

Research Article

Effects of Different Pollination Treatments on Nutrition Changes of the Ovary in Chinese Chestnut (*Castanea mollissima* Blume)

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Abstract: Chinese chestnut (*Castanea mollissima* Blume) has noteworthy ecological, economic and cultural importance in the Northern Hemisphere. The low yield of chestnut often affect the economic efficiency. Ovary development is an important step in nut production. Changes in nutrient contents during ovary development in chestnut cultivar ‘Yanshanzaofeng’ have not been thoroughly investigated. In this study, cultivar ‘Yanshanzaofeng’ and ‘Dabanhong’ were used as material. About 50~100 pollinated female inflorescences were picked every five days (5, 10, 15, 20, 25, 30, 35, 40, 45, 50 days) to determine N, P, K, fat, total soluble sugar, crude protein and starch contents. The results indicated that the contents of total soluble sugar, starch and fat increased constantly in ovaries after self-and cross-pollination, but protein, N and K contents first increased in 20 DAP (day after pollination) and after that decreased in the stage of young fruit development. The changes of P has two peak values, one was in 40 DAP and the other was in 50 DAP. P and crude protein were not significantly after pollination treatments. However, N, sugar, starch, fat and K were significantly higher in cross-pollination treatment it seems that these nutrient has a decisive role during ovary development in chestnut. The characteristics of these nutrition changes provide a basis information for spraying N, P, K etc during ovary development and may have the potential to improve nut yield.

Keywords: *Castanea mollissima* blume, nutrition, ovary, pollination

INTRODUCTION

Chestnut is widely distributed in the northern hemisphere, including East Asia, North America and the Mediterranean countries, especially China (Engin, 2007; Serdar *et al.*, 2011). Chestnut is a deciduous tree that native to china, which belongs to the genus of *Castanea*. *Castanea mollissima* Blume is distributed in 24 provinces in China (Giancarlo and Daniela, 2005), from south to north, they are Taiwan, Hainan Island, Yunnan, Jiangsu, Hubei, Sichuan, Anhui, Jilin, Hebei, Shandong, Beijing, Tianjin and so on (Zou *et al.*, 2013a). It is one of important economic forest trees in China (Yuhe *et al.*, 2005). At present, chestnut forests cover an area of over 20×10^6 ha (Zou *et al.*, 2013a). In contrast to the majority of fruit nuts, chestnuts are characterized by low fat and protein contents but high carbohydrate and moisture content (Hernández Suárez *et al.*, 2012). Chinese chestnut has two major functions which are economic and ecological protection. It is not only the main economic source of income for farmers in the mountainous areas, but also plays an important role in greening barren hills, keeping water and soil

(Shi and StÖsser, 2005; Martín *et al.*, 2012; Zou *et al.*, 2014a).

Chestnut flowers are not self-compatible, so two trees are required for pollination. After pollination, the pollen germination on the stigma, while the pollen tube growth requires a large amount of nutrients, like mineral elements (Tian and Yuan, 2000; Guan *et al.*, 2000; Xiao and Peng, 2008; Li *et al.*, 2012; Jia and Zhang, 2012). The pollen tube grows down the style and into the embryo sac, where fertilization takes place. After this, a seed may develop along with the fruit. Nutrition plays an important role in the process of chestnut reproductive development, which influence the fruit set, fruit development and the formation of yield. At present, there were some published accounts of the nutrient contents change rule during the development of chestnut seeds. Zhou *et al.* (1994) reported that the content changes of carbohydrate, protein and amino acid in the process of development of *C. mollissima* ‘Ershuizao’. Du *et al.* (1995) described the contents of amino acid, reducing sugar, starch, mineral elements and the activity of the H_2O_2 in the empty and normal chestnut of *C. mollissima* ‘Ershuizao’. Zhou *et al.*

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(2000, 2002) investigated the changes and relationships among the main nutrient contents in ovary of *C. mollissima* 'Qingmaozao', he suggested that empty burr mainly due to the serious lack of sugar, starch, protein, amino acid, P and K nutrients in the ovary. Yet there is a little available information about the changes of main nutritional substances in the ovary with self- and cross-pollination of *C. mollissima* 'Yanshanzaofeng'. Thus, in this study is to determine the changes in nutrients in ovary of Chinese chestnut after self- and cross-pollination and provide basic information in relation to the seed production which may make an effective chemical measures to increase the yield in Hebei Province.

MATERIALS AND METHODS

Study site: The study takes place in Qianxi county, Hebei province, Northern China (118°12'E, 40°21'N). It has a warm semi-humid climate, with an annual average temperature of 10.9°C and an annual average precipitation of 744.7 mm, which is suitable for Chinese chestnut growth (Guo and Zou, 2014). The soil is rich, available N, P and K content are 75, 24 and 234 mg/kg, respectively (Li *et al.*, 2013).

