

代谢组与转录组联合分析揭示山核桃中褪黑素合成调控新机制

陈文超¹ 张佳琦¹ 郑杉¹ 王占旗² 徐川梅¹ 张启香¹ 吴家胜¹ 娄和强^{1*}

(1.浙江农林大学 杭州 311300; 2.湖州师范学院 湖州 313000)

摘要:【目的】解析山核桃中褪黑素合成通路,探究其合成调控分子机制。【方法】以山核桃三个不同发育时期的种仁为实验材料进行转录组与代谢组测定。生信分析挖掘山核桃中褪黑素合成代谢通路。通过进化树分析,蛋白同源性比对以及烟草亚细胞定位实验验证候选基因功能。进一步相关性分析挖掘褪黑素合成基因调控因子,使用双荧光素酶系统以及酵母单杂交系统对转录因子进行功能验证。【结果】代谢组数据表明山核桃中存在褪黑素及其合成代谢中间产物:色氨酸,色胺,5-羟色氨酸,血清, N-乙酰血清以及褪黑素;从转录组中筛选到褪黑素合成通路基因,最终确定 *CcTDC1*, *CcASMT1*, *CcCOMT1*, *CcSNAT1* 以及 *CcT5H1* 五个候选基因,烟草亚细胞定位发现 *CcTDC1*, *CcASMT1*, *CcCOMT1* 定位于细胞质和细胞核, *CcSNAT1* 定位于叶绿体, *CcT5H1* 定位于内质网;全转录组转录因子与褪黑素合成基因共表达分析发现,多数转录因子为激素响应因子;双荧光素酶实验结果显示 *CcEIN3* 可以调控 *CcTDC1* 的表达, *CcAZF2* 可以调控 *CcASMT1* 的表达,酵母单杂交实验进一步验证 *CcEIN3* 与 *CcAZF2* 可以结合 *CcTDC1* 和 *CcASMT1* 的启动子片段;对山核桃进行激素处理,通过 qRT-PCR 发现 *CcEIN3* 与 *CcAZF2* 能够响应乙烯和 ABA。

【结论】本研究鉴定了山核桃中褪黑素合成通路基因 *CcTDC1*, *CcASMT1*, *CcCOMT1*, *CcSNAT1* 以及 *CcT5H1*, 首次发现激素调控褪黑素合成的新机制,验证乙烯响应因子 *CcEIN3* 与 ABA 响应因子 *CcAZF2* 可以分别调控褪黑素合成基因 *CcTDC1* 和 *CcASMT1* 的表达从而影响褪黑素的合成,丰富了我们对褪黑素和其他植物激素之间相互作用的理解。

关键词: 山核桃;褪黑素;合成通路;新调控机制

Metabolite profiling and transcriptome analyses reveal novel regulatory mechanisms of melatonin biosynthesis in hickory

Abstract: 【Objective】 To analyze the synthesis pathway of melatonin in hickory and explore the regulatory mechanisms. 【Method】 The seed kernel of three different developmental periods of hickory was used for transcriptome and metabolome determination. The melatonin synthesis pathway was determined by bioinformatics analysis. The function of candidate genes were verified by evolutionary tree, protein homology alignment, and tobacco subcellular localization experiments. The transcription factors regulating melatonin synthesis were mined by correlation analysis and the dual-luciferase system as well as the yeast one-hybrid system was using for functional validation. 【Result】 The metabolome indicated the presence of melatonin and its intermediates in hickory: L-tryptophan, L-tryptamine, 5-hydroxytryptophan, serotonin, N-acetylserotonin and melatonin; Melatonin synthesis genes were selected from the transcriptome, *CcTDC1*, *CcASMT1*, *CcCOMT1*, *CcSNAT1* and *CcT5H1* were finally

identified as candidate functional genes, the subcellular localization showed that *CcTDC1*, *CcASMT1*, *CcCOMT1* were localized to the cytoplasm and nucleus, *CcSNAT1* was localized to the chloroplasts, *CcT5H1* was localized to the ER; Co-expression analysis of all transcription factors with melatonin synthesis genes found that most transcription factors were hormone-responsive factors; The results of the dual-luciferase assay showed that *CcEIN3* can regulate the expression of *CcTDC1*, *CcAZF2* can regulate *CcASMT1* expression, yeast one-hybrid experiments further verify that *CcEIN3* and *CcAZF2* can bind to the promoter of *CcTDC1* and *CcASMT1* respectively; Hormone treatment of hickory revealed *CcEIN3* and *CcAZF2* respond to ethylene and ABA. **【Conclusion】** This study identified the melatonin synthesis pathway in hickory, a new molecular mechanism was discovered that plant hormones can regulate melatonin synthesis, and verify that the ethylene response factor *CcEIN3* and the ABA response factor *CcAZF2* can regulate the expression of melatonin synthesis genes *CcTDC1* and *CcASMT1* to affect the synthesis of melatonin, which enriched our understanding of the interaction between melatonin and other phytohormones.

Key words: hickory; melatonin; synthesis pathway; new regulation mechanism