Journal of Plant Physiology and Breeding

2022, 12(1): 109-122 ISSN: 2008-5168

Research paper

Floral morphology, pollen quality, and self-(in) compatibility in three natural *Prunus* interspecific hybrids

Yasamin Shamsolshoara[1], Rahim Gharesheikhbayat[2*], Seied Mehdi Miri[1], Mohiedin Pirkhezri[2], and Daryoush Davoodi[3]

Received: March 14, 2022 Accepted: May 13, 2022
[1]Department of Horticulture, Karaj Branch, Islamic Azad University, Karaj, Iran
[2]Temperate Fruits Research Center, Horticultural Science Research Institute, AREEO, Karaj, Iran
[3]Department of Nanotechnology, Agricultural Biotechnology Research Institute of Iran (ABRII), AREEO, Karaj, Iran
*Corresponding author; Email: rahim2002bayat@gmail.com

Abstract
The occurrence of inter-specific hybrids between two species in nature has the potential ability to develop new fruit trees with desirable traits. In this study, flower morphology, viability, and *in vitro* germination of pollen grains and self-(in) compatibility, using fruit set (%) and fluorescence microscopy methods were examined in three promising natural *Prunus* interspecific hybrids [*P. armeniaca* × *P. salicina* (Bavanat) and *P. cerasifera* × *P. armeniaca* (Shiraz and Shahriar)]. Bavanat and Shiraz had the highest flower diameter, while the highest number and length of stamens, as well as stamen number/pistil length ratio, belonged to Shahriar. The highest and lowest pollen viability and *in vitro* germination were observed in Shahriar and Bavanat, respectively. In general, sucrose concentrations of 150 to 250 g/l increased *in vitro* pollen germination rate, although concentrations of more than 150 g/l had a negative effect on Bavanat. The three-year average (2018-2020) of final fruit set by self-pollination ranged from 0% in Shiraz to 1.3% in Shahriar. A strong positive correlation was found between the occurrence of twin pistils and the mean temperature from July-August in the previous year. According to field self-pollination and fluorescent microscopy, all three genotypes were considered self-incompatible and needed pollinizer varieties.

Keywords: fluorescent microscopy; fruit set; inter-specific hybrid; *in vitro* germination; pollen viability

How to cite: Shamsolshoara Y, Gharesheikhbayat R, Miri SM, Pirkhezri M, and Davoodi D, 2022. Floral morphology, pollen quality, and self-(in) compatibility in three natural *Prunus* interspecific hybrids. Journal of Plant Physiology and Breeding 12(1): 109-122.

Introduction
The genus of *Prunus*, from the Rosaceae family, comprises a large number of temperate fruit crops grown worldwide (Burgos *et al*. 2007; Cici and Van Acker 2011). *Prunus* includes over 200 species, while commercial stone fruits belong to *Amygdalus*, *Cerasus*, and *Prunus* subgenera (Gharaghani and Solhjoo 2021). Stone fruits such as apricot (*P. armeniaca*), cherries (*P. avium* and *P. cerasus*), peach (*P. persica*), and plums (*P. domestica*, *P. salicina*, *P. cerasifera*) play a significant role in human nutrition and health (Lara *et al*. 2020).

Several natural and artificial intra- or inter-specific crosses within *Prunus* have resulted in new commercial cultivars or rootstocks (Hummer and Janick 2009; Cici and Van Acker 2011; Das *et al*. 2011). The use of *Prunus* interspecific hybrids can improve the fruit quality or increase the resistance to biotic and/or abiotic stresses (Minev and Balev 2002; Shamsolshoara *et al*. 2021). One of the proceedings in breeding efforts is to utilize *Prunus* interspecific hybrids such as Plumcots (apricot × European plum), Aprium (plumcot × apricot), pluot (plumcot × plum), and Tanasgol (apricot × plum) as a parent (Minev and Balev 2002; Okie and

Hancock 2008; Hummer and Janick 2009; Das *et al*. 2011; Gharaghani *et al*. 2017).

The *Prunus* cultivars are mainly gametophytic self- or cross-incompatible, so they need a specific pollinizer to produce commercial fruit yield (Imani *et al*. 2015; Piri *et al*. 2022). Gametophytic self-incompatibility is genetically controlled by a polymorphic locus (S-locus), encoding two linked genes including *S-RNase* and S-haplotype-specific F-box (*SFB*), which are expressed in the pistil and pollen, respectively (Abdallah *et al*. 2019). Determining self-incompatibility in *Prunus* is important for orchard design (Najafi *et al*. 2015). Fluorescence microscopic observation of pollen tube growth in the pistils is a reliable technique for identifying self-(in) compatibility in various fruit trees (Milatović *et al*. 2018). Also, determining the pollen quality (pollen viability and vigor) contributes to incompatibility studies (Sulusoglu and Cavusoglu 2014).

