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The relationship between reproductive growth and blank fruit formation in *Corylus heterophylla* Fisch

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ABSTRACT

The reason leading to the high blank fruit ratio of *Corylus heterophylla* Fisch was elucidated by investigating pollen compatibility, ovule and embryo development. It was showed that the female flowers bloomed in the middle of April and the ovary did not develop until 1 month later. In late May, two ovules were found in an ovary. On 22 June, the ovule wall differentiated into integument, and nucellus endosperm were clearly observed in the ovules, suggesting the starting of fertilization event. Globular, heart, torpedo and cotyledon embryo developed step by step from 28 June to 12 July. The ovule grew rapidly in filled fruit since 12 July. Ovule in blank fruit ceased growth from 12 July although the full embryo with cotyledon could be observed. The blank fruit could be distinguished from the filled one for its undeveloped ovule and large amount of parenchyma. There was no significant difference in ovary size between the filled and empty nuts, but the weight of blank nut was only about one half of the filled one. It is concluded that formation of blank fruit of *C. heterophylla* Fisch is closely related to embryo abortion, but not incompatibility between the pollen and stigma.

1. Introduction

Hazelnut is an edible woody species which has great economic value in mountainous areas. Blank fruit is a popular phenomenon in Europe hazelnut cultivars. The year-to-year variation of blank fruit ratio in Tonda Gentile delle Langhe was from 1 to 45%. In Turkey, the percentage of blank nuts varied from 5.52 to 11.64% (Beyhan and Marangoz, 2007). For hazelnut cultivar Gasavay, a minimum of 5.5 and a maximum of 17.1% blank fruit ratio was reported (Mehlenbacher et al., 1993). At present, *Corylus heterophylla* Fisch is one of the most important Corylaceae plants in the hazelnut germplasm in China. High blank fruit ratio of *C. heterophylla* Fisch has been observed in Northeast China for decades. The explanation for blank fruit formation is ambiguous. In Europe, a number of studies suggested that self-incompatibility is often associated with a higher frequency of blanks (Beyhan and Marangoz, 2007; Erdogan and Shawn, 2000; Erdogan and Mehlenbacher, 2001). However, it was found that infertile fruit dropped during early fruit development stage, and could not reach the size of normal nut (Lagerstedt, 1977). For the fertilization occurred during the period between mid-May and the beginning of June, namely, 3.5–5 months after pollination in hazelnut (Beyhan and Marangoz, 2007), some researchers mentioned that abnormal environment factor could lead to the formation of blank fruit (Silva et al., 1996, 2001a). Nevertheless, the reason for blank fruit remains unclear. The objective of this study is to investigate the relationship between pollination, ovule development and blank fruit formation in *C. heterophylla* Fisch grown in Northeast China.

2. Materials and methods

2.1. Materials

C. heterophylla Fisch production amounts for nearly 90% of China's total, and its cultivation and management are backward. No pollination cultivars are planted in its orchard for decades of years, and its fruit originated from self-fruitfulness. Compared with Europe hazelnut cultivars, blank fruit ratio of *C. heterophylla* Fisch is high and production per unit area is low, however, its cold resistance is extremely strong. Hybridization was conducted between Europe hazelnut (*Corylus avellana* L.) and wild *C. heterophylla* Fisch germplasm, several *C. heterophylla* Fisch × *C. avellana* L. hybrid cultivars were acquired, and cultivation and extension were carried out for their traits of high production and good cold resistance in Northeast China in recent years. *C. heterophylla* Fisch and 5 *C. heterophylla* Fisch × *C. avellana* L. hybrid cultivars were used as study materials in the present study.

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2.2. Pollen collection and pollination treatment

Field experiment was conducted in the Siping, Jilin province (43°09′20″N, 124°30′16″E) in 2009 and 2010. In early April, more than 20 0.5-m-long twigs attached with male flower cluster were cut from the six 10-years trees of *C. heterophylla* Fisch, cv. Dawei, cv. Pingdinghuang, cv. Yuzhui, cv. Bokehong and cv. Jinling of *C. heterophylla* Fisch × *C. avellana* L. hybrid. Then the twigs were transferred to laboratory within an hour. The ends of the twigs were soaked in beakers filled with some tap water, and then cultured in GXZ-160 incubators to accelerate male flower blooming. The temperature of the incubator was set to 25 °C and light intensity at 900 μmol m^{-2} s^{-1}. Pollen grains from the 6 genotypes were collected with smooth sulfuric acid paper 4 days later and were dried and preserved in 4 °C refrigerator for several days. When the styles of *C. heterophylla* Fisch protruded 2–6 mm, more than 100 earlier bagged female flower stigmas of *C. heterophylla* Fisch were pollinated with the 6 kinds of pollen grains, respectively, on a sunny day without wind for self-compatibility and cross-compatibility analysis. Forty-eight hours after pollination, the pollinated female clusters were sampled, fixed in FAA solution [70% ethanol:glacial acetic acid:formalin (18:1:1, v/v/v)] for 3 days, and then stored in 70% alcohol at 4 °C.

