Pollination, Fertilization, and Embryo Development in Southern China Fresh-eating Jujube

Fengxia Shao and Sen Wang

Central South University of Forestry and Technology, Changsha 410004, China; Key Lab of Non-wood Forest Products of State Forestry Administration, Central South University of Forestry and Technology, Changsha 410004, Hunan, China; and Engineering and Technology Research Institute of Jujube Industry in Southern China, Central South University of Forestry and Technology, Changsha 410004, Hunan, China

Zhiming Liu

Department of Biology, Eastern New Mexico University, Portales, NM 88130

Rongyan Hong and Tianjiao Zhao

Central South University of Forestry and Technology, Changsha 410004, China; Key Lab of Non-wood Forest Products of State Forestry Administration, Central South University of Forestry and Technology, Changsha 410004, Hunan, China; and Engineering and Technology Research Institute of Jujube Industry in Southern China, Central South University of Forestry and Technology, Changsha 410004, Hunan, China

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Abstract. To explore the reasons for seed abortion in southern China fresh-eating jujube, improve its reproductive biology, and provide a theoretical basis for the crossbreeding of jujube, we carried out self-pollination and cross-pollination experiments with *Ziziphus jujuba* Mill. ‘Zhongqiusucui’ as the female parent. We observed the process of pollen tube growth in pistil and embryo development by fluorescence microscopy and paraffin section methods. The results show there were self- and cross-incompatibilities during pollination and fertilization, and there were no significant differences in pollen germination and pollen tube growth between self-pollination and cross-pollination. It took at least 4 hours for pollen and stigma to recognize each other, 6 hours for pollen to germinate on the stigma, and 12 hours for the pollen tube to penetrate the mastoid cells of the stigma. After 48 hours of pollination, the pollen tube reached one third of the style. The pollen tube remained stagnant 72 to 120 hours after pollination, and remained at one third of the stylar canal. Simultaneously, the pollen tubes on the stigma twisted and interacted with each other, and expanded into a spherical shape. A few pollen tubes reached the ovary and completed fertilization. However, some early globular embryos degenerated before forming into globular embryos and resulted in the formation of empty embryo sacs, which leads to seed abortion. In conclusion, the poor pollination and fertilization, and the blocked development of the embryo resulted in seed abortion in *Z. jujuba* ‘Zhongqiusucui’.

Chinese jujube (*Ziziphus jujuba* Mill.), which belongs to the Rhamnaceae family, is estimated to have been cultivated for more than 7000 years and is one of the most economically important fruit trees in China (Li et al., 2007). It is widely distributed and rich in germplasm resources (Liu, 2000), and has high nutritional and medicinal value as it contains several biologically active components required by the human body, including vitamin C, phenolics, flavonoids, triterpenic acids, polysaccharides, and microelements (Gao et al., 2013; Liu et al., 2015). Its fruit can be consumed fresh, dried, or processed into various jujube products (Qu and Wang, 1993). In southern China, the climate is

characterized by high temperature and humidity in summer. Under these geographic and climate conditions, southern China fresh-eating jujube fruit has many advantages, such as a high sugar content, rich flavor, good texture, high yield, strong adaptability, and easy storability and transportability. It has important nutritional and economic value. Although China has abundant jujube germplasm resources for fresh eating, there are only a few fresh jujube germplasms suitable for planting in southern China, with about 60 varieties at present. At the same time, Chinese southern fresh-eating jujube has disadvantages, such as severe abscission of flowers and fruit along with the ease with which the

fruit can crack. Therefore, it is urgent that new varieties are cultivated with excellent integrated characteristics suitable for the geographic and climatic conditions in south China. Crossbreeding is the primary means by which jujube trees have been bred to date, and this method is likely to continue to prove useful for a long time to come (Liu et al., 2014). However, seed abortion is a common phenomenon in Chinese jujube, which prevents the formation of viable seeds, restricts crossbreeding efficiency, and presents a challenge in obtaining hybrid progeny. Thus, it is critical we acquire an understanding the causes of seed abortion, which is necessary for breeding new cultivars and improving the jujube industry, especially in southern China.