Plantation characteristic: Test cultivar was 'Yanshanzaofeng', which was firstly selected from Yan Mountain Region and became an improved varieties in 1989. It was famous for ripening in the early September and developed quickly in Qianxi county (Zou *et al.*, 2013b). Plantations were installed in 2002, grafted after two years later and were then 11 years old in 2012 at the time of sampling. Plantations were routinely management, with a spacing of 4 m×6 m, average height of 3.6 m and average crown width of 4.1 m×4.3 m.

Sampling and measurement: Self-pollination with *C. mollissima* 'Yanshanzaofeng'. For cross-pollination (*C. mollissima* 'Yanshanzaofeng' × *C. mollissima* 'Dabanhong'), *C. mollissima* 'Dabanhong' was used as pollen source. A total of 2000 bisexual inflorescences, borne on 100 trees, were selected for the study. Every year in June 1st to June 3rd before blossom, bisexual inflorescences were covered parchment paper bags (25×30 cm) to prevent fertilization by external pollens, meanwhile, the inflorescences were emasculated. After pollination (June 11th to 14th, 2012 and June 16th to 19th, 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.

Both combinations pollinated 1000 female inflorescences. About 50~100 female inflorescences were picked every five days (5, 10, 15, 20, 25, 30, 35, 40, 45, 50 days) since pollinated till the embryo

maturation at the beginning of August. All samples were kept in ice-box and brought to the laboratory of Chestnut Research and Development center of Qianxi. Stripped the burr, all ovaries were steamed at 105°C for 30 minutes, then dried at 80°C to a constant weight. Grinded samples to pass a 40-mesh screen for nutrient analysis.

Samples were analyzed for N, P, K, fat, total soluble sugar, crude protein and starch. Mineral nutrients measurement: the stored samples (0.200 g) were digested by H₂SO₄-H₂O₂ nearly 2 h, diluted to 50 mL with double distilled water. Total N was determined by automatic kjeldahl procedure (Kjeltec Auto ANT-300 Analyzer). P was determined by Mo-Sb colorimetry. K was determined by atomic absorption spectrophotometer. Organic nutrients measurement: the stored samples (1.500 g) was used to determine the fat content by Soxhlet extraction method. Total soluble sugar was determined by anthrone colorimetric method (fluorescence spectrophotometer) with defatted samples (0.100 g). To determine the content of starch by the samples of defatted and removal sugar (0.100 g) (Ulger *et al.*, 2004; Huan *et al.*, 2014). Method of protein determination was the same as N determination. All samples of the same index were determined for three times.

Statistical analysis: Statistical analysis was performed using SPSS 18.0 and Excel 2003 software, graphics production by OriginPro 8.5 software.

RESULTS

The results in Fig. 1 show that the content of total soluble sugar in ovary were increased generally after self- and cross-pollination. In the ovary of self-pollination, total soluble sugar content reached the highest value 17.47% in 50 days, while in the ovary of cross-pollination, it reached the highest value 18.47% in 45 days and these differences were significant.

The results in Fig. 2 show that the starch content of cross-pollination in ovary was higher than in self-pollination ovary in most sampling dates. In ovary of cross-pollination, the content of starch increased very slowly from 5 to 20 days but dramatically increased afterwards. However, in ovary of self-pollination, the content of starch increased very slowly from 5 to 30 days. The starch content reached the highest in the 50 days, the starch content of cross-pollination was 11.70% higher than that of the self-pollination was 9.35% and the difference was significant.

The results in Fig. 3 show that the protein content increased from 5 to 20 days after self- and cross-pollination and decreased afterwards. In the 20 days after pollination, the protein content reached the highest value, self-pollination was 16.75% higher than that of

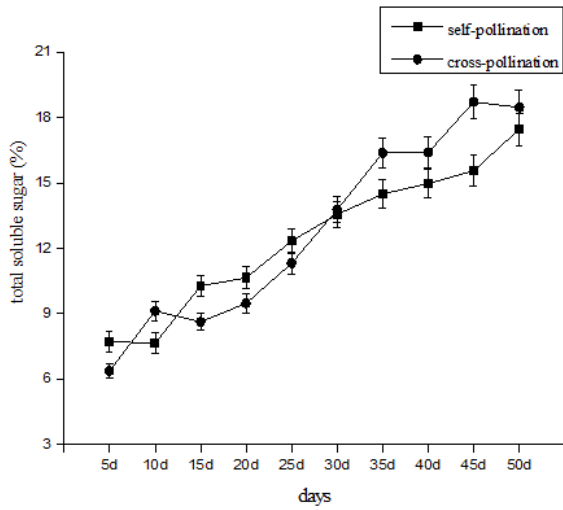


Fig. 1: The content of total soluble sugar in ovary after self- and cross-pollination