2.3. Compatibility analysis using a fluorescence microscope

The stigmatic styles were detached from the pollinated and prefixed female cluster, washed in distilled water for several times, and then squashed in aniline blue solution (0.1 g aniline blue + 0.071 g K_3PO_4 + 100 ml distilled water) (Mehlenbacher, 1997; Alireza et al., 2004). Pollen germination ratio and pollen tube length stigma was measured and calculated with a fluorescence microscope (DM AE31EF-INV-5000C) to determine compatibility reaction between pollen and stigma. Compatible crosses produce masses of long tubes, whereas incompatible crosses form very short tubes which often terminate in a pronounced blub and do not penetrate or adhere to the stigmatic surface.

2.4. Calculation of compatibility index, fruit setting ratio and blank fruit ratio

During late August, compatibility index, fruit setting ratio and blank fruit ratio were calculated with these formulas: compatibility index = number of fruit after artificial pollination × 100%/number of pollination female flower cluster; fruit setting ratio = number of fruit cluster after artificial pollination × 100%/number of pollination female flower cluster; blank fruit ratio = number of blank fruit/total fruit number after artificial pollination.

2.5. Microstructure observation of ovule and embryo

From April 20 to August 22, flower clusters or fruit clusters were collected from *C. heterophylla* Fisch pollinated with its own pollen in above mentioned orchard every 10 days from the end of artificial pollination until harvest. During the rapid embryo development stage, samples were collected every other day. Parts of the samples were fixed in FAA fixation solution. The samples were dehydrated in 70, 80, 90 ethanol for 3 h respectively, then the samples were dehydrated in absolute ethanol two times for 3 h. After that, they were transferred to a mixed solution of ethanol and xylene three times for 3 h (three parts ethanol + one part xylene, two parts ethanol + two parts xylene and one part ethanol + three parts xylene). After that, the samples were put into absolute xylene and paraffin was added gradually. Finally, the samples were kept in melted paraffin four times for 3 min. Changing the paraffin, the same operations were applied for another four times. Samples were embedded in paraffin, sectioned at 10 μm in a rotary microtome and stained with hematoxylin and eosin solution.

2.6. Characterization of female flower and fruit development

From May 20 to August 22, fruit clusters were collected from *C. heterophylla* Fisch pollinated with its own pollen. Fresh weight and diameter of the shell, parenchyma, ovary and ovule were measured every 10 days until harvest. Shell and parenchyma weight ratio was calculated by the following formula: shell weight ratio = shell weight × 100%/ovary weight; and parenchyma weight ratio = parenchyma weight × 100%/ovary weight. Fruit structure was recorded using stereomicroscope or digital camera.

2.7. Statistical analysis

Statistical analyses were performed with SAS System 8.0. The means were compared using Duncan's Multiple Range Test at 5% level. Values expressed as a percentage were transformed by calculating the angular transformation (arcsin value of the square root).

3. Results

3.1. Self-compatibility and cross-compatibility analysis

In Fig. 1, it was shown that the stigma of hazelnut is in cylinder shape. No pollen grain or pollen tube was observed on non-pollination stigma (Fig. 1A). When the pollen grains of *C. heterophylla* Fisch were used, about 40 pollen grains germinated on stigma, and the pollen tubes penetrated the surface of front stigma, stretched into the bottom (Fig. 1B). When the pollen grains of cv. Dawei, cv. Pingdinghuang, cv. Yuzhui, cv. Bokehong and cv. Jinling of *C. heterophylla* Fisch × *C. avellana* L. hybrid were applied, large amount of pollen grains and pollen tube could also be observed on front stigma (Fig. 1C–G). On the posterior part of the stigma, no pollen grain could be identified, but several parallel bundle of the pollen tube was obvious on stigma pollinated by pollen grains from all the genotypes (Fig. 1H).

The pollen germination ratios of 6 combinations were more than 95%, and each of pollen tube length was longer than 1.3 mm (Table 1). So, when pollen grains of 6 genotypes were applied on stigmas of *C. heterophylla* Fisch, respectively, it seemed that there was no obstacle during pollen germination process induced by self-incompatibility or cross-incompatibility. There were 3–6 fruits in one fruit cluster, and flower and fruit drop occurred during 20 April to late May and early June to late July respectively. Compatibility index of 6 pollination combinations was from 2.63 to 3.12, and fruit setting ratio was from 34.7 to 37.4%, while blank fruit ratio was from 34.4 to 38.7%. It showed that pollination of 5 *C. heterophylla* Fisch × *C. avellana* L. hybrid cultivars could not promote fruit setting ratio and reduce blank fruit ratio significantly compared with self-pollination on *C. heterophylla* Fisch.