Studies on seed abortion in jujube have been reported. Shan et al. (2009), Liang (2013), and Li and Wei (2014) conducted general surveys on seed abortion in different *Z. jujuba* germplasms. Li et al. (2016) observed the dynamic of seed abortion in jujube. Qi and Liu (2004) studied the change of endogenous hormones of jujube fruit with seed abortion. Huang et al. (2016) and Hou (2018) identified the *S-RNase* genes at the self-incompatibility loci in different jujube cultivars. These studies were helpful in determining the factors that affected seed abortion in jujube and provided references for breeding in *Z. jujuba*. However, most existing studies have focused on jujube varieties in the northern production area in China, and few on cultivars in southern China. The jujube industry in southern China started relatively late compared with northern China, and there are few studies on the mechanism of seed abortion. Thus, its mechanism of seed abortion has not been fully clarified. What remains unclear is whether the reasons for seed abortion in southern China fresh-eating jujube are consistent with other jujube varieties that have been reported. *Z. jujuba* Mill. ‘Zhongqiusucui’ is the major cultivar and is cultivated in a large area in southern China. It has become the main industry in some areas in southern China. However, it has a high seed abortion rate ($\approx 95\%$) under natural pollination according to our previous investigation (data to be published). Thus, we took *Z. jujuba* Mill. ‘Zhongqiusucui’ as the research object in this study to explore the mechanism of seed abortion.

Any disruption of the pollen, embryo sac, embryo, and endosperm developmental process could result in seed abortion (Liang et al., 2005). The embryological mechanism of seed abortion is divided into four aspects: male sterility, female abortion, pollination, and fertilization failure caused by self-incompatibility or cross-incompatibility, and embryo abortion (Liang et al., 2005). According to the results of previous studies, the pollen of *Z. jujuba* Mill. ‘Zhongqiusucui’ was fertile and its pollen germination rate reached 48.27% during the sepal flattening stage (Shao et al., 2020), and its stigmas had strong receptivity during a specific period (Shao et al., 2019a). A normal mature embryo sac

Fig. 1. Flower and style structure in *Z. jujuba* 'Zhongqiusucui'. (A) Morphological characterization of jujube flower. (B) The style characterization via scanning electron microscopy (SEM). (C) The unpollinated stigma via SEM, and a lay of papillary cells on it (arrow). (D) Cross-section of medial style. (E) Longitudinal section of style. Sty, style; Sti, stigma; SC, styler canal; PC, papillary cell; Ov, ovule. Bar = 100 μ m.

could also be formed (Shao et al., 2019b). Therefore, the reason why the normal viable seed could not form may be in the processes of pollination, fertilization, and embryo development. Thus, we studied systematically the process of pollination, fertilization, and embryo development to provide a theoretical basis and reference for exploring the mechanism of seed abortion and to establish an efficient, assisted-breeding technology system.

Materials and Methods

Experimental site

The experimental site was the jujube experimental base of Central South University of Forestry and Technology, Qidong County, Hunan Province, China (lat. 26.78°N, long.

112.12°E). This area has a subtropical climate. It is cold in winter, hot and humid in summer, and has sufficient and unstable heat, and abundant rainfall with uneven seasonal distribution. The annual average temperature is 17.9 °C, the annual minimum temperature is -6.6 °C, the annual accumulated temperature with daily average temperature is ≥ 10 °C, the annual sunshine hours are 1580 h, the annual precipitation is 1100 to 1250 mm, and the frost-free period is 282 d.

Experimental cultivars

The experimental jujube cultivars were *Z. jujuba* Mill. 'Zhongqiusucui', *Z. jujuba* Mill. 'Qidongxiaoza', and *Z. jujuba* Mill. 'Yueguang'. Five-year-old jujube trees were selected, and their water and fertilizer management were the same.

