

Evaluation of the Reproductive Process as the Cause for Low Fruit Set in Two Japanese Plum Cultivars

M. E. Guerra and M. López-Corrales
Departamento de Hortofruticultura.
Centro de Investigación Agraria 'Finca
La Orden-Valdesequera'. A-V. Km 374,
Guadajira, 06187 Badajoz
Spain.

A. Wünsch and J. Rodrigo Unidad de
Fruticultura, Centro de Investigación y
Tecnología Agroalimentaria de Aragón
(CITA). Av. Montañana 930, 50059
Zaragoza
Spain

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Abstract

The reproductive process has been evaluated to ascertain the causes of low fruit set in two Japanese plum cultivars, 'Sweet August' and 'Rubirosa'. In order to evaluate pollen germination, pollen-pistil incompatibility and ovule viability, different experiments were performed in orchard conditions. Pollen viability was determined by assessing the percentage of *in vitro* pollen germination. The self-(in)compatibility of both cultivars and their cross-compatibility with 'Champion' were examined by self- and cross-pollination experiments followed by observation of pollen tube growth under the microscope. Likewise, the *S-RNase* alleles of each cultivar were determined by PCR amplification of the *S-RNase* gene. Finally, ovule development was examined under the microscope. While 'Sweet August' behaves as self-incompatible, 'Rubirosa' appears to be self-compatible. The observation of pollen germination and pollen tube growth in the different crosses analyzed showed that fruit set was not limited by the absence of compatible pollen. However, a high percentage of degenerated ovules were observed in flowers from both cultivars. This premature ovule degeneration is likely to be the cause of low fruit set in both cultivars.

INTRODUCTION

Some Japanese plum-type cultivars are particularly prone to erratic fruit set showing very low or even null fruit set for reasons that are not clear (Okie and Weinberger, 1996; Hartmann and Neumüller, 2009). In *Prunus*, as in other fruit tree species, a number of factors intervene in the reproductive process including pollen viability, successful pollen transfer, pollen-pistil incompatibility, synchrony between pollen tube arrival to the ovule and embryo sac maturation, fertilisation of at least one ovule and initial zygotic viability (Pimienta and Polito, 1982; Rodrigo and Herrero, 1998). Although these processes have been analysed in different *Prunus* sp, the reproductive behaviour of Japanese plum-type cultivars is not known and this may be one of the reasons of the lack of fruit set obtained in some new cultivars.

In this work, the reproductive process has been evaluated to ascertain the causes of lack of fruit set in two new Japanese plum-type cultivars, Sweet August and Rubirosa, and in the productive cultivar Simka that was used as a control. The determination of fruit set following controlled self- and cross-pollinations in orchard conditions was combined with observations of pollen germination, pollen tube growth, pollen-pistil incompatibility

and ovule development under the microscope and with *S*-genotyping of the cultivars by PCR.

MATERIALS AND METHODS

Pollination experiments

Both low producing cultivars 'Rubirosa' and 'Sweet August' were cross-pollinated with 'Champion', and the control cultivar 'Simka' with 'Songold'. The three cultivars were also self-pollinated and an additional population of open-pollinated flowers was used as control in each cultivar. Pollen used in each pollination treatment was obtained from flowers in balloon stage by removing anthers and placing them on paper at room temperature for 24 h until their dehiscence. Pollen was then sieved through a fine mesh and stored at -20°C until required. To ascertain final fruit set, weekly counts were carried out of flowers and developing fruit from anthesis to harvest in each pollination treatment. Pollen viability of each cultivar was evaluated by *in vitro* culture. The pollen was scattered on a solidified germination medium (Hormaza et al., 1996) in polystyrene Petri dishes and germinated for 24 h at 20°C. Preparations were stained with 1% (v/v) aniline blue in 0.1 N K₃PO₄ to stain callose (Linskens and Esser, 1957) and observed under a microscope equipped with UV epifluorescence. A pollen grain was considered viable when its growing pollen tube was longer than the pollen grain diameter.

Pollen-pistil incompatibility

In order to study the self-(in)compatibility of the cultivars and the compatibility relationships in the crosses, pollen tube growth in flowers of each cross was observed under the microscope. For this purpose, around 30 flowers from each treatment were collected 4 d after pollination and fixed in alcohol: acetic acid (3:1). For microscope preparation, the fixed pistils were washed three times for 1 h with distilled water and left 24 h in 5% (w/v) sodium sulphite at 4°C. These pistils were autoclaved during 8 min at 1 kg/cm² in 5% sodium sulphite to soft the tissues. The pollen tube growth was monitored with the same staining procedure used to determine pollen viability.