3.2. Observation of kernel filling, blank fruit formation process and blank fruit ratio

At middle April when male and female flowers began to bloom, only two stigmas were found in a female flower, and no ovary was observed below the stigmas. The two stigmas connected with each other at the bottom. The ovary occurred 1 month later. In middle May, ovary is visible and half of it is wrapped by the leaf under the ovary. In late May, two ovules in ovary could be observed under light microscope after hand section (Fig. 2A), and their diameter is about 0.3 mm. There were about 3–7 fruits in a cluster (Fig. 2B). In July, the wall of ovary differentiated into the exocarp (soft shell) and endocarp (parenchyma tissue), and most of the inside ovary

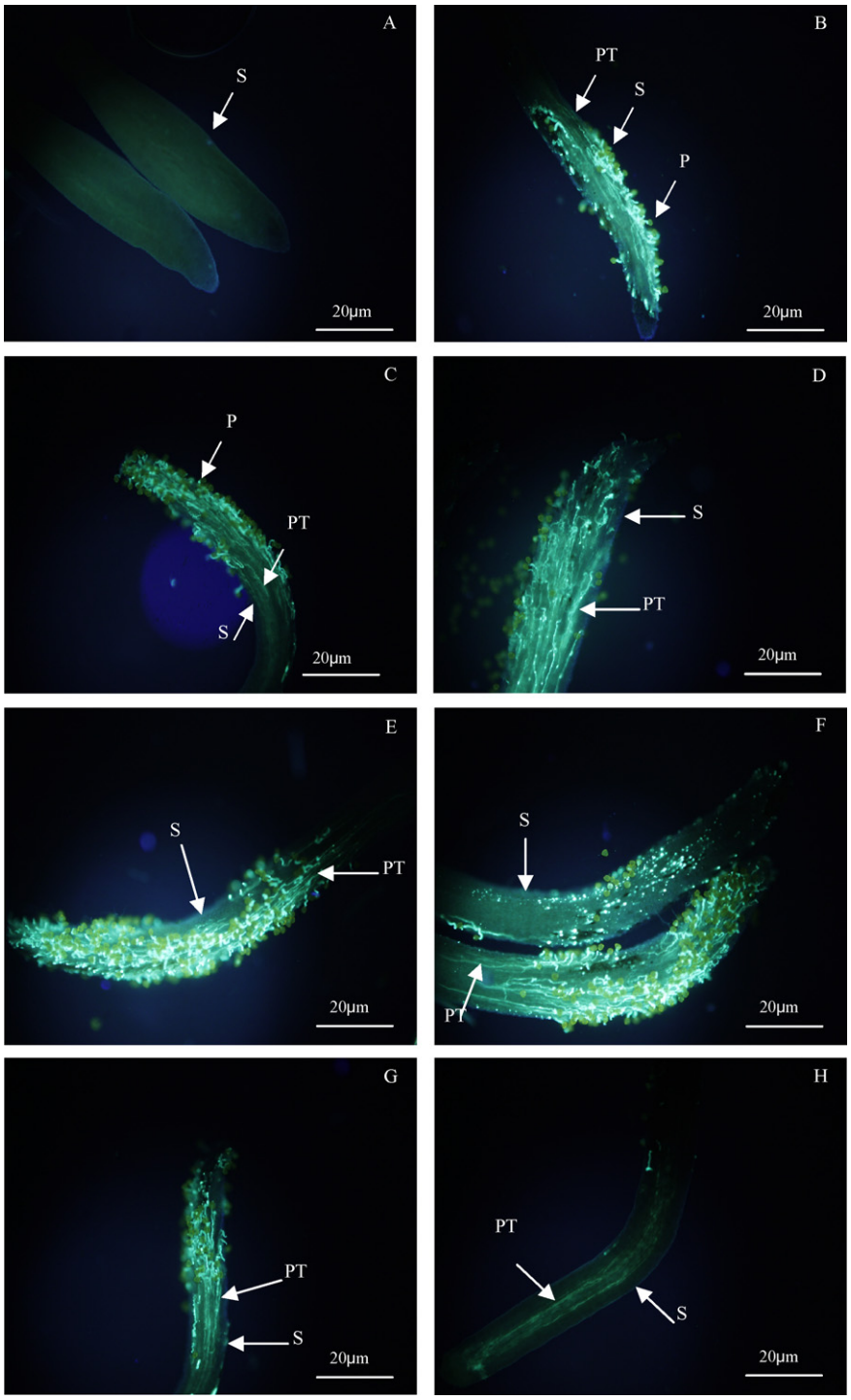

Fig. 1. Self-compatibility and cross-compatibility analysis of *Corylus heterophylla* Fisch. (A) Control, non-pollination stigma; (B) stigma pollinated by pollen grains of *Corylus heterophylla* Fisch; (C–G) stigma pollinated by pollen grains of *Corylus heterophylla* Fisch × *Corylus avellana* L. hybrid (C: cv. Dawei; D: cv. Pingdinghuang; E: cv. Yuzhui; F: cv. Bokehong; G: cv. Jinling); (H) bottom of the pollinated stigma. S: stigma; P: pollen; PT: pollen tube.