Hand pollination

This study took *Z. jujuba* Mill. 'Zhongqiusucui' as the female parent in both self-pollination and cross-pollination combinations. They were *Z. jujuba* Mill. 'Zhongqiusucui' $\times$ *Z. jujuba* Mill. 'Zhongqiusucui', *Z. jujuba* Mill. 'Qidongxiaoza' $\times$ *Z. jujuba* Mill. 'Zhongqiusucui', and *Z. jujuba* Mill. 'Yueguang' $\times$ *Z. jujuba* Mill. 'Zhongqiusucui'.

Jujube flowers are hermaphroditic and are composed of a flower stalk, calyx, petals, flower disks, stamens, and pistils (Fig. 1A). The flowering time of the jujube cultivars tested is day-flowering type. We selected strong jujubes bearing shoots with less flowering buds than buds, removed all the opening flowers, and covered the shoots with net bags from 1700 to 1800 HR before the day we conducted pollen collection and hand pollination. Flowers at the sepal flattening stage

and petal-anther separating stage were collected in weighing bottles and stored at 4 °C (within 24 h) to be used for pollination. At the floral bud-cracking stage, we took off the bags from the bearing shoots of *Z. jujuba* Mill. 'Zhongqiusucui', removed stamens from their flowers, and covered them with the bags again. At the early flowering stage of the female parent, hand pollination was performed. When pollinating, we dipped pollen grains using a small, soft writing brush; gently brushed them on stigmas; marked on the flower handle with a marking pen; and then immediately covered them with the bag. Then, 40 to 50 flowers were collected in sequence at 2, 4, 6, 8, 12, 24, 36, 48, 60, 72, 96, and 120 h after pollination. Forty to 50 jujube fruit were taken once a day from 6 to 15 d after hand pollination and once every 5 d from 15 d after pollination. The samples were immersed immediately in formalin-acetic acid-alcohol (FAA) fixative (50% ethanol:acetic acid:formaldehyde = 90:5:5) and placed in a vacuum to pull air out of the tissue. The samples were then stored at 4 °C.

Microscopic observation

Process of pollination. The fixed samples collected at 2 to 120 h after pollination were rinsed with distilled water and softened with 10 mol/L sodium hydroxide solution for 10 h. After washing with distilled water, they were stained with 0.1% aqueous aniline blue solution for 6 h, imprinted, and observed using a Leica DMI8 inverted microscope (Leica, Wetzlar, Germany) to detect germination of the pollen on the stigma, and growth of the pollen tubes in the style. Photographs were taken using the Leica DMI8 inverted microscope.

Process of fertilization and embryo development. The fixed samples collected 6 d after pollination were used to observe

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S.W. is the corresponding author. E-mail: csuftwangsen@163.com.

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Fig. 2. Pollen tube growth during self-pollination. (A) No pollen on the stigma 2 h after pollination. (B) Pollen grains on the stigma after pollination for 4 h. (C) Pollen germinated after pollination for 6 h, and the pollen tube (arrowhead). (D) Eight hours after pollination, pollen tubes (arrowhead) are elongated but do not extend into the stigma. (E) Twelve hours after pollination, pollen tubes (arrowhead) have grown into the stigma. (F) Twenty-four hours after pollination, the pollen tubes (arrowhead) continue to extend into the style. (G) Thirty-six hours after pollination, the pollen tubes (arrowhead) is growing upward while extending into the style. (H) Forty-eight hours after pollination, the pollen tubes (arrowhead) extend upward and are twisted. (I) Sixty hours after pollination, the pollen tubes (arrowhead) extend upward and are twisted. (J) Seventy-two hours after pollination, the pollen tubes (arrowhead) interact with each other and have expanded into a spherical shape. (K) Ninety-six hours after pollination, the pollen tubes (arrowhead) interact with each other and have expanded into a spherical shape. (L) One hundred twenty hours after pollination, the pollen tubes (arrowhead) interact with each other and have expanded into a spherical shape. (M) The pollen tubes (arrowhead) have reached the ovary. (N) Callose in the stylar canal (arrowhead). (O) The pollen tubes (arrowhead) are twined around the style. Ov, ovule; Po, pollen; PT, pollen tube; Ca, callose. Bar = 100 μ m.

the process of fertilization and embryo development. The routine paraffin section method (Li, 2009) was used to generate sections of 8- to 12- μ m thickness. Sections were stained using the modified Ehrlich's hematoxylin staining method (Xu et al., 2008) and were mounted with neutral gum. A Leica DMI8 inverted microscope was used to observe the processes of fertilization and embryo development. Photographs were taken to record the observations.

