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Effects of different pollination treatments in genotypes of *Prunus salicina* Lindl.

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Summary: The low productivity in the Japanese plum (*Prunus salicina* Lindl) is related with self-incompatibility characteristics, so other species or varieties that act as pollinators need to be present to improve fruit production. The objective of this work was to study the efficiency of pollination in different genotypes of *P. salicina* using treatments of natural self-pollination, cross-pollination with *P. armeniaca* cv. Giada and open pollination. These treatments were evaluated through viability techniques and *in vitro* and *in vivo* germination of pollen grains; the growth of pollen tubes along the pistil was also observed. Genotypes used in this study showed differences for each one of the pollination treatments. Some genotypes showed signs of self-sterility and interincompatibility with *P. armeniaca* cv. Giada, while others showed partial self-fertility characteristics or pseudocompatibility. Moreover, some genotypes showed a higher affinity coefficient with cv. Giada and these will be indicating a possible intercompatibility. These studies will be an important contribution breeding and selection of intra and intercompatible genotypes to be used in commercial orchards.

Key words: incompatibility, natural self-pollination, open pollination, pollen viability, *Prunus salicina*

Introduction

Most Japanese plums (*Prunus salicina* Lindl*)* are characterized by being self-incompatible or partially self-fertile (*Szabó & Nyéki*, 2000). However, *Palara* et al. (1990) observed some self-fertile varieties of *P. salicina*. The incompatibility of a cultivar could be modified by the environment; thus, the cv. Santa Rosa was defined as self-fertile (*Gautier*, 1977), partially self-fertile (*Bellini & Bini*, 1978; *Costa & Grandi*, 1982; *Szabó*, 1989) and self-incompatible (*Albertini*, 1978). In the genus *Prunus*, self-incompatibility is frequent. Characteristics of self-incompatibility have also been observed in most almond (*Pimienta* et al., 1983) and sweet cherry (*Nyéki & Soltész*, 1996) varieties.

Incompatibility is generated by the presence of allele S, found in all pollen grains. If this allele is also present in the pistil, incompatibility is produced (*Shivanna*, 2003). Incompatibility can be gametophytic or sporophytic, whether it is manifested in the style during the growth of pollen tubes or whether inhibition is produced on the surface of the stigma, respectively (*Shivanna*, 2003).

In natural self-pollination studies of *P. salicina*, low percentages of set fruits were found in relation to open pollination studies. The latter, in turn, showed lower values of set fruit than those from cross pollination studies (*Keulemans*, 1991, 1994). Independently of the pollination treatments, when a great quantity of pollen is found on the stigma the competition can occur and a higher growth rate in the tubes is observed (Thomson, 1989). More vigorous pollen grains stand out for the higher growth rate of pollen tubes, reaching ovule fertilization in less time (*Tanksley* et al., 1981). After the arrival of pollen to the stigma the process of fertilization is not only influenced by physiological conditions but also by compatibility characteristics (*Brewbaker*, 1957).

Species with compatibility problems need the presence of other species or varieties that act as pollen donors, allowing them to obtain a good fruit production (*Nyéki & Soltész*, 1996; *Sansavini* et al., 1981). In this study, *Prunus armeniaca* cv Giada was selected to be used as a pollinating variety of *P. salicina* genotypes. It was selected because of its close location, its flowering, coincident with that of the studied genotypes, and for having a very high frequency of pollinating insects.

The objective of this work was to study the efficiency of pollination in different genotypes of *P. salicina* using natural self-pollination treatments, cross-pollination treatments with *P. armeniaca* cv. Giada and open pollination treatments.

Materials and methods

Pollination trials were carried out during two consecutive years (2004–2005) in six 9 year old genotypes of *P. salicina*, identified as 2, 3, 8, 10, 111 and 1153, obtained by crossing. Trees are in an experimental orchard, together with other numerous genotypes of Japanese plum, apricot and peach

trees, belonging to the Faculty of Agronomy of the University of Florence in the "Azienda Montepaldi", S. Casiano Val di Pesa, Florence, Italy (43º 40' N; 11º 09' E; 230 m a.s.l.).

The quality of pollen grains of *P. salicina* and *P. armeniaca* cv Giada genotypes was evaluated through *in vitro* viability tests, according to Greissl's test (1989), and *in vitro* germination tests, according to Dafni (1992).