Table 1
Self-incompatibility and cross-incompatibility analysis of *Corylus heterophylla* Fisch.

Combination	Pollen germination ratio (%)	Pollen tube length (mm)	Compatibility index	Fruit setting ratio (%)	Blank fruit ratio (%)
A × A	97.4 ab	1.53 ab	2.72 bc	35.6 b	37.9 ab
A × B	96.8 ab	1.47 bc	2.81 abc	37.4 ab	36.4 abc
A × C	98.5 ab	1.38 c	2.54 c	34.4 b	34.8 bc
A × D	97.6 ab	1.62 a	2.92 ab	36.9 ab	36.5 abc
A × E	99.3 a	1.61 a	3.12 a	39.4 a	34.4 c
A × F	96.4 b	1.54 ab	2.63 bc	34.7 b	38.7 a

Different letters within the same column indicated significant difference at 5% level by LSD. A: *Corylus heterophylla* Fisch; B: *Corylus avellana* L. hybrid (cv. Dawei); C: *Corylus avellana* L. hybrid (cv. Pingdinghuang); D: *Corylus avellana* L. hybrid (cv. Yuzhui); E: *Corylus avellana* L. hybrid (cv. Bokehong); F: *Corylus avellana* L. hybrid (cv. Jinling).

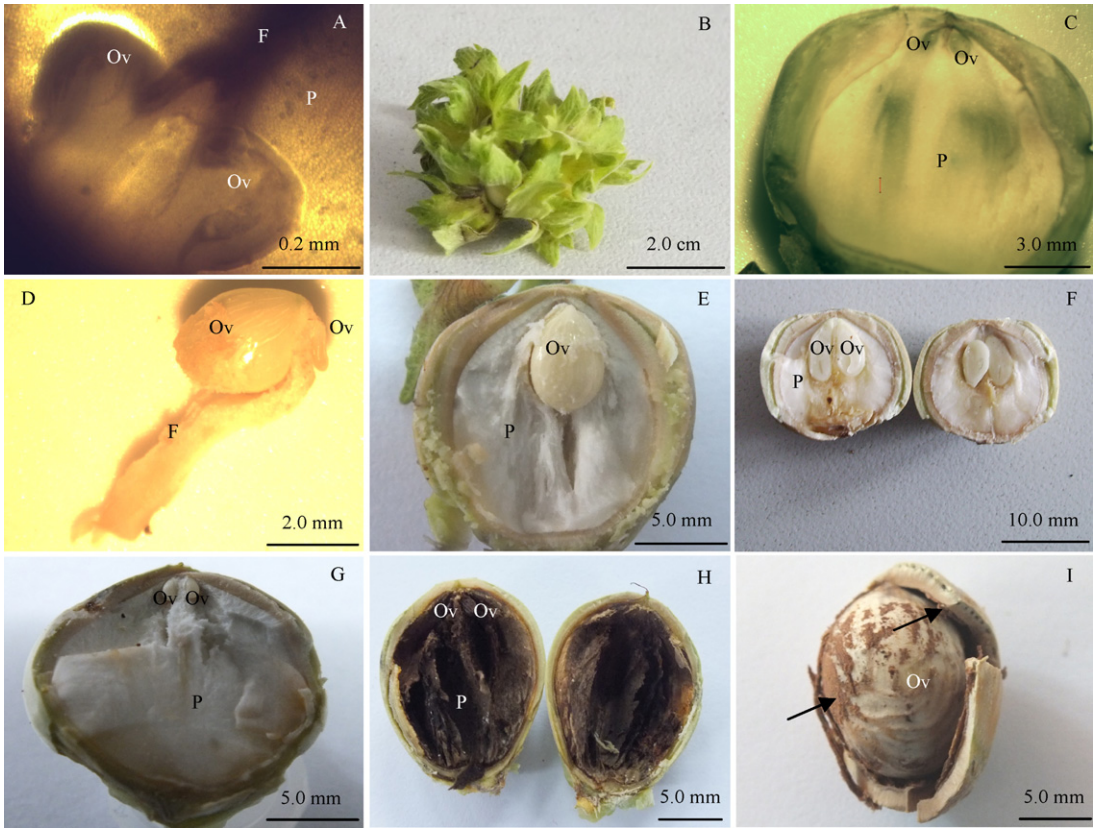

Fig. 2. Ovule filling and blank fruit formation. (A) Two ovules formed in an ovary, 22 May; (B) fruit cluster, 23 June; (C) fruit at early development stage, with one developed and one undeveloped ovule. Most space inside the fruit shell was filled with parenchyma, 2 July; (D) two ovules attached with funiculus, one ovule developed (left) and the other one (right) cease development, 12 July; (E) rapid expanding ovule, only one ovule developed in one kernel, 2 August; (F) double-kernel fruit, two well developed ovules, 2 August; (G) blank fruit. Two ovules ceased development and most of the inside shell was filled with parenchyma, 2 August; (H) blank fruit at harvest. Two undeveloped ovules were left inside the shell, 22 August; (I) normal filled kernel at harvest. Blown parenchyma residue was shown by arrows, 22 August. P: parenchyma; F: funiculus; Ov: ovule.