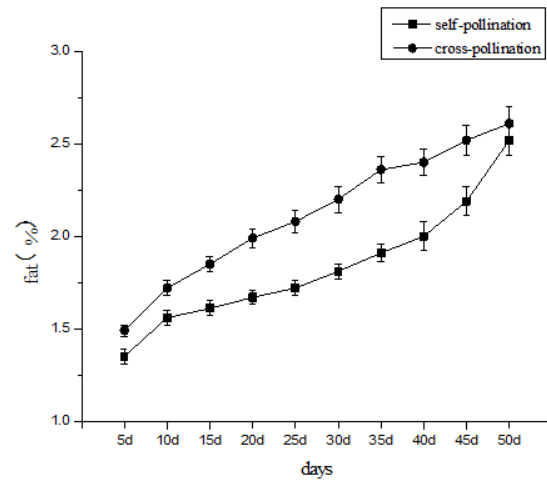


Fig. 4: The content of fat in ovary after self- and cross-pollination

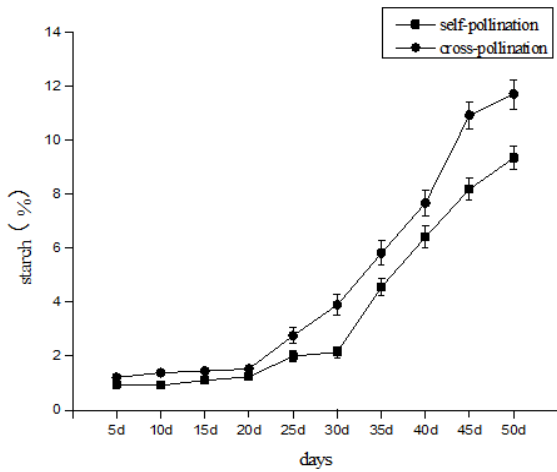


Fig. 2: The content of starch in ovary after self- and cross-pollination

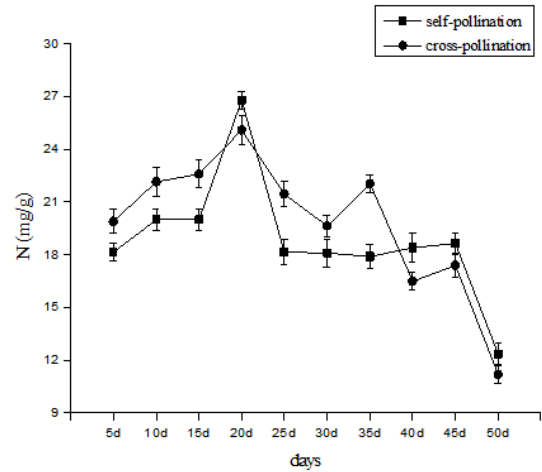


Fig. 5: The content of N in ovary after self- and cross-pollination

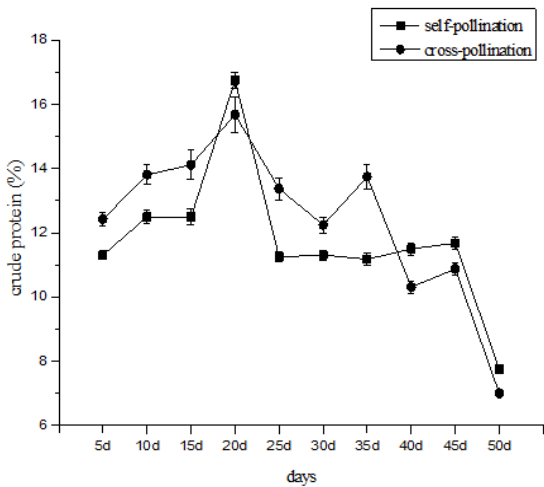


Fig. 3: The content of crude protein in ovary after self- and cross-pollination

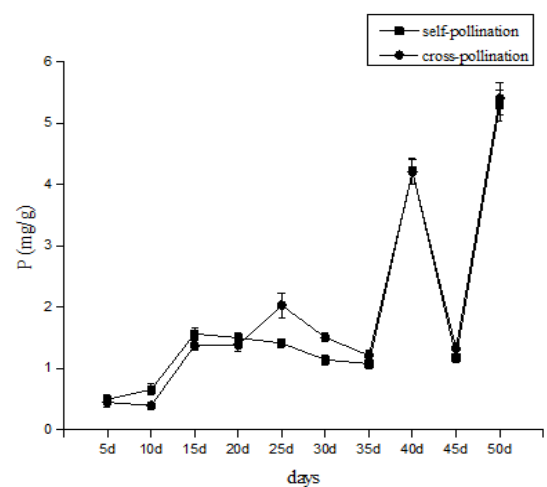


Fig. 6: The content of P in ovary after self- and cross-pollination

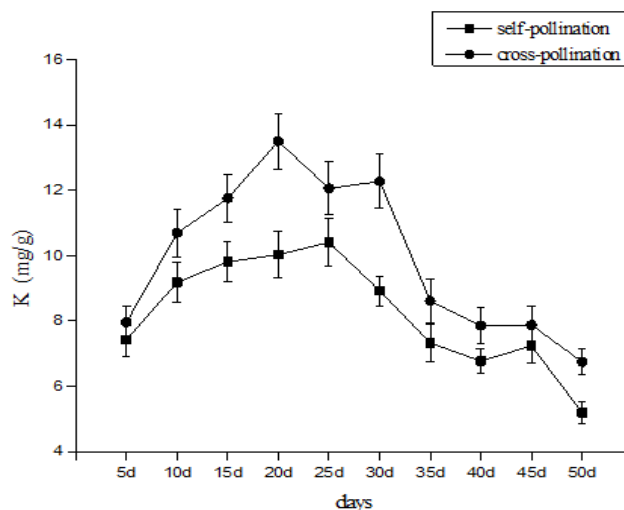


Fig. 7: The content of K in ovary after self- and cross-pollination

the cross-pollination was 15.68%, but these differences were not significant.

The results in Fig. 4 show that the fat content in ovary increased gradually after self-and cross-pollination. However, the fat content in the ovary of cross-pollination was higher than self-pollination from 5 to 50 days and these differences were significant.

The results in Fig. 5 show that the N content increased from 5 to 20 days after self-and cross-pollination and decreased afterwards. The N content reached the highest value in 20 DAP, it was 26.8 mg/g higher in self-pollination than that of cross-pollination was 25.1 mg/g and these differences were significant.

The results in Fig. 6 show that there were no noticeable changes in the amount of P content in ovary after pollination treatments. However, the change of P content in ovary were two peak values after self-and cross-pollination, one was in 40 days and the other was in 50 days. There were no significant differences in the amount of P content in ovary of self-and cross-pollination.

The results in Fig. 7 show that K contents in ovary of cross-pollination were greater than self-pollination after pollination from 5 to 50 days and these differences were significant. The content of K in ovary of cross-pollination increased from 5 to 20 days and then decreased, the highest K content was 13.50 mg/g in 20 DAP. However, self-pollination increased from 5 to 25 days and then decreased, the highest K content was 10.41 mg/g in 25 DAP.

DISCUSSION

It is a complex physiological procedure for flowering and fruiting, which needs large amounts of mineral and organic nutrients (Lv *et al.*, 2012; Zou *et al.*, 2014b). The protein, starch and carbohydrate

