

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

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Introduction

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. Thus, revealing the molecular mechanism of *C. mollissima* 'Tanqiao' mixed catkins differentiation is of great significance for improving its fruit yield.



Objective

- Clarify the cytological process of female and male flower development of *C. mollissima* 'Tanqiao'.
- Explore the change characteristics of hormone content during the development of male and female flowers of *C. mollissima* 'Tanqiao'.
- Screen several genes related to the differentiation of male and female flowers in the mixed catkins of *C. mollissima* 'Tanqiao'.

Materials and Methods

➢ Materials

Different stages of male and female flowers in *C. mollissima* 'Tanqiao' as shown in Fig. 1 and Fig. 2.

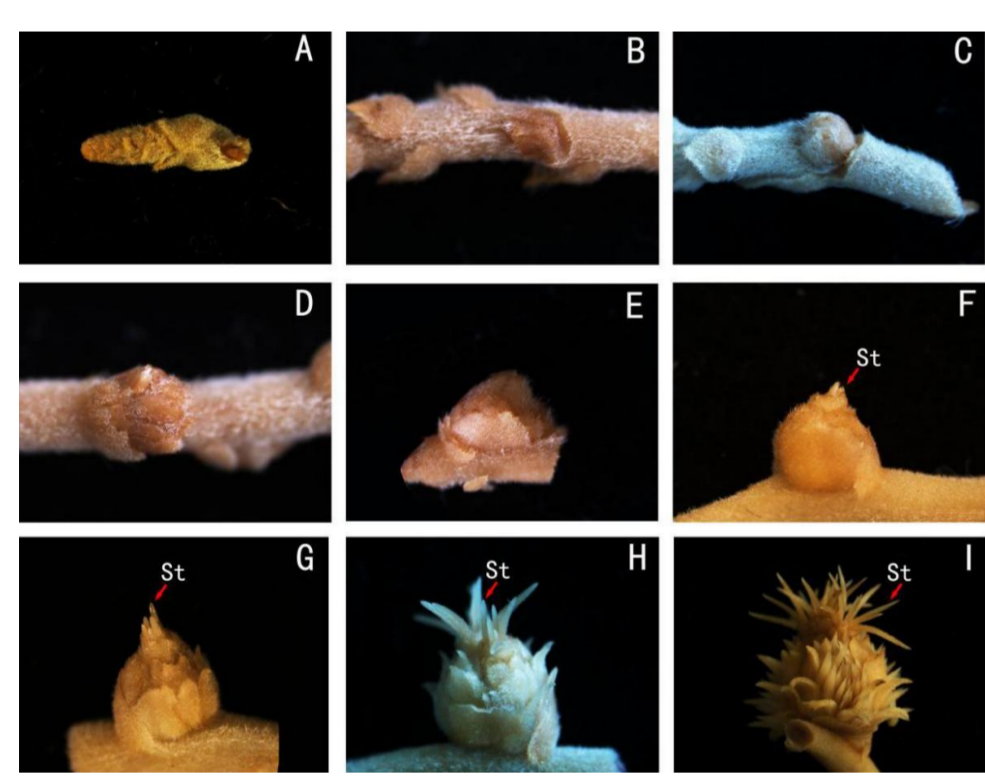


Fig.1 Female flower material of different stage

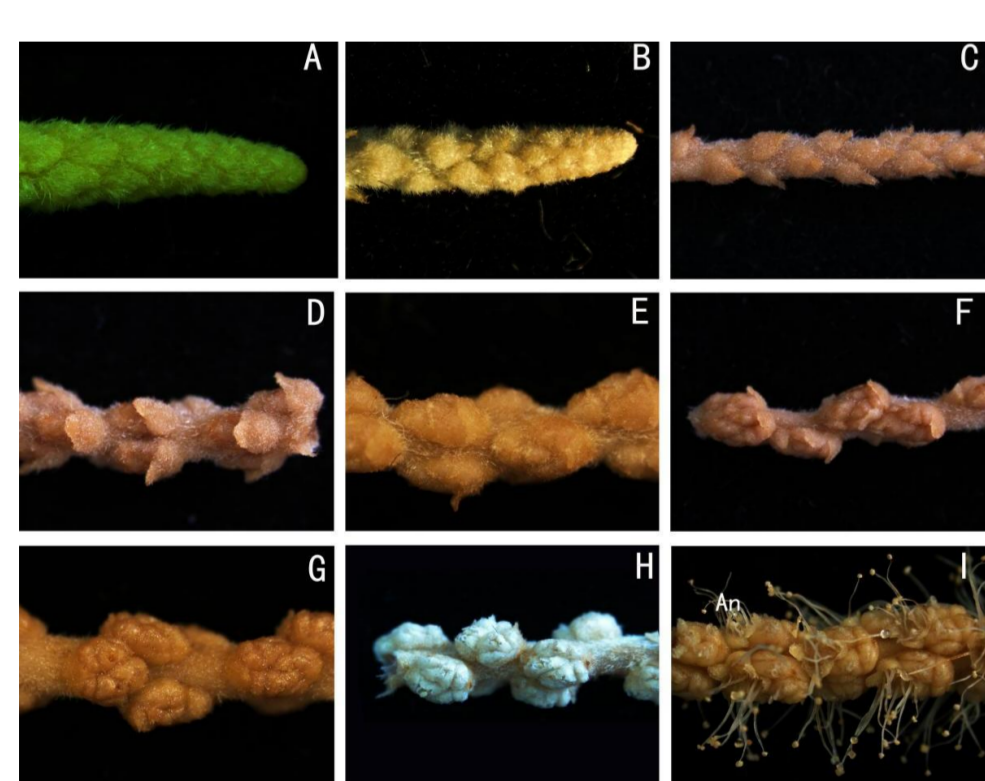


Fig.2 Male flower material of different stage

➢ Methods

The development of *C. mollissima* 'Tanqiao' mixed catkins 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. Furthermore, we carried out RNA-Seq assays aimed at identifying differentially expressed genes responsible for male and female flower development in *C. mollissima*.

Result

- The female flower differentiation of *C. mollissima* 'Tanqiao' was divided into 8 stages (Fig.3A), and the male flower could be divided into 9 stages (Fig.3B).
- The development of male and female flowers of *C. mollissima* 'Tanqiao' is closely related to plant hormone synthesis and transduction. The high contents of Salicylic acid (SA), gibberellin (GA₃) and abscisic acid (ABA) promoted male flowers development (Fig.4A, B, C), while the dynamic changes of Zeatin (ZT) and jasmonic acid (JA) promoted female flowers morphogenesis (Fig.4D, E).
- The JA synthesis gene *AOS3*, the ZT-related gene *CKX3*, and *UGTK4/5* were crucial genes in the development of female flower regulation. The GA biosynthesis-related genes *GA₃OX1*, *GA₂OX2*, ABA synthesis pathway genes *CYP707A1/2*, and SA signaling gene *TGA10* were the key genes in regulating male flower development (Fig.5).
- Through WGCNA analysis, 10 genes were excavated, among which *IDD7* gene may be the key gene for female flower development of *C. mollissima* 'Tanqiao' (Fig.6).