space was occupied by white parenchyma tissue. Two small ovules were located in the apex of the ovary. Funiculus connected with the two ovules was imbedded by parenchyma and was hardly recognized (Fig. 2C). At this stage, the two ovules have completed revolution process to an anatropous position, but were significantly different in size. In most cases, only one ovary could develop into edible kernel. The difference in development was obvious on 12 July (Fig. 2D). The developed ovule began to swell rapidly and its size was dozen times larger than that of the non-developed one. Fig. 2E shows the rapid expanding ovule in the ovary. The ovary was gradually occupied by the developing ovules, and parenchyma began to lose water. However, the majority of inside ovary space was still filled by parenchyma at this stage. Occasionally, double developed ovule in an ovary could be observed (Fig. 2F). On 22 July, the ovule of the blank fruit was very small with the diameter less than 2 mm and was close. Most of the inside ovary space was filled with loose parenchyma (Fig. 2G). At the stage near harvest, parenchyma, funiculus and ovule in blank fruit began to lose water and withered, and the color of them changed into black or brown. Small ovule residue was left in the apex of ovary (Fig. 2H). For the filled normal nuts, the ovule and embryo growth completed and the parenchyma cells were gradually replaced by the kernel which filled most of the ovarian cavity (Fig. 2I). The parenchyma tissues withered, were gradually pressed against the shell or outer ovary wall, and appeared as a brown layer of fibers upon ovule maturity. Some of the withered parenchyma was left inside the shell, while others wrapped the filled ovule. In most cases, there was only one kernel in an ovary. The non-developed ovule residue was left in ovary, which was always located on funiculus near the apex of developed ovule. So, there are two ovules in the ovary of hazelnut. If one of the ovules is filled, single kernel forms; if both ovules are filled, twin kernels form; if neither ovule is filled, a blank fruit forms.

3.3. Microstructure observation of ovule and embryo

For hazelnut *C. heterophylla* Fisch, the ovule wall differentiated into integument, and nucellus endosperm formation and enlargement inside the ovules was clearly observed on 17 June (Fig. 3A). On 22 June, mature embryo sacs were visible (Fig. 3B). So, pollination of hazelnut *C. heterophylla* Fisch occurs at April, while fertilization was completed in late June. Ovule size varied from 2.0 to 3.0 mm in 20 days after fertilization. Based on the observation in the year of 2009 and 2010, the embryo development was classified into following stages (Table 2):

Stage I (globular embryo): on 28 June, embryo in ovule was visible clearly. Embryo at this stage was ellipsoid shaped. The diameter of embryo was about 0.2 mm. The diameter of outside ovule was about 2 mm. The diameter of ovary was about 6.0 mm, equivalent to about 30% of the maximum ovary diameter (Fig. 3C).
Stage II (heart-shaped embryo): on 2 July, about 5 days after stage I, the appearance of globular embryo turned into heart shape (Fig. 3D). Diameter of the heart shaped embryo and the outside ovule did not increase obviously compared with that of stage I. However, diameter of ovary increased to 12.0 mm.
Stage III (torpedo embryo): on 7 July, the embryo was torpedo-shaped in appearance. Diameter of the torpedo-shaped

