Self-compatibility and cleistogamy in Japanese plum

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Abstract

Most Japanese plum cultivars are self-incompatible and to produce fruits require cross-pollination with compatible cultivars coincident at flowering. However, a small number of Japanese plum cultivars have been described as self-compatible. In this work, self-(in)compatibility and cleistogamy have been analyzed in the Japanese plum cultivar 'African Rose' by combining controlled pollinations and microscopic observation of flowers. One tree was enclosed in a mesh cage before blooming to avoid pollination by insects. Several branches in the caged tree and also in trees outside the cage were selected and marked as a control. The experiment was made during two years. Self-incompatibility was evaluated by controlled self-pollinations in a set of flowers in the caged tree. Fruit set and fruit drop were characterized in both self-pollinated and control flowers until harvest. Additionally, cleistogamy was evaluated in another set of branches, by determining the presence of pollen grains on the stigma and pollen germination in flower buds collected at four phenological stages before flower opening. The presence of pollen tubes reaching the ovary in pistils from self-pollinated flowers, and fruit set observed after self-pollination as well as the showed that 'African Rose' behaves as self-compatible. The synchrony between anther dehiscence and stigma receptivity before flower opening, which resulted in self-pollination without pollinator intervention, indicates that this could be the first reported case of cleistogamy in Japanese plum.

Keywords: 'African Rose', buds, flowers, fruit set, pollination

INTRODUCTION

In the last years, an intense varietal renewal is taking place in Japanese plum-type cultivars (hybrids of *Prunus salicina* Lindl.) (Guerrero et al., 2018). Most breeding programs share some common objectives as self-compatibility. Although most cultivars grown nowadays are self-incompatible (Okie and Weinberger, 1996), a small number of cultivars have been described as self-compatible (Guerra and Rodrigo, 2015; Guerra et al., 2020). Self-compatible cultivars are highly appreciated by producers, because they do not require cross-pollination and this facilitates crop management (Herrera et al., 2021), and also by breeders to use them as parents to pass on self-compatibility to the new releases (Nicolás-Almansa et al., 2020).

Flowers of both self-incompatible and self-compatible plum cultivars require the presence of pollinating insects, mainly honeybees and bumblebees, to transfer pollen grains to the stigma of flowers (Benachour and Louadi, 2013). In this work, self-compatibility of the Japanese plum-type cultivar 'African Rose' has been analyzed, exploring its ability to self-pollinate in the absence of pollinating insects.

MATERIALS AND METHODS

Pollinations in the field

Two trees of the Japanese plum cultivar 'African Rose' were selected from a collection

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held at CICYTEX-La Orden in Guadajira (Extremadura), located in the main plum growing area in Spain. To avoid the arrival of pollinating insects, one tree was enclosed in a 0.8-mm mesh cage before bloom. In marked branches, a set of 1000-1500 flowers were self-pollinated every other day until all flowers opened using a thin paintbrush (Guerra et al., 2010). The rest of flowers were left un-pollinated. Additionally, a group of 1000-1500 flowers was left for open-pollination in another tree not covered, which were used as control. The flowers from each pollination treatment were weekly counted until the establishment of the fruit set. The experiment was made during two years.

To determine self-compatibility, 30 flowers were collected at D stage (Baggiolini, 1952) one day before anthesis, emasculated and placed on wet florist foam in the laboratory and kept at room temperature. The next day the emasculated flowers were self-pollinated by hand with pollen previously obtained (Guerra et al., 2020). At least 72 h after pollination the pistils were fixed in acetic acid:ethanol (95%) (1:3) during 24 h, and conserved at 4°C in 75% ethanol (Williams et al., 1999). For observation of callose plugs in pollen tubes, the pistils were stained with 1% (v/v) aniline blue in 0.1 N K_3PO_4 (Guerrero et al., 2020). Pollen tube growth along the style was observed under a microscopy Leica DM2500 (Leica Microsystems CMS GmbH, Wetzlar, Germany), equipped with UV epifluorescence with a BP340-390 exciter filter and a LP425 barrier filter.

