

Study on Characteristics of *in situ* Pollen Germination and Pollen Tube Growth of Loquat

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Abstract: The characteristics of pollen germination and pollen tube growth were studied by using 'Dawuxing' as test material. The results showed that the stigma receptivity lasted about 7 days and the pollen germination time was various among stigmas and there were long intervals among different stigmas (3-4 days). The rate of pollens whose tubes reached upper styles to germinated pollens was significantly higher than those reaching in open pollination and self-pollination. But there was no obvious difference in cross-pollination. The percentage of ovules in which pollen tube growth was normal in cross-pollination was obviously higher than that in self-pollination. The differences of pollen tube growth were remarkable in normally growing ovules, lightly degenerated ovules and completely degenerated ovules and also between the ovules of fallen flowers and normal sear flowers. Therefore, it could be concluded that 'Dawuxing' was self-incompatibility to some extent and pre-fertilization barrier was the basic reason for serious embryo abortion and the abundant genetic diversity of plants from degenerate seeds.

Key words: Loquat, pollen germination, pollen tube growth

INTRODUCTION

There are 5 ventricles in the each fruit and 2 seeds in a ventricle in loquat (*Eriobotrya japonica* Lindl). In the process of fruit growth, most seeds degenerated for various reasons and just 3 or 4 seeds can be mature. The degenerate seeds have a high genetic diversity, which offers valuable materials for breeding of loquat (Deng *et al.*, 2007). The embryo logic mechanisms can be divided into 4 types, the male sterility, female sterility, pollination and fertilization failure, embryo abortion. Embryo abortion varies in abortion time and anatomy characteristics among species (Liang *et al.*, 2005). In recent years, the fluorescence microscopy was applied to study on the characteristics of the pollen germination and pollen tube growth in Japanese apricot (Tao *et al.*, 2004), pear (Chen *et al.*, 2004; Zhang and Shin, 2000), Chinese cherry and sweet cherry (Li *et al.*, 2007), by many researchers. And these researches afforded many valuable embryology data to study on fertility, embryo abortion and reasonable utilization of breeding materials in fruit crops. But there is still few report about pollen germination and pollen tube growth in loquat at present.

In the present study, the characteristics of pollen germination and pollen tube growth were studied after pollinated in natural conditions and self- or cross-pollination by using 'Dawuxing' as test material. It was aimed at to offering embryology data to study sterility, embryo abortion and seed degradation in loquat.

MATERIALS AND METHODS

The 10 years old 'Dawuxing' plants were used as experimental materials, whose average pollen viability was 64.88% for 3 years and the number of normal seeds, low-grade degeneration seeds and abnormality seeds were 2.47, 0.34 and 7.19 in a fruit, respectively and the study was conducted in well developed bearing branches and inflorescences in the plants, which planted in the loquat orchard of Biotechnology Research Center for Horticulture of Sichuan Agricultural University. The pollinated cultivar was 'Longquan No. 1' with 72.83% pollen viability. The flowers were sampled at balloon stages, the pollens were collected and stored in -20°C refrigerator after pollens scatteration by cultivation in a 25°C constant temperature box.

Pollen germination and pollen tube growth in open pollination: On a sunny forenoon in full bloom, 270 flowers were labeled which were just at bloom. About 30 flowers from them were sampled to observe their state of pollen germination for 7 days. On the 9th day (after the 9th day, the rate of embryo with pollen tube tending to be stable according to pre-experiment), 30 flowers were sampled to observe the pollen germination on stigmas, pollen tube growth in styles and the pollentube growth in embryos. And on the 25th day (the sepals just furred and anthers withered and the embryos were differentiation in size could be identified by naked eyes in a flower and the embryos were classified normal growth, low-grade degeneration and abnormality embryos, which were displayed in Plate 1a-c), 30 flowers were sampled to observe the pollen tube growth in embryos in fallen flowers and normal flowers, respectively. The materials were brought to laboratory with ice pot, then the styles were cut with a blade and the embryos were picked out with a dissecting needle and these styles and embryos were placed in FAA (38% formaldehyde: acetic acid: 70% ethanol = 5:5:90).

