杨树 YABBY 转录因子家族的全基因组分析及 *PtoYABBY6* 和 *PtoYABBY7* 的功能研究

韩开元[1] 郝颖颖[1] 陈仲[1*]

（1.北京林业大学 北京市海淀区清华东路 35 号 100083）

摘 要: 【目的】鉴定杨树 YABBY 转录因子家族，揭示毛白杨 YABBY 转录因子成员 *PtoYABBY6* 和 *PtoYABBY7* 在营养生长和生殖生长过程中的功能，为解析毛白杨 YABBY 转录因子调控杨树开花以及侧生器官生长发育的分子机制奠定前期理论基础。【方法】对杨树 YABBY 转录因子家族进行系统的全基因组分析。参照课题组前期的研究，毛白杨花芽发育过程分为 8 个时期：时期 1. 成花诱导期；时期 2. 花原基形成期；时期 3.器官发生期；时期 4. 伸长期；时期 5. 孢子形成期；时期 6、 7. 休眠期；时期 8. 小孢子发生期。分别取毛白杨成熟叶片、及毛白杨无菌组培苗的根、茎、叶，另外在树冠外围采取八个发育关键时期的雌雄花芽为组织定量材料，进行基因表达模式的分析。并结合公开的 RNA-Seq 数据探讨了 *YABBY* 基因对激素和干旱胁迫响应的表达模式。在分析的基础上我们对“生殖 *YABBY* 基因”成员 *PtoYABBY6* 和 *PtoYABBY7* 进行了功能探究。【结果】杨树中共鉴定出 13 个候选 *YABBY* 基因，命名为 *PtrYABBY1-PtrYABBY13*，其编码序列长度分布在 498-657 范围内。 除 PtrYABBY2 之外，其余 PtrYABBY 蛋白均在 N 端存在完整的 C2C2 锌指结构；所有 PtrYABBY 蛋白的 C 端存在 YABBY 结构域。通过多物种氨基酸序列比对和系统进化分析将其划分为 5 个亚家族，这些亚家族成员具有保守的基因结构和基序。启动子分析发现多种顺式调控元件，结果表明 *YABBY* 基因可能参与植物发育以及对各种植物激素和非生物胁迫的响应。公开的 RNA-Seq 数据和实时定量 PCR 分析的表达表明，杨树 *YABBY* 基因在器官以及对激素和干旱胁迫的响应中具有不同的表达模式。随后，我们对雌花芽发育过程中显著表达的 *PtoYABBY6* 和 *PtoYABBY7* 进行了功能探究。过表达 *PtoYABBY6* 的转基因拟南芥在营养生长阶段叶面积变小，在生殖生长阶段会导致早花表型。RT-qPCR 结果表明细胞分裂素 A 型反应调节因子 *ARR5*、*ARR6* 和 *ARR7* 表达下调，细胞周期基因 *CYCB1;1* 和 *CYCD3;1* 表达下调，*TCP4* 表达上调，与开花相关的基因 *FT* 表达升高， *FLC* 表达降低。而过表达 *PtoYABBY7* 的转基因拟南芥叶片背面轴极性发生改变，并且会导致晚花的表型。RT-qPCR 结果表明开花途径整合子 *FT* 极显著下调， 与叶片远轴极性相关的 *FIL*、 *KAN* 基因表达上调，与叶片近轴极性相关的 *REV*、*PHV* 基因表达下调。【结论】在全基因组水平上鉴定了杨树 YABBY 转录因子家族成员，毛白杨 *PtoYABBY6* 和 *PtoYABBY7* 基因参与营养生长和生殖生长过程，主要表现在叶片发育和开花时间的改变，初步揭示了 *PtoYABBY6* 和 *PtoYABBY7* 基因功能。

关键词： 杨树；YABBY 转录因子；系统发育分析；营养生长；生殖生长

Genome-wide analysis of the YABBY transcription factor family in poplar and functional studies of *PtoYABBY6* and *PtoYABBY7*