Pollen germination and pollen tube growth

To evaluate the presence of pollen on the stigma before flower opening, another set of flower buds at D stage of Baggiolini (1952), corresponding with 57 BBCH stage (Meier, 2001), was collected. Four stages of flower bud development were herein established from incipient petal appearance to the balloon stage (Figure 1).

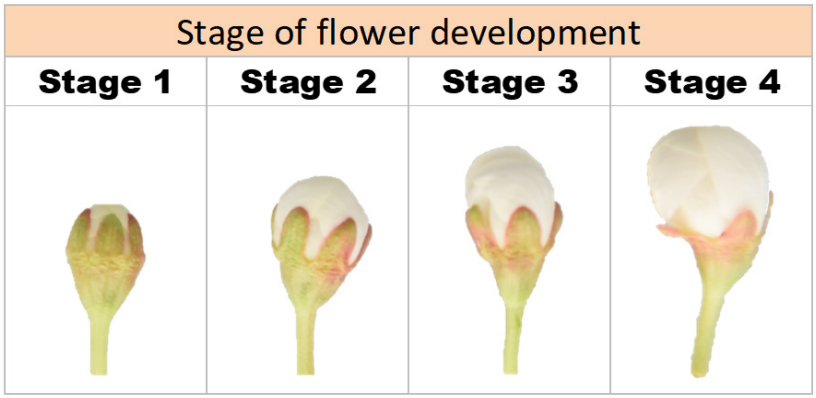

Figure 1. Stages of flower bud development herein established. Stage 1: White tip, first appearance of the petals emerging between the sepals. Stage 2: More appreciable emerging petals, but shorter than the sepals. Stage 3: Petal length greater than that of the sepals. Stage 4: Balloon stage, petals extended and rounded but flower still closed.

A group of 25-61 flower buds at each stage was collected and kept at 4°C until evaluation. Every flower bud was dissected and observed under a Nikon SMZ800N stereo microscope (Nikon Metrology Europe N.V., Leuven, Belgium), determining the presence of pollen grains on the stigma surface and the stage of development of the anthers.

Another group of 10-23 flower buds at each stage was collected and immediately fixed in 95% ethanol:acetic acid (3:1) during 24 h and conserved at 4°C in 75% ethanol (Williams et al., 1999). For histochemical preparations, the pistil from each flower bud was extracted and stained with 1% (v/v) aniline blue in 0.1 N K_3PO_4 to stain callose (Guerrero et al., 2020). Pollen grains on the stigma, pollen germination and pollen tube growth along the style were observed under the same microscope used for self-(in)compatibility determination.

RESULTS AND DISCUSSION

Self-compatibility

Some pollen tubes reached the ovary in most of the self-pollinated pistils (62%), confirming the self-compatibility of 'African Rose' (Guerra et al., 2020). The percentage of fruit set obtained in the field pollinations was lower in self-pollinated flowers inside the cage than in open-pollinated flowers outside the cage (control) (Figure 2). However, both populations of flowers showed a percentage of fruit set higher than that reported for other Japanese plum cultivars, which usually ranges from 2% to 6% (Guerra et al., 2010; Jia et al., 2008). Since the number of flowers produced in Japanese plum-type cultivars is much higher than in other *Prunus* spp., a fruit set of 5% usually results in adequate yield (Hartmann and Neümuller, 2009; Okie, 2006; Nyéki et al., 1997; Guerra and Rodrigo, 2015).

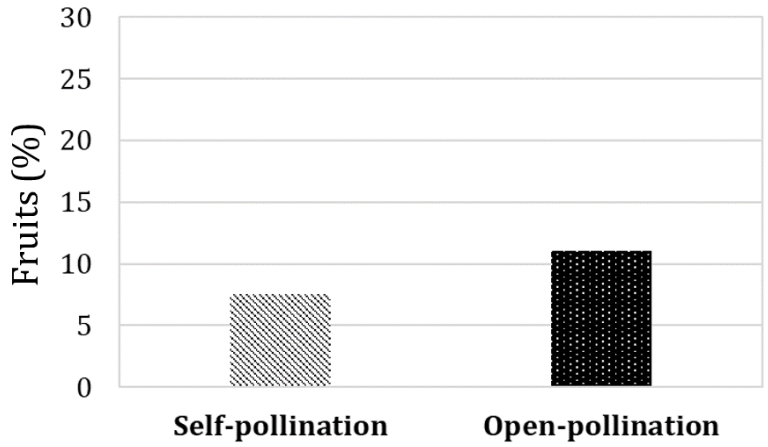

Figure 2. Percentage of fruit set in self-pollinated and open-pollinated flowers of the Japanese plum-type cultivar 'African Rose'. Average values for two years.