Iran is one of the centers of origin, diversity, and cultivation of *Prunus* species and interspecific hybrids in the world (Gharaghani *et al*. 2017; Shirani Rad *et al*. 2017). Our knowledge of pollen biology and (in) compatibility of some of these hybrids are very limited. So, the present study aimed to identify the flower morphology, pollen quality, and self-(in) compatibility under field and laboratory conditions of three promising natural *Prunus* interspecific, which could be considered in *Prunus* breeding programs for yield and fruit quality. This work aimed to achieve more information about these hybrids and assess their potential to be introduced as cultivars. Our studied genotypes were tested for the first time.

Materials and Methods

Plant materials

This research was carried out on three 7-year-old natural interspecific hybrids of diploid (2n = 2x = 16) *Prunus* species (Shamsolshoara *et al*. 2021) (Table 1) grown in the collection of Horticultural Science Research Institute, Karaj, Iran. Six trees for each genotype were studied over three years (2018-2020). The mean temperature during flower bud differentiation (July-August (Westwood 1978) is presented in Table 2.

Table 1. Geographical information of genotypes of the genus *Prunus* used in this study

Genotype	Species	Location	Latitude (N)	Longitude (E)	Altitude (m)
Bavanat	*P. armeniaca* × *P. salicina*	Bavanat, Fars	N 30º 27' 14"	E 53º 39' 01"	2265
Shiraz	*P. cerasifera* × *P. armeniaca*	Shiraz, Fars	N 29º 39' 13"	E 52º 28' 53"	1500
Shahriar	*P. cerasifera* × *P. armeniaca*	Shahriar, Tehran	N 35º 39' 35"	E 51º 03' 33"	1024

Table 2. Mean monthly temperatures during July-August on the experimental orchard of the Horticultural Science Research Institute, Karaj, Iran (2017-2019)

Month	Temperature (˚C)		
	2017	2018	2019
July	28.9	31.9	29.1
August	27.4	28.4	26.8
Mean	28.1	30.1	27.9

Flower morphology

Several flower characters including pedicel length, open flower diameter, stamen number, stamen length, pistil length, twin pistils, and stamen number/pistil length ratio were recorded. Flowers were collected in full bloom (10 flowers from 10 branches per genotype). Flower dimensions were measured using a digital caliper (with an accuracy of 0.01 mm) (Rodrigo and Herrero 2002).

Pollen preparation

Branches with unopened flowers were isolated and placed in 5% sucrose solution at room temperature until flower opening (Milatović and Nikolić 2007). Then, pollen grains were sampled from dehisced anthers and stored at 4 ˚C (Barzamini and Fotouhi Ghazvini 2017).

Pollen viability

Pollen viability was determined using IKI (iodine potassium iodide) solution (1 g KI and 0.5 g I dissolved in 100 ml distilled water) (Bolat and Pirlak 1999). To characterize viability, about 40-60 pollen grains from four different areas of the slide were counted in a light microscope within five min after pollen staining. Pollen grains stained dark were counted as alive, while light or non-staining pollens were as dead (Sulusoglu and Cavusoglu 2014).

Pollen germination

Pollens were dispersed on germination media containing 0.1 g/l calcium nitrate, 0.05 g/l boric acid, different concentrations of sucrose (50, 100, 150, 200, and 250 g/l) solidified with 10 g/l agar (Agar-Agar, Merck) and incubated at 22-24 ˚C (Asma 2008; Sharafi 2011). The germinated pollens (pollen tube length ≥ pollen grain diameter) were counted using a light microscope 24 h after incubation (Milatović *et al*. 2015).

Self- and open pollination in the field

Self- and open-pollination were carried out on four branches of a tree (six trees for each genotype) in four directions. Shoots with flower buds at the balloon stage were covered with cloth bags in early April, while open pollination was done by shoots without bagging. The flowers were hand-pollinated in the full bloom stage twice with an interval of two days. The initial and final fruit sets were calculated two and eight weeks after full bloom, respectively (Zarrinbal *et al*. 2018).

Self-incompatibility assay by fluorescence microscopy

Branches with flower buds were isolated at the balloon stage and placed in a 5% (w/v) sucrose solution at room temperature. The opened flowers were self-pollinated by brush. The pistils (eight pistils for each genotype) were fixed in formaldehyde acetic acid (FAA) and stained using the aniline blue (Milatović and Nikolić 2007). Pollen tube growth was observed via a fluorescent microscope (Eclipse TE300, Nikon) at 24, 48, 72, and 96 h after pollination.

Statistical analysis

The data obtained for the characteristics of floral morphology and pollen viability were analyzed based on completely randomized design in 2020.

Three slides with 150-250 pollen grains were used to determine pollen viability for each genotype. The three-year data for twin pistils (%) were analyzed based on the split-plot design with three replications with 30 pistils for each replicate. The pollen germination study was performed as a factorial experiment (genotypes and sucrose levels as factors) based on completely randomized design with five replications and was analyzed using two-year data (2019-2020).

