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Research Article

Changes of Endogenous Hormone Levels during Ovary Growth and Development after Self-and Cross-pollination of Chestnut (*Castanea*) Cultivar 'Yanshanzaofeng'

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Abstract: Chinese chestnut trees spread all over the world produce nut and chestnuts that have considerable economical value. Ovary development is an important step in nut production. Normal ovary development probably requires the coordinated action of plant hormones. Endogenous hormone status of chestnut tree, particularly IAA, IBA, GA_3 and ZT, influences chestnut ovary development as well as nut yield. Changes in endogenous hormones during ovary development in chestnut cultivar 'Yanshanzaofeng' have not been thoroughly investigated. In this study, cultivar 'Yanshanzaofeng' and 'Dabanhong' were used as materials. About 50~100 pollinated female inflorescences were picked every five days (5, 10, 15, 20, 25, 30, 35, 40, 45 and 50 days, respectively) to determine the IAA, ABA, GA_3 and ZT levels by HPLC. The results showed that there were differences between self-and cross-pollination endogenous hormone change rules during the development of ovary. The IAA and GA_3 contents in ovary of self-pollination were significantly higher than cross-pollination during pollination and fertilization period, which suggested that lower IAA and GA_3 acts as a positive regulator of fertilization. In the first 40 DAP, ZT level in ovaries of self-and cross-pollination almost have the same change rule, but changed dramatically in the last 10 DAP, which suggested that ZT serves as a positive regulator for young fruit. ABA level in ovary of self-pollination was significantly higher than cross-pollination in the first 30 DAP. It seems that high ABA level was probably related with self-incompatibility in chestnut. The findings of this study could lay the scientific basis for spraying GA_3, IAA and ZT during ovary development and may have the potential to improve nut yield.

Keywords: *Castanea mollissima* blume, endogenous hormone, ovary, pollination

INTRODUCTION

Chestnut is one of the most important food and timber and have been cultivated for thousands of years in the Northerm Hemisphere (Payne *et al*., 1990). China is considered as a gene centre for the genus *Castanea* (Shi and Stősser, 2005). In China, this species is largely distributed in Jilin, Hebei, Shandong, Sichuan, Hubei, Anhui, Jiangsu and Yunnan Provinces and Hainan Island and is also found in Taiwan Island (Feng *et al*., 2013a). Chinese chestnut production is particularly important source of income in rural regions, especially in Yan Mountain Region of Northern China (Wang *et al*., 2012). Moreover, Chinese chestnut plays an important role in keeping soil and greening barren hills (Martín *et al*., 2012; Feng *et al*., 2014).

Chestnut is a monoecious species and there are staminate and pistillate flowers on the current season's growth (Bounous and Marinoni, 2005). The chestnut self-fertilization rate was less than 1% (Mckay, 1942). Self-pollination can seriously reduce chestnut production (Guo *et al*., 2013; Lyu *et al*., 2013). Chestnuts exhibits a set of floral features that would tend to promote out crossing (Klinac *et al*., 1995). Cross-pollination with other cultivars have to be provided to ensure satisfactory yields (Shi and Stősser, 2005). Ovary development is induced after pollination and successful fertilization of the egg cells in the ovules. Plant hormones are considered to be important mediators of the fruit developmental signal after pollination (Vriezen *et al*., 2008). Normal ovary development probably requires the coordinated action of plant hormones. Hormone imbalance often causes the flower and fruit abscission. Endogenous hormone status of chestnut tree, particularly IAA, IBA, GA_3 and ZT, influences chestnut fruit development as well as nut yield.

At present, the dynamic change rules of endogenous hormone levels during the process of fruit development have been investigated by many scholars.