Cleistogamy

During field experiments to evaluate self-compatibility, a number of fruits were observed in un-pollinated branches of the caged tree in both years. This encouraged us to explore if these fruits were the result of accidental pollination, or whether the flowers had been pollinated with their own pollen by contact between the dehisced anthers and the stigma without the mediation of pollinating insects. All the flower buds of the four stages analyzed showed undehisced anthers, but in different proportions depending on the stage (Table 1). Dehisced anthers were observed in an increasing proportion from stage 2 (33.3%) to 4 (100%), corresponding to three to one day before anthesis (Table 1). Dehisced anthers before flower opening have been reported in flowers of almond [*Prunus dulcis* (Mill.) D.A. Webb], in which the maximum proportion of dehisced anthers was observed one day before anthesis (Soodan et al., 1989).

Table 1. Anther traits observed under stereo microscope in flower buds of 'African Rose' at four different stages before anthesis.

Observations	Stage of flower development			
	Stage 1	Stage 2	Stage 3	Stage 4
Number of flowers	25	51	54	61
Anthers per flower (average)	25	28	27	27
Flowers with some dehisced anthers (%)	0	33.3	74.1	100
Flowers with pollen grains on the stigma (%)	0	11	55.6	100

To evaluate pollen germination and pollen tube growth, pistils of the four stages were analyzed under the microscope. Stigmas with germinating pollen grains were observed from stage 3, showing that the stigmas were receptive at least two days before anthesis (Table 2). A similar behavior was reported in almond, with receptive stigmas from at least two days before anthesis (Soodan et al., 1989). The synchrony between dehisced anthers and receptive stigmas observed in 'African Rose' led to self-fertilization as reported in self-fertile cultivars of almond (Soodan et al., 1989) and peach (*Prunus persica* L.) ('Glowing Star' and 'VABM29') (Sherif et al., 2015).

Table 2. Floral traits related to pollen germination and pollen tube growth observed under stereo microscope in flower buds of 'African Rose' at four different stages before anthesis.

Observations	Stage of flower development			
	Stage 1	Stage 2	Stage 3	Stage 4
Number of flowers	10	18	23	14
Flowers with germinated pollen grains on stigma (%)	0	0	4.3	57.1
Percentage of style traveled by the longest pollen tube	0	0	10	100

A proportion of the cleistogamous flowers that were pollinated with their own pollen before anthesis resulted in fruits, since the caged trees showed a yield similar to that of the control trees. Once the flowers opened, they had the opportunity for outcrossing. This type of cleistogamous behavior has been defined as preanthesis cleistogamy (Culley and Klooster, 2007). Cleistogamy has not been previously described in Japanese plum, although it has been reported in some accessions of other *Prunus* species, such as 'Stella' sweet cherry (*Prunus avium* L.) (Békefi, 2004), 'SB13,25-75' almond (Gradziel and Kester, 1998) and 'Babygold 5' and 'Babygold 7' peach (Nyéki et al., 1997). In peach, self-fertility has been related to alterations of jasmonic acid content due to biotic and abiotic stresses (Sherif et al., 2015). Further studies are needed to determine whether the cleistogamous behavior observed in 'African Rose' depends on internal or external factors.

CONCLUSIONS

The fruit set obtained in self-pollinated flowers as well as the observation of pollen tubes reaching the ovary in pistils from self-pollinated flowers showed that 'African Rose' behaved as self-compatible. The synchrony between anther dehiscence and stigma receptivity before flower opening, which resulted in self-pollination without pollinator intervention, indicates that this could be the first reported case of cleistogamy in Japanese plum.

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