S-allele genotyping of the cultivars analysed was carried out by PCR. *S*-allele PCR analysis was carried out from genomic DNA isolated from young leaves following the protocol described by Hormaza (2002). *S-RNase* typing was carried out by *S-RNase* PCR amplification using primers pairs Pru C2- PCER, and Pru T2-PCER (Tao et al., 1999; Yamane et al., 2001) according to Guerra et al. (2009).

Ovule development

Ovule viability was determined by the presence of callose deposits in the chalaza of degenerating ovules (Pimienta and Polito, 1982; Rodrigo and Herrero, 1998) in the same pistils previously evaluated for pollen-pistil incompatibility. Preparations were monitored with the same staining procedure used to observe pollen germination and pollen tube growth.

RESULTS AND DISCUSSION

The percentage of fruit set in both 'Rubirosa' and 'Sweet August' was very low or null in all the pollination treatments and lower than expected for Japanese plum (Sapir et al., 2008; Beppu et al., 2010; Guerra et al., 2010) (Fig. 1), thus reflecting the records of

lack of fruit set for these cultivars in previous seasons. Open- and self-pollination resulted in lack of fruit set in 'Rubirosa' and 'Sweet August' whereas for 'Simka' fruit set was 6% and 4% for each treatment respectively (Fig. 1). In the cross-pollinations, 'Rubirosa' and 'Sweet August' also displayed very low fruit set, ranging from 0% to 1%, but in 'Simka' fruit set was 5%.

All the cultivars analysed had viable pollen, and 'Champion' was coincident at blooming with 'Rubirosa' and 'Sweet August'. Therefore pollen viability did not seem affect the lack of fruit set. To evaluate if the lack of fruit set in 'Rubirosa' and 'Sweet August' was due to lack of pollination with compatible pollen, pollen-pistil incompatibility was analysed. In all self-pollinated flowers of 'Sweet August' pollen tube growth was arrested in the style and no pollen tubes reached the base of the style, indicating that this cultivar is self-incompatible. On the other side, a variable percentage of pistils of 'Rubirosa' (36%) showed pollen tubes growing along the pistil and reaching the base of the style after self-pollination, indicating self-compatibility in this cultivar.

Cross-pollinations displayed variable percentages of pistils with pollen tubes reaching the base of the style. While some pistils of 'Sweet August' x 'Champion' (66%) had pollen tubes at the base of the style, pollen tubes did not reach the base of the style in the pistils analysed from the cross 'Rubirosa' x 'Champion'. Thus, all the crosses appeared to be were cross-compatible except 'Rubirosa' x 'Champion' that was cross-incompatible. The *S-RNase* genotypes of 'Champion' (*ScSe*), 'Rubirosa' (*ScSe*) and 'Sweet August' (*ScSh*) have been reported for the first time, and the *S-RNase* genotype of 'Simka' (*SeSk*), and 'Songold' (*ShSk*) agree with previous studies (Beppu et al., 2003; Guerra et al., 2009). *S-RNase* typing by PCR confirmed the cross-compatibility relationships of all the crosses because all the parental combinations had different *S*-alleles and only the cross 'Rubirosa' x 'Champion' was expected to be cross-incompatible because both cultivars have the same *S*-genotype.

Once discarded other factors involved in the reproductive process, the presence of degenerated ovules in flowers from pollination treatments without fruit set and the absence of degenerated ovules in flowers from fruit-setting treatments allowed identifying ovule degeneration as the cause of lack of fruit set in the two cultivars in these orchard conditions. Thus, both ovules were degenerated showing callose in the chalaza (Fig. 2A) in a variable percentage (50%-100%) of self-, cross- and open-pollinated flowers from 'Rubirosa' and 'Sweet August' (Fig. 1). On the other hand, all the pistils from 'Simka' presented at least one ovule well developed with no callose in the chalaza (Fig. 2B).

The study of different factors intervening in the reproductive process allowed dismissal of pollen viability and pollen-pistil incompatibility as the causes of lack fruit set in the two Japanese plum-type cultivars evaluated. However, premature degeneration of the ovules was identified as the factor causing female sterility and therefore lack of fruit set in these cultivars.

