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THE PISTIL STRUCTURE IN LOQUAT [*ERIOBOTRYA JAPONICA* (THUNB.) LINDL.]

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Abstract

In this study the pistil structure of loquat (*Eriobotrya japonica* (Thunb.) Lindl.) was investigated for the first time through free-hand section and observation on seed set under manually controlled pollination. The results showed that 5 styles with stigmas, free at base, inserted on the upper surface of the ovary. The ovary consisted of 5 fused carpels; the septums, originating from the carpellary margins, divided the ovary into 5 locules, with each locule containing a placenta to which the 2 ovules attached; the locules were lined by a thick cuticle. In the hand-pollination to the only one stigma left (4 styles were removed), seeds were produced only in one locule (fertile locule), while in other 4 locules all the 8 ovules were unfertilized and wilt. 76.67% fertile locules produced two normal seeds and 23.33% fertile locules produced only one normal seed and the other degenerated. Therefore, it can be concluded that 5 styles and 5 locules had a one-to-one relationship in both morphological and reproductive characteristics in loquat.

Introduction

Loquat (*Eriobotrya japonica* (Thunb.) Lindl.), an important perennial evergreen fruit crop that originated in China, is well-known all over the world for high nutrient, medicinal, economic and ecological values（Xu *et al*., 2007; Hussain *et al*., 2009; Hussain *et al*., 2011b）and is widely cultivated between 20° and 35° latitude, including Japan China, india, Pakistan, Spain and so on (Badenes *et al*., 2000; Hussain *et al*., 2007; 2011a).

The understanding of pistil structure and reproductive characteristics is crucial for understanding the reproduction biology and sterility. However, in loquat, the pistil structure is not yet well understood, compared to other species of family Rosaceae. Up to now, our limited knowledge about the pistil of loquat is contributed by Wilson (1920) and Qiu & Zhang (1996): the former described indistinctly that loquat had five pistils that joined toward the base, while the latter described that the loquat pistil consisted of five styles with papillate stigmas, an ovary with five locules and two ovules situated in each locule. Especially, it remains uncertain regarding the structure of styles, and so far a popular belief is that the five styles connate at base (Cai, 2000; Huang, 2003). Furthermore, the relationship between five styles and five locules, pistil structure and seed set, as well as seed abortion are unknown.

In the present study, the relationship between the pistil structure and seed reproduction was addressed for the first time in loquat. The objectives of this study were to investigate the loquat pistil structure through free-hand section and observation on seed set in hand-pollination after removal of 4 styles (only one style left) and to clarify the morphological and reproductive characteristics of loquat.

Materials and methods

The study was carried out in November 2009 and May 2010 in the loquat orchard of the Biotechnology Research Center for Horticulture, Sichuan Agricultural University, Ya'an, China. In November 2009, flowers of cv. 'Dawuxing' at fully open development stage were collected to make free-hand section for observation of pistil structure. The samples were sectioned transversely and longitudinally. Observation was performed under an anatomical lens (14X). Images were captured by LY-WN-HP SUPER CCD imaging system (Chengdu Liyang Precision Machinery Co., LTD, Chengdu, China).

Pollen collected from cv. 'Zaozhong 6' was used to pollinate the flowers of 'Dawuxing' under orchard conditions in November 2009 (the manual control-pollination process is shown in Fig. 1). To prevent insect pollination and self-pollination before experiment, the flower buds at balloon stage (petals were slightly closed and anthers were not split yet) were selected strictly. After removing all the stamens and 4 styles, a large quantity of pollen grains were applied onto the remaining stigma to ensure that the ovules were fertilized successfully (Fig. 1). At the same time, hand pollination to all the 5 stigmas left was conducted as contrast. On May 21, 2010, 90 fruits from hand pollination to one stigma and 30 fruits from hand pollination to 5 stigmas were harvested and seed sets were investigated, respectively and images were captured digitally using a digital camera.

Results

Free-hand section and lens observation were conducted and the anatomical structure of pistil in loquat is shown in Fig. 2. The 5 styles with stigmas, free at base, inserted into the upper surface of the ovary. The ovary consisted of 5 fused carpels. The septums originating from the carpellary margins divided the into 5 locules, with each locule containing a placenta to which the 2 ovules attached. The locules themselves were lined by a thick cuticle. Each one of the 5 styles led to one of the 5 locules.

Manual control-pollination in the field was conducted to clarify the reproductive relationship between 5 styles and 5 locules in loquat. With the control (all the 5 styles were left), in all the 30 fruits, there were seeds in every locule, including normal seeds and degenerative seeds to various degrees, the seed distribution pattern was shown in Fig. 3 a & b.

