs E0 的 DEGs 显著富集在核糖体、谷胱甘肽代谢及苯丙烷生物合成途径中；E4 vs E1 的 DEGs 显著富集在其他次级代谢物的生物合成途径及折叠、分类和降解途径；E6 vs E4 的 DEGs 显著在苯丙烷类生物合成、次生代谢物的生物合成及角质、木栓素和蜡的生物合成,，具有催化功能和结合功能。进一步对不同时期上调 DEGs 进行 KEGG 分析，结果显示，E1vsE0 的上调 DEGs 主要富集在五个通路里，共有 40 个 DEGs 显著富集在植物激素信号转导路径（$P < 0.01$, $Q < 0.05$）中，主要包括生长素信号转导路径中的 AUX1、AUX/IAA、ARF、SAUR，细胞分裂素信号转导路径中的 CRE1、AHP、A-ARR 及赤霉素信号转导路径中 GID、DELLA、TF。E4 vs E1 的上调 DEGs 主要富集在内质网蛋白质加工、苯丙类生物合成以及谷胱甘肽代谢通路中；E6 vs E4 的上调 DEGs 主要富集在苯丙类生物合成、次生代谢物的生物合成和内质网蛋白质加工等路径中。E6vsE4 和 E4vsE1 中分别有 16 和 25 个 DEGs 上调并显著富集在苯丙烷生物合成信号转导路径中，这部分基因可能参与根系细胞壁的生物合成过程。E4vsE1 中共有 57 个 DEGs 上调并显著富集在内质网蛋白质加工过程中，这些基因的高表达可能为根系细胞膜的生物合成提供原料。尾叶桉无性系在转移至生根培养基上之后差异表达基因数量呈逐渐减少的趋势，说明在转移至 IBA 培养基上 1d 时是根系启动发育的关键期。生长素、赤霉素、细胞分裂素信号转导路径中的表达模式在启动期呈上升趋势，说明根系的启动受植物激素调控。在根系生长期，苯丙烷生物合成相关基因与内质网蛋白质加工相关基因呈上调趋势，说明这类基因可能参与根系细胞壁及细胞膜的生物合成。

Transcriptome analysis of root development of Eucalyptus urophylla tissue culture seedlings in different periods

Abstract: To study the differentially expressed genes (DEGs) in different periods during the root development of Eucalyptus urophylla tissue culture seedlings, providing data basis for mining functional genes related to rooting of Eucalyptus urophylla. A tissue culture clone (E) of E.urophylla was used as the material. According to the morphological changes of the base during root development, 2 mm materials at the base of 0d (E0), 1d (E1), 4d (E4) and 6d (E6) were selected for transcriptome sequencing analysis. Gene differential expression analysis, GO analysis and KEGG analysis were used to obtain candidate functional genes that promote root development of E.urophylla. A total of 7944 DEGs were obtained, among which E1 (root development 1d) had the highest number of DEGs compared with control (E0), with a total of 5902 DEGs. The second was E4 vs E1 with 2840 DEGs. E4 vs E1 had the least DEGs (1094). GO analysis showed that DEGs were significantly involved in metabolic processes, cellular

processes and biological regulation, with the function of catalytic activity and binding functions 1 day after transfer to IBA medium. KEGG analysis showed that DEGs of E1 vs E0 was significantly enriched in ribosome, glutathione metabolism and phenylpropanoid biosynthesis. DEGs of E4 vs E1 were significantly enriched in biosynthesis of other secondary metabolites and folding, sorting and degradation. The DEGs of E6 vs E4 were significantly enriched in phenylpropanoid biosynthesis, biosynthesis of secondary metabolites, and cutin, suberine and wax biosynthesis. Further KEGG analysis of up-regulated DEGs at different periods showed that E1vs E0 up-regulated DEGs were mainly enriched in five pathways, and a total of 40 DEGs were significantly enriched in plant hormone signal transduction ($P < 0.01$, $Q < 0.05$), mainly including AUX1, AUX/IAA, ARF, SAUR in auxin signal transduction pathway, CRE1, AHP, A-ARR in cytokinine signal transduction pathway, GID, DELLA, TF in gibberellin signal transduction pathway. The up-regulated DEGs of E4 vs. E1 were significantly enriched in protein processing in endoplasmic reticulum, phenylpropanoid biosynthesis and glutathione metabolism pathway. The up-regulated DEGs of E6 vs. E4 were significantly enriched in the pathways of phenylpropanoid biosynthesis, biosynthesis of secondary metabolites and protein processing in endoplasmic reticulum. In the libraries of E6 vs. E4 and E4 vs. E1, 16 and 25 DEGs were up-regulated and significantly enriched in phenylpropanoid biosynthesis, which may be involved in the biosynthesis process of root cell wall. A total of 57 DEGs of E4 vs. E1 were up-regulated and significantly enriched in endoplasmic reticulum protein processing. The high expression of these genes indicating that they may provide materials for the biosynthesis of root cell membranes. The number of DEGs of E. urophylla decreased gradually after being transferred to rooting medium, indicating that 1d on IBA medium was the key period for root initiation and development. The expression patterns of auxin, gibberellin and cytokinin signal transduction pathways showed an upward trend during the initiation period, indicating that the initiation of root system was regulated by plant hormones. In the root growth period, the genes related to phenylpropanoid biosynthesis and endoplasmic reticulum protein processing were up-regulated, indicating that these genes may be involved in the biosynthesis of root cell wall and cell membrane.