

Full Length Research Paper

Types of aborted seed and quality evaluation of 'Wuheli' litchi (*Litchi Chinensis* Sonn.)

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Accepted 16th April, 2012

This study investigated types of aborted seed, embryo abortion period and fruit quality changes during the development process of 'Wuheli' litchi. 'Wuheli' litchi has four types of seeds: normal seeds, later aborted seeds, middle aborted seeds and early aborted seeds (similar to aborted ovules). The three types of abnormal seeds resulted from embryo abortion, whose pyrenes, similar to an aborted ovule, are not caused by parthenocarpy. Middle aborted (when abortion occurs to globular embryos and heart-shaped embryos) and late aborted (when abortion occurs during rapid growth period of torpedo embryos and cotyledons) seeds consist of the highest number of arils and aril ratio. Their fruits also contain a higher proportion of Total Soluble Solids (TSS) and a lower percentage of titratable acidity (TA). In terms of the characteristics of aborted seeds of 'Wuheli' litchi, early aborted seeds (when abortion occurs before formation of globular embryos) have no effect on improving the aril ratio of fruits.

Key words: 'Dazao', embryogenesis, shriveled seed, parthenocarpy, seedlessness.

INTRODUCTION

Litchi chinensis Sonn. is a member of the family Sapindaceae, evergreen, sub-tropical economic fruit tree that originated in the tropical and subtropical regions of Southern China. Litchi fruit has a bright color, fine and juicy arils, and is very popular among consumers (Li, 2008). Seedlessness has long been the objective of fruit breeding while the greatest difficulty in achieving it lies in the interdependence between seed development and fruit growth (Varoquaux et al., 2000). Regular fruit trees lack a fertilization function during blooming periods, resulting in aborted carpels and fallen petals. However, some breeds possess the ability of parthenocarpy or stenospermocarpy through which carpels can continue normal development without double fertilization or seed development (Koltunow et al., 1998). However, this type of fruit usually has a smaller size or poorer quality, causing low prices. Among current litchi cultivars, a few also have the ability of parthenocarpy, such as

'Hexiachuan', 'Nandao Wuheli', 'Maohangnuo', 'Jiaxiangli', and 'Jiagualu' (Li and Zheng, 2004). In addition to the aforementioned parthenocarpy or stenospermocarpy that can result in seedless fruits, other factors like embryo sac abortion, self-incompatibility, polyploidy, abnormal climatic conditions and the use of plant growth regulators may all lead to seedless fruits (Ye et al., 2009).

Embryogenesis is an important factor associated with output quantity and quality of litchi (Lu, 2001). In the development process of litchi fruits, partial seeds that become smaller and dry due to abortion or underdevelopment are called aborted seeds or shriveled seeds. For example, fruits from 'Nuomici' and 'Guiwei' are usually larger than the ones produced by parthenocarpy and consist of a higher proportion of shriveled fruits, which are widely appreciated among cultivators and consumers (Xiang et al., 2001).

In the process of fruit development, seed development is a strong sink. Normally developed seeds show strong competitiveness for assimilation, consuming most nutrients and preventing arils or other edible parts from full development. On the other hand, in fruits derived from

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aborted seeds, seeds and arils do not compete against each other for assimilation (Huang et al., 2001). Seed development not only affects aril ratios but also has impact on the quality of edible parts. According to the study of Singh (2005), comparison of quality between normal mango fruit and fruit from insufficient embryogenesis, the latter has a higher proportion of total soluble solids (TSS), TSS/acid ratio and a larger amount of total sugars than the former. Among current litchi cultivars, fruit embryogenesis can be characterized by three features; seeds with normal embryogenesis, shriveled seeds with completely embryo abortion, and normally developed and shriveled seeds from partial embryo abortion (Lu, 2001). In a natural environment, 'Wuheli' litchi contain various types of embryogenesis. In addition to normal seeds, there are also shriveled seeds and seeds whose ovules seem to be completely developed. As a result, this study examined the parthenocarpy ability, ovule tissue section, seed development types, and fruit quality in terms of the polymorphism of seed development of 'Wuheli' litchi.

MATERIALS AND METHODS

The research subject was the 'Wuheli' litchi preserved in the Fengshan Tropical Horticultural Experiment Branch, which was introduced from Guangxi province in China and whose mature fruits contain various seed types. We used the cultivar 'Dazao' as the control group, which is a large-seed cultivar with normal seed development, with an age of eight to ten years.