When only one style was left, there were seeds only in one locule (fertile locule) per fruit, while all the 8 ovules in other 4 lobules were unfertilized and wilt (Fig. 3 c & d and e & f). 76.67% fertile locules produced two normal seeds (seed set pattern I) (Fig.3 c & d, Table 1); while 23.33% fertile locules had only one normal seed with the other one degenerated (seed set pattern II) (Fig.3 e & f, Table 1). Seed degradation rate was 11.67% when only one style left (Table 1).

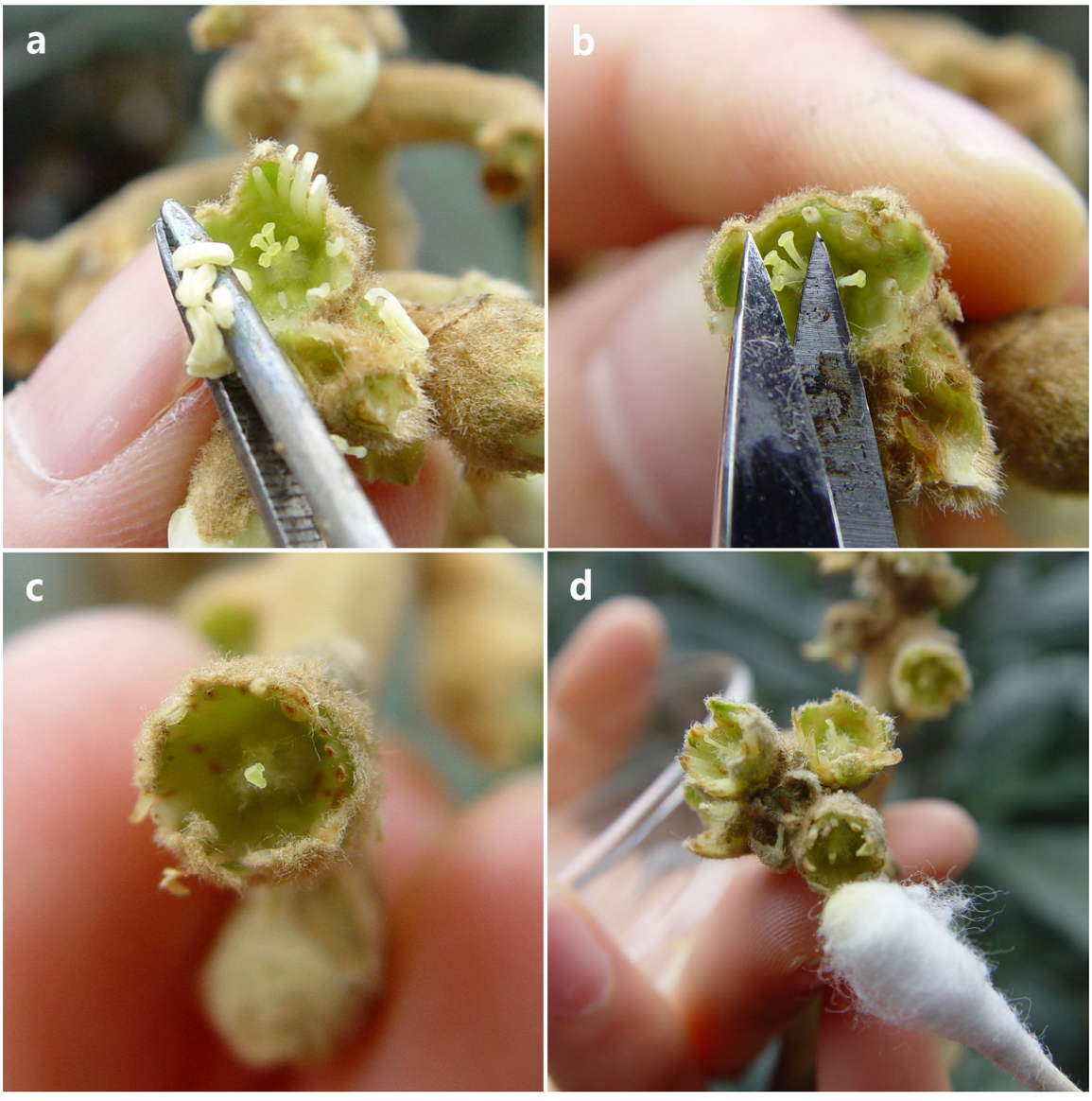

Fig.1. Illustration of the manual control-pollination process. (a) Removing stamens. (b) Cutting off 4 styles randomly. (c) Leaving one style only. (d) Hand-pollination.

