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Full Length Research Paper

Pollen viability and longevity in some selected genotypes of peach, plum, prune and sour cherry

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In stone fruit trees such as peach, plum, prune and sour cherry, pollen performance includes pollen quantity produced in a flower and pollen quality consists of viability, longevity, morphological homogeneity, pollen germination and pollen tube growth rate which are very important component of fertilization and fruit setting. However, study of main pollen traits is one of the most important approaches for the stone fruit trees growers and breeders. In this research, main pollen traits including germination, tube growth and longevity were investigated in some favorable selected genotypes of four *Prunus* species including peach, plum, prune and sour cherry. Pollen traits of five genotