Physiological Characteristics and Transcriptome Analysis of Mixed Catkins Differentiation of *Castanea mollissima*

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Abstract

Castanea mollissima is an important monoecious fruit crop with high economic and ecological value in China. It has unisexual catkins and mixed catkins, in which the mixed catkins can differentiate into male and female flowers. As we know, chestnut has an imbalance of the ratio of male and female flowers, contributing to the low yield of this species. However, the mechanisms involved in the development of mixed catkins are still not clear.

In this paper, the mixed catkins development of *Castanea mollissima* 'Tanqiao' were investigated by the microscope method. We measured the eight types of hormones by the high performance liquid chromatography and enzyme-linked immunosorbent assay during its differentiation. Futhermore, we carried out RNA-Seq assays aimed at identifying differentially expressed genes responsible for male and female flower development in *C. mollissima*. The results showed that female flower differentiation was divided into 8 stages, including flower cluster bract primordium differentiation stage, flower cluster primordium differentiation stage (B0), sepal primordium differentiation stage, stamen primordium differentiation stage (B1), pistil primordium differentiation stage (F1), pistil development stage (F2), flowering stage (F3) and ovary formation stage (F4). Compared to the female flower, the male flower could be divided into 9 stages, including flower cluster bract primordium differentiation stage, flower cluster primordium differentiation stage, sepal primordium differentiation stage, stamen primordium differentiation stage(M1), pistil primordium differentiation stage, stamen elongation stage, anther formation stage (M2), anther development stage (M3) and flowering stage (M4).

The contents of jasmonic acid (JA) and zeatin (ZT) in female flowers were higher than those in male flowers from F1 to F4. The content of gibberellin (GA_3) in male flowers began to increase exponentially in the F1 stage, and the highest content in the M4 stage was more than 10,000 ng/g. The contents of salicylic acid (SA) and abscisic acid (ABA) in male flowers were significantly higher than those in female flowers. In the M3 stage, the SA and ABA content reached the highest value of 329.56 ng/g and 1216.56 ng/g, respectively. These results suggested that the high contents of SA, GA and ABA promoted male flowers development, while the dynamic changes of ZT and JA promoted female flowers morphogenesis.

A total of 34,053 unigenes were obtained, and 24,861 unigenes were successfully annotated against the public database. Among them, 971 genes were differentially expressed in both male and female flowers at four stages. KEGG enrichment analysis

revealed that the most representative pathway was the plant hormone synthesis and transduction. Thus, we excavated 20 highly expressed phytohormone-related genes, the expression of 9 genes were higher in the female flower and 11 genes were higher in the male flower, respectively. These findings indicated that JA synthesis gene *AOS3*, the ZT-related gene *CKX3*, and *UGTK4/5* were crucial genes in the development of female flower regulation. Similarly, the GA biosynthesis-related genes *$GA_3OX1/2$*, ABA synthesis pathway genes *CYP707A1/2*, and SA signaling gene *TGA10* were the key genes in regulating male flower development. Besides, transcription factors played an important role in flower development, such as *KUA1*, *AMS*, *NAC056*, *AIL1*, and *MYB26*, which were highly up-regulated during flower development. The weighted gene co-expression network analysis revealed that Zinc finger protein transcription factor *IDD7* was the pivotal factor in regulating female flower development. These genes would be useful for understanding mixed catkins development in *C.mollissima*, and help researchers to regulate the mixed catkins through plant biotechnology in future.

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