Analysis of the data was carried out by SPSS ver. 22 software. Means were compared according to Duncan's Multiple Range Test at the 1% level of probability. Pearson correlation coefficients were calculated to determine the interrelationships among floral characteristics, pollen quality, and fruit set.

Results

The flowers of all three genotypes were bisexual and had five white petals and five bright red sepals. In Bavanat, the petals were rolled inward. Bavanat blossoms were borne singly or doubly at a node, while Shiraz and Shahriar flowers were in small clusters on short spurs (Figure 1). Flower characteristics are shown in Table 3. Pedicel length varied from 2.0 mm (Shiraz) to 9.5 mm (Shahriar). The highest flower diameter was found in Shiraz and Bavanat (2.8 and 2.9 cm, respectively), while they had the lowest stamen number/flower. Stamen length ranged from 5.4 mm (Bavanat) to 15.6 mm (Shahriar). Pistil length was 7.6 mm in Shahriar, while in Bavanat it was 14.8 mm. The stamen number to pistil length ratio varied from 1.7 in Bavanat to 3.8 in Shahriar. Pistil and fruit twins occurred in all three genotypes (Figure 2). The highest and lowest average percentage of twin pistils were obtained in 2019 (30.3%) and 2018 (10.8%) (Table 4). Twin pistils had a significant positive correlation with temperature (r = 0.92). The order of pistil twinning in the genotypes was as follows: Shiraz > Bavanat > Shahriar (Table 4). A significant negative correlation of twin pistils with stamen number, self-pollinating fruit set, and open-pollinating final fruit set was observed, while it was not correlated to other measured traits (Table 5).

Table 3. Flower morphological characteristics of three *Prunus* interspecific hybrids in 2020

Genotype	Pedicel length (mm)	Flower diameter (cm)	Stamen number	Stamen length (mm)	Pistil length (mm)	Stamen number/ pistil length
Bavanat	4.9 b	2.9 a	25.3 b	5.4 c	14.8 a	1.7 c
Shiraz	2.0 c	2.8 a	23.9 b	7.7 b	10.2 b	2.3 b
Shahriar	9.5 a	2.4 b	28.8 a	15.6 a	7.6 c	3.8 a

Values with different letters in each column are significantly different at $p \leq 0.01$ according to Duncan's Multiple Range Test

Table 4. Occurrence of twin pistils (%) in three *Prunus* interspecific hybrids in 2018-2020

Genotype	Year			Mean
	2018	2019	2020	
Bavanat	10.6 de	20.2 bc	17.6 bcd	16.1 b
Shiraz	13.6 cde	49.3 a	22.1 b	28.3 a
Shahriar	8.3 e	21.3 b	11.6 de	13.7 b
Mean	10.8 c	30.3 a	17.1 b	

Values with different letters over three years are significantly different at $p \leq 0.01$ according to Duncan's multiple range test.

Table 5. Pearson correlation coefficients of twin pistils with floral characteristics, pollen quality, and fruit set of three *Prunus* interspecific hybrids

Trait	FD	SN	SL	PL	SN/PL	PV	*Ivit*PG	SIFS	SFFS	OIFS	OFFS	*Iviv*PG	PTGBS
TP	0.47^{ns}	-0.82^{*}	-0.44^{ns}	-0.00^{ns}	-0.39^{ns}	-0.02^{ns}	-0.22^{ns}	-0.78^{*}	-0.97^{**}	-0.64^{ns}	-0.74^{*}	-0.14^{ns}	-0.63^{ns}

FD: flower diameter, SN: stamen number, SL: stamen length, PL: pistil length, SN/PL: stamen number/pistil length ratio, PV: pollen viability, *Ivit*PG: *in vitro* pollen germination, SIFS: self-pollination initial fruit set, SFFS: self-pollination final fruit set, OIFS: open-pollination initial fruit set, OFFS: open -pollination final fruit set, *Iviv*PG: *in vivo* pollen germination (on the stigma), PTGBS: pollen tube growth at the base of the style

ns, * and **: non-significant and significant at $p \leq 0.05$ and $p \leq 0.01$, respectively