In vivo germination and growth of pollen tubes was studied for each one of the genotypes and treatments with the addition of aniline blue (Martín, 1959) using an IMT-2 Olympus epifluorescence microscope (Tokyo, Japan). The quantity of germinated pollen grains in relation to total pollen grains sticked to the stigma (%) and the quantity of pollen tubes present according to the portion of the pistil in relation to total germinated pollen grains (%) were evaluated to estimate pollen tube growth. Thus, the pistil was divided into five portions: stigma, first third of the style (1/3), second third of the style (2/3), third third of the style (3/3), and ovary. Viability of ovules was also evaluated according to Martín´s technique (1959).

Pollination studies for each genotype were carried out on floral buds that had not begun anthesis yet. In the natural self-pollination treatment, branches with buds were isolated with paper bags, while in the open pollination treatment branches were only identified. In the cross pollination trial, buds were emasculated and then pollinated manually with pollen from *P. armeniaca* cv. Giada. These branches were also covered with paper bags to avoid the entrance of unknown pollen.

In all treatments, flowers were collected 10 days after the beginning of studies and fixed in FAA solution (formol, 100 ml; ethyl alcohol, 500 ml; acetic acid, 50 ml; distilled water, 350 ml).

Results

Primary ovules of pistils collected from the different genotypes of *P. salicina* were still viable 10 days after the beginning of all treatments, assuring, in some way, the possibility of fertilization.

Evaluation of pollen grains

Viability and *in vitro* and *in vivo* pollen germination studies for each genotype, carried out during 2004 and 2005, showed the same tendency and, consequently, the same differences between them. For that reason, only results obtained in 2005 were analyzed.

Viability of pollen grains of the different genotypes of *P. salicina* and *P. armeniaca* cv. Giada is observed in *Figure 1*. The quantity of viable pollen grains of *P. armeniaca* cv. Giada was significantly higher (92.42%) than that of *P. salicina* genotypes. No significant differences were found among genotypes 8, 10, 111 and 1153 in the percentage of viable pollen grains. However, all of them were significantly different from genotypes 2 and 3 (*Figure 1*). The highest quantity of non-viable pollen grains was observed in genotypes 2 and 3 (74.26% and 71.65% respectively, *Figure 1*).

Pollen viability evaluated through *in vitro* germination

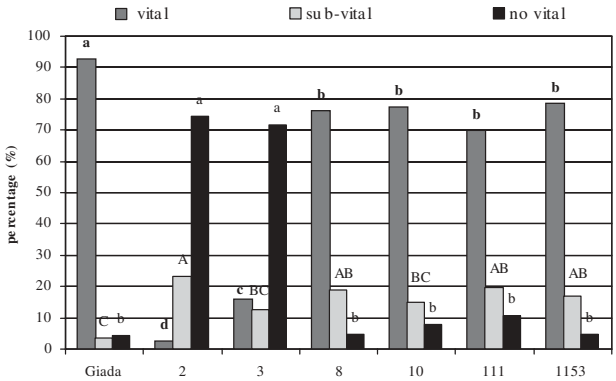

Figure 1: Pollen grains viability of six *Prunus salicina* Lindl genotypes (S2, S3, S8, S10, S111 and S1153). Columns with different bold lower-case (vital) or upper-case (sub-vital) or in normal print letters (no vital) indicate significant differences for each variable studied between genotypes. Results were analyzed by the χ^2 Test (P< 0.05).

for all these genotypes was similar to that obtained using Greissl´s technique (1989); however, *P. armeniaca* cv. Giada did not show significant differences with genotypes 8, 10, 111 and 1153 of *P. salicina*. Pollen grain germination of genotypes 2 and 3 was significantly lower (*Figure 2*)

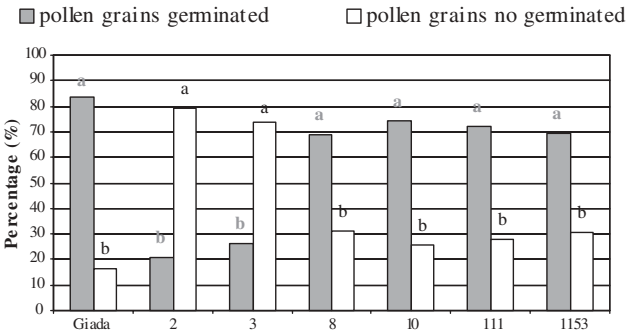

Figure 2: *In vitro* germination of the pollen grains of the *P. armeniaca* cv. Giada and *P. salicina* genotypes. Values followed by different letters for each one of the variable studied show significant differences. Results were analyzed by the χ^2 Test (P 0.05).