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Zhou *et al*. (1999, 2000a, b) reported the relationship between endogenous hormone levels and fruit diameter growth of *C. mollissima* 'Bokeyouli', speculated that the endogenous hormone level and balance in ovary made the differences in the fruit growth process of empty shell and normal chestnut. Zhou *et al*. (2002) observed the contents of endogenous hormones and nutrients of the ovary, suggested that the declining of endogenous GA_{1+3}, iPAs and the ascending of endogenous ABA lead to the low levels of reducing sugar, starch, protein, amino acid, phosphorus and potassium in ovary, which consequently cause an empty shell. Liu *et al*. (2004) investigated the relationship between calcium and endogenous hormones in ovary of pear; found that IAA and GA_3 could probably take part in the process of calcium transportation from tree to ovary during the pre-and post-fertilization stage. Zeng *et al*. (2006) noted the dynamic change of endogenous hormones during the chestnut embryonic development in Hunan, found that iPAs ascending and ABA decreased after blossom which was beneficial to the normal growth of chestnut embryo. However, there is a little available information about the dynamic change rules of endogenous hormones in cultivar 'Yanshanzaofeng'. *C. mollissima* 'Yanshanzaofeng' was first selected in Qianxi County and became an populization varieties since 1989 in Yan mountain region. It was famous for ripening in the early September and developed very quickly for it covered about 45,000 ha in Qianxi County (Feng *et al*., 2013b). Guo *et al*. (2013) descried the change rules of endogenous hormones and polyamine levels of chestnut ovary of 'Yanshanzaofeng', but it only limited to pollination and fertilization period , about 2 weeks after pollination (Feng *et al*., 2014). Thus, in this study, through the treatment of self- and cross-pollination, 4 hormone levels were investigated in order to observe endogenous hormones levels during the growth and development of ovaries, which make reasonable management by plant growth regulators during ovary development and may have the potential to improve fruit yield.

MATERIALS AND METHODS

Plant material and treatment: Twelve-year-old trees of the Chinese chestnut (*C. mollissima* 'Yanshanzaofeng') growing at Hanerzhuang village, Hebei province (118°12′E, 40°21′N). Experiment trees in the orchard with moderate tree vigor have no diseases and pests, with the planting spacing of 4×6 m. The management of chestnut orchard were followed under the standard fertilization practices and supplemental irrigation condition (Guo and Zou, 2014).

Self-pollination with *C. mollissima* 'Yanshanzaofeng'. For cross-pollination (*C. mollissima* 'Yanshanzaofeng' ×*C. mollissima* 'Dabanhong'), *C. mollissima* 'Dabanhong' was used as pollen source. Before blossom (1^{st} to 3^{rd} June, 2013), bisexual inflorescences were covered parchment paper bags (25×30 cm) to prevent fertilization and the male inflorescences were emasculated. After pollination (16^{th} to 19^{th} June, 2013), the inflorescences were covered with parchment paper bags and marked to avoid the disturb of other pollen. The bags would not demolished until the end of June that all the chestnut male flowers were withered around the study site.

Sampling and pretreatment: Both combinations pollinated over 1000 female inflorescences. About 50~100 female inflorescences were picked every five days (5, 10, 15, 20, 25, 30, 35, 40, 45 and 50 days) since pollinated till the embryo maturation at the beginning of August (Feng *et al*., 2014). All samples were kept in ice-box and brought to the laboratory of Chestnut Research and Development center of Qianxi. Stripped the burr quickly, the ovaries were bagged with silver paper, pre-cooling at -20°C for 6 h, then processing 24 h by freezing dryer (FD-1B-50, Biocool, Beijing, China) and then stored at -70°C for further analysis.