Parthenocarpy ability

Male flowers were removed from inflorescences of 'Wuheli' and 'Dazao' with only female flowers remaining. Inflorescences were bagged in order to prevent pollination from external pollen parents. We observed the development of bagged female flowers and documented the number of fruit setting of each inflorescence. Ten inflorescences from each cultivar were bagged for emasculatation, and each inflorescence was repeated once.

Seed development type

'Wuheli' and 'Dazao' litchi were grown in a field, and open for pollination. The day on which half of the female flowers of a plant bloomed was chosen as the day after full female bloom. Samples were collected every seven days after that day, with 50 fruits each time to measure their length, width, weight, seed, peel, and aril weight; seed development was observed by a stereomicroscope and photographed. Observations on embryo sac development were conducted by paraffin section. Materials cut into an appropriate size were soaked in the FAA solution (Formalin + glacial acetic acid + ethanol), and were dehydrated by t-butanol and TBA-series after being vacuumed and stabled. Following infiltration of paraffin, embryo sacs were embedded and trimmed, and a 10 μ m continuous section was cut by a rotary microtome and put up. When the section was completely dry, the paraffin was dissolved and the section stained by Safranin-fast green double staining (4% Safranin O in 50% alcohol; 2% fast green in 95% alcohol) and was then observed and photographed with an optical microscope.

Determination of fruit quality

Litchi fruits were harvested 84 days after blooming of female flowers for determination of fruit quality, which included TSS, titratable acidity (TA) and Vitamin C content. The measurement of TSS was carried out by extracting arils and juices of fruits and testing the total soluble solids contained by a hand-refractometer (Atago); the unit was degrees Brix ($^{\circ}$ Brix). The measurement of TA used an automatic potentiometric titrator to examine 5 mL of filter juice, titrating it with 0.1 N NaOH solution until a pH value of 8.1 was reached. As malic acid is the major acid in mature litchi fruits (Paull et al., 1984), it was measured to represent TA. The determination of Vitamin C was based on Kampfenkel et al. (1995). We placed 2.0 g arils in 5 ml of 6% trichloroacetic acid solution to homogenize it and then conducted centrifugation of 12,000 g for 20 min. This method adopted the principle of transforming Fe^{3+} to Fe^{2+} by Vitamin C in the acid solution. Fe^{2+} tends to combine with 2'-bipyridyl, forming a pink substance and achieving the strongest absorbance at wavelength of 525 nm. We used Vitamin C solutions to set up a standard curve so as to calculate the Vitamin C content in the samples.

Statistics

ANOVA analysis was conducted on the experimental data by the statistical analysis system (SAS) and its significance level within 5% deviation was confirmed by Duncan's multiple range test.

RESULTS

Parthenocarpy ability

This study conducted emasculatation on bagged inflorescences of 'Dazao' and 'Wuheli' litchi and observed the parthenocarpy ability of female flowers in an environment without pollen parents. The results suggest that, when not pollinated, female flowers of both 'Dazao' and 'Wuheli' tend to fall within four to five days of blooming. In a natural environment, ovaries of 'Dazao' and 'Wuheli' do not set fruits or continue developing to become fruits without pollination.

Fruit development and seed types

The two fruit cultivars were harvested on the 84th day after blooming; their seed types are presented in Figure 1. 'Dazao' fruits had normal seeds, with an average weight of 3.48 g while seeds of 'Wuheli' presented four different development types, with an average weight of 5.78 g for normal seeds, 0.73 g for later aborted seeds, 0.30 g for middle aborted seeds and 0.1 g for early aborted seeds. According to records on the weight development of fruits, seeds, peels and arils, the development of 'Dazao' fruits display a single sigmoid growth curve. Fruit weights increased from the 28th day after blooming and grew rapidly from the 42nd day. During the early development of fruits, mostly peels and seeds developed. Peel weights grew only gradually between the first day and the 84th day after blooming.

