Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2014, 6(1):686-690

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Observation on the pistillate differentiation of Chestnut (*Castanea*) cultivar 'Yanshanzaofeng'

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ABSTRACT

Chinese Chestnut (Castanea mollissima Blume.) is a widely distributed nut tree and well known for its ecological and economic value. Seed yield, the main factor determining Chinese chestnut production, is highly associated with flower development, especially with the number of female flowers. However, little effort has been devoted to the role of pistillate flower differentiation in this species. We tried to examined the female flower morphological characterization in chestnut cultivar 'Yanshanzaofeng' by light and scanning electron microscopy. The results showed that the time from the appearance of female inflorescence axis primordial to the maturity of pistil needed about two months in Qianxi county. Pistillate inflorescences were difficult to recognize from lateral bud primordia during their early development. The inflorescences expanded rapidly and produced individual pistillate flowers in early May. The process of pistillate differentiation could be divided into seven stages, which including pistillate initial differentiation stage, pistillate primordium differentiation stage, alabastrum primordium differentiation stage, stigma primordium differentiation stage, stigma elongation stage, ovary formation stage and the blooming stage. Based our results, we may spraye the plant growth regulator in late April, which could be promoted more female flowers differentiation in Chestnut (Castanea) cultivar 'Yanshanzaofeng'.

Key words: *Castanea mollissima* Blume., Yanshanzaofeng, female flower, differentiation

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INTRODUCTION

Chinese chestnut (*Castanea mollissima* Blume.) has been cultivated for food three thousand years ago [1]. Chinese chestnut trees perform best in well-drained, loamy to sandy loam soils. Chestnuts prefer soils that are somewhat acidic (pH 5.5~6.5). With both good clod hardiness and rather drought tolerant, Chinese chestnut is largely distributed in Jilin, Hebei, Shandong, Sichuan, Hubei, Anhui, Jiangsu, Yunnan Provinces, Hainan and Taiwan Island [2]. Chestnuts are low in fat compared with other nuts and are receiving attention from the health food industry. Chinese chestnut production is particularly important source of income in rural regions, especially in northern China [3].

Chinese chestnuts are monoecious, bearing both staminate (male) and pistillate (female) flowers on the same tree [4]. The proportion of female to male flowers usually reach to 1:2000 to 1:3000, which has became a major constraining factor for chestnut production [5]. Female reproductive success was the key for fruit [6-7]. Thus, an understanding of the female flower differentiation is of basic importance to achieve high quality and consistently high production in chestnut orchards. In recently years, there have been some reports on the characteristics of the flower differentiation [8-11] and male flower morphology [12-15] in *Castanea*. However, there was little description on pistillate morphological characterization of this cultivar 'Yanshanzaofeng'. In this paper, we tried to provide a definitive record of the process of female flower differentiation in this cultivar, which may obtain a basis information for appropriate management during flowering period and have the potential to improve nut yield.

EXPERIMENTAL SECTION

2.1 Sampling

Floral buds of the chestnut cultivar 'Yanshanzaofeng' at successive developmental stages were collected from three 12-year-old trees in Qianxi county, Hebei Province, China (40°21′57″N, 118°12′17″E), at approximately 163 meters above sea level. This site is located in the warm temperate zone and semi-humid region, with a mean annual precipitation of 744.7mm and a mean annual temperature of 10.9℃ [15]. The samples were collected about every week from April 2012 to June 2013.

2.2 Fixation of samples

Collection materials were fixed for 24h in FAA (formalin: glacial acetic acid: 70% ethyl alcohol=1: 1: 16, v/v), then transferred to 70% ethanol, and were stored at 4℃ prior to sectioning [16].

2.3 Light Microscopy

Light Microscopy(LM). The materials were dehydrated in a graded ethanol series from water through 30-50-70-85-95-100% ethanol, embedded in paraffin with point 56~60℃, and sectioned at a thickness of 10μm by Leica RM2265 (Germany). The sections were stained with Safranin-O/Fast green or haematoxylin-eosin [15-16]. Observation and photomicroscopy of sections were carried out using a microscope (BX51, Olympus, Japan).

2.4 Scanning Electron Microscopy

Scanning Electron Microscopy (SEM). The materials were dehydrated through an ethyl-alcohol series [17] and then by freeze-drying treatment (Hitachi ES-2030, Japan). The materials were mounted on SEM stubs, coated with gold-palladium (Hitachi E-1010, Japan), and examined with a SEM (Hitachi S-3400N, Japan) [15].

