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The Relationship between Styles and Locules in Loquat and its Relation to Seed Set and Abortion

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Abstract: This study investigated the relationship between styles and locules through observation seed set under manual control-pollination in loquat (*Eriobotrya japonica* (Thunb.) Lindl.). After hand-pollination with removal of 4 styles (only 1 style left), accordingly there are seeds only in one locule per fruit with 2 types of seed set patterns: 76.67% fruit had 2 normal seeds which are in accord with pattern I; 23.33% had only 1 normal seed but the other one degenerated which are in accord with pattern II. While all the ovules in other 4 lobules are unfertilized and in wilts; seed degradation rate is 11.67%.

Key words: Eriobotrya japonica, style, locule, seed set, seed abortion, China

INTRODUCTION

Loquat is an important perennial evergreen fruit tree that originated in China and it is widely cultivated between 20° and 35° latitude (Lin et al., 2004). It is well-known all over the world for high nutrient, economic and ecological values. There are 10 ovules per fruit in loquat but usually only about 3 or 4 seeds mature, the others degenerate in various degrees and the abortion rate of seed was about 60-70% per fruit (Deng et al., 2007). The seed deterioration mechanism of loquat had been recently focused on by many researchers (Deng, 2008; Jaun et al., 2009). Earlier, observed some abnormalities in pollen mother cells meiosis such as chromosomes bridges, chromosomes fragment and lagging chromosomes in loquat and in the study of Jaun et al. (2009), about 3.9% of pollen mother cells had non-division or nonsynchronous division at meiosis which may cause zygotic infertility and seed abortion. In Deng (2008)'s research, the unnormal pollen-tube growth and embryonic development would result in degenerative seeds.

Pistil is the female floral organ involved in sexual reproduction and consists of the stigma which receives pollen grains and provides a favourable environment for germination, the style which supports pollen tube growth and the ovary, containing the ovules (Shivanna, 2003). The understanding of loquat female reproductive organs and reproductive characteristics can provide basic theory for the study of seed degradation mechanism. Loquat pistil consists of 5 styles with papillate stigmas, an ovary with 5 locules and 2 ovules situated in each locule

(Qiu and Zhang, 1996). However, its pistil structure is not yet well undersood. Especially, the relationship between 5 styles and 5 locules and its relation to seed set and abortion are unknown. The objectives of this study were to clarify the relationship between styles and locules through observation seed set of hand-pollination after removal of 4 styles (only 1 style left) in loquat and also, the seed set and abortion of loquat was discussed.

MATERIALS AND METHODS

The study was carried out in November, 2009 and May, 2010 in loquat orchard of Research Center for Horticulture Biotechnology, Sichuan Agricultural University, Ya'an, 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 was shown in Fig. 1a-f). 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.

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 1 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.

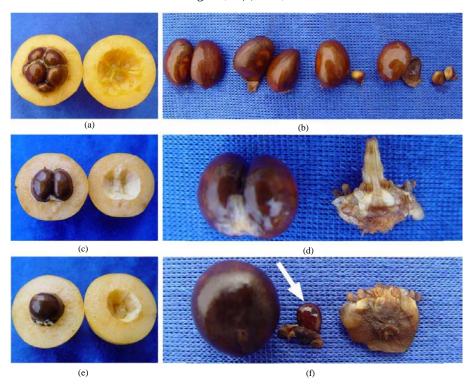


Fig. 1: Seed set patterns under manual control-pollination; a and b) seed set pattern when all the 5 styles left, c and d) seed set pattern I when only one style left, e and f) seed set pattern II when only one style left (arrow showing degenerative seed)

RESULTS AND DISCUSSION

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 locules including normal seeds and degenerative seeds to various degrees, the seed distribution pattern was shown in Fig. 1a and b.

When only 1 style left, there were seeds only in 1 locule (fertile locule) per fruit while all the 8 ovules in other 4 lobules were unfertilized and wilt (Fig. 1c-f). About 76.67% fertile locules produced two normal seeds (seed set pattern I) (Fig. 1c and d, Table 1) while 23.33% fertile locules had only 1 normal seed with the other one degenerated (seed set p attern II) (Fig.1 e and f, Table 1). Seed degradation rate was 11.67% when only 1 style left (Table 1). The female floral organ is the sexual reproductive structure of plants and has attrscted almost full attention of horticulturalists (Suxia et al., 2009; Cousin and Maataoui, 1998; Owens et al., 1995). Based on the observation of reproductive characteristics of hand-pollination after removal of 4 styles (only 1 style left), the pistil structure of loquat (Eriobotrya japonica

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

	Seed set pattern I			Seed set pattern II		Degenerative seed	
Fruit	Seed	-					
no.	no.	No.	Percentage	No.	Percentage	No.	Percentage
90	180	69	76.67	21	23.33	21	11.67

(Thunb.) Lindl.) can be described clearly that the 5 styles and 5 lobules have the relationship of 1-1 correspondence at reproductive characteristic in loquat. This results can supplement the cognition of pistil structure in loquat and it is the 1st time to clarify the relationship between styles and lobules.

The previous studies on seed abortion in loquat focused mainly on meiosis of pollen mother cells (Juan *et al.*, 2009) or on pollen tube growth by fluorescence microscopy and embryonic development by paraffin section (Deng, 2008) at the cytological level. In the current study, the relationship between pistil structure and seeds abortion was discussed firstly in loquat.

In this study, in the hand-pollination to the flowers with only 1 style left, 23.33% fruit had only 1 normal seed in one locule while the other one degenerated. Seed degradation rate in loquat was 11.67% when only 1 style left while the seed abortion rate was about 60-70% when

5 styles left (Deng et al., 2007). Based on the relationship between styles and locules revealed in the present study, researchers can attribute the seed deterioration mechanism most preferably to the 1-1 relationship between the 5 styles and 5 locules in reproductive characteristic 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. Moreover, there may be another explanation for the origin of the degenerative seed beside the normal seed in the same lobule (Fig. 1f).

The ovule may not be fertilized and haploid embryo may be stimulated by plant growth substances such as gibberellin, auxin, cytokinin and so on which were secreted by the normal embryo in the same locule (Tianzhong and Zhihong, 2008). This hypothesis can be supported indirectly by the research of Wang *et al.* (2005) in which a substantial proportion of haploids were identified in the plants regenerated from degenerative seeds of loquat. So in the next study, researchers will culture *in vitro* the seeds (Fig. 1f), save embryo into seedlings and determine the number of chromosomes in order to verify the hypothesis directly and obtain haploids.

CONCLUSION

The results demonstrated that the 5 styles and 5 lobules have the relationship of 1-1 correspondence at reproductive characteristics in loquat and its relation to seed set and abortion was discussed.

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