Morphological observation of style. *Z. jujuba* 'Zhongqiusucui' flowers at the early flowering stage were collected and fixed with 2.5% glutaraldehyde and FAA fixative. The test materials fixed with 2.5% glutaraldehyde for 24 h were washed with phosphate buffered saline and then distilled water, dehydrated sequentially with different concentrations of ethanol solution (30%, 50%, 70%, and 100%, v/v), dried at the critical

point, and gold-coated before viewing. The style and stigma were observed using a scanning electron microscope (JSM-6390LV, JEOL, Japan). The samples fixed with FAA were used to observe the anatomic structure of the style. The routine paraffin section method (Li, 2009) was used and the staining method of sections was the same as noted previously.

Results

Style structure. Based on the observations of the jujube stigmas during all flowering stages, we did not observe any exudate secretion; therefore, the stigmas were dry. Stigmas of the jujube flower were covered with a layer of stigmatic papilla cells (Fig. 1C), which expanded to a maximum area during flowering to receive as many pollen grains as possible, like a pollen trapper. The style was split into two lobes, and its

length was about 500 to 800 μ m (Shao et al., 2019a). The upper two thirds of the style separated from each other and the lower third began to connect (Fig. 1B). The jujube style was hollow and there was a stylar canal in the middle of it (Fig. 1D and E), which was surrounded by a layer of special passage cells. From the top to the bottom of the style, the gap of the stylar canal got larger and larger, extending all the way to the ovary (Fig. 1E). The stylar canal of each style was interconnected with one ventricle of the ovary.

Pollen germination and pollen tube growth in the style. In general, after pollen grains fall onto the stigma, it begins to hydrate, germinate, and grow pollen tubes. Then, the pollen tubes penetrate into the space of mastoid cells, extend into the stigma, and grow downward along the stylar canal. In our results, there were no significant

Fig. 3. Pollen tube growth during cross-pollination. (A) Two hours after pollination, there is no pollen on the stigma. (B) At 4 h after pollination, pollen adheres to the stigma. (C) At 6 h after pollination, the pollen is germinating (arrowhead). (D) At 8 h after pollination, the pollen tubes (arrowhead) are elongated. (E) At 12 h after pollination, the pollen tubes (arrowhead) have grown into the stigma. (F) At 24 h after pollination, the pollen tubes (arrowhead) continue to extend into the style. (G) At 36 h after pollination, the pollen tubes (arrowhead) extend upward while growing into the style. (H) At 48 h after pollination, the pollen tubes (arrowhead) extend upward and are twisted. (I) At 60 h after pollination, the pollen tubes (arrowhead) extend upward and are twisted. (J–L) At 72 to 120 h after pollination, the pollen tubes (arrowhead) are twisted, interact with each other, and have expanded into a spherical shape. (M) The pollen tubes (arrowhead) have reached the ovary. (N) The pollen tube (arrowhead) is twined around the style. Ov, ovule; Po, pollen; PT, pollen tube. Bar = 100 μ m.

differences in the germination of pollen grains on the stigma after self-pollination and cross-pollination. At 2 h after self-pollination and cross-pollination, there were almost no pollen grains adhering to the stigma (Figs. 2A and 3A), indicating that pollen and stigma had not interacted and recognized each other, and thus pollen grains could easily fall off the stigma at this moment. The pollen grains adhering to the stigma were observed 4 h after pollination, but had not yet germinated (Figs. 2B and 3B). They started to germinate at 6 h after pollination, and the pollen tubes were short (Figs. 2C and 3C). Thereafter, the pollen tube continued to extend, and its blue or green fluorescence could be observed clearly using the microscope (Figs. 2D and 3D). The pollen tubes penetrated the papilla cells and extended into the style 12 h after pollination (Figs. 2E and 3E). As the pollen tubes extended toward the stylar canal, they stretched upward and began to twist (Figs. 2F and 3F).