RESULTS AND DISCUSSION

Flower buds were initiated during late summer on shoot growth above the development. During the following spring, new shoots emerged from these buds with catkins appearing midway along the shoot. Initial development of inflorescences primordium was observed in material collected in late April (Fig.1, A, B). The inflorescence dramatic changes occurred during the last week of April and the first week of May. The inflorescence elongated rapidly, with the apex appearing pointed. As the axis elongated, it produced a number of bracts in a spiral arrangement. It produced one or two big bracts near the inflorescence base (Fig.1, C). The pistillate inflorescences were identifiable in early May, which this time was called the female flower primordium differentiation phase (Fig.1, D). After the hemispherical base of female flower clusters primordium appeared, there were some small protrusions around the base in the sepal primordial period. With the growth and development of sepal primordial, which forming pistillate flowers. After the alabastrum primordium appeared at the mid-May (Fig.1, E, F), it developed rapidly, there were 6~9 small protrusions differentiation by the top of pistil primordial (Fig.1, G), namely the stigma primordial period (Fig.1, H). Subsequently, the stigma primordial gradually developed elongation through cell divisions, forming style and stigma. Stigma gradually elongated and deep to the bracts (Fig.2, A, C), this time was called the stigma elongation stage (Fig.2, B), which lasted from late May to early June. With the continuous growth of the stigma (Fig.2, E), the ovary at the base also began to develop (Fig.2, D). When the female flower dehisced on the mid-June (Fig.2, G), whereas the ovary was not mature and the ovules were just beginning to develop (Fig.2, H). The three female flowers at least were bisexual, but the filaments did not attain the length as they did in the male flowers. The young anthers were hidden within the perianth (Fig.2, F) and although pollen may be formed in them they did not normally dehisce. Within 7 ~ 8 weeks after they were initiated, pistillate flowers were structurally mature and stigmas were receptive—usually less than a week after beginning of anthesis of the staminate flowers on the same tree.

The combination of light microscopy and scanning electron microscopy has made in possible to gain a unique perspective on the development of female flower primordial in Chinese chestnut. This study of the 'Yanshanzaofeng' cultivar showed that the process of female flower differentiation was similar to that of other species of *Fagaceae* [18-19]. The extemal morphology of pistillate flower-bud could indirectly reflected the changes of internal structure. Thus, knowledge of the time of female flower differentiation in chestnut (*Castanea*) cultivar 'Yanshanzaofeng' may prove useful for the implementation of strategies aimed at manipulating nut production. However, the process of female flower differentiation was influenced by many factors such as environment, variety, tree age, density, growth regulator, nutrition and so on [20]. We should be performed to get more details in the critical stage of flower-bud differentiation and control more female flower differentiation by the plant growth regulator in the field.