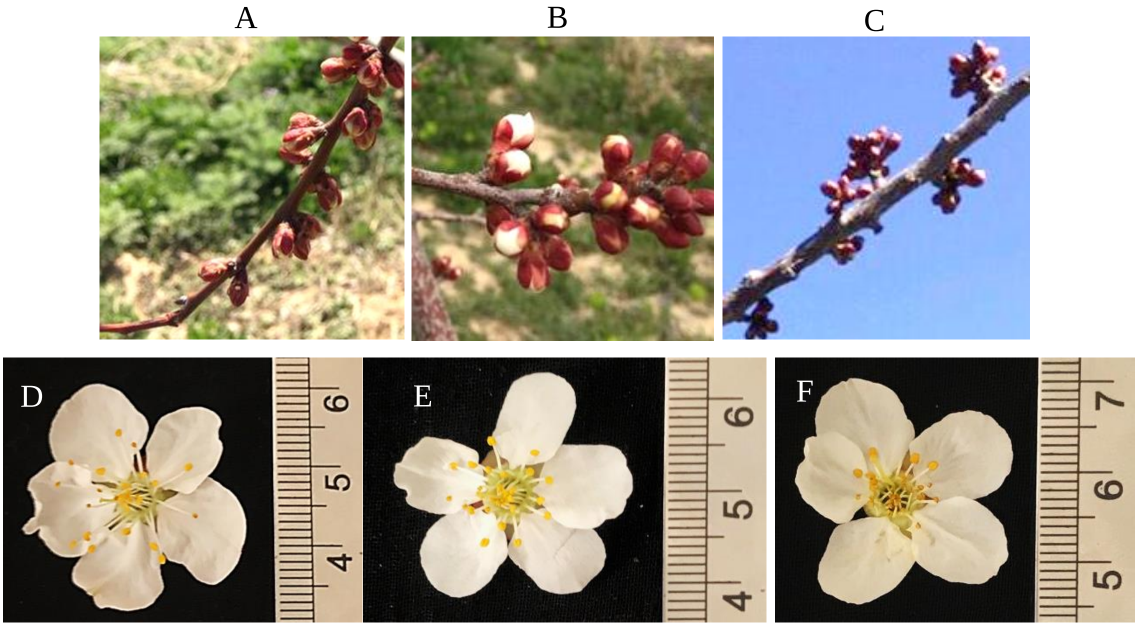

Figure 1. The blooms (top) and flowers (bottom) of three *Prunus* interspecific hybrids in 2020. A-D) Bavanat (*P. armeniaca* × *P. salicina*), B-E) Shiraz (*P. cerasifera* × *P. armeniaca*), and C-F) Shahriar (*P. cerasifera* × *P. armeniaca*).

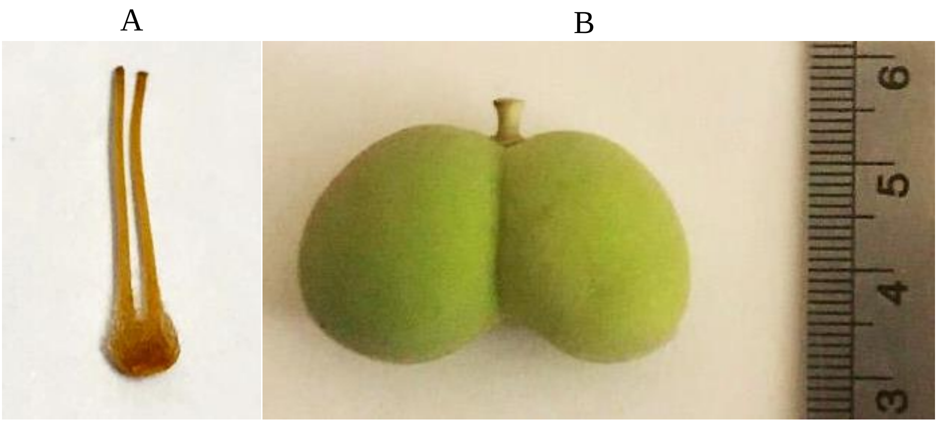

Figure 2. Twin pistil (A) and fruitlet (B) in Bavanat genotype in 2019

The three *Prunus* interspecific hybrids showed significant differences in pollen viability (Table 6). Shahriar had the highest pollen viability (21.3%), while the lowest one (6.4%) was found in Bavanat.

The genotypes exhibited different *in vitro* pollen germination rates (Figures 3 & 4). Similar to the pollen viability test, Shahriar and Bavanat had the highest (21.6%), and the lowest (3.3%) pollen germination, respectively. The effect of sucrose concentration on pollen grain germination was statistically significant ($p \leq 0.01$). Pollen germination percentage increased up to 150 g/l sucrose, although the response of the genotypes was different after this concentration. In Bavanat, increasing sucrose concentration by more than 150 g/l affected negatively the pollen germination rate. In Shiraz and Shahriar, the highest pollen germination was found with 100-250 and 200-250 g/l sucrose, respectively.

Initial and final fruit set by self- and open-pollination differed among genotypes in 2018-2020 (Table 7). Initial fruit set by self-pollination varied from 1.5% (Bavanat in 2019 and Shiraz in 2020) to 6.6% (Bavanat in 2018), while the final fruit set ranged from 0.0% in Shiraz (2018-2020) to 1.5% in Sharhiar (2020). Moreover, the initial and final fruit sets varied from 7.7% (Bavanat in 2019) and 2.6% (Shiraz in 2019) to 22.3 and 18.1% (Sharhiar in 2020), respectively, by open