At 24 to 72 h after pollination, the pollen tubes twisted and interacted, and ultimately expanded into a spherical shape on the stigma (Figs. 2E–L and 3E–L). The degree of distortion of the pollen tube from self-pollination was greater than that from cross-pollination. During the process of growth of the pollen tube in the stylar canal, it was only observed that the pollen tube grew to one quarter to one third of the style, but not the entire style. Anatomic observation of the style showed that there was a large amount of callose in the stylar canal that blocked the growth of the pollen tubes (Fig. 2N). However, it was observed in the anatomy of the ovary that a small part of the pollen tubes (4% to 6%) grew to the base of the style and reached the ovary at 72 h after pollination (Figs. 2M and 3M). At 120 h after pollination, the ovary began to expand while the pollen tubes still twined on the stigma (Figs. 2L and 3L). During the process of pollination, some pollen grains germinated on the style and

their pollen tubes wrapped around the style (Figs. 2O and 3N).

Based on these results, most pollen tubes in self-pollination and cross-pollination stopped growing in the stylar canal and could not reach the base of the style. However, a few of them could reach the ovary.

Fertilization process and embryo development. The jujube pistils have two ovaries, two anatropous ovules, inner and outer integuments, and thick nucellus. The mature embryo sac contains seven cells and eight nuclei: two dipole nuclei located in the center of the embryo sac, three antipodal cells at the chalazal end, and one egg cell and two helper cells at the micropyle end. Antipodal cells disappear after the embryo sac matures (Shao et al., 2019b).

The two polar nuclei began to fuse to form the secondary nucleus in the embryo sac at 72 h after pollination (Fig. 4A). After pollination for 96 h, one sperm fused with the secondary nucleus in the embryo sac and

Fig. 4. Fertilization and embryo development after self- and cross-pollination in *Z. jujuba* 'Zhongqiusucui'. (A) Two polar nuclei fused to form a secondary nucleus (arrowhead). (B) Primary endosperm nucleus (arrowhead). (C) The sperm and the egg began to fuse (arrowhead). (D) The zygote has formed (arrowhead). (E) Three-celled proembryo (arrowhead). (F) Early globular embryo (arrowhead). (G) Globular embryo. (H) Heart-shaped embryo. (I) Torpedo-shaped embryo. (J) Mature embryo. Co, cotyledon; Pl, plumule; PA, plumule axis; Ra, radicle. Bar = 50 μ m.

Fig. 5. The process of embryo and seed abortion after self- and cross-pollination in *Z. jujuba* 'Zhongqiusucui'. (A) Degeneration of early globular embryo (arrowhead). (B) Degenerated embryo sac degenerated. (C) Degenerated ovule. (D) The integument has begun to degenerate. (E) The egg apparatus and other cells in the embryo sac have disintegrated. (F) The nucellar tissue and ovule structure have begun to degenerate. (G) The entire ovule has degenerated. (H) One ovule has aborted (arrowhead), but the other did not abort in the same ovary. Bar = 50 μ m.

formed the primary endosperm nucleus (Fig. 4B), and the other sperm moved to the vicinity of the egg cell (Fig. 4C). After pollination for 120 h, another sperm fused

with the egg cell, forming a zygote (Fig. 4D). The zygote did not divide immediately; it began to divide after 4 to 5 d of dormancy. It first divided into a two-cell proembryo, then a

three-cell proembryo (Fig. 4E), then a four-cell proembryo, and formed an early globular embryo (Fig. 4F) after pollination for 10 d. Then, the embryo body grew in length, width,

and thickness, and formed into a round shape 20 d after pollination (a globular embryo) (Fig. 4G). During this process, the suspensor elongated rapidly and increased its width, and the suspensor was vacuolized. The globular embryo developed further. Its tissues began to differentiate, the suspensor cell gradually degenerated, protuberances appeared on both sides of the flat top of the embryo body (that is, the cotyledon primordium), and a depression formed in the middle. The embryo body was heart-shaped (Fig. 4H). The two cotyledons of the heart-shaped embryo continued to protrude upward, and the radicle and hypocotyl grew correspondingly, as the embryo became torpedo-like (Fig. 4I). The torpedo-shaped embryo developed further and formed into a mature embryo (Fig. 4J). It took about 20 to 25 d from the early globular embryo to the mature embryo.