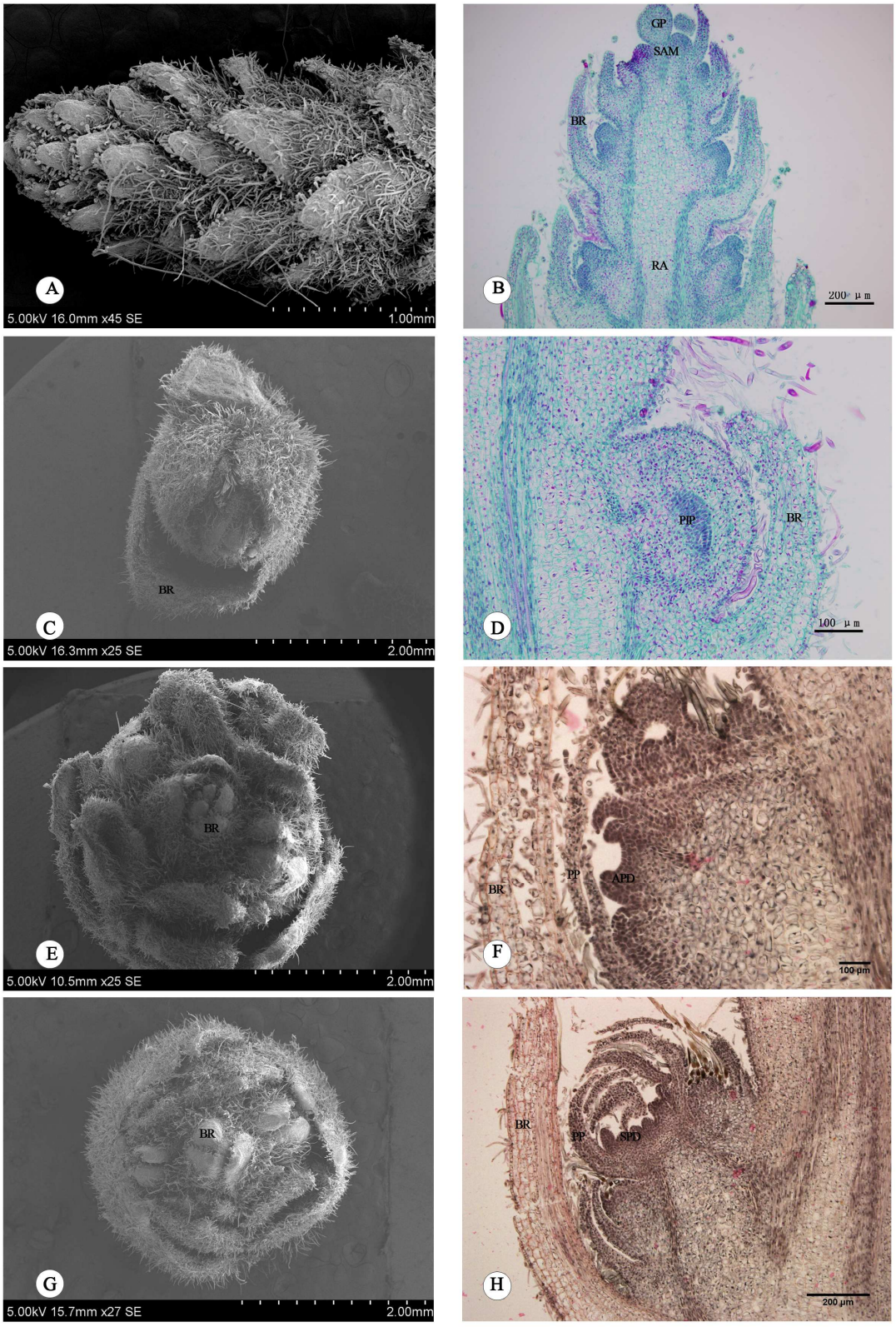

Fig.1 The process of pistillate inflorescence differentiation in *Castanea mollissima* 'Yanshanzaofeng' (A) The differentiated inflorescence observed by the SEM. (B) Details of the initial differentiation stage showing the growing point, the shoot apical meristem, the rachis and bracts. (C) Details of the initial differentiation stage showing the two bracts by the SEM. (D) Details of the female primordium differentiation stage showing the pistil primordium and the bracts. (E) The alabstrum primordium differentiation stage by the SEM. (F) Details of the alabstrum primordium differentiation stage showing the bracts, the petal primordium and the alabstrum primordium. (G) The stigma primordium differentiation stage by SEM. (H). Details of the stigma primordium differentiation stage showing the bracts, the petal primordium and the stigma primordium

(APD= alabstrum primordium; BR=bract; GP=growing point; PP= petal primordium; PIP= pistil primordium; RA= rachis; SAM= shoot apical meristem; SPD= stigma primordium)