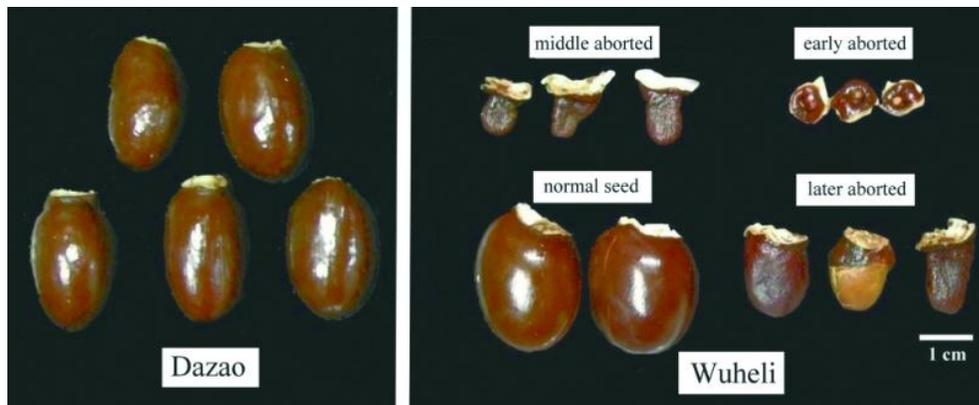


Figure 1. Seed types of 'Dazao' and 'Wuheli' fruits on the 84th day after blooming. Normal seeds of 'Wuheli' weigh more than 1 g, while late aborted seeds are between 0.5 g and 1 g, middle aborted seeds between 0.2 g and 0.49 g and early aborted seeds under 0.2 g.

Seed weights only increased slowly between the 14th and the 35th day after blooming and developed more rapidly between the 35th and the 63rd day. Afterwards, seed development matured gradually such that increase in seed weight became minor. Aril developed gradually from the 35th day after blooming. Aril weight increased slowly between the 35th and the 63rd day after blooming and grew rapidly between the 63rd and 84th day (Figure 2A).

Seed development of 'Wuheli' litchi can be categorized into four types: normal seeds, with weight greater than 1 g on the 84th day after blooming; later aborted seeds, with weight between 0.5 g and 1 g on the 84th day; middle aborted seeds, with weight between 0.2 and 0.49 g respectively, on the 84th day and early aborted seeds, with weight under 0.2 g on the 84th day. All four types presented a single sigmoid growth curve (Figure 2B, C, D and E). Seed weights grew slowly between the 14th and the 63rd day after blooming but rapidly from the 63rd day. Peel weights only increased slowly. Arils of normal, late aborted and middle aborted fruits commenced rapid development from the 56th day after blooming while the aril development of early aborted seeds only accelerated from the 63rd day after blooming. Weights of normal seeds increased slowly between the 35th and 49th day after blooming (Figure 2F) but rapidly from the 49th day. Weights of the three other types of seeds showed only a slow and gradual trend of increase.

We used tissue embedding sections and a stereomicroscope to observe embryo development after fertilization and embryo abortion. Embryo development of large-seed 'Dazao' is shown in Figure 3. Ovules of female flowers are anatropous ovules of a polygonum type with embryo sacs covered by inter integuments and with an ovum close to the micropyle and the outside layer covered by outer integuments (Figure 3A). Between the 7th and 14th day after blooming during embryo development, fertilized zygotes in embryo sacs showed no apparent changes as zygotes in this period were in

dormancy (Figure 3B). Embryos became globular (Figure 3C and D) between the 14th and 21st day after blooming, became heart-shaped (Figure 3E) on the 28th day and took the form of torpedo embryos (Figure 3F) on the 35th day. Observations on embryo development of 'Wuheli' showed that normal ovules became globular embryos on the 28th day after blooming (Figure 4A). Ovule sections on the 28th day also showed zygotes that are barely developed (Figure 4B), which possibly indicated the earliest embryo abortion. In addition, abortion divisions (Figure 4C) and abnormal embryos that did not develop inside an embryo sac but outside the micropyles (Figure 4D) were also found, which may be an indicator for middle and late abortion. From the 35th day after blooming, the abnormally developed embryos were able to continue developing. Observations using a stereomicroscope found that the inner seed cavity of early aborted seeds were already significantly browned and had no obvious embryo structure on the 63rd day after blooming (Figure 4E). Abnormal embryos developed inside embryo sacs also appeared browned and water-soaked, with an anomalous shape (Figure 4F).

Quality characteristics

Properties and quality of the 'Dazao' and 'Wuheli' fruits studied are presented in Table 1. 'Dazao' is a large-seed cultivar, whose average fruit weight was approximately 19.51 g and aril weight 12.83 g, with an aril ratio of 65.64%, 18.5°Brix of TSS, and 0.22% of TA. Among 'Wuheli' fruits, the shriveled ratio was 11.5% of large-seed normal fruits, 25.4% of late aborted fruits, 29.5% of middle aborted fruits and 33.6% of early aborted fruits, with a total ratio of 88.5%. Fruit weights, diameters and transverse diameters of normal, late aborted and middle aborted fruits were larger than early aborted fruits. Normal, late aborted and middle aborted fruits all weighed approximately 29 g, belonging to large fruits.