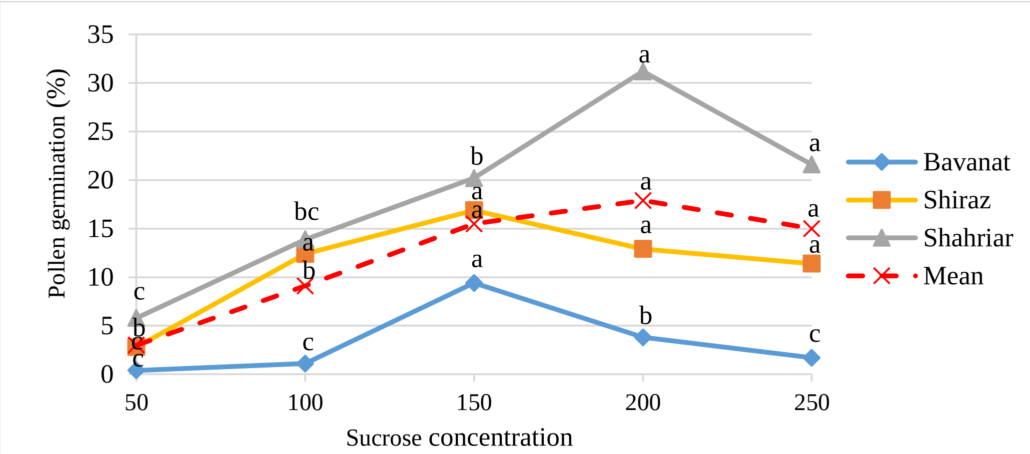

Figure 3. Pollen germination of three *Prunus* interspecific hybrids at different sucrose concentrations (mean of 2019-2020).

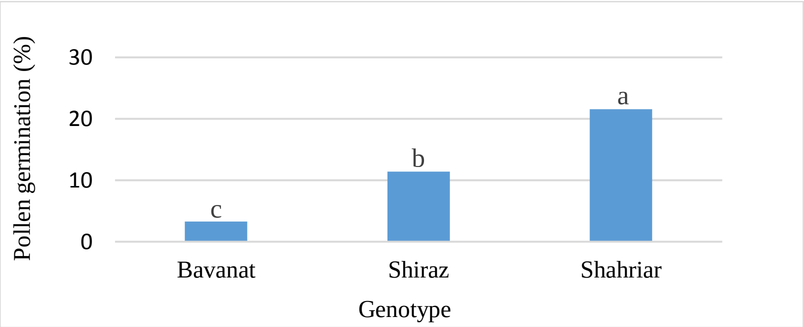

Figure 4. *In vitro* pollen germination of three *Prunus* interspecific hybrids (mean of 2019-2020); Values with different letters are significantly different according to Duncan's Multiple Range Test.

Table 6. Pollen viability of three *Prunus* interspecific hybrids in 2020

Genotype	Pollen viability (%)
Bavanat	6.4 c
Shiraz	15.6 b
Shahriar	21.3 a

Values with different letters in the column are significantly different at $p \leq 0.01$ according to Duncan's Multiple Range Test.

Table 7. Initial and final fruit set of three *Prunus* interspecific hybrids following self- and open-pollination in 2018-2020

Genotype	2018†		2019				2020				Mean			
	SP		SP		OP		SP		OP		SP		OP	
	IFS (%)	FFS (%)	IFS (%)	FFS (%)	IFS (%)	FFS (%)	IFS (%)	FFS (%)	IFS (%)	FFS (%)	IFS (%)	FFS (%)	IFS (%)	FFS (%)
Bavanat	6.6	1.2	1.5	0	7.7	2.7	5.8	1.2	15.3	7.9	4.7	0.8	11.5	5.3
Shiraz	2.4	0.0	3.0	0.0	11.8	2.6	1.5	0.0	11.0	4.6	2.3	0.0	11.4	3.6
Shahriar	3.9	1.1	3.2	1.4	14.5	10.3	3.3	1.5	22.3	18.1	3.5	1.3	18.4	14.2

†Fruit set was not realized by open-pollination in 2018; SP: self-pollination, OP: open-pollination, IFS: initial fruit set, FFS: final fruit set

pollination. On the average of three years, the lowest and highest percentage of the final fruit set was related to Shiraz (0.0 and 3.6% by self- and open-pollination, respectively) and Shahriar (1.3 and 14.2% by self- and open-pollination, respectively).

Self-(in) compatibility could be determined by fluorescent microscopic observation of pollen tube growth in the self-pollinated pistils. In all of the three genotypes, the pollen tube did not reach the ovary, and only 2.3% of the pollen grains in Shahriar penetrated the base of the style after 96 h (Table 8).

Significant positive correlations of the final fruit set following self-pollination with stamen number and pollen tube growth were observed (Table 9). The final fruit set following open pollination had a significant positive correlation with stamen number, stamen length, stamen number/pistil length ratio, in vitro pollen germination, in vivo pollen germination, and pollen tube growth, whereas it showed a negative correlation with the flower diameter.