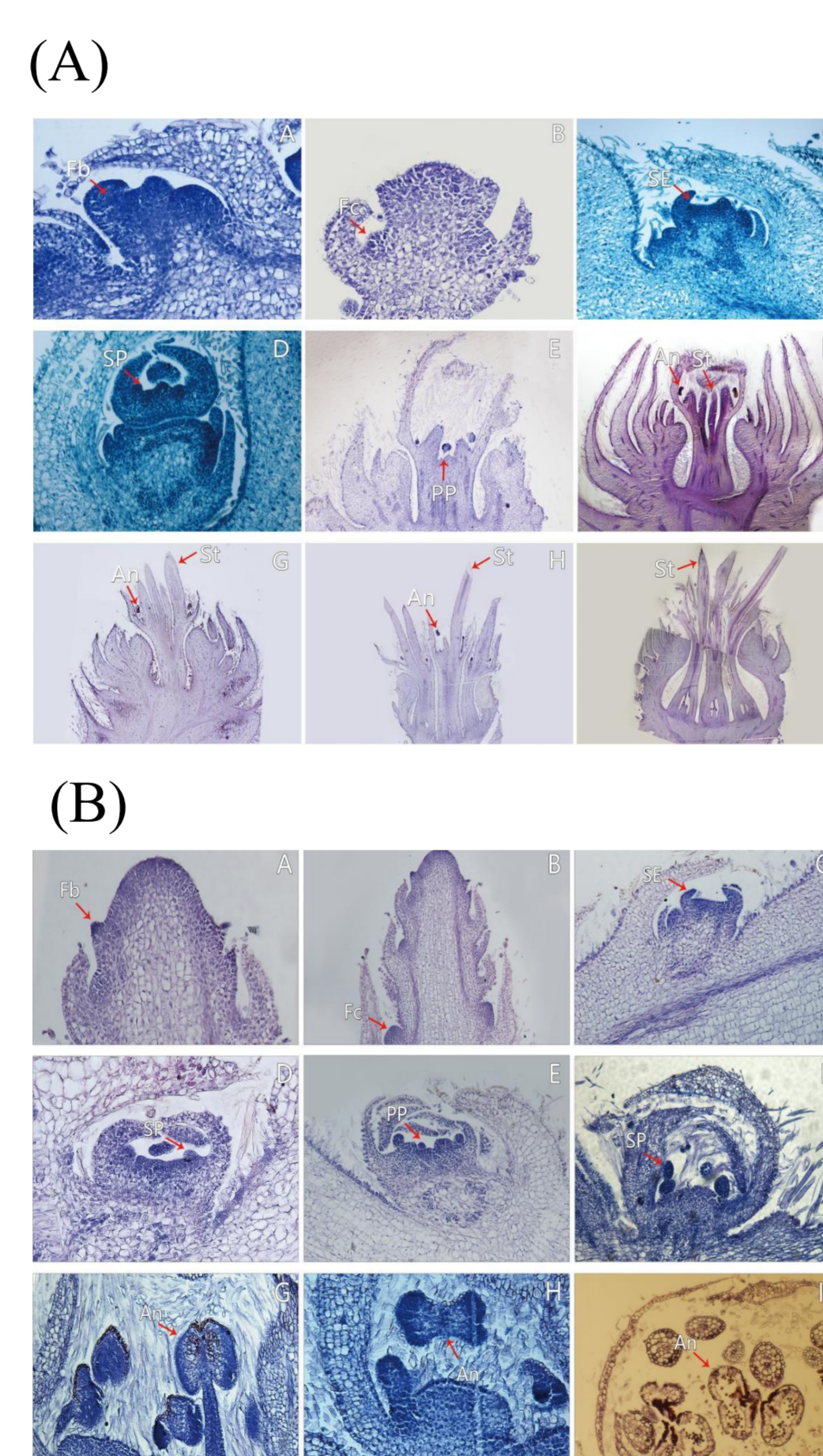


Fig.3 Cytological structure of the differentiation process of female(A) and male(B) flowers