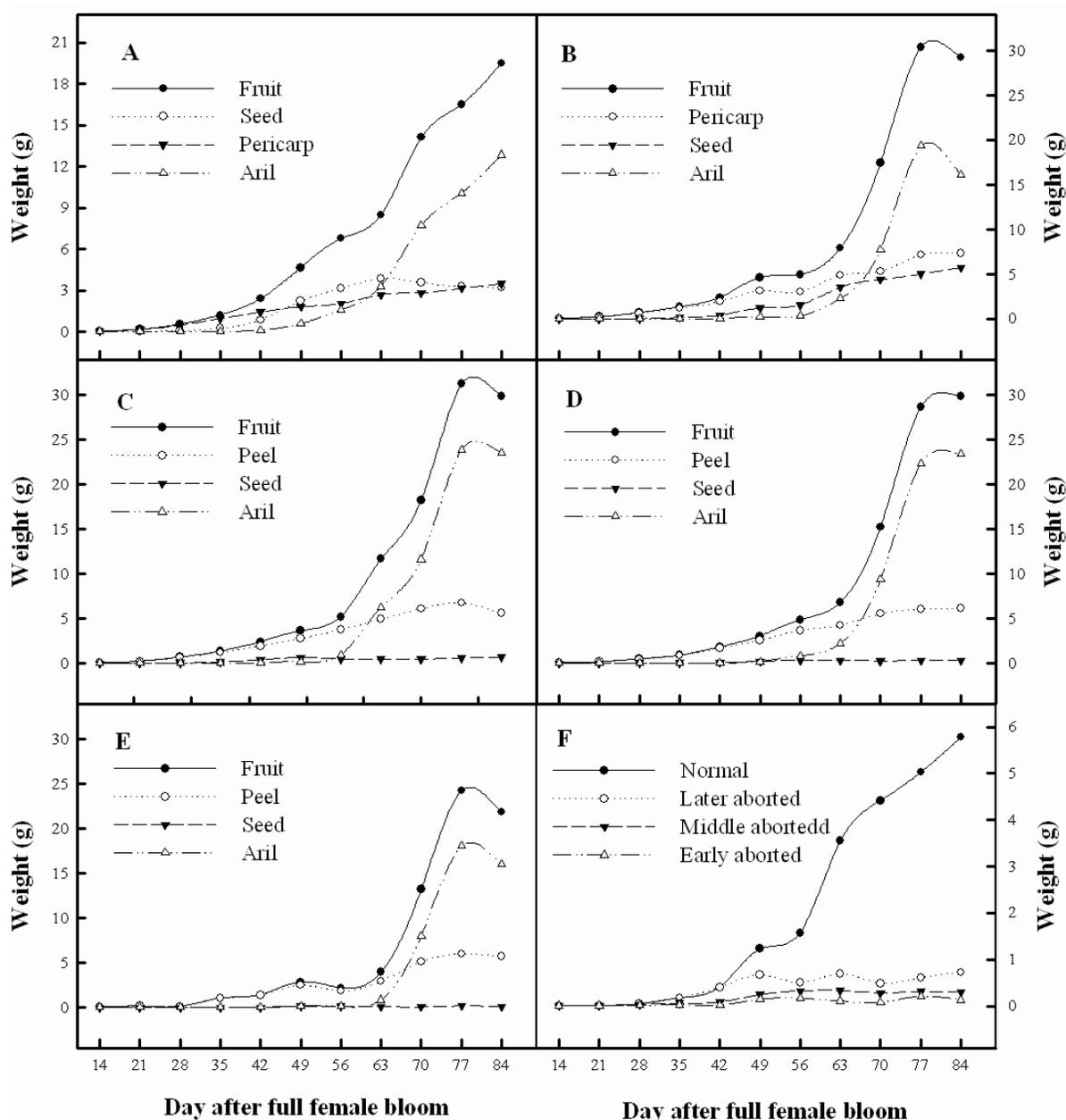


Figure 2. Weight changes of fruit, peels, seeds, arils of 'Dazao' and 'Wuheli'. A is 'Dazao'; B represents normal fruit of 'Wuheli'; C represents late aborted fruit of 'Wuheli'; D represents middle aborted fruit of 'Wuheli'; E represents early aborted fruit of 'Wuheli'. F represents weight changes of various types of 'Wuheli'.