Pollen germination and pollen tube growth in self-or cross-pollination in Dawuxing: At balloon stage, 70 flowers were labeled and isolated with sulfuric paper bags, in which 30 flowers were self-pollinated ('Dawuxing'×'Dawuxing') and cross-pollinated ('Dawuxing'×'Longquan No. 1'), respectively in the morning of the 3rd day after anthesis, the rest 10 flowers were left as control materials, opened. The pollinated flowers were sampled on the 5th day after pollination.

Pollen germination and pollen tube growth: The method applied to observe the pollen germination and pollen tube growth was according to the describing by Du Yuhu *et al.* (2007), meanwhile some steps of the method was altered. These materials were successively fixed more than 48 h in 1 mol L⁻¹ NaOH (25°C about 72 h) and 0.1% aniline blue (compound in 0.1% K₃PO₄). The pollen germination and pollen tube growth was observed with fluorescence microscope (Olympus-DP70). The data were analyzed by Shortest Significant Ranges (SSR).

RESULTS AND DISCUSSION

Comparison of pollen germination on stigma at different floral stages: On the first day of flowering, few styles of 'Dawuxing' were pollinated. With the growth of flower, the rate of style with germination pollen increased gradually, which was up to 90% on the 5th day after flowering. And the rate of style with pollen tube tended to be stable afterwards. The period of pollination and pollen germination focused on the 2nd to 4th day after flowering.

The data showed that the stigma receptivity could last about 7 days and the pollination and pollen germination time varied, most intervals were 3-4 days (Fig. 1).

Comparison of pollen germination on stigma and pollen tube growth between self-pollination and cross-pollination: The pollen germination on stigma in cross-pollination was better than self-pollination in 'Dawuxing' (Plate 1d-e). And pollen tubes reaching upper style, middle style and bottom style in cross-pollination were significantly more than those in self-pollination. Pollen tubes reaching upper style were significantly more than those reaching middle style and bottom style in self-pollination, meanwhile there was no obvious difference in cross-pollination (Fig. 2a). The percentage of ovules in which pollen tube growth was normal in cross-pollination was higher obviously than that in self-pollination, but there was no obvious difference between cross-pollination and self-pollination (Fig. 2b; Plate 1f-i). Thus, it could be concluded that 'Dawuxing' was self-incompatible to some extent. And the key parts were the middle of style and micropyle.

Comparison of pollen germination on stigma and pollen tube growth in open pollination: The pollen germination was better after being pollinated in natural condition, in which pollen tubes reaching upper styles were significantly more than those reaching middle styles and bottom styles. The pollen tube growth was bated seriously in style, only about 30% pollen tubes could reach the base of styles (Fig. 3a; Plate 1j-l). And pollen tubes were found in about 85% tested ovules, the rate of ovules with pollen tubes was significantly higher than that with normally growing pollen tubes. Meanwhile, the pollen tube grew along the integument or became bending in more than 70% ovules, which was indicative of pre-fertilization barrier.

Comparison of pollen tube growth at different development stages: The differences of pollen tube growth were remarkable in different development stage embryos whose ovules grew normally, low-grade degenerated and abnormally. The rate of ovule with normal pollen tubes was extremely significantly different