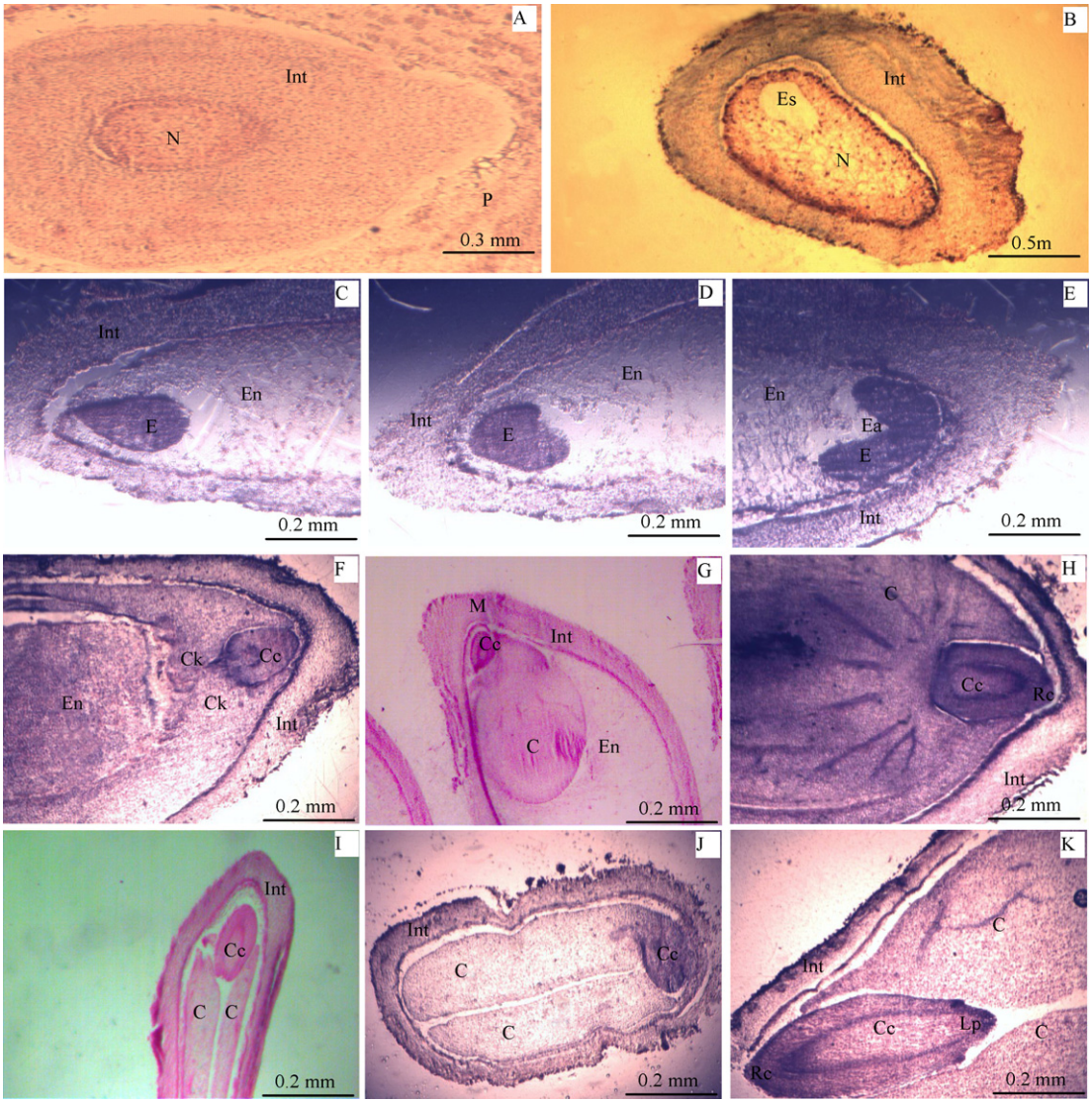

Fig. 3. Microstructure of the ovule and embryo. (A) Nucellus endosperm formed, 17 June. (B) Nucellus with embryo sacs, 22 June. (C) Globular embryo and cellular endosperm in the ovule, 28 June; (D) heart shaped embryo and cellular endosperm in the ovule, 2 July; (E) torpedo embryo and cellular endosperm in the ovule, 7 July; (F) developing cotyledon embryo and endosperm in the ovule, 12 July; (G) developing cotyledon embryo and endosperm in the ovule, 15 July; (H) two cotyledons in the embryo were shown. Endosperm began to degenerate, 22 July; (I) the ovule in the blank fruit in which complete embryo formed. No endosperm in the ovule could be observed, 22 July; (J) the small ovule in the one-kernel fruit in which complete embryo formation. No endosperm in the ovule could be observed, 2 August; (K) large ovule in the one-kernel fruit in which complete embryo formed. No endosperm in the ovule could be observed. M: micropyle; N: nucellus endosperm; Es: embryo sacs; E: embryo; C: cotyledon; En: endosperm; Int: integument; Ea: embryonal axis; Rc: root cap; Ck: cotyledonary knots; Cc: central cylinder; Lp: leaf primordial; P: parenchyma.

embryo increased significantly compared with that of heart shaped embryo (Fig. 3E). Diameter of the ovule and ovary increased rapidly, and they are about 3.0 mm and 15.0 mm respectively.

Table 2
Observation times of the fertilization and embryo development stages in the year of 2009 and 2010.

Stages	2009		2010	
	Date	Ovary size (mm)	Date	Ovary size (mm)
Fertilization	17 June	3.4 e	22 June	3.6 e
Stage I (globular embryo)	24 June	4.9 d	28 June	5.1 d
Stage II (heart shaped embryo)	30 June	6.7 c	2 July	7.2 c
Stage III (torpedo embryo)	3 July	8.0 b	7 July	8.7 b
Stage IV (cotyledon embryo)	8 July	10.2 a	12 July	10.6 a

Different letters within the same column indicated significant difference at 5% level by LSD.