Natural self-pollination

The number of pollen grains sticked to the stigma and the quantity of pollen germinated in the pistils of self-pollinated flowers were different for each one of the studied genotypes. Although in genotypes 2 and 3 there were pollen grains on the stigma, they did not germinate *in vivo* in the pistils of collected flowers and there were significant differences with the rest of the evaluated genotypes (*Figure 3* C). In genotypes 8, 10, 111 and 1153, a great quantity of pollen grains was found sticked on the stigma. In genotypes 8 and 10, the highest percentages of pollen grains germinated *in vivo* were 68.29%

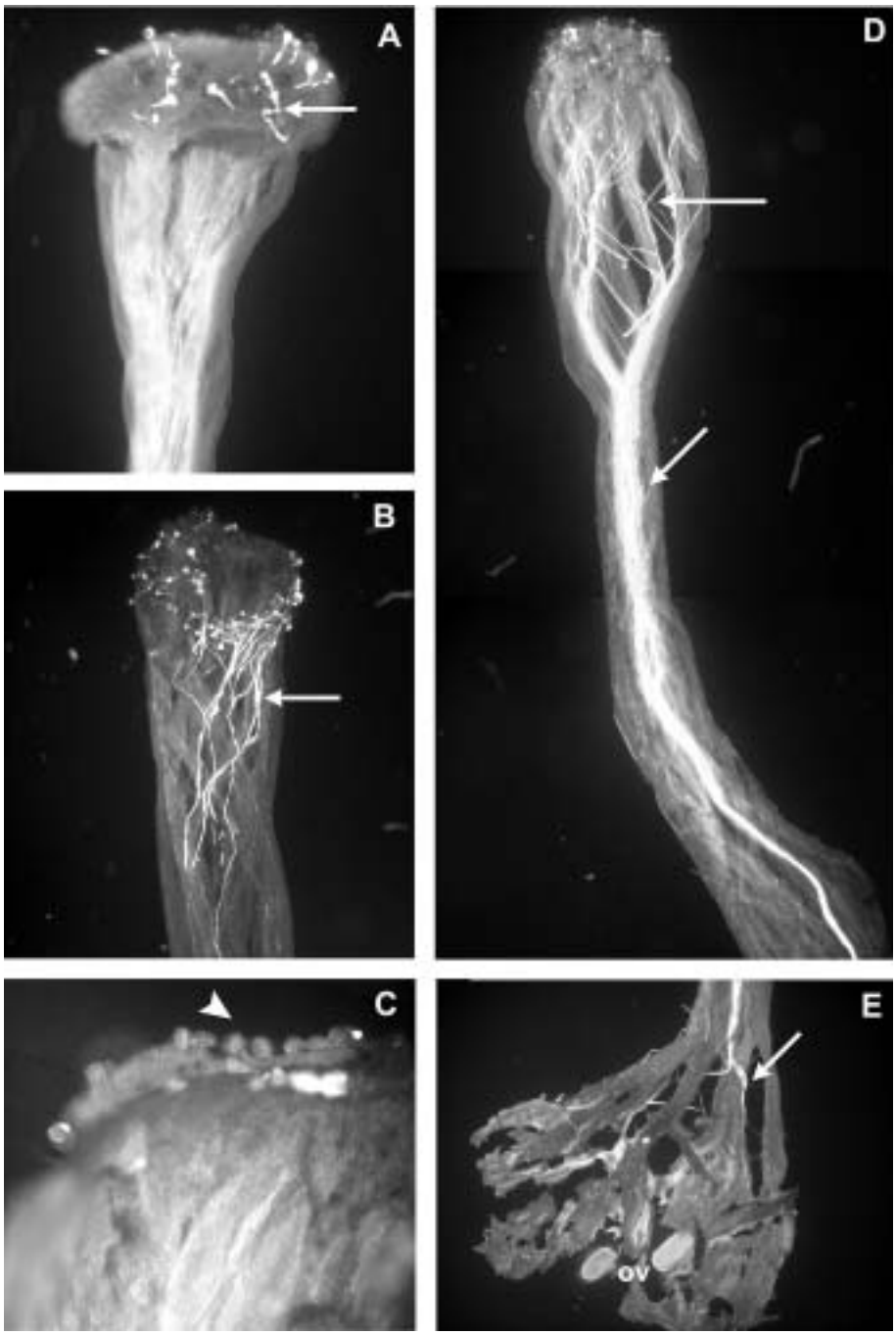

Figure 3: Growth of pollen tubes through the style in genotypes of *P. salicina*.
A: pollen tube in the stigma only in the open pollination treatment (100x).
B: pollen tube in the 2/3 of the style in the self-pollination treatment (100x). C: pollen grains no show pollen tubes on the stigma in the self-pollination (400x). D: pollen tubes were founded in the 1/3 to 3/3 of the style in the cross-pollination treatments (100x). E: only few tubes penetrated in the ovary at 10 days after cross-pollination treatment (100x). Pollen tube (arrow), pollen grain (arrow head) and ovule (ov).

and 62.76%, respectively *(Table 1)*. In genotypes 111 and 1153, intermediate values were registered (*Table 1*).

In the genotypes 8 and 10, a higher percentage of pollen tubes in the stigma was observed in relation to other selections of *P. salicina* for both years. In these genotypes, 84.18% and 72.67% germinated pollen grains were registered in relation to total pollen grains deposited in the stigma for 2004, while at the following year, values observed were 100% and 89.47%, respectively (*Figure 4*). Genotypes 111 and 1153 of *P. salicina* were significantly different, showing a higher quantity of pollen tubes at the beginning and at half of the style in both years (*Figures 3* B; 4), while in genotypes 2 and 3 there were no pollen tubes in any sector of the pistil (*Figure 4*).