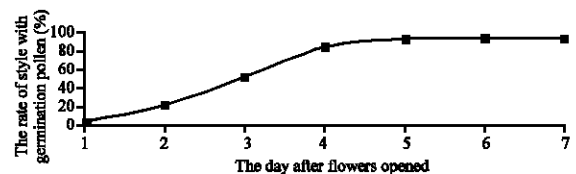


Fig. 1: Comparison of pollination rate on stigma at different floral stages

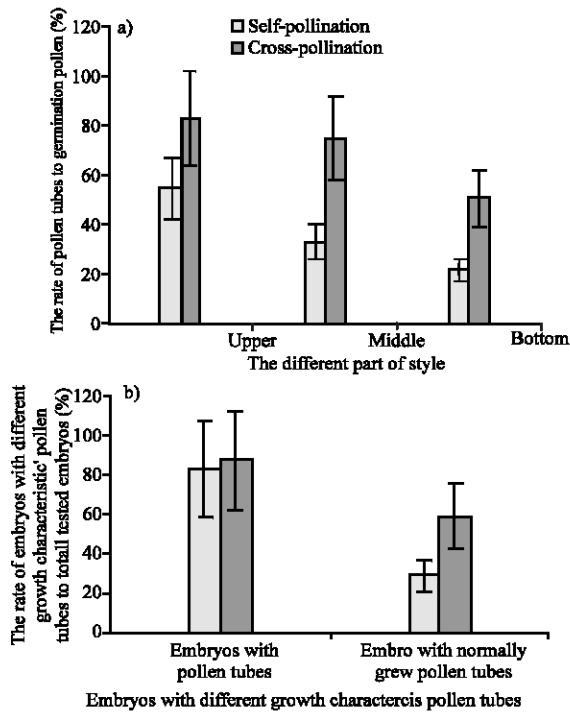


Fig. 2: Comparison of pollen germination and pollen tube growth, a) The ratio of pollen tube in the different parts of styles to germinated pollens; b) The ratio of embryos with pollen tubes or normally growing pollen tubes to total embryos

between normal growth embryos and low-grade degeneration embryos. And in no instance did we observe any normal pollen tubes penetrating to the abnormal ovules (Plate 1m-t). There was no significant difference in the rate of ovules with pollen tubes between fallen flowers and normal sear flowers, but their rates of ovules with normal pollen tubes were significantly different. The results indicated that only a few ovules could be fertilized, while most ovules failed to be fertilized because of pre-fertilization barrier, which resulted in low-grade degeneration or abnormality (Fig. 4).

Effect of pollen germination time on stigma on pollen tube growth: The stigma receptivity could last 7 days and the pollination and pollen germination time varied among stigmas and the intervals were 3-4 days. However, pollen tube reached to the bottom of style in 48 h (the data were rectified in other study). The characteristics of pollen tube growth were diverse in styles or ovules, which was likely due to the differences in ribonuclease (S-RNases), receptor kinase, G-protein, Ca^{2+} and so on (Sun *et al.*, 2001).

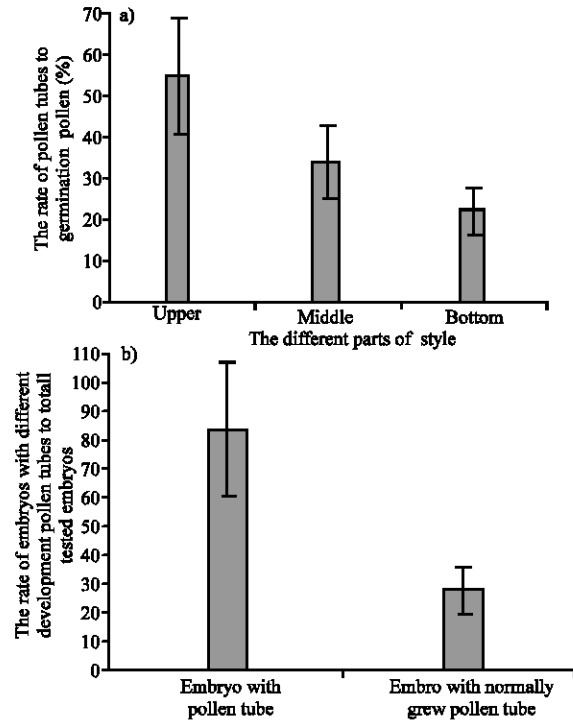


Fig. 3: Comparison of pollen germination and pollen tube growth in pollination, a) The ratio of pollen tube in the different parts of styles to germinated pollens; b) The ratio of embryos with pollen tubes or normally growing pollen tubes to total embryos