Table 8. Pollen tube growth in the style of three *Prunus* interspecific hybrids following self-pollination (mean of 2019-2020)

Genotype	Time after self - pollination (h)	Pollen number on the stigma surface	Germinated pollen (%)	Pollen tubes (%) in pistils					Pistils (%) with at least one pollen tube at the base of the style	Self-(in) compatible
				Upper third of the style	Middle third of the style	Lower third of the style	Base of the style	Ovary		
Bavanat	24	6.0	6.2	100.0	0.0	0.0	0.0	0.0	0.0	-
Shiraz	24	4.4	28.6	90.0	10.0	0.0	0.0	0.0	0.0	-
Shahriar	24	7.0	30.3	82.3	17.6	0.0	0.0	0.0	0.0	-
Bavanat	48	5.5	36.3	87.5	12.5	0.0	0.0	0.0	0.0	-
Shiraz	48	3.9	48.4	80.0	20.0	0.0	0.0	0.0	0.0	-
Shahriar	48	6.5	48.1	80.0	20.0	0.0	0.0	0.0	0.0	-
Bavanat	72	6.0	56.2	59.2	33.3	7.4	0.0	0.0	0.0	-
Shiraz	72	6.7	68.5	48.6	35.1	16.2	0.0	0.0	0.0	-
Shahriar	72	7.9	76.1	41.7	39.6	18.7	0.0	0.0	0.0	-
Bavanat	96	8.6	72.4	46.0	42.0	12.0	0.0	0.0	0.0	SI
Shiraz	96	7.2	82.7	43.7	35.4	20.8	0.0	0.0	0.0	SI
Shahriar	96	11.5	92.4	34.1	41.2	22.3	2.3	0.0	37.5	SI

SI: self-incompatible

Table 9. Pearson correlation coefficients among floral characteristics, pollen quality, and fruit set of three *Prunus* interspecific hybrids

Trait	FD†	SN	SL	PL	SN/PL	PV	*Ivit*PG	SIFS	SFFS	OIFS	OFFS	*Iviv*PG
SN	-0.89**											
SL	-1.00**	0.88*										
PL	0.88*	-0.57^{ns}	-0.89**									
SN/PL	-0.99**	0.84*	1.00**	-0.92**								
PV	-0.89**	0.59^{ns}	0.90**	-1.00**	0.93**							
*Ivit*PG	-0.96**	0.74*	0.97**	-0.97**	0.98**	0.98**						
SIFS	0.19^{ns}	0.27^{ns}	-0.21^{ns}	0.63^{ns}	-0.27^{ns}	-0.61^{ns}	-0.44^{ns}					
SFFS	-0.66^{ns}	0.93**	0.64^{ns}	-0.23^{ns}	0.59^{ns}	0.25^{ns}	0.44^{ns}	0.61^{ns}				
OIFS	-0.98**	0.96**	0.97**	-0.77*	0.95**	0.78*	0.89**	0.01^{ns}	0.80*			
OFFS	-0.94**	0.99**	0.93**	-0.67^{ns}	0.91**	0.69^{ns}	0.82*	0.15^{ns}	0.87*	0.99**		
*Iviv*PG	-0.93**	0.68*	0.95**	-0.99**	0.96**	0.99**	0.99**	-0.51^{ns}	0.36^{ns}	0.85*	0.77*	
PTGBS	-0.98**	0.96**	0.97**	-0.77*	0.96**	0.79*	0.89**	0.00^{ns}	0.79*	1.00**	0.99**	0.86**

†FD: flower diameter, SN: stamen number, SL: stamen length, PL: pistil length, SN/PL: stamen number/pistil length ratio, PV: pollen viability, IvitPG: in vitro pollen germination, SIFS: self-pollination initial fruit set, SFFS: self-pollination final fruit set, OIFS: open-pollination initial fruit set, OFFS: open -pollination final fruit set, IvivPG: in vivo pollen germination (on the stigma), PTGBS: pollen tube growth at the base of the stylens, * and **: non-significant and significant at $p \leq 0.05$ and $p \leq 0.01$, respectively

Discussion

The evaluation of flower characteristics showed morphological variation among the three studied genotypes. Floral traits are largely controlled by genetic factors (Li *et al*. 2022), although environmental conditions may also play an important role (Azizi-Gannouni and Ammari 2020). The morphology of Bavanat blossoms and flowers showed some similarities with apricot (single or double flower buds, rolled inward petals, and bright red sepals). Furthermore, Shiraz and Shahriar showed some phenotypic traits of the maternal parent (cluster flower buds on short spurs) and paternal parent (bright red sepals). Some parents' morphological and pomological intermediate traits had already been observed in these genotypes (Shamsolshoara *et al*. 2021). Zhang and Gu (2015) noted that flowers of *P. tomentosa* × *P. salicina* progenies had some characteristics intermediate between their parents.