Field cross-pollinations with P. armeniaca cv Giada

Germination and growth of cv. Giada pollen tube on pistils of the different genotypes of *P. salicina* was different. In genotypes 2 and 3, a higher quantity of pollen grains was found sticked to the stigma; however, very few of them germinated (*Table 1*). On the contrary, in genotypes 8, 10, 111 and 1153, a lower quantity of pollen grains was observed on the stigma, many of which germinated. These values were significantly higher than those from genotypes 2 and 3 (*Table 1*).

In genotype 1153, pollen tubes grew along the pistil, and some of them reached the ovary 10 days after the beginning of the treatment (*Figures* 3D-E and *5*). For that reason, this genotype showed a lower quantity of pollen tubes on the stigma (9.2%). On the contrary, genotype 111 showed the highest proportion of pollen tubes on the stigma (90.3%). In genotypes 8, 10 and 1153, the highest proportion of pollen tubes was observed in the first third of the style, with values of 59.20%, 50% and 61.8%, respectively (Figure 5). In pistils of genotype 10, the same proportion of pollen tubes was observed in the stigma and in the first third of the style. The highest proportion of pollen tubes in genotypes 2 and 3 did not grow further than the stigma (*Figure 5*).

Open pollination

Pistils also showed different results in the quantity of pollen grains sticked to the stigma and in the percentage of *in vivo* germination, according to the studied genotype. Genotypes 8 and 1153 showed the highest percentages of

Table 1: Number pollen grains sticked on the stigma and number of pollen grains germinated *in vivo* of the several *P. salicina* genotypes in the self-pollination, cross-pollination with cv. Giada and open pollination treatments. The observation were realized after 10 days of beginning the treatments. Values followed by different letters show significant differences for each one of the treatments. Results were analyzed by the χ^2 Test (P 0.05).