Meanwhile, early aborted fruits weighed approximately 21.87 g, which were apparently impacted by the early embryo abortion. In terms of seed weight, normal 'Wuheli' seeds weighed approximately 5.87 g, while aborted seeds weighed less than 1 g. Normal seeds weighed significantly more than other aborted seeds. Arils of late and middle aborted fruits weighed significantly more than arils of normal and early aborted fruits such that normal fruits had a relatively lower aril ratio of 53.93%. The aril ratios were approximately 78% of late and middle aborted

fruits and 73% of early aborted fruits. Concerning the quality of 'Wuheli', TSS of early aborted fruits was significantly lower than fruits of the other three types, while TA and Vitamin C content in normal fruits were significantly higher than in aborted fruits.

DISCUSSION

With regards to fruit breeding, shriveled seeds caused by

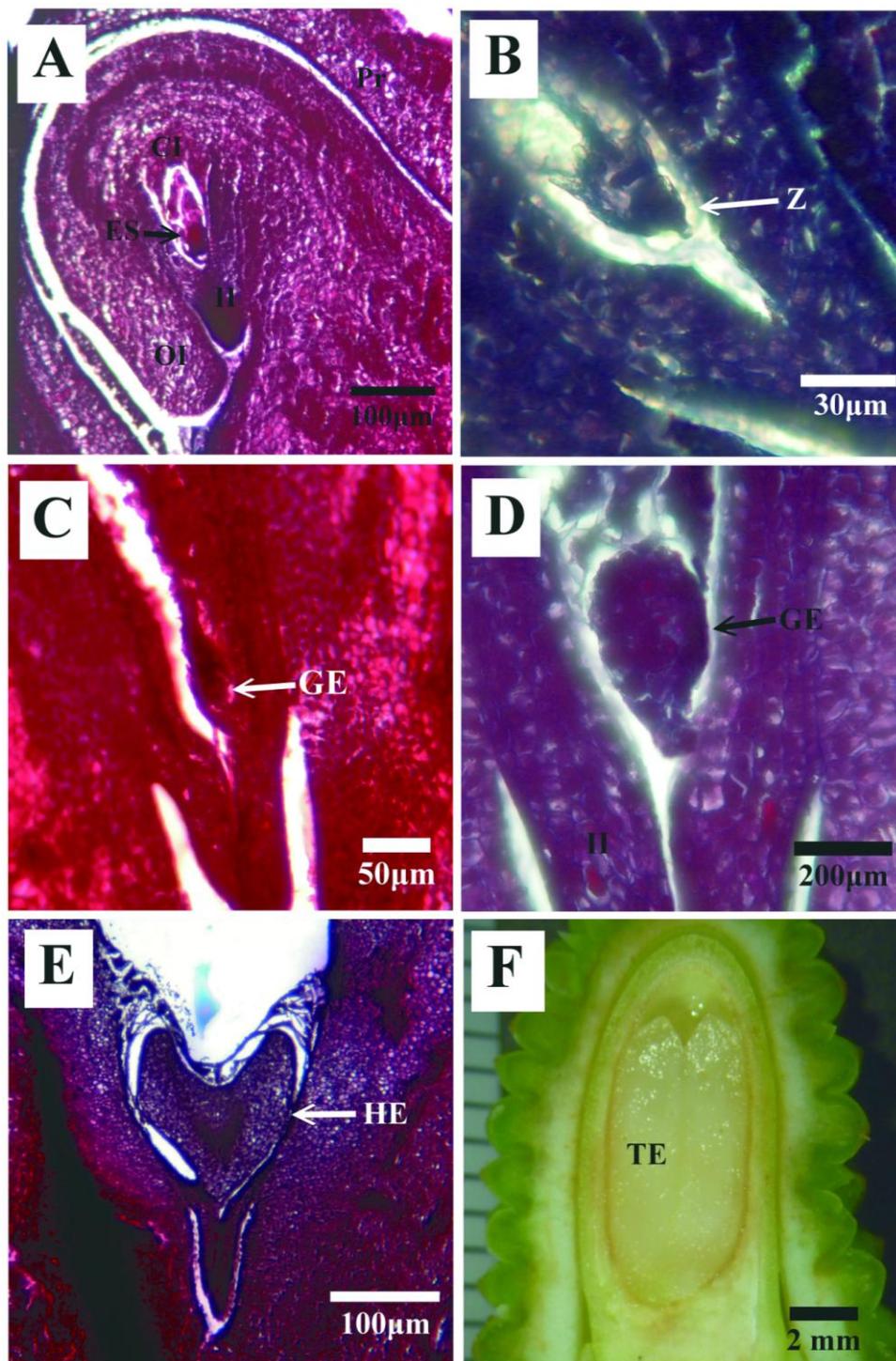