Bavanat and Shiraz had the largest flower size. Flower size is generally considered to be the most important factor for pollinators. Larger flowers generally attract more pollinators, which could directly affect flower pollination and therefore fruit set (Azizi-Gannouni and Ammari 2020). However, in our study, a negative relationship was found between flower diameter and open-pollinating fruit set. This may be due to the longer pistil in larger flowers, which results in more time required for the pollen tube growth from stigma to the ovules and a reduction in the effective pollination period, which ultimately reduces the chance of fertilization and fruit set. On the other hand, more and taller stamens in Shahriar might favor for a successful cross pollination.

Suranyi (1976) reported that the stamen number/pistil length ratio in the self-sterile *Prunus* cultivars was higher than that in self-fertile ones. In disagreement with Suranyi (1976), this ratio was higher in Shahriar compared to Bavanat. Therefore, the relationship between stamen number/pistil length ratio and self-sterility or -fertility may be a genotype-dependent trait.

Fruit twinning has been reported among stone fruits. They have fewer fruit sets and are not desired in the market due to their shape (Jia *et al*. 2013; Wu *et al*. 2019), which was also observed in our results. Pistil twinning is due to the abnormal formation of pistil primordia during flower bud differentiation in the previous warm summer (Beppu and Kataoka 2011). A higher occurrence of twin pistils in all genotypes was found in 2019. The mean temperature from July-August 2018 was 2.0 ˚C and 2.2 ˚C higher than at the same time in 2017 and 2019, respectively.

Determining pollen viability by IKI staining is an easy and reliable method to select a suitable pollinizer when establishing orchards (Sulusoglu and Cavusoglu 2014). In the present study, pollen viability was very low, so, all genotypes need suitable pollinizers. The natural hybrids examined here are believed to be the result of crossbreeding between *Prunus* species, with at least one parent being *P. cerasifera*. Thus, disruption of meiosis is very likely to occur during pollen grain formation. It should be noted that, based on previous karyological studies of similar hybrids, the parents have morphologically distinct chromosomes (Zarifi and Gharesheikhbayat 2018). This can result in the production of abnormal pollen grains with a low germination rate, or even not at all. Due to open pollination in orchards, these hybrids are productive trees.

Sucrose concentrations affected pollen germination of studied genotypes. *In vitro* pollen tube growth is achieved in sugar-containing media (Sulusoglu and Cavusoglu 2014). Generally, sucrose, as a carbohydrate, is used to supply energy and osmotic potential for *in vitro* pollen germination (Tushabe and Rosbakh 2021). Similar to our results, previous studies have shown that the highest *in vitro* germination of stone fruit pollens was obtained with sucrose concentrations at 150 or 200 g/l (Bolat and Pirlak 1999; Sulusoglu and Cavusoglu 2014). However, in Bavanat, pollen germination decreased at sucrose concentrations of more than 150 g/l, which is similar to the report of Asma (2008) on apricot, who observed that the concentration of 200 g/l sucrose has an inhibitory effect on pollen germination rate.

Fruit set depends on a large number of factors such as environmental conditions, training and pruning, rootstock, thinning, pollen source, and nutrition of the tree (Nikolić and Milatović 2010; Ahmadpoor *et al*. 2022). In Bavanat, the average fruit set ratio was 5.3%. Similarly, Yaman and Uzun (2020) reported fruit set ratios of 0.6 to 6.2% in *P. armeniaca* × *P. salicina* hybrids.

Successful fertilization is essential for fruit set in stone fruits (Fernandezi Marti *et al*. 2021; Jamshidi *et al*. 2021). One of the most important problems in fruit growing is self-incompatibility as it limits the design of monoculture orchards and requires suitable pollinizers to achieve high fruit yield. Self-(in) compatibility has been studied by calculating the percentage of final fruit set after controlled self-pollination (Zarrinbal *et al*. 2018). In our study, controlled self-pollination under orchard conditions resulted in a very low fruit set, while open pollination increased the final fruit set by 2.6 to 12.0 folds. A fruit set of 5-15% for plums and 20-25% for apricot is essential to have adequate yield (Westwood 1978; Fotirić Akšić *et al*. 2022). Different classifications have been proposed to determine *Prunus* species or cultivars as self-incompatible (Nikolić and Milatović 2010). Based on fruit set percentage following self-pollination, Iliev (1985) considered plum varieties up to 20% fruit set as self-incompatible, while Nyéki and Szabó (1996) classified 0%, 0.1-1%, and 1.1-10% fruit set as entirely self-incompatible, self-incompatible, and partially self-fertile, respectively. In apricots, fruit set of less than 3-5% following controlled self-pollination is referred to as self-incompatibility (Burgos *et al*. 1997; Audergon *et al*. 1999; Muñoz-Sanz *et al*. 2017). Based on these criteria, the studied genotypes were self-incompatible. This phenomenon was expected, as most diploid plums (*P. salicina* and *P. cerasifera*) and Iranian-Caucasian apricots (unlike European apricot) are self-incompatible (Hegedüs and Halász 2006; Herrera *et al*. 2018) and our studied genotypes inherited this trait from their parents. Karayiannis and Tsaftaris (1999) found that about half of the apricot hybrids resulting from a *PPV* (plum pox virus) resistance breeding program inherited the self-incompatibility trait.