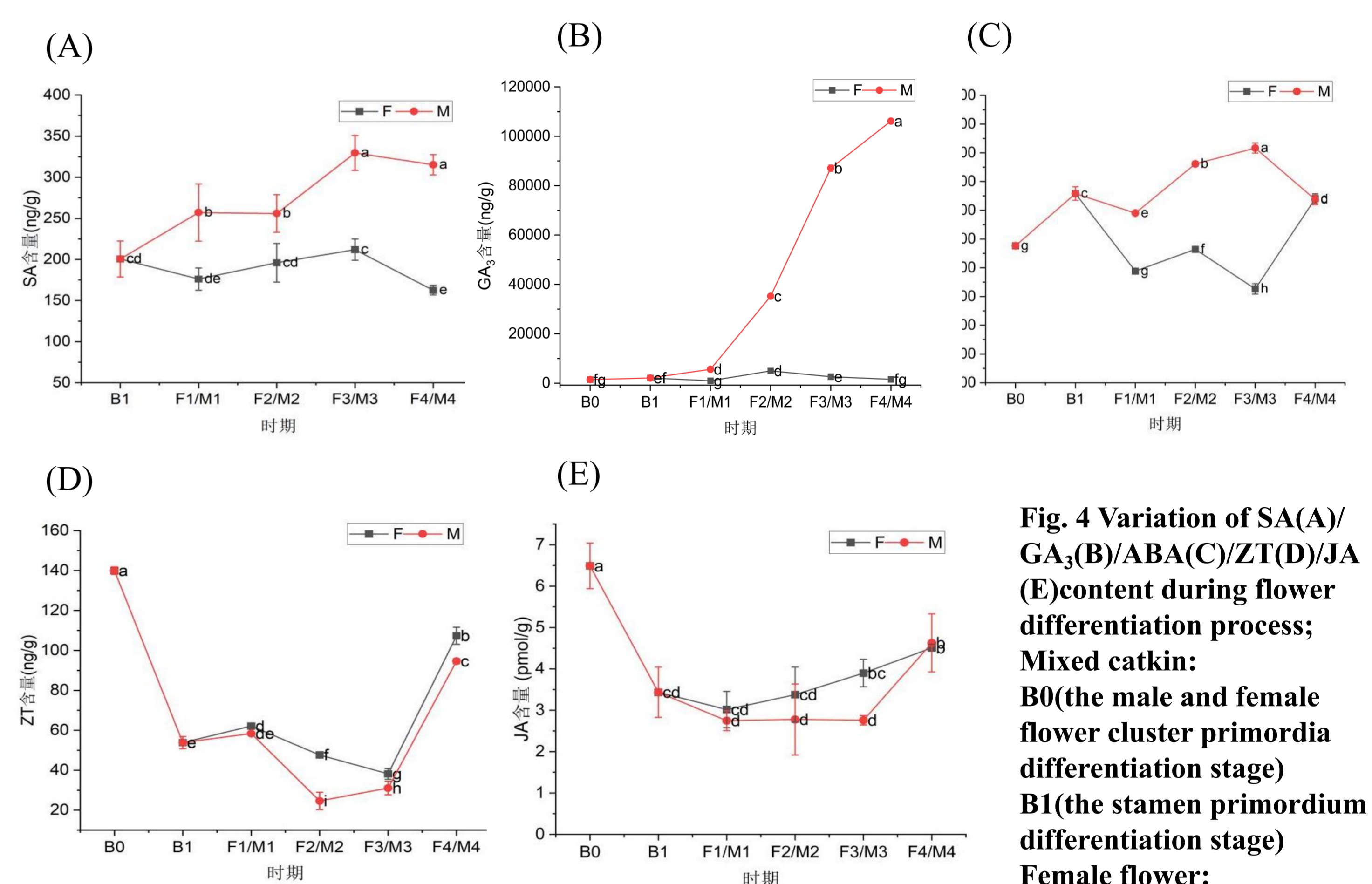


Fig. 4 Variation of SA(A)/GA₃(B)/ABA(C)/ZT(D)/JA(E) content during flower differentiation process; Mixed catkin: B0(the male and female flower cluster primordia differentiation stage) B1(the stamen primordium differentiation stage) Female flower: F1(Basal differentiation stage) F2(pistil elongation stage) F3(flowering stage) F4(ovary formation stage) Male flower: M1(stamen primordia differentiation stage) M2(anther development stage I) M3(anther development stage II) M4(male flower flowering stage).