Figure 3. Paraffin section and stereomicroscope observation of 'Dazao' embryo development; A is an ovule in blooming period; B is the 7th day after blooming; C the 14th day; D the 21st day; Figure 3E the 28th day; and F the 35th day. CI; Chalaza, Z; Zygote, ES; Embryo sac, II; Inter integuments, OI; Outer integuments, Pr; pericarp, GE; Globular embryo, HE; Heart embryo, TE; Torpedo embryo.

embryo abortion have always been breeders' main concern. The problem of large or multiple seeds among

various commercial fruits (such as litchi, longan, mango and citrus fruit) is a factor impacting their quality

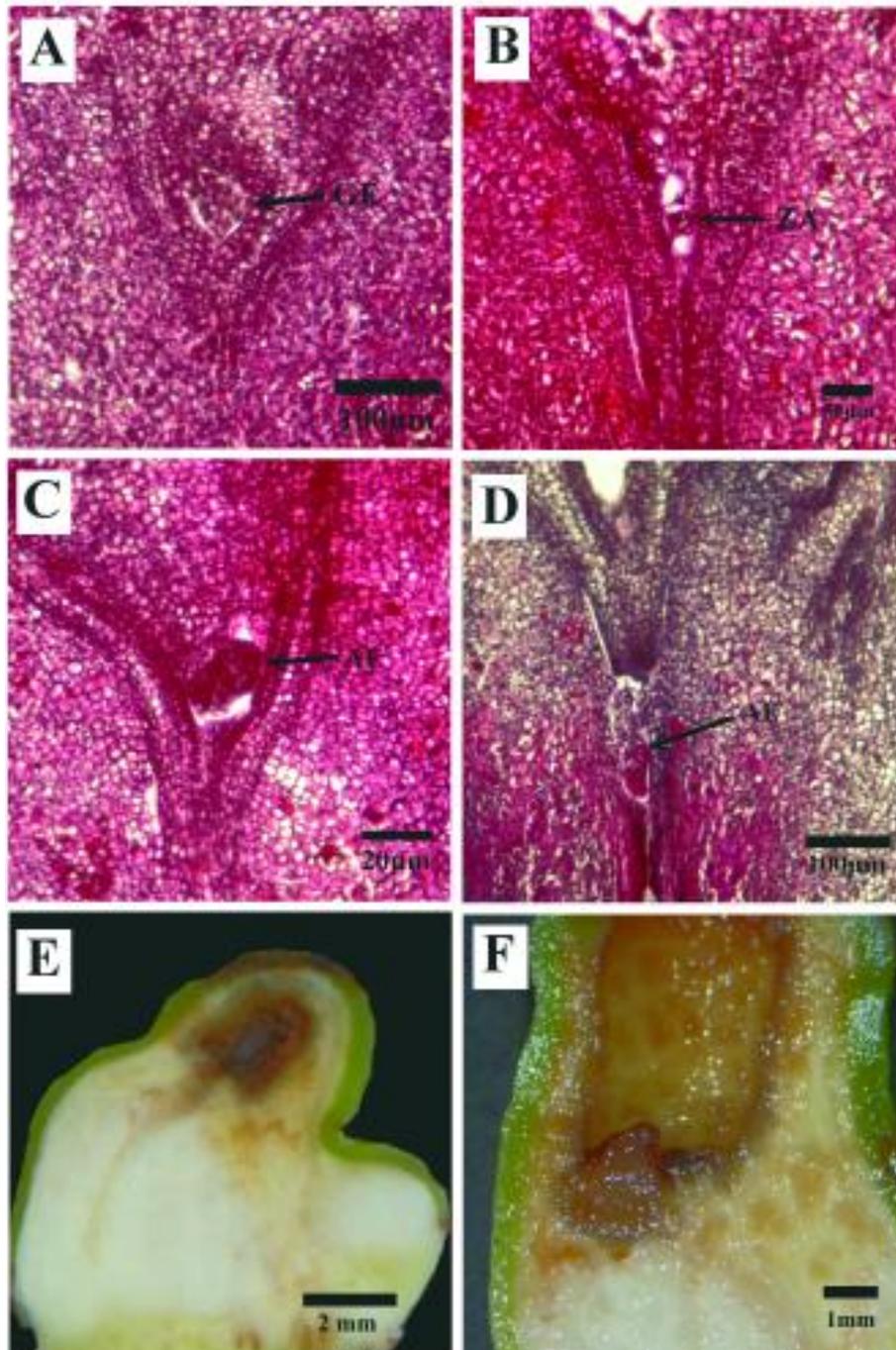


Figure 4. Paraffin section and stereomicroscope observation of 'Wuheli' embryo development; A, B, C and D show the 28th day after blooming; E and F the 63rd day after blooming. ZA; zygote aborted, II; inter integuments, GE; globular embryo, AE; abnormal embryo.