During embryo development and differentiation, it was observed that some embryos (30% to 40%) degenerated after the formation of the early globular embryo, but before the formation of the globular embryo (Fig. 5A). After 15 d of fertilization, the embryo degenerated completely and an empty embryo sac was formed (Fig. 5B). After embryo sac abortion, nucellar cells gradually atrophied and the integument cells disintegrated and degenerated gradually. The nuclear membrane and nucleolus of the integument cells disappeared and the cells lost their original shape. Then, the structure of the integuments became loose and the entire ovule degenerated gradually (Fig. 5C and D).

Because the growth of pollen tubes in the stylar canal was blocked, there were many unfertilized ovules. Over time, the oocytes, polar nucleus, synergids, nucellar cells, and structure of the embryo sac degenerated gradually, leading to a hollow in the entire ovule cavity that was surrounded by a few layers of integument cells (Fig. 5E and F). Finally, the entire ovule atrophied and degenerated (Fig. 5G).

In general, both ovules aborted at the same time, but we also observed that one ovule developed normally and the other one degenerated in the same ovary (Fig. 5H).

Discussion

Seed abortion is common in many plants. Embryo dysplasia is the main cause for seed abortion. Any aspect of embryo development, such as the development of pollen, embryo sac, embryo, and endosperm, might cause seed abortion (Liang et al., 2005). The results of previous studies have shown that the *Z. jujuba* 'Zhongqiusucui' pollen was fertile and the stigma had strong receptivity (Shao et al., 2019a, 2020). Therefore, our study observed the process of pollination, fertilization, and embryo development.

It is an important reproductive process for plants that pollen grains adhere to and germinate on the stigma, and that the pollen tubes ultimately grow into the style. In our study, whether by self-pollination or cross-pollination, it took at least 4 h for pollen and

stigma to recognize each other, at least 6 h for the pollen to germinate on the stigma, 12 h for the pollen tube to penetrate the mastoid cells and grow into the style, 24 h for the pollen tube to reach one quarter of the style. Zhang et al. (2004) observed that pollen tubes grew to one third of the style after pollination for 16 h, one half of the style after 24 h, and to the base of the style and entered the ovary after 48 h in *Z. jujuba* 'Wuhezao'. Wang (2008) studied the pollination and fertilization characteristics in different cross-pollination combinations of *Z. jujuba* 'Dongzao', *Z. jujuba* 'Pingguozao', *Z. jujuba* 'Lajiaozaao', and *Z. jujuba* 'Shenglizao', and more, and found that the time for mutual recognition between pollen and stigma was at least 5 h. The pollen then started to germinate on the stigma at 14 to 20 h after pollination, and the pollen tube extended to one third to one half of the style 24 h after pollination. These results indicate that the characteristics of pollen germination and growth on the stigma are different in different species and in different hybrid combinations in jujube fruit. In our study, regardless of self-pollination or cross-pollination combinations, the pollen tubes began to distort 48 h after pollination, and the pollen tubes on the stigma continued to elongate, twist, and interact, leading to a swelling spherical area on the stigma. Only a few pollen tubes were observed growing to the base of the style and entering the ovary. The growth of most pollen tubes was inhibited in the pistillary cord. A large amount of callose formed in the pistillary cord, demonstrating self- and cross-incompatibility, which is consistent with the results of Asatryan and Telzur (2013). The self- and cross-incompatibility of *Z. jujuba* 'Zhongqiusucui' belonged to prezygotic incompatibility (Chen et al., 2012; Sage et al., 1999, 2006), similar to *Z. jujuba* 'Huizao' and *Z. jujuba* 'Dongzao' (Hou et al., 2019). In our study, the reasons for incompatibility of the two cross-pollination combinations may lie in different compatibility in different jujube cultivars. In the future, it is necessary to explore the compatibility degree between *Z. jujuba* 'Zhongqiusucui' and other different jujube varieties, and to select a suitable cross-pollination combination.