Stage IV (cotyledon embryo): at this stage, diameter of the embryo increased rapidly; meanwhile, endosperm in ovule degenerated quickly. On 12 July, endosperm near the two ovules degenerated first, accompanied by the growth of the cotyledon (Fig. 3F). Then, the embryo with the cotyledon turned into egg shape (Fig. 3G), and the embryo was surrounded by un-degenerated endosperm. Finally, the inside space of ovule was occupied completely by the embryo containing the cotyledon, and all the endosperm degenerated and disappeared (Fig. 3H and K).

Blank fruit could be distinguished from the normal filled fruit after July 12. The size of two ovules in blank fruit was less than 3 mm during the fruit development. Neither of the two ovules could develop into edible kernel. The embryo with cotyledon was visible clearly in Fig. 3I. However, the embryo development ceased after the endosperm in the ovule degenerated. Two small residual ovules could be found in a blank fruit at harvest. In filled fruit with one kernel, embryo with cotyledon was also visible in the smaller

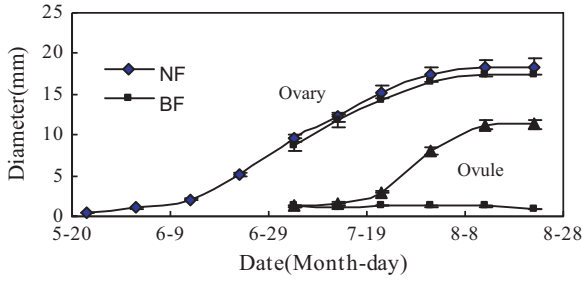

Fig. 4. Change in the diameter of the ovary and ovule in normal fruit and blank fruits. NF: normal fruit; BF: blank fruit.

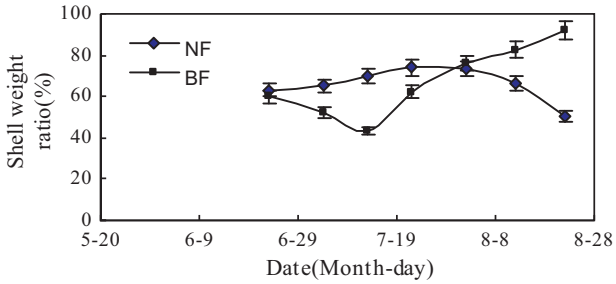

Fig. 6. Change of the shell weight ratio of the ovary and ovule in the normal fruit and blank fruits. NF: normal fruit; BF: blank fruit.

ovule (Fig. 3J). Similarly, the smaller ovule cease development and a withered ovule residue were left on the funiculus. Therefore, the fertilization process has been completed in both ovules in blank fruit and in the smaller ovule in one kernel fruit. Lack of fertilization is not the reason leading to blank fruit. Similarly, the reason leading to one kernel fruit is the abortion of one of the two embryos in the ovule.

3.4. Growth dynamics of ovary and ovule

The growth of the ovary and ovule in normal fruits showed a sigmoid curve (Fig. 4). The rapid diameter increasing stage of the ovary and the ovule was from 12 June to 22 July and from 12 July to 12 August, respectively. It took only 1 month for the ovule to finish rapid growth and reach mature. When ovule began to grow rapidly, the ovary reached 66% of its full size. So, ovule grew much slower than ovary did. The diameter of the blank fruit was very close to that of the normal fruit during the fruit development stage. It was very difficult to distinguish the blank fruit from the normal fruit if only the fruit size was compared. However, the ovule size of the blank fruit was less than 3 mm during the whole ovule development stage, much smaller than that of the normal fruit ($P < 0.05$).

The growth pattern of ovary and ovule weight in normal fruit is similar to that of the diameter. Normal ovary weight maintains unchanged in 20 days near harvest (Fig. 5). It was significantly higher than that of blank fruit ($P < 0.05$) after 12 July. The weight of blank fruit was only about one half of the normal fruit. The ovule weight in blank fruit has little effect on that of total ovary.

Shell weight ratio and parenchyma weight ratio were calculated and were shown in Figs. 6 and 7. Shell weight ratio increased slowly before 2 August, and then declined dramatically, corresponding to the rapid growth of ovule (Fig. 5). In the blank fruit, shell weight ratio declined from 22 June to 12 July, corresponding to the increase of parenchyma, then increased until harvest. At harvest the shell weight accounted for more than 90% of total fruit weight. From 22 June, parenchyma weight ratio of blank fruit was significantly higher than that of normal fruit ($P < 0.05$).

4. Discussion

Nut and kernel defects are serious problems for the hazelnut production. These problems include blanks, brown stain disorder, doubles, moldy kernels, kernels with black tips, shriveled kernels and poorly filled nuts (Mehlenbacher et al., 1993). Blank nut formation is a common phenomenon for Europe hazelnut cultivars during fruit development, and their blank fruit ratio seldom is greater than 15%. However, for *C. heterophylla* Fisch in Northeast China, more than 30% blank nut ratio was found in the year of 2009 and 2010, and lead to serious production loss. Clarifying the reasons for the empty nut formation and finding solutions to reduce the blank ratio is a very urgent task in hazelnut production.