(Hasegawa et al., 2003; Stern and Gazit, 2003). Litchi embryo development is impacted by both heredity and environmental factors. Among environmental factors, plant hormone regulation is the key. Climate changes and nutrient content affect embryo development or abortion through different concentrations and balances of various

types of plant hormone (Gao et al., 2001; Li and Zheng, 2004; Lu, 2001). Regulation of cell division and the physiological and biochemical reactions that control cell development are all dominated by genetic heredity and plant hormone. While cytokinin (CTK) is transported into embryos or seeds during the development process, seed

Table 1. Fruit properties and quality of different types of seeds of 'Dazao' and 'Wuheli'¹.

Cultivar	'Dazao'		'Wuheli' ^{2,3}		
	Normal	Normal	Later aborted	Middle aborted	Early aborted
Seed type					
Ratio of the seed type (%)	99.9	9.6	23.8	47.6	19.0
Fruit weight (g)	19.51	29.24 ^a	29.87 ^a	29.89 ^a	21.87 ^b
Transversal Diameter (mm)	30.58	36.36 ^a	38.54 ^a	38.95 ^a	34.10 ^b
Longitudinal diameter (mm)	36.08	38.87 ^a	38.14 ^a	37.77 ^a	34.10 ^b
Pericarp weight (g)	3.48	7.34 ^a	5.65 ^b	6.19 ^a	5.72 ^b
Aril weight (g)	12.83	16.12 ^b	23.50 ^a	23.39 ^a	16.01 ^b
Seed weight (g)	3.20	5.78 ^a	0.73 ^b	0.30 ^c	0.13 ^c
Aril ratio (%)	65.64	53.93 ^b	77.21 ^a	78.15 ^a	73.30 ^a
Total soluble solids (°Brix)	18.5	15.4 ^a	14.9 ^a	15.1 ^a	13.2 ^b
Titrateable acidity (%)	0.22	0.57 ^a	0.41 ^b	0.44 ^c	0.47 ^b
Ascorbic acid (mg/100 g)	28.63	30.59 ^a	24.71 ^c	26.18 ^b	23.53 ^c

¹. Fruits were harvested on the 84th day after blooming; ². Seeds that weighed more than 1 g were normal seeds, between 0.5 and 1 g were later aborted seeds, between 0.2 and 0.49 g were middle aborted seeds and less than 0.2 g were early aborted seeds; ³. Duncan's multiple range test was used to analyze the significance level with 5% of deviation ($\alpha = 0.05$, $n = 10$).

development regulates the transmission of CTK in fruits and positively influences cell division and expansion around the seeds. Volume expansion of cells determines the final sizes of fruits. Auxin is an important substance that regulates cell expansion of fruits; it improves the extensibility of cell walls and increases or maintains the absorption of water and solutes in cells. In addition to synthesizing auxin to regulate the division of surrounding cells, embryos or seeds that are undergoing development also produce signaling molecules to regulate the expansion of surrounding cells as well as their sink vitality. Auxin and signal molecules need to cooperate in order to increase cell volumes; this explains why the use of auxin alone does not expand fruit cells *in vitro* (Gillaspy et al., 1993).

Chen and Lu (2001, 2002) examined changes in plant hormone content during ovule development of litchi and found that ovules of aborted fruits have a lower content of indole acetic acid (IAA) and CTK than normal ovules do. In addition, ovules of aborted fruits have a significantly higher content of p-Hydroxybenzoic acid (p-HBA), which is a very powerful growth inhibitor, than normal fruits. In terms of heredity, pollen parents have a significant impact on embryo development of plants. Comparing fruits derived from self-pollination and cross-pollination shows that a smaller number of fruits and seeds result from fertilization through self-pollination, with a higher abortion rate and weaker vitality of future generations (Stephenson, 1981). Crossbred fruits and seeds from "Floridian" and "Mauritus" litchi are larger than self-bred ones. Researchers believe that pollen parent is a factor associated with aborted fruits and seeds (Stern et al., 1993). Embryo development of litchi is not controlled by a single allele; instead, embryo development is regulated by different alleles. Mutations can result in abortions at different times (Lu, 2001).