In the unfertilized ovary, the embryo sac structure degenerated gradually over time, and the entire ovule eventually degenerated. However, the unfertilized ovary could still develop into fruit. Studies showed that as long as pollen germinated on the stigma, the ovary could enlarge and develop into fruit even if it was unfertilized (Zhongchuan, 1979). First, after the pollen germinated on the stigma, the pollen tubes produced a kind of hormone that acted on the style and ovary. Because of the chain stimulation of this hormone, the hormone secretion in the style and ovary increased, which promoted development of the ovary. In addition, the germination of pollen on the stigma could activate auxin synthetase in the style or ovary, which promoted ovary development (Zhongchuan, 1979). Thus, the unfertilized ovary devel-

oped into fruit, and then the fruit with abortive seeds formed in jujube.

Both in the self- and cross-pollination combinations, a few pollen tubes reached the ovary and completed fertilization. Embryo development in *Z. jujuba* 'Zhongqiusucui' was the same as that in other dicotyledon plants, progressing through proembryo, globular embryo, heart-shaped embryo, torpedo-shaped embryo, and cotyledon embryo until fully mature. However, only a few jujube varieties can complete the development to form a mature embryo. In the combination of *Z. jujuba* 'Huizao' × *Z. jujuba* 'Wuhezao' with *Z. jujuba* 'Wuhezao' as the female parent, the sperm cells arrived at the ovary and proceeded with double fertilization after 72 h of pollination (Zhang et al., 2004). Then, the primary endosperm nucleus was formed after 5 d, the zygote was formed after 6 d, the globular embryo was formed after 20 d, and then the mature embryo was formed after going through the heart-shaped embryo and torpedo-shaped embryo stages (Zhang et al., 2004). Embryo development of *Z. jujuba* 'Jinai No.1', *Z. jujuba* 'Dongzao', and *Z. jujuba* 'Liuyuexian' (Jin, 2003) was consistent with that of *Z. jujuba* 'Wuhezao'. In our study, at 120 h after pollination, sperm and egg cells fused to form a zygote. After dormancy for 4 to 5 d, they divided and formed an early globular embryo. The final mature embryo was finished after the development stages of the globular embryo, heart-shaped embryo, and torpedo-shaped embryo. It was found that some early globular embryos degenerated during the process of differentiation, and degenerated completely to form an empty embryo sac after 15 d of fertilization. Li et al. (2016) observed that the embryo of five abortive jujube varieties—*Z. jujuba* 'Taigusuanzao', *Z. jujuba* 'Xiangfenyuanzao', *Z. jujuba* 'Lengbaifu', *Z. jujuba* 'Liuyuexian', and *Z. jujuba* 'Hupingzao'—began to abort in large numbers at the globular embryo at about 28 d after anthesis, which was similar to the results of our study.

Chen et al. (2014) studied the pollination and fertilization of *Z. jujuba* 'Jinsixiaozao' and *Z. jujuba* 'Wuhezao', and found that the early degeneration of the embryo and endosperm after double fertilization eventually led to seed abortion, which occurred during the globular embryo period. These results were similar to ours. *Z. jujuba* 'Liao', *Z. jujuba* 'Pingguozao', and *Z. jujuba* 'Ruanmizao' began to abort during the globular embryo and the heart-shaped embryo stage (Jin, 2003), *Z. jujuba* 'Xiongxingbuyu No.1' aborted in the heart-shaped embryo stage (Li et al., 2016), and the abortion of *Z. jujuba* 'Dongzao' occurred during the torpedo-shaped embryo stage (Liang et al., 2005), which was quite different from our results. The reason for these differences may lie in the different jujube cultivars.