The relationship of fertility to the pollen germination and pollen tube growth: The pollen germination in the stigma in cross-pollination was better than self-pollination (Plate 1d, e). The rates of pollen tubes reaching upper style, middle style and bottom style to germinated pollens in cross-pollination were significantly higher than that of self-pollination. Meanwhile, the rate was not obviously different between upper style and middle style in cross-pollination (Plate 1f, g), the rate of pollen tubes to germinated pollens was remarkably different between upper style and middle style in self-pollination (Fig. 2) and many pollen tubes stopped growth in the upper or middle of style (Plate 1h). And pollen tubes reaching upper style to germinated pollens were significantly more than those reaching middle style and bottom style, which showed that the pollen tube growth was bated seriously in style and ‘Dawuxing’ was self-incompatible to some extent (Fig. 3-a, b; Plate 1d, f, g, j-l). The phenomenon may be ascribed to the inhibition of production of S-gene in style, higher concentration of *S. glycoprotein* (Zhang and Hiratsuka, 2000), lack of inclusions of promotion substances for pollen tube growth (lipid and

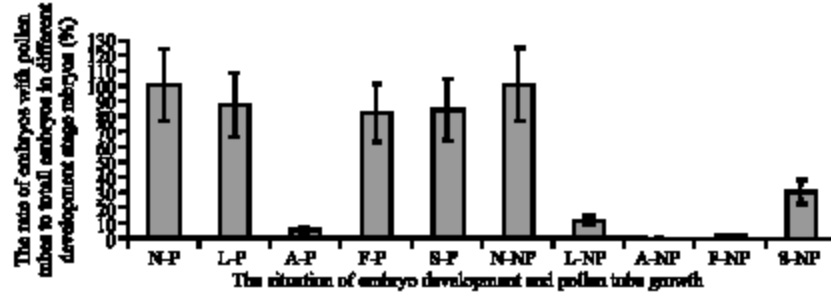


Fig. 4: Comparison of pollen tube growth in different development stage embryos, p-ovules with pollen tubes; NP-ovules with normally growing pollen tubes; N, normal growth ovules; L, low-grade degeneration ovules; A, abnormality ovules; F, fallen flowers' ovules; S, normal growth sear flowers' ovules