Fruit development can be divided into three stages. The first stage is ovary development and the fruit setting process that determines further development or abortion. The second stage is cell division of fruits, and the third stage starts from volume expansion of fruit cells after division till fruit volumes reach the final fruit sizes. At this stage, because cells contain strong sink activity during expansion, obvious state and physiological changes occur (Gillaspy et al., 1993). Within two to three days of pollination, female litchi flowers produce zygotes and free nucleus through double fertilization, while nucellar cells and micropyle plugs start to dissociate. Divisions occur within zygotes after five days of dormancy. Within 15 days of pollination, zygotes divide into dozens of cell embryos. The original embryos experience organ differentiation and turn into globular embryos 20 days after pollination, form heart-shaped embryos by 25 days and torpedo embryos in 30 days, with their endosperms gradually degenerated and free nucleus mostly absorbed by embryo development. After the 35th day after pollination, embryos continue to develop, with cotyledons growing rapidly. From the 55th day on, developed cotyledons fill up seed cavities and differentiate radicles and plumule. The basic development of embryos is completed in approximately 60 days. Arils develop rapidly from the 42nd day after blooming (Gao et al., 2001; Stern and Gazit, 2003; Wang and Qiu, 1997). According to investigations, 'Dazao' is a typical litchi cultivar with normal embryo development while embryo abortions of 'Wuheli' litchi result in significantly different fruit development behavior due to different stages of abortion. It is assumed that middle aborted fruits from 'Wuheli' litchi are caused by abnormal or aborted globular or heart-shaped embryos while late aborted fruits tend to result from abnormal or aborted torpedo embryos or embryos in the cotyledon period. Also, 'Wuheli' litchi contains ovules

that seem to be undeveloped. Based on the results concerning parthenocarpy, non-pollination results in ceased development of pistils and thus, fallen petals. As a result, the ovule-type seeds of 'Wuheli' litchi that seem undeveloped are in fact an early embryo abortion after pollination. The embryos cease to develop during the early stage of zygote division after fertilization, significantly oppressing ovule development and causing ovules to have nearly no seeds after fruits mature.

The consumable part of litchi fruit is the aril. As seeds and arils compete for assimilation, no-seed fruits or aborted seeds may reduce seeds' competition for assimilation and assist arils in acquiring assimilation, promoting full development of arils (Huang et al., 2001). The relation between embryo abortion stage and aril development of 'Wuheli' litchi suggests that embryo abortion of litchi fruits in the early stage of development may hinder synthesis of hormone and signal molecules and impact cell division and expansion of ovules and arils, resulting in arils inferior to middle and late aborted shriveled fruits. During the process of embryo development, cotyledons expand by quickly accumulating nutrients and carbohydrates during the rapid growth period, with their weight growing rapidly as well. At this time, compared with arils, an embryo is a powerful sink for nutrition, having a strong need for nutrients and carbohydrates. Abortion or abnormal development occurring to globular embryo or heart-shaped embryo of litchi or during the rapid growth period of cotyledons may reduce the competitiveness of embryo development for nutrients, allowing arils to obtain more carbohydrate and nutrient supplies, enhancing cell division and development of arils and improving aril content and aril ratio. Therefore, the structural development of fruits is significantly impacted by embryo development. In terms of improving aril ratio, neither normal nor early aborted embryos have a positive impact.

Fruit development is clearly impacted by seed development, as embryos in seeds provide peels and arils with plant hormone and relevant growth substances necessary for cell division. Early aborted embryos may decrease supplies of relevant growth hormones, hindering the structural development of fruits and further impacting fruit size and aril content. A middle or later abortion of embryos provides fruits with relevant growth hormones in the early stage of fruit development, allowing fruit structure to fully develop. Furthermore, embryo abortions occurring in the heart-shaped period or the rapid growth period of cotyledons may compromise embryos' ability to compete for carbohydrates, so that arils can obtain sufficient nutrient for cell division and growth. Therefore, the early abortions of embryo provide small seeds, but have no effect on improving the aril ratio of fruits.

ACKNOWLEDGMENTS

Thanks to Fengshan Tropical Horticultural Experiment Branch for providing litchi material that was used in this study.

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