compounds play an important role in the development of chestnut ovary. After self-and cross-pollination, from 5 to 50 days, the total soluble sugar, starch and fat accumulated in ovary and the content of protein in ovary first increased but then decreased gradually. These indicated that the ovary growth needs the accumulation of nutrient before fruit mature. N is an important component of growth and development material, such as protein, coenzyme, chlorophyll, nucleic acids, vitamins, hormones, alkaloids and so on (Zou *et al.*, 2014b). K takes part in carbohydrate metabolism, transport and promotes young fruit development and the opening of K^+ channels can also promote plant absorption of mineral elements (Cao *et al.*, 2012). After self-and cross-pollination, both N and K content in ovary firstly increased and then decreased in the stage of young fruit development. This showed that we should add N and K fertilizer for young fruit growth and development. P plays an important role in the regulation of vegetative and reproductive growth. There were no noticeable changes in the amount of P content in ovary after pollination treatments. The change of P content in ovary were two peak values after self-and cross-pollination, one was in 40 days and the other was in 50 days. It suggested that P was important for young fruit development. P and crude protein were not significantly after pollination treatments. However, N, sugar, starch, fat and K were significantly higher in cross-pollination treatment it seems that these nutrient has a decisive role during ovary development in chestnut. Zhou *et al.* (2000, 2002) investigated the changes and relationships among the main nutrient contents in ovary of *C. mollissima* 'Qingmaozao', he found that empty burr mainly due to the serious lack of sugar, starch, protein, amino acid, P and K nutrients in the ovary. Thus, foliar application of nitrogen, phosphorus and potassium elements etc is essential during the ovary development period in chestnut.

CONCLUSION

This study provided basic information on the main nutrition changes in the ovary of Chinese chestnut after self- and -cross-pollination. We may spray the N, P, K etc during ovary development and have the potential to improve nut yield in Hebei province.

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