Extract endogenous hormones: The methods used for the extraction and analysis of endogenous hormone followed the protocol of Lyu *et al*. (2013) with minor modifications. IAA, GA_3, ABA and ZT extraction method: Accurate 0.200 g lyophilized plant sample was quickly grinded into homogenates under ice bath, immersed in 10 mL cold 80 % (v/v) methanol at 4°C for 12~24 h. Particulates were removed by centrifugation at 12000 rpm for 15 min at 4°C and the supernatant was collected. Added 1~2 drops of ammonia, total volume was reduced to a half by a rotary evaporator, then added 1~2 mL of ultrapure water, for IAA, GA_3 and ABA, it was then adjusted to pH 2.5~3.0 with 2 N HCl and extracted with equal volume of ethyl acetate for 3 times and transferred to an Eppendorf tube. Added 1~2 drops of ammonia and dried at 40°C, then stored at -20°C. For ZT, it was then adjusted to pH 7.5~8.0 with 2 N NaOH and extracted with equal volume of saturated N-butanol for 3 times and transferred to an Eppendorf tube. Added 1~2 drops of ammonia and dried at 60°C, then stored at -20°C. The stored sample was dissolved in 1.0 mL methanol and ultrapure water (1:1, v/v, ultrapure water pH = 7.0) and then line-loaded onto a 0.22 mm thick filtration membranes (Jinteng, Tianjin, China). Finally, the purified sample was got.

Determined by HPLC: IAA, ABA, GA_3 and ZT were determined by Agilent 1100 HPLC equipped with a capillary column (ZORBAX Eclipse×DB-C18, Analytical 4.6×250 mm 5-Micron). Samples (5 μL) were injected into the column at 30°C, the flow rate was 1 mL/min. For the detection of IAA, ABA and

GA_3, the mobile phase was methanol and glacial acetic nm, while for ZT, the mobile phase was methanol and ultrapure water (40:60, v/v) and the detection wavelength was 267 nm. All samples of the same index were determined for three times.

Data analysis: Statistical analysis was performed using SPSS 18.0 and EXCEL 2003 software, graphics production by origin Pro 8.5 software.

RESULTS

Dynamic change of IAA level during fruit growth and development with self-and cross-pollination: The dynamic change of IAA level in ovary was depicted in Fig. 1. The IAA level in ovary of self-pollination was significantly higher than cross-pollination in the first 25 DAP. After self-pollination, IAA level in ovary has two peak values, one was on 15 DAP and the other was in 35 DAP, the values were acid (40:60, v/v) and the detection wavelength was 260 117.06 and 85.28 μg/g FW, respectively. IAA level in ovary of cross-pollination has three peak values; they were on 10, 30 and 40 DAP, respectively.

Dynamic change of ABA level during fruit growth and development with self-and cross-pollination: The dynamic change of ABA level in ovary was depicted in Fig. 2. In the first 30 DAP, the ABA level in ovary of self-pollination was significantly higher than cross-pollination, while 35 to 50 DAP, was on the contrary. The ABA level in ovary of self-pollination was increased first and attained a peak value on 15 DAP with the content of 141.15 μg/g FW and decreased till 40 DAP, attained a valley value 56.70 μg/g FW and increased gradually to a stable level. The ABA level in ovary of cross-pollination attained the peak values of 109.75, 99.87 and 95.87 μg/g FW on 20, 30 and 50 DAP, respectively.