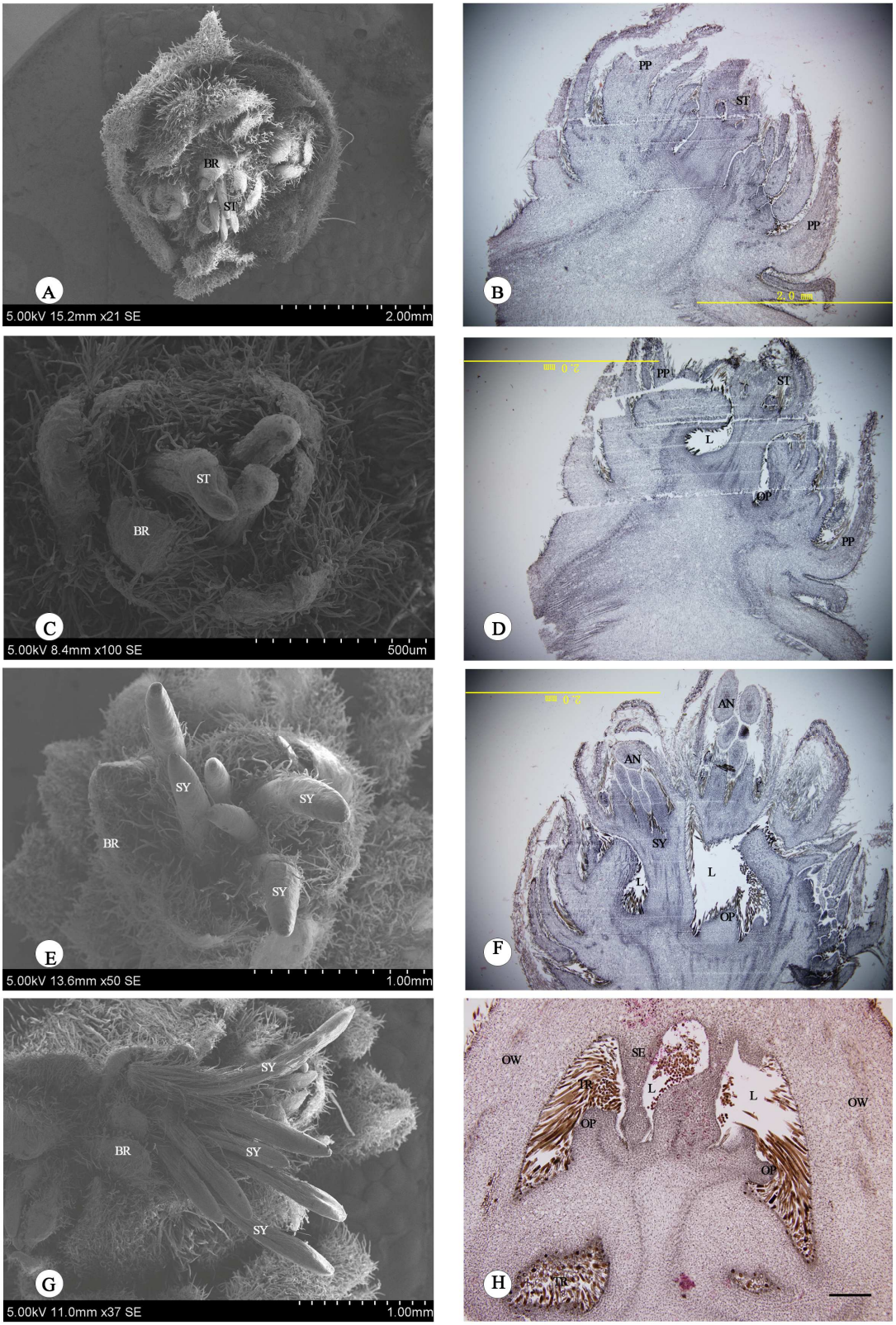

Fig.2 The process of pistillate inflorescence differentiation in Castanea mollissima 'Yanshanzaofeng' (A) The stigma elongation stage showing the stigma and the bract by the SEM. (B) Details of the stigma elongation stage showing the stigma and the petal primordium. (C) High magnification of A showing the stigma and the bract. (D) Details of the ovary formation stage showing the ovule primordium, the pistil primordium, the stigma and locule. (E) The ovary formation stage showing the style and bract by the SEM. (F) Details of the blooming stage showing the ovule primordium, the young anther, the style and locule. (G) The blooming stage showing the style and the bract by the SEM. (H). Details of the ovary showing the ovule primordium, the ovary wall, the septum, trichome and locule
(AN=anther; BR=bract; L=locule; OW=ovary wall; OP=ovule primordium; PP=petal primordium; SE=septum; SP=stamen primordium; ST=stigma; SY=style; TR=trichome)

CONCLUSION

This study provides basic information on the female flower differentiation of *C. mollissima*. The time from the appearance of female inflorescence axis primordial to the maturity of pistil need about two months in Qianxi county. It could be divided into seven stages, including the pistillate initial differentiation stage, pistillate primordium differentiation stage, alabastrum primordium differentiation stage, stigma primordium differentiation stage, stigma elongation stage, ovary formation stage and the blooming stage. The results can be used as a reference for spraying plant growth regulator on the late April, and it can promote more female flowers differentiation in chestnut (*Castanea*) cultivar 'Yanshanzaofeng'.

Acknowledgments
The authors received assistance from many individuals during the course of the work and express appreciation to all, especially Peng Xie, Huan Xiong, Xiao-Na Zhang, Ya-Qin Peng and Li-Yuan Wang. We wish to thank Jun-Na Shi for her advice on scanning electron microscopy observations. The project was partly supported by the National Science and Technology Support (Grant No. 2013BAD14B0402), the Public Welfare Project of Forestry Industry (Grant No. 201204401), and the Doctoral Foundation of Ministry Education (Grant No. 20120014110011).

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