

榿树小孢子叶球快速伸长和开裂的分子调控

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摘要:【目的】挖掘榿树小孢子叶球发育过程中的快速伸长以及最终开裂的分子机制。【方法】在2月-4月每7天采集一次样品直至开裂,共采集8个时期的样品。对8个时期的小孢子叶球样品分别进行形态测量和转录组测序。通过WGCNA分解将转录组数据与形态特征相结合挖掘关键的模块和基因,接着通过榿树小孢子叶球的瞬时表达验证关键基因的功能,然后基于WGCNA相关性分析,并结合双荧光素酶试验、酵母单杂交试验等验证关键基因的调控。【结果】(1)通过对榿树小孢子叶球形态测量显示,小孢子叶球在XBZ1-XBZ5期间处于缓慢增长期,在XBZ5-XBZ8时期处于快速伸长期,并在XBZ8时期开始开裂。

(2)转录组数据和形态数据的WGCNA分析结果显示与快速伸长相关的有6个模块,其中turquoise模块具有最多的关联基因,这其中包括了WOX, PIF4等转录因子,并构建了转录因子调控网络。(3)在小孢子叶球内瞬时表达TgPIF4能显著促进小孢子叶球伸长。基于WGCNA的关联性分析挖掘TgPIF4潜在的靶基因,并结合双荧光素酶试验、酵母单杂交试验和荧光定量PCR发现TgPIF4能结合*TgTIR2*和*TgNADP-ME*的启动子调控它们的表达参与榿树小孢子球的快速伸长。(4)在小孢子叶球的开裂期,茉莉酸、茉莉酸甲酯、木质素和木质素单体显著上升,通过WGCNA分析关联到red模块最相关,模块内的phenylpropanoid pathway和linolenic acid metabolism分别有17和15个基因在开裂期显著上升。(5)通过双荧光素酶试验筛选到TgWRKY1和TgWRKY2能分别激活*TgPAL*和*TgJMT*启动子驱动的LUC活性,并且酵母单杂交试验结果显示TgWRKY1和TgWRKY2能分别和*TgPAL*启动子的P1段和*TgJMT*启动子的P2段直接结合。在小孢子叶球内分别瞬时表达TgWRKY1和TgWRKY2,能分别提高TgPAL和TgJMT的表达量,进而提高木质素和茉莉酸甲酯的含量,促进小孢子叶球提早开裂。【结论】本研究发现在快速伸长期TgPIF4能调控*TgTIR2*和*TgNADP-ME*的表达,促进榿树小孢子叶球的快速伸长。在开裂期TgWRKY1和TgWRKY2能分别调控*TgPAL*和*TgJMT*的表达,提高木质素和茉莉酸甲酯的含量,促进小孢子叶球的开裂。

关键词:榿树; 小孢子叶球; 快速伸长; 开裂; PIF4

Molecular regulation of *Torreya grandis* staminate strobilus rapid elongation and dehiscence

Abstract: **【Objective】** Exploring the molecular mechanisms of rapid elongation and final dehiscence during the development of staminate strobilus in *Torreya grandis*. **【Method】** From February to April, we collected samples every 7 days for a total of 8 periods. Staminate strobilus samples at eight stages underwent morphological measurement and transcriptome sequencing. Key modules and genes are extracted by WGCNA analysis by fusing transcriptome data with morphological traits. Then, to confirm their function, the main genes' transient expression in the *T. torreya*'s staminate strobilus was utilised. Finally, the regulatory function of important genes was confirmed using WGCNA correlation analysis in conjunction with luciferase assays and yeast one hybrid. **【Result】** (1) *T. grandis*' staminate strobiles were measured for their shape, and it was discovered that they went through three stages of growth: gradual growth from XBZ1 to XBZ5, rapid elongation from XBZ5 to XBZ8, and dehiscence during XBZ8. (2) Six modules relevant to rapid elongation were identified by WGCNA analysis of the transcriptome and morphological data; the turquoise module had the highest number of connected genes, including transcription factors like WOX and PIF4, and it was used to create a transcription factor regulatory network. (3) When compared to the control, transient expression of TgPIF4 in staminate strobilus dramatically improved staminate strobilus elongation. Potential TgPIF4 target genes were discovered using WGCNA association analysis. TgPIF4 was discovered to bind to the promoters of *TgTIR2* and *TgNADP-ME* to regulate their expression and promoted the rapid elongation of staminate strobiles in *T. grandis*, according to the results of luciferase assays, yeast one hybrid and RT-PCR. (4) Jasmonic acid, methyl jasmonate, lignin, and lignin monomer levels increased considerably in the staminate strobilus during the dehiscence stage. They were most closely associated to the red module according to WGCNA analysis. At the dehiscence stage, 17 and 15 genes from the phenylpropanoid pathway and linolenic acid metabolism, respectively, increased considerably in the red module. (5) Through the dual luciferase assay, TgWRKY1 and TgWRKY2 were screened to activate the LUC activity driven by *TgPAL* and *TgJMT* promoters, respectively. Yeast one hybrid experiment findings also demonstrated that TgWRKY1 and TgWRKY2 could bind to P1 promoter of *TgPAL* and P2 promoter of *TgJMT*, respectively. Transient expression of TgWRKY1 and TgWRKY2 genes in staminate strobiles can respectively enhance the expression of *TgPAL* and *TgJMT* genes, resulting in an increase in the concentration of lignin and Methyl jasmonate. This increase in concentration can promote the dehiscence of staminate strobiles. **【Conclusion】** This study discovered that TgPIF4 can regulate the expression of *TgTIR2* and *TgNADP-ME* during the rapid elongation stage, promoting the rapid elongation of *T. grandis*' staminate strobilus. TgWRKY1 and TgWRKY2 can regulate the expression of *TgPAL* and *TgJMT* during the dehiscence stage, increase the concentration of lignin and methyl jasmonate, and promote the dehiscence of staminate strobilus in *T. grandis*.

Key words: *T. grandis*; staminate strobilus; rapid elongation; dehiscence; PIF4.