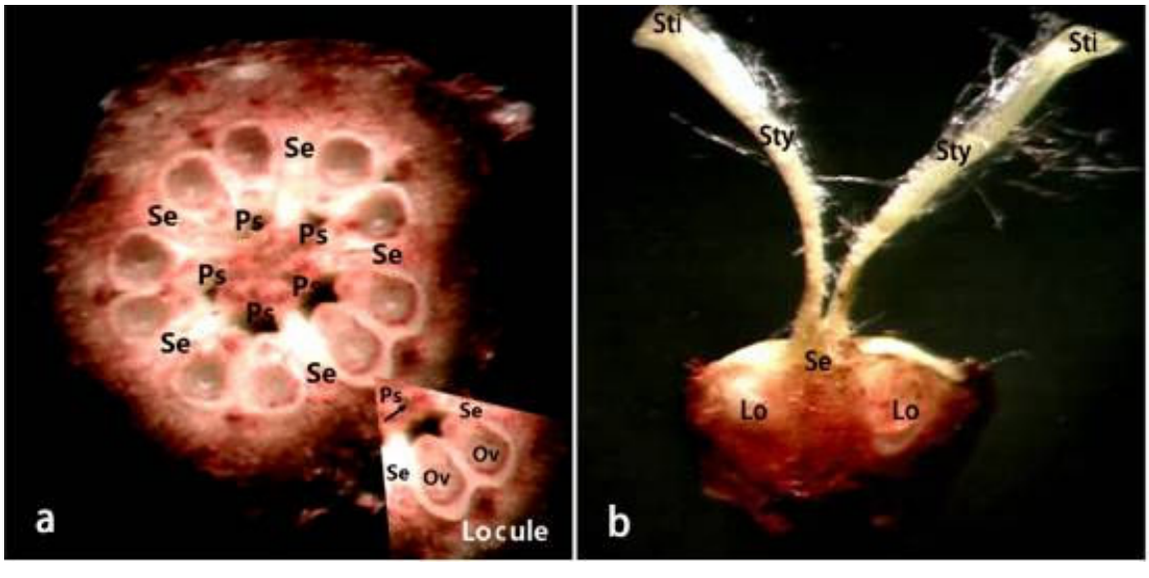

Fig. 2. Free-hand section of the pistil in loquat (14X). (a)Transverse section (Se=septum; Ov=ovule; Ps=position inserted by the style). (b) Longitudinal section (Sti=stigma; Sty=style; Se=septum; Lo=locule).