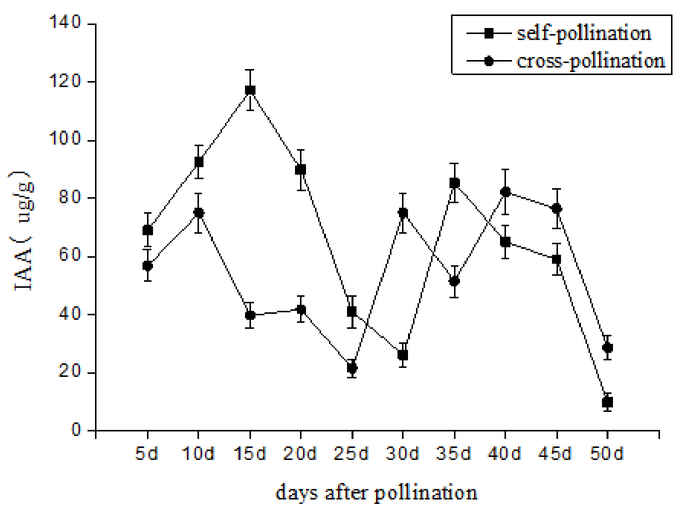

Fig. 1: IAA level in the ovary of self-and cross-pollination

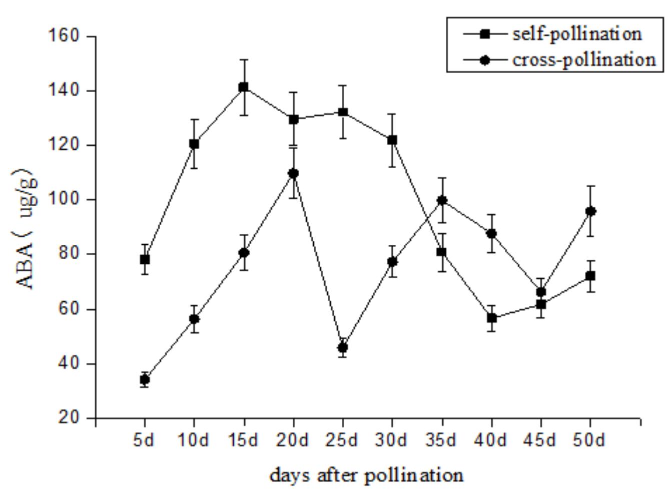

Fig. 2: ABA level in the ovary of self-and cross-pollination

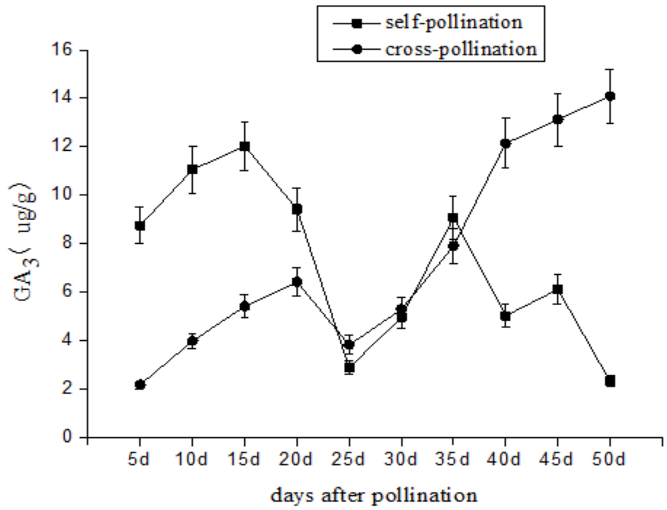

Fig. 3: GA_3 level in the ovary of self-and cross-pollination

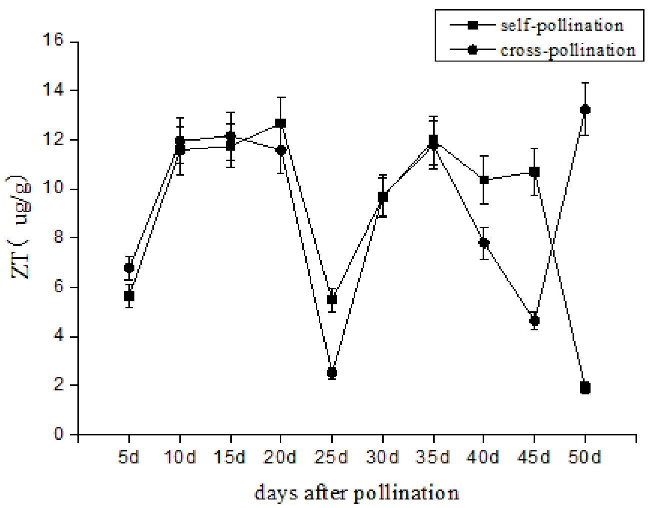

Fig. 4: ZT level in the ovary of self- and cross-pollination

Dynamic change of GA_3 level during fruit growth and development with self-and cross-pollination: The dynamic change of GA_3 level in ovary was depicted in Fig. 3. GA_3 level in ovary of self-pollination was significantly higher than cross-pollination in the first 20 DAP, while 40 to 50 DAP, was on the contrary. After self-pollination, GA_3 level in ovary attained peak values of 12.03, 9.08 and 6.12 μg/g FW on 15, 35 and 45 DAP, respectively. After cross-pollination, GA_3 level in ovary increased in the first 20 DAP and attained a peak value of 6.42 μg/g FW, then decreased, attained a valley value on 25 DAP, at last increased continuously and attained the highest value 14.10 μg/g FW on 50 DAP.

Dynamic change of ZT level during fruit growth and development with self-and cross-pollination: The dynamic change of ZT level in ovary was depicted in Fig. 4. In the first 40 DAP, ZT level in ovaries of self- and cross-pollination nearly have the same change rule, both ovaries of self-and cross-pollination attained a valley value on 25 DAP, which were 5.49 and 2.50 μg/g FW, respectively. On 45 DAP of cross-pollination, it attained the second valley value of 4.63 μg/g FW and then increased dramatically to get the highest value of 13.23 μg/g FW on 50 DAP. However, from 35 to 50 DAP of self-pollination, the ZT level decreased gradually and last attained the lowest value of 1.90 μg/g FW.

DISCUSSION

Endogenous hormones play an essential role in the procedures of pollen germination, pollen tube growth, fertilization and embryo development (Bing and Zhang, 2012). Here, we observed that the IAA and GA_3 contents in ovary of self-pollination were significantly higher than cross-pollination during pollination and fertilization period, which suggested that lower IAA and GA_3 acts as a positive regulator of fertilization. Nie and Liu (2002) also showed that pollination and fertilization promoted the activation of IAA, GA_3 and CTK, which was beneficial to the development of ovary and fruit.