	Natural self-pollination		***Cross-pollination***		***Open pollination***	
Genotypes of *P. salicina*	Nº pollen grains sticked on the stigma	Pollen grains germinated (%)	Nº pollen grains sticked on the stigma	Pollen grains germinated (%)	Nº pollen grains sticked on the stigma	Pollen grains germinated (%)
2	36 c	0 d	633 a	17.69 b	0 e	0 d
3	20 d	0 d	538 b	17.28 b	108 d	28.70 bc
8	287 a	68.29 a	141 d	38.29 a	479 c	53.65 a
10	290 a	62.76 a	354 c	41.80 a	910 a	30.00 b
111	113 b	20.35 c	490 b	36.12 a	485 c	18.96 c
1153	285 a	46.66 b	298 c	43.95 a	730 b	49.86 a

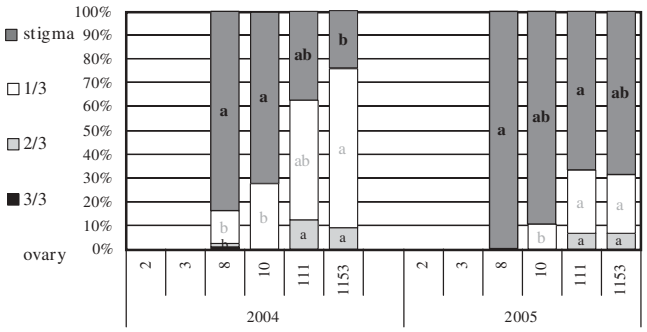

Figure 4: Growth of pollen tubes in the style for six genotypes of *P. salicina* at 10 days following self-pollination for two years (2004–2005). The value were expressed in percentage. The percentage were obtained of the total of pollen grains germinated. Values followed by different letters for each one of the variable studied show significant differences. Results were analyzed by the χ^2 Test (P0.05).

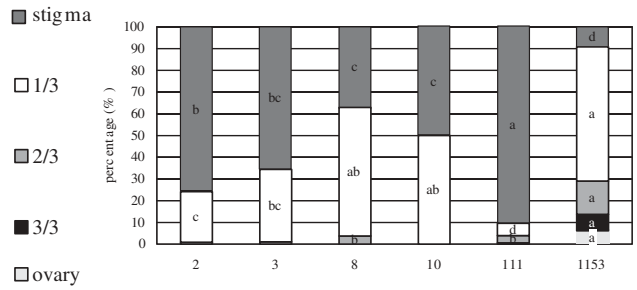

Figure 5: Growth of pollen tubes in the style for six genotypes of *P. salicina* at 10 days following *cross-pollination* with *P. armeniaca* cv. Giada. The value were expressed in percentage. The percentage were obtained of the total of pollen grains germinated. Values followed by different letters for each one of the variable studied show significant differences. Results were analyzed by χ^2 Test (P0.05).

pollen grain germination (55.7 and 49.8 %, respectively). Genotype 111 showed the lowest germination value, while intermediate values were observed in genotypes 3 and 10 (*Table 1*). The quantity of flowers in genotype 2 was not enough to carry out the open pollination treatment.

Pollen tubes only grew around the stigma in genotypes 3 and 10 (*Figure* 3A), while they grew up to 2/3 of the style in genotype 1153. In genotypes 8 and 111, a lower proportion of pollen tubes was observed distributed in the first third of

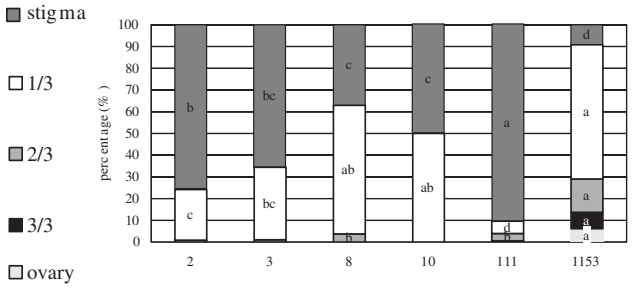

Figure 6: Growth of pollen tubes in the style for six genotypes of *P. salicina* at 10 days following open pollination treatment. The value were expressed in percentage. The percentage were obtained of the total of pollen grains germinated. Values followed by different letters for each one of the variable studied show significant differences. Results were analyzed by the χ^2 Test (P 0.05).

the style (5.06% and 26.09% respectively, *Figure 6*).