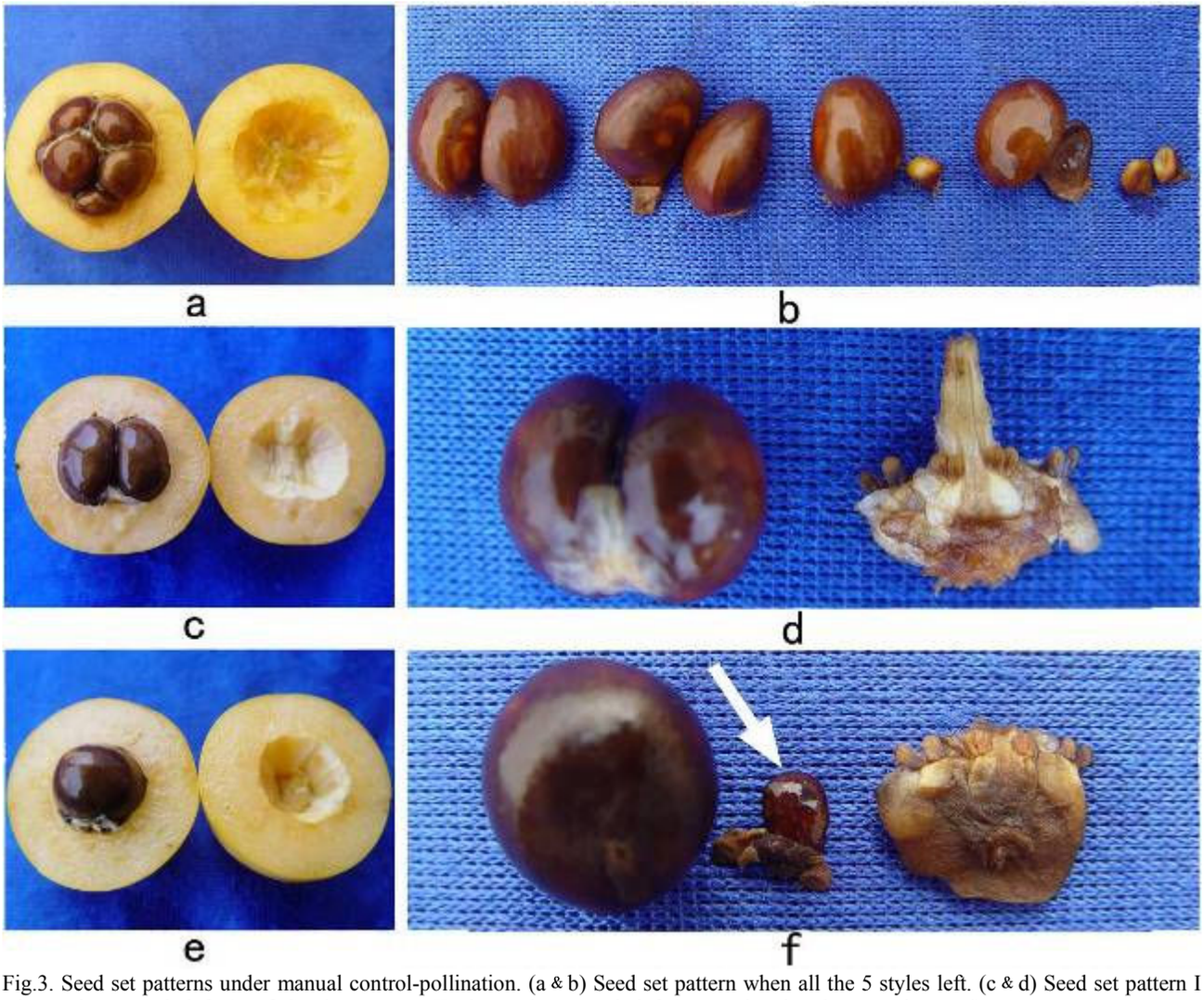

Fig.3. Seed set patterns under manual control-pollination. (a & b) Seed set pattern when all the 5 styles left. (c & d) Seed set pattern I when only one style left. (e & f) Seed set pattern II when only one style left (arrow showing degenerative seed).

Table 1. Seed set patterns and degenerative seeds in 90 fruits derived when only one style left.

Fruit number	Seed number	Seed set pattern I		Seed set pattern II		Degenerative seed	
		number	%	number	%	number	%
90	180	69	76.67	21	23.33	21	11.67

Discussion

Pistil (female floral organ) is the sexual reproductive structure of plants, and has attracted almost full attention of botanists (Susan, 1994; Owens *et al*., 1995; Fabrizio *et al*.,1996; Marianne & Mohamed, 1998; Xua *et al*.,2009). Despite the important values of loquat, the reproductive biology of this species remains relatively unknown. Especially, little is known of the morphological and reproductive relationship between the five styles and 5 locules, pistil structure and seed set, as well as seed abortion. In the present study, the pistil structure was experimentally examined in loquat for the first time. It was also the first time that the relationship between the pistil structure and seed abortion was addressed in loquat.

Based on the observation of free-hand section and seed set in hand-pollination after removal of 4 styles (only one style left), the pistil structure of loquat can be described clearly. The 5 styles were free at base, which corrects the popular belief (Huang，2003). And also, the 5 styles and 5 locules have a one-to-one relationship in both the morphological and reproductive characteristics, which can be useful for understanding the reproduction biology and its sterility in loquat.

Since there are 5 locules in the ovary and 2 ovules in each locule, the seeds may be as many as ten if all the ovules develop to seeds. But usually several of the ovules are aborted and not more than three to five seeds develop, the others degenerate to certain degrees, and the abortion rate of seed is about 60-70% (Deng *et al*., 2007). The previous studies on seed abortion in loquat were focused all on meiosis of pollen mother cells (Lv, 1984; Liang *et al*., 1999; Yan *et al*., 2009). In this study, in the hand-pollination to the flowers with only one style left, 23.33% fruit had only one normal seed in one locule, while the other one degenerated. Similarly, stone fruit species have 2 ovules but only one is capable of fertilization. One ovule (primary or functional ovule) has the ability to

develop into a seed while the secondary ovule is mostly underdeveloped and aborts prematurely (Eaton, 1959; Pimienta & Polito, 1982). In the present study, seed degradation rate in loquat was 11.67% when only one style left, while the seed abortion rate was about 60-70% when 5 styles left (Deng *et al*., 2007). Based on the pistil structure revealed in the this study, we can attribute the seed deterioration mechanism most preferably to the 5 styles free at base and the one-to-one relationship between the 5 styles and 5 locules in both the morphological and reproductive characteristics in loquat. Deng *et al*., (2011) found that there were significant differences in length of the 5 styles, and the development and receptivity of the 5 stigmas, which would lead to differences in pollen germination on the 5 stigmas, as well as pollen tube growth in the 5 styles in a flower. The fertilization and development of the ovules in each locule differ certainly, the seeds in different locules undergo different resource environment, and even the 2 seeds in the same locule are still faced with resource contention, thus leading to degradation inordinately. This would account for the high rate and various degrees of seed degradation in loquat.

Conclusion

In this study, based on the observation of free-hand section and seed set in hand-pollination after removal of 4 styles (only one style left), the pistil structure of loquat (*Eriobotrya japonica*(Thunb.)Lindl.) can be described clearly for the first time: the 5 styles were free at base and had a one-to-one relationship between the 5 styles and the 5 locules in both the morphological and reproductive characteristics, which would account for the high rate and various degrees of seed degradation in loquat.

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