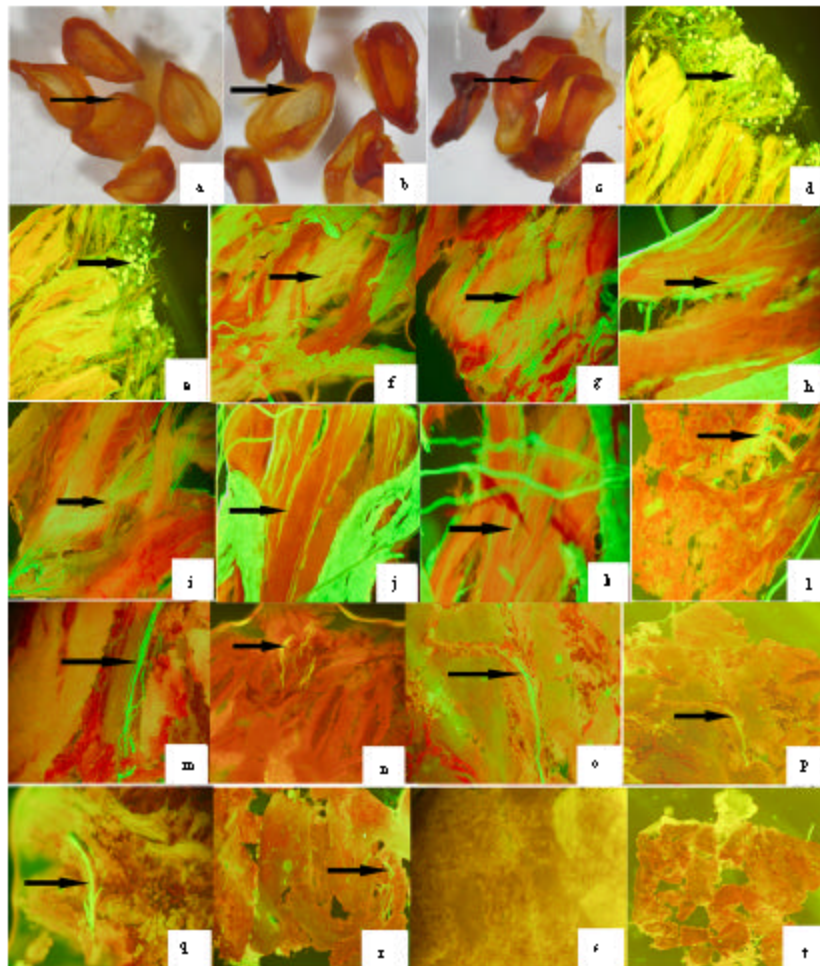


Plate 1: a-c: Pollen germination on stigma and Pollen tube growth in middle part and bottom part of style in self-pollination($\times 40$, $\times 100$, $\times 100$); d-f: Pollen germination on stigma and pollen tube growth in middle part and bottom part of style in cross-pollination($\times 40$, $\times 100$, $\times 100$); g-i: Pollen germination on stigma and pollen tube growth in middle part and bottom part of style in open pollination ($\times 100 \times 100$, $\times 100$); j-l: Normal growth, low-grade degeneration and abnormality embryos (25 days after florescence) ($\times 40$, $\times 40$, $\times 40$); m-p: Pollen tube growth in normally growing embryos($\times 100 \times 100$, $\times 100$, $\times 40$); q, r: Pollen tube growth in low-grade degeneration embryos ($\times 100$, $\times 40$); s, t: Pollen tube growth in abnormality embryos($\times 100$, $\times 40$); The arrows indicate germinated pollens (a, d), pollen tubes (b, c, e-i, m-r), different development stage embryos whose ovules grew normally, low-grade degenerated and abnormally (j-l). No arrow indicates no germinated pollens or pollen tubes

phospholipids etc) in style (Hakan Demirköser *et al.*, 2007). So, study of biochemistry experiment on styles and ovules would be of great significance for research of breeding and embryo abortion in loquat.

The relationship between pollen tube growth in ovules and fertility and abundant genetic diversity of plants from degenerate seeds: On the 9th day after flowering, the rate of ovules with pollen tube was about 80%, but the ovules with normal pollen tube were less than 30%. And the percentage of ovules whose pollen tubes grew normally was in accordance with that of mature fruit. The differences of pollen tube growth were remarkable in a cultivar whose ovules grew normally (Plate 1m-p), partly normally (Plate 1q, r) and abnormally (Plate 1s, t) after the 25th day flowering. In about 60% ovules, the pollen tubes grew along the integument or bending (Plate 1q, r), which may result from the micropyle, the key site of self-incompatibility, or higher Ca²⁺ concentration in a suitable range in integument which led pollen tube growth to changed direction (Malho and Trewavas, 1996; Zhao *et al.*, 2005) and resulted in pollen tubes being unable to penetrate ovule. Combined with 3.2, it was concluded that 'Dawuxing' was self-incompatible to some extent, as in other cultivars reported by Spanish scholars (Lin *et al.*, 2003). And it had been found that pollen tubes could penetrate into ovary, but failed to fertilize the ovules. We found that pollen tubes could grow along the integument, but also failed to fertilize the ovules.

The ovules of fertilization failure (Plate 1q-t) and apomixes in normally growth fruit could continue to develop due to enough nutrition matter and hormones, but these ovules developed to degenerative seeds with abundant genetic diversity, including aneuploid, polyploidy and polyembryony seedling (Hanna, 1995). So, the study on degenerative ovules was of great significance for researching breeding and embryo abortion in loquat.

CONCLUSION

The characteristics of pollen tube growth had high diversity in styles and ovules, attributed to many inclusions, which resulted from long pollen germination intervals among different stigmas. 'Dawuxing' was self-incompatible to some extent, the middle of style and the micropyle were the key site of self-incompatibility, the loquat seeds were degenerative to some extent and there was abundant genetic diversity in the resultant plants, which was ascribed to pre-fertilization barrier and self-incompatibility.

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