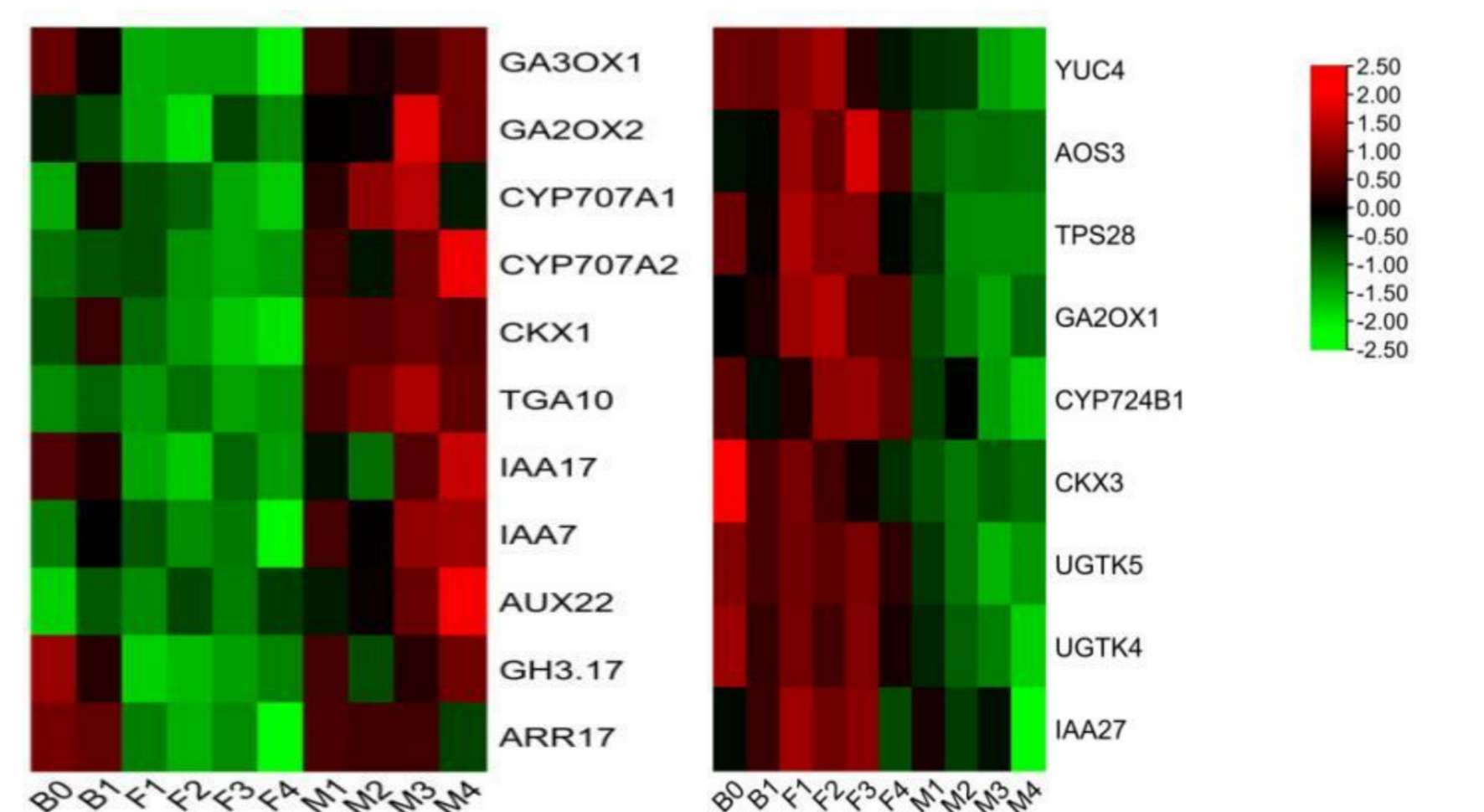


Fig.5 Heat map of differential gene expression trend of hormone synthesis and signal transduction genes related to flower development