Discussion

The efficiency of pollination in *P. salicina* is genetically determined (*Niesenbaum & Casper*, 1994). Pollen tubes in *P. salicina* reach the embryo sac in 4 days when temperatures are between 20–21Cº, while with mean temperatures of 10–11 Cº this occurs 8 days after pollination (*Bubán*, 1996). The highest growth rate of pollen tubes suggests a higher vigour of grains (*Tanksley* et al., 1981). No fertilization of the studied genotypes was observed 10 days after the beginning of treatments. This delay could be due to genetic factors or to the low temperatures registered during the period of pollination-fertilization of the two years of study (*Ontivero* et al., 2005). According to *Jefferies* et al. (1982b), mean temperatures of around 5 ºC would delay fertilization for up to 16 to 20 days.

In *in vitro* techniques, a correlation between viability and germination of pollen grains of *P. armeniaca* and *P. salicina* was observed. According to results obtained in this study, pollen grains produced by genotypes 8, 10, 111 and 1153 are more viable and significantly superior than those produced by genotypes 2 and 3. As has already been studied, these problems are due to diverse anomalies occurring during the

process of microsporogenesis (*Ontivero* et al., 2005).

In natural self-pollination treatments, pollen grains did not germinate on pistils of genotypes 2 and 3 due to their low viability, while they did germinate when they were seeded *in vitro*. This was due to the fact that conditions of temperature and humidity at the laboratory were better for pollen grain germination of these genotypes; however, their percentage of germination was very low. In genotypes 8, 10, 111 and 1153, differences observed in relation to percentages of germination and growth rate of pollen tubes could be due to compatibility problems, since the quantity of pollen grains sticked to the stigma was similar in all of them. Although in the natural self-pollination treatment genotypes 8 and 10 showed the highest quantity of pollen grains germinated 10 days after the beginning of treatments, most pollen tubes were located in the stigma, with a low probability of reaching the viable ovule. This would indicate some type of incompatibility. In genotypes 111 and 1153 of *P. salicina*, the percentage of germination was lower; however, pollen tubes reached 2/3 of the style: this could be due to a higher affinity with their own pollen. This higher affinity could be expressed through a higher growth rate of pollen tubes in relation to the other genotypes. In natural self-pollination treatments, differences observed among genotypes in relation to pollination efficiency could be attributed to the different germination rates of pollen grains because of a higher viability and compatibility characteristics (*Tanksley* et al., 1981). Since no pollen tubes were found in ovules of the different genotypes 10 days after the beginning of treatments it is possible that these genotypes would show pseudocompatibility characteristics (*Williams & Maier*, 1977).

In the cross-pollination treatments with *P. armeniaca* cv. Giada, genotypes 2 and 3 showed a higher percentage of cv. Giada pollen grains sticked on the stigma; however, their lower percentage of germination could be due to interspecific incompatibility characteristics (*Bubán*, 1996). In genotypes 8, 10 and 1153, the higher presence of cv. Giada pollen tubes at the beginning of the style would indicate a higher growth rate of pollen tubes due to a positive interaction between pollen tubes and the style tissue (*Mulcahy & Mulcahy*, 1985). Genotype 1153 was the only one in which pollen tubes of cv. Giada reached the ovary by having a higher germination rate due to a higher coefficient of affinity (*Bubán*, 1996). This could demonstrate intercompatibility characteristics between genotype 1153 of *P. salicina* and that of cv. Giada (*P. armeniaca* L.).

The quantity of pollen grains sticked on the stigma was different among genotypes of the open pollination treatment. These differences could be due to a different floral morphology that could or could not favour the reception of their own pollen and to the quality of rewards offered to pollinating insects. The presence of pollinating insects would indicate that grains present on stigmas of the different genotypes could come from different species distributed in the fruit orchard. For that reason, in the open pollination treatment, the differences observed among genotypes of *P. salicina* in relation to the quantity of germinated grains and the growth rate of pollen tubes could be due to different affinity coefficients.

Conclusion

Pollination studies carried out on different genotypes of *P. salicina* showed that genotypes 2 and 3 presented signs of self-sterility and interincompatibility with *P. armeniaca* cv. Giada, while genotypes 8 and 10 could present partial self-fertility or pseudocompatibility, and genotypes 111 and 1153 could also be considered as pseudocompatible but with a higher affinity coefficient with the cv. Giada, assuming the existence of intercompatibility between them. These preliminary results should be corroborated with genetic studies to determine the degree of compatibility in genotypes of *P. salicina*. Moreover, these studies will contribute to the selection of inter e intra-compatible genotypes that could be used to create commercial varieties.

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