The results of our study are similar to those conducted on other fruit trees. For example, the reason for the formation of seedless fruit in 'Wuhebai' grape is that its immature embryo no longer continued to

divide and differentiate, but gradually degenerated 70 d after anthesis (Wang et al., 1992). In the cross combination of the seedless grape and Chinese wild grape, the embryo began to abort when it developed to globular embryo and early heart-shaped embryo stage. In general, the endosperm aborted first and the nutrition supply was insufficient, which led to embryo abortion (Wang et al., 2005). In our study, the reasons for embryo abortion during the early globular embryo stage need to be explored further.

The characteristics of pollination, fertilization, and embryo development were not only related to differences in plant cultivars, but were also influenced by the climate and nutrient conditions during the flowering period (Yan et al., 2010). Meteorological factors during the flowering phase played a determining role in pollination and fertilization, but temperature was the most important environmental factor affecting stigma receptivity, pollen germination, pollen tube growth, and ovule life span (Yang et al., 2015). High temperatures (20 to 30 °C) reduced stigma receptivity in *Prunus avium* L. (Hedhly et al., 2003). Suitable temperatures (15 to 20 °C) accelerated pollen germination and pollen tube growth in *Armeniaca vulgaris* Lam. and *P. avium*, but too-high temperatures decreased the pollen germination rate (Hedhly et al., 2004; Pirlak and Bolat, 2002). At low temperature (5 °C), the pollen germination rate was greater, and more pollen tubes grew to the base of the style (Hedhly et al., 2004; Pirlak and Bolat, 2002). Studies showed that the ovule life of *Cerasus pseudocerasus* Lindl. and *Prunus salicina* Lindl. decreased with an increase in temperature (Cerovic et al., 2000). The ovule lifetime in *P. avium* was only 1 to 2 d at 20 °C, but it extended to 5 d at 5 °C (Sanzol and Herrero, 2001). In our research, the blooming period of jujube flowers was from June to July, when the temperature was greater than 25 °C and sunny days prevailed. Moreover, hand pollination was carried out at around 11:00 AM, when the temperature was high. Whether high temperature has a certain effect on the duration of stigma receptivity, pollen germination rate, pollen tube growth, and ovule life remain to be studied.

In addition to temperature, the nutritional level of the tree is also of great significance to pollination, fertilization, and embryo development. Research has shown that soluble starch, soluble sugar, potassium (K), boron (B), nitrogen (N), calcium, and magnesium (Mg) were involved in pollination and fertilization in *Castanea mollissima* BL., and needed to be supplemented in during full-blossom period (Lyu et al., 2012). During ovule formation and embryo sac development, the supply of exogenous nutrients such as N, phosphorus, K, B, zinc, and Mg effectively reduced the rate of embryo abortion in *C. mollissima*. (Du et al., 2006). The lifetime of ovules in *Malus domestica* was prolonged by spraying N fertilizer on the leaves after harvest in autumn (Jin, 2001). Defoliation in autumn decreased the carbohydrate accumu-

lation in *P. avium*, thus shortening the life span of ovules (Sanzol and Herrero, 2001), which also confirms the significance of tree nutrition level. Therefore, we can consider improving the nutritional level of trees to promote fruit set rate and seed rate in *Z. jujuba*.

Overall, the main reasons for seed abortion in *Z. jujuba* 'Zhongqiusucui' could be summarized as follows. One was poor pollination and fertilization. During the processes of pollination and fertilization, there were self-incompatibility and cross-incompatibility, where the unfertilized ovule aborted eventually. Second, the development of the embryo was blocked. Some early globular embryos degenerated before forming into globular embryos, and thus formed empty embryo sacs, leading to seed abortion. In addition, the climatic conditions of pollination and fertilization, and tree nutrition should also be considered. Seed abortion in jujube is a complex physiological phenomenon. In the future, we should deeply explore the compatibility and embryo abortion mechanism, and make clear the compatibility degree among different varieties to select a suitable cross-pollination combination to provide theoretical and practical guidance for crossbreeding in jujube. At the same time, we can study the genes and mechanisms related to seed abortion in jujube at the molecular biology level. In our study, the key period of seed abortion in *Z. jujuba* 'Zhongqiusucui' was defined, which laid an important foundation for further exploration of its molecular mechanism.

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