It is known that there is self- and cross-incompatibility trait in most of hazelnut cultivars (Thompson, 1979a; Mehlenbacher and Smith, 2006; Erdogan and Shawn, 2000; Erdogan et al., 2005; Germain, 1994). Self-incompatibility is often associated with a higher frequency of blank fruits (Beyhan and Marangoz, 2007; Erdogan and Mehlenbacher, 2001). Incompatibility is shown to be the sporophytic type and is under the control of a single locus with multiple alleles. The S-alleles of several hazelnut cultivars were described by Thompson (1979b) and Mehlenbacher and Thompson (1988). Compatibility and incompatibility reaction is easily distinguished using fluorescence microscope. In the incompatibility reaction, germinated pollen grains form short tubes that fail to penetrate the stigmatic surface. The tubes of compatible pollen grains penetrate the stigmatic surface and develop a mass of long parallel tubes with strong fluorescing callose plugs (Alireza et al., 2004). For *C. heterophylla* Fisch in Northeast China, no pollination trees has been arranged in early established hazelnut orchard for decades of years, and more than 30% blank nut ratio was found in the year of 2009 and 2010. In the present study, pollen grains of self- or cross-germinated well and the pollen tubes penetrate stigma surface easily (Fig. 1; Table 1). It seemed that there was no obstacle during pollen germination process induced by self-incompatibility or cross-incompatibility. Therefore, we concluded that pollen incompatible obstacle is not the reason inducing high blank fruit ratio in *C. heterophylla* Fisch.

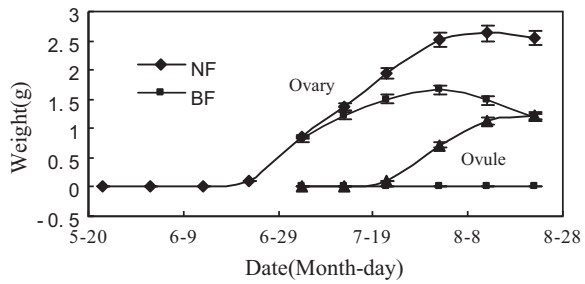

Fig. 5. Change in the fresh weight of the ovary and ovule in the normal fruit and blank fruit. NF: normal fruit; BF: blank fruit.

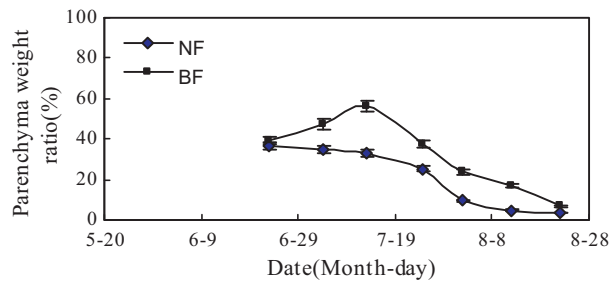

Fig. 7. Change of the parenchyma weight ratio of the ovary and ovule for the normal fruit and blank fruits. NF: normal fruit; BF: blank fruit.

There are two phenomena associated with seedlessness. Parthenocarpy is common in fruit production without fertilization, and seedless *Citrus* cultivars are typically parthenocarpic. Other species, such as most seedless grape cultivar, produce seedless fruits due to post-fertilization embryo abortion (Polito, 1999). Fruit set requires fertilization and, to some degree, embryo growth. Pollination and fertilization must occur for growth of the embryo and the ovule. Without fertilization, the embryo and the seed do not grow (Silva et al., 2001b). For most of the plant species, fertilization is completed within several days after pollination (Kaufmanea and Rumpunen, 2001; Dorcey et al., 2009; Mehmet, 2011). However, pollination and fertilization of hazelnut are two individual events, and it takes hazelnut dozens of days to complete fertilization process (Me et al., 1989; Hampson et al., 1993). Thompson (1979b) and Me et al. (1989) previously reported that fertilization was difficult to observe, and the first divisions of the free nuclear endosperm, which occurred a few days after fertilization, was regarded as evidence of this event (Solar and Stampar, 2001). In the present study, pollination of *C. heterophylla* Fisch occurred in middle of April, and fertilization was accomplished in middle of June. The ovary size of the blank fruit was closed to that of the filled ones (Fig. 4) and its ovule diameter was less than 3 mm through the whole fruit development. One month after fertilization, the ovary of blank fruit was filled with large amount of parenchyma (Fig. 2G), and embryo with cotyledon was observed in the ovules. However, the cotyledon could not be further filled after endosperm was used up. The parenchyma and ovules began to lose water and withered before harvest. Finally no filled edible ovule in ovary could be found at harvest. So, it is embryo abortion that leads to the blank fruit formation in hazelnut. The reason resulting in the cease of embryo development needs further research.

Acknowledgements

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