Abstract: 【Objective】To identify the poplar YABBY transcription factor family and reveal the functions of poplar YABBY transcription factor members *PtoYABBY6* and *PtoYABBY7* in the process of vegetative and reproductive growth, in order to lay a theoretical foundation for analyzing the molecular mechanism of YABBY transcription factors in regulating the flowering and lateral organ growth and development of poplar. 【Method】 A systematic genome-wide analysis of the poplar YABBY transcription factor family was carried out. Referring to the previous research of the group, the development of poplar flower buds was divided into eight periods: Period 1. flower induction; Period 2. floral primordium formation; Period 3. organogenesis; Period 4. elongation; Period

5. spore formation; Periods 6 and 7. dormancy; and Period 8. microsporogenesis. Mature leaves of *Populus tomentosa*, and roots, stems and leaves of *Populus tomentosa* aseptic group-cultivated seedlings were taken respectively, in addition, eight male and female flower buds at critical periods of development were taken at the periphery of the canopy as the tissue quantitative materials for the analysis of gene expression patterns. We also explored the expression pattern of *YABBY* gene in response to hormone and drought stress in combination with publicly available RNA-Seq data. On the basis of this analysis, we investigated the functions of *PtoYABBY6* and *PtoYABBY7*, members of the "reproductive *YABBY* genes".【Result】A total of 13 *YABBY* genes, named *PtrYABBY1-PtrYABBY13*, were identified in poplar, and their coding sequences were distributed in the range of 498-657 in length. Except for PtrYABBY2, all PtrYABBY proteins have a complete C_2C_2 zinc finger structure at the N-terminal end, and all PtrYABBY proteins have a YABBY structural domain at the C-terminal end. All PtrYABBY proteins have a YABBY domain at the C-terminus. They were classified into five subfamilies by multi-species amino acid sequence comparison and phylogenetic analysis, and the members of these subfamilies have conserved gene structures and motifs. Promoter analysis revealed multiple cis-regulatory elements, and the results suggest that the *YABBY* gene may be involved in plant development and response to various phytohormones and abiotic stresses. Publicly available RNA-Seq data and expression analyzed by real-time quantitative PCR indicated that poplar *YABBY* genes have different expression patterns in organs and in response to hormones and drought stress. Subsequently, we functionally explored *PtoYABBY6* and *PtoYABBY7*, which are significantly expressed during female bud development. Transgenic Arabidopsis overexpressing *PtoYABBY6* had smaller leaf area during the nutrient growth stage and resulted in an early flowering phenotype during the reproductive growth stage.RT-qPCR results showed down-regulation of the expression of the cytokinin A-type response regulators *ARR5*, *ARR6*, and *ARR7*, down-regulation of the expression of the cell cycle genes *CYCB1;1* and *CYCD3;1*, and up-regulation of the expression of *TCP4*, and up-regulation of the flowering-related genes related to flowering, *FT* expression was elevated, and *FLC* expression was decreased. The transgenic Arabidopsis leaves overexpressing *PtoYABBY7* had altered abaxial polarity and resulted in a late-flowering phenotype. RT-qPCR results showed that the integrator of the flowering pathway *FT* was significantly down-regulated, and the expression of *FIL* and *KAN* genes, which were related to the distal polarity of leaves, were up-regulated, while the expression of *REV* and *PHV* genes, which were related to the proximal polarity of leaves, were down-regulated.【Conclusion】Members of the poplar YABBY transcription factor family were identified at the genome-wide level. The poplar *PtoYABBY6* and *PtoYABBY7* genes are involved in the processes of vegetative and reproductive growth, which are mainly manifested in the alteration of leaf development and flowering time, and the functions of the *PtoYABBY6* and *PtoYABBY7* genes were preliminarily revealed.

Key words: Populus; YABBY transcription factor; Phylogenetic analysis; Nutritional growth; Reproductive growth