Observation of pollen tube growth in the style using fluorescence microscope is known as a consistent, reliable, and accurate technique to characterize self-(in) compatible phenotypes and confirm the fruit set evaluation (Zarrinbal *et al*. 2018). Similar to the studies of Nikolić and Milatović (2010), Zarrinbal *et al*. (2018), and Guerrero *et al*. (2020), our microscopic observations confirmed the results of the final fruit set by self-pollination in the field. The gametophytic incompatibility often occurs in the upper third of the style and is determined by the stop of pollen tubes growth and swelling of their tips due to greater accumulation of callose (Nikolić and Milatović 2010), which was also confirmed in our study.

Conclusions

This study showed variations among floral traits of three studied *Prunus* interspecific hybrids. The viability and *in vitro* germination of pollen grains were very low. Results of the final fruit set and pollen tube growth observation using fluorescence microscopy after self-pollination revealed the self-incompatibility of all three genotypes, as, all genotypes need suitable pollinizers. Therefore, fruit sets and fluorescence microscopy techniques are useful tools for the characterization of self-incompatibility studies.

Conflict of interest

The authors declare that there is not any conflict of interest regarding the publication of this manuscript.

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بررسی مورفولوژی گل، کیفیت دانه گرده و خود(نا)سازگاری در سه دورگ بین‌گونه‌ای جنس *Prunus*

یاسمین شمس الشعرا[1]، رحیم قره شیخ بیات[2*]، سید مهدی میری[1]، محی الدین پیرخضری[2] و داریوش داوودی[3]

۱ - گروه باغبانی، دانشکده کشاورزی و منابع طبیعی، دانشگاه آزاد اسلامی واحد کرج، کرج

۲ - پژوهشکده میوه‌های معتدله و سردسیری، موسسه تحقیقات علوم باغبانی، سازمان تحقیقات، آموزش و ترویج کشاورزی، کرج

۳ - بخش تحقیقات نانوتکنولوژی، پژوهشگاه بیوتکنولوژی کشاورزی ایران، سازمان تحقیقات، آموزش و ترویج کشاورزی، کرج

مسئول مکاتبه؛ Email: rahim2002bayat@gmail.com

چکیده

پیدایش دورگ‌های بین‌گونه‌ای به صورت طبیعی می‌تواند به طور بالقوه دستیابی به نمونه‌هایی از درختان میوه با صفات ارزشمند را ممکن سازد. مورفولوژی گل، زنده‌مانی و جوانه‌زنی درون شیشه‌ای دانه‌های گرده و خود(نا)سازگاری با مطالعه درصد تشکیل میوه و روش تعقیب لوله گرده با میکروسکوپ فلورسانس بعد از خودگرده افشانی کنترل شده در سه دورگ طبیعی بین‌گونه‌ای جنس *Prunus* شامل: [*P. armeniaca* × P. *salicina* (بوانات) و *P. cerasifera* × *P. armeniaca* (شیراز و شهریار)] مورد بررسی قرار گرفت. دورگ‌های بوانات و شیراز بیشترین قطر گل را داشتند، درحالی که بیشترین تعداد و طول پرچم و نسبت تعداد پرچم به طول مادگی متعلق به دورگ شهریار بود. بیشترین و کمترین زنده‌مانی دانه گرده و جوانه‌زنی درون شیشه‌ای به ترتیب در دورگ‌های شهریار و بوانات مشاهده شد. به طور کلی، غلظت‌های ساکارز ۱۵۰ تا ۲۵۰ گرم در لیتر سرعت جوانه‌زنی دانه گرده در شرایط درون شیشه‌ای را افزایش داد، هرچند غلظت‌های بیش از ۱۵۰ گرم در لیتر بر دورگ بوانات اثر منفی داشت. میانگین سه ساله (۲۰۱۸-۲۰۲۰) تشکیل میوه نهایی توسط خودگرده‌افشانی از صفر درصد در دورگ شیراز تا ۱/۳ درصد در دورگ شهریار متغیر بود. همبستگی مثبت معنی‌داری بین دوقلوزایی مادگی و میانگین دمای تیر-مرداد سال قبل مشاهده شد. نتایج آزمایش با میکروسکوپ فلورسانس نشان داد که هیچ لوله گرده‌ای ۹۶ ساعت پس از خود گرده افشانی به تخمدان نرسیده است. بر اساس هر دو روش، هر سه ژنوتیپ خودناسازگار در نظر گرفته شدند که برای تولید میوه نیاز به ارقام گرده‌زا دارند.

واژه‌های کلیدی: تشکیل میوه؛ جوانه‌زنی درون شیشه‌ای؛ زنده‌مانی گرده؛ میکروسکوپ فلورسنت؛ هیبرید بین‌گونه‌ای