High ABA level was a negative factor in the process of pollen germination, which could cause flower abscission of incompatibility pollination in poplar (*Populus* L.) (Zhang *et al*., 2014). ABA was found to inhibit the pollen germination in vitro of pear (*Pyrus serotina*) (Zhang *et al*., 2003). Similarly, It was obvious that ABA content of self-incompatible apple cultivars increased significantly after self-pollination 12~48 h (Xuemei *et al*., 2009). High ABA level was not only related to incompatibility pollination (Qi *et al*., 2007; Xiao-Ling *et al*., 2009), but also the key factor for plant programmed cell death (Li *et al*., 2010). In present paper, we observed that the ABA level in ovary of self-pollination was significantly higher than cross-pollination in the first 30 DAP. It was obvious that high ABA level was probably related with self-incompatibility. Similar observations were noted in *Lilium* (Xiao-Ling *et al*., 2009), *Malus* (Xuemei *et al*., 2009) and *Populus* (Zhang *et al*., 2014).

ZT can promote cell division and inhibit bisexual inflorescences procedure cell death in chestnut (Wang, 2012). Here, ZT level in ovary of self-pollination was significantly lower than cross-pollination during young fruit development, the results suggested that higher ZT maybe act as a positive regulator of young fruit.

CONCLUSION

The results indicate that there were differences between self-and cross-pollination endogenous hormone change rules during the development of ovary. The lower IAA and GA_3 have a decisive role during pollination and fertilization period, while higher ZT acts as a positive regulator for young fruit. High ABA level was probably related with self-incompatibility in chestnut. The characteristics of these endogenous hormones changes provide a basis information for spraying GA_3, IAA and ZT during ovary development and may have the potential to improve nut yield.

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ABBREVIATIONS

HPLC : High performance liquid chromatography
DAP : Days after pollination
IAA : Indole-3-acetic acid
ABA : Abscisic acid
GA_3 : Gibberellic acid
ZT : Trans-Zeatin

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