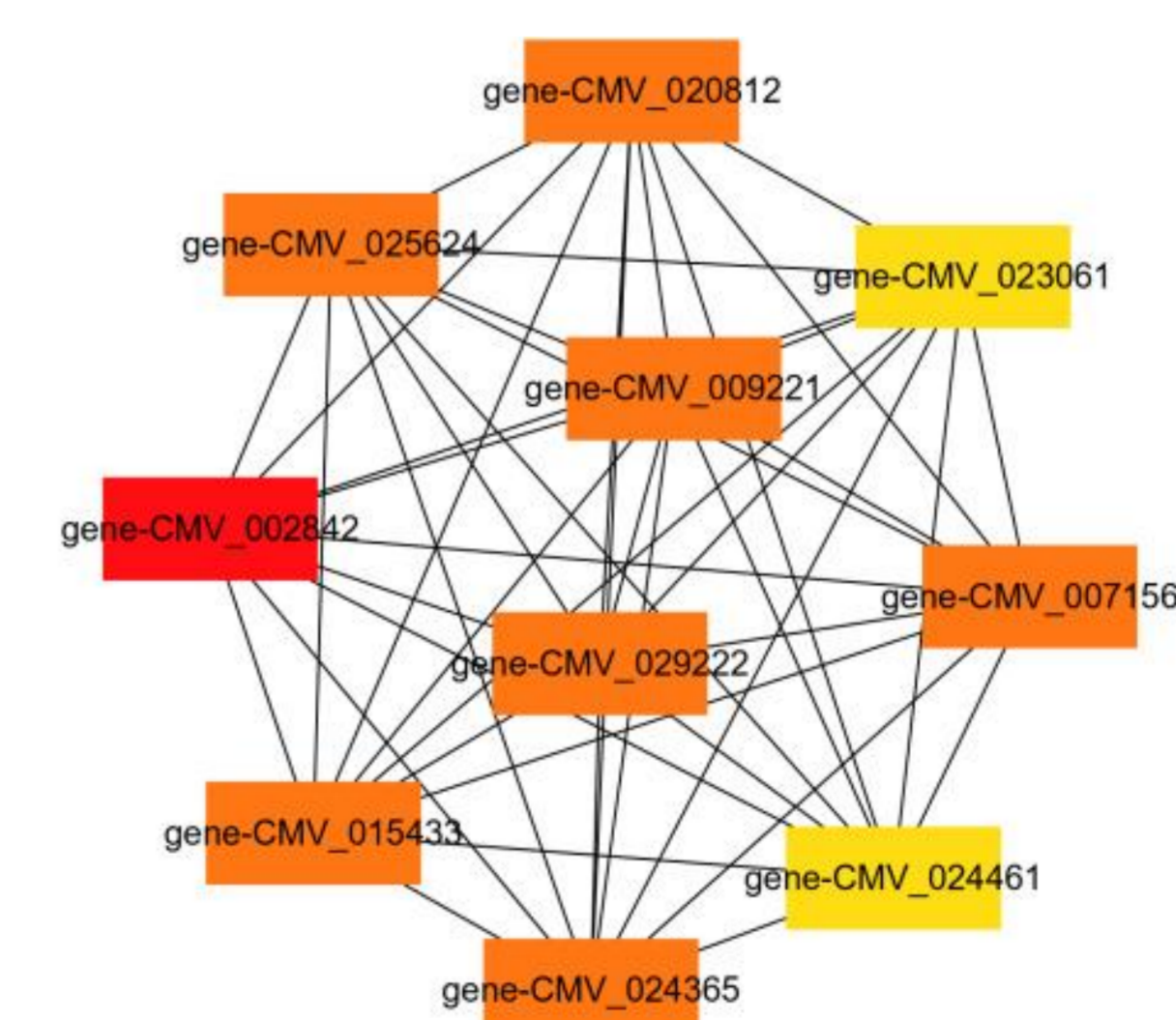


Fig.6 Core gene coexpression network; CMV_002842 (*IDD7*).

Acknowledgements

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Conclusion

- Our experimental results indicated plant hormone SA, GA played an major role in the process of male flower development. ZT and JA yet played an important role in the process of female flower development.
- Zinc-finger protein transcription factor *IDD7* and phytohormone-related genes *CYP707A1/2*, *TGA10*, *AOS3*, *UGTK4/5*, *GA₃OX1*, *GA₂OX2*, *CKX3* were high related to the differentiation of male and female flowers in the mixed catkins of *C. mollissima* 'Tanqiao'.