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Figures

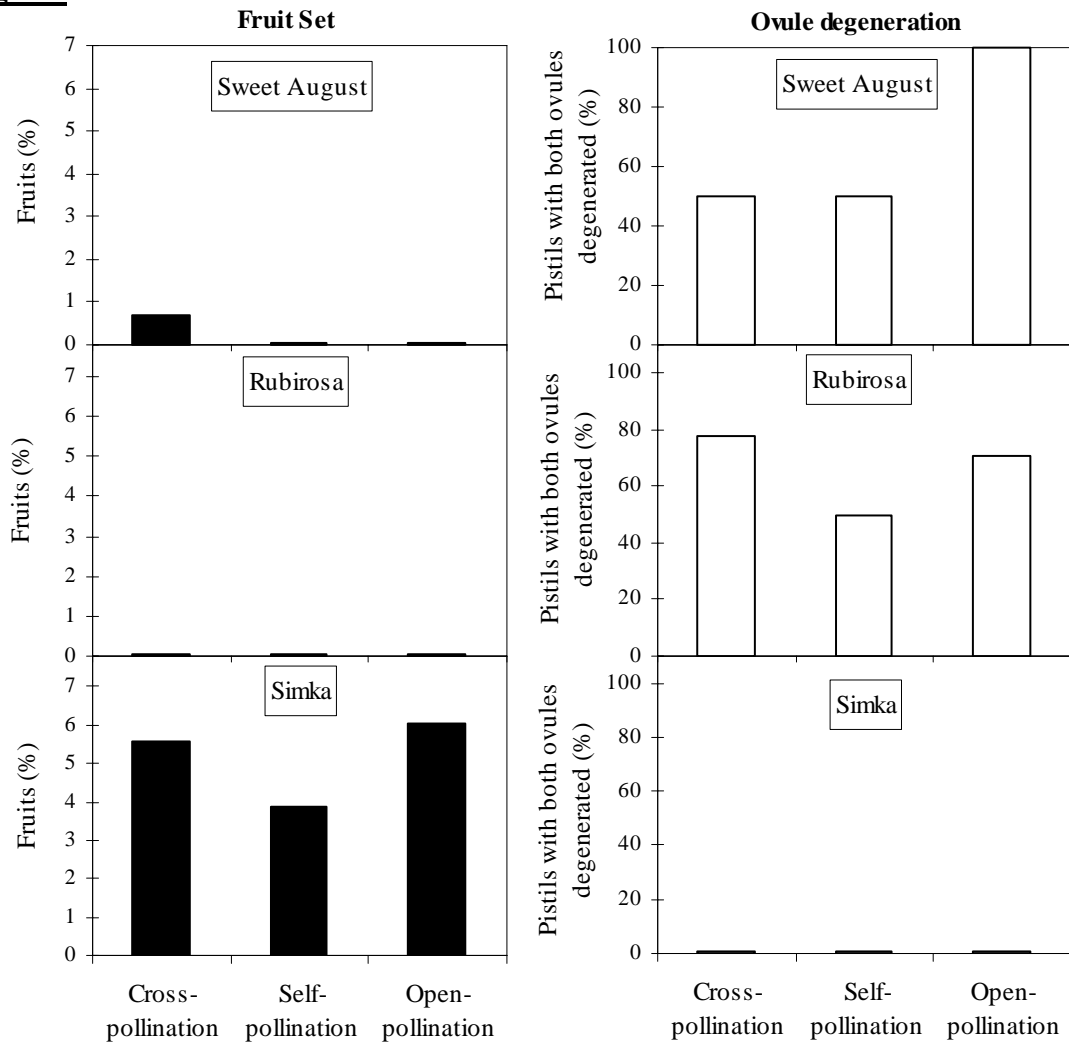


Fig. 1. Percentage of fruit set and percentage of pistils with both ovules degenerated in flowers from different pollination treatments (cross-, self- and open-pollinations) in the low producing Japanese plum cultivars Sweet August and Rubirosa and the control cultivar Simka.

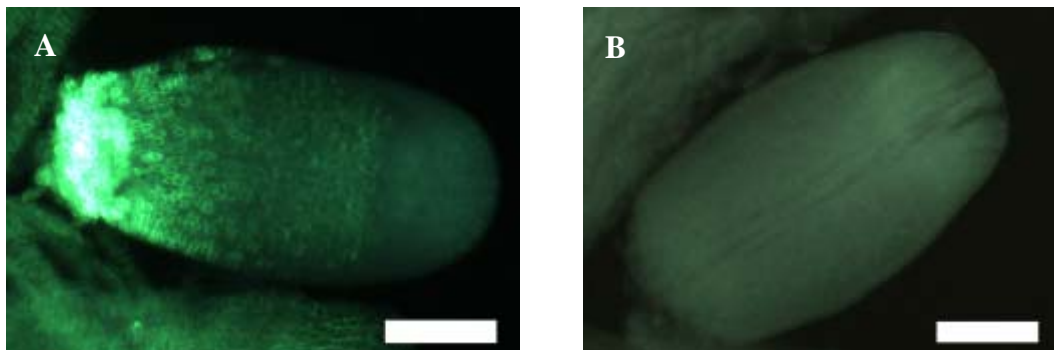


Fig. 2. Ovule development in Japanese plum flowers. (A) Degenerated ovule with callose accumulation at the chalazal end. (B) Well developed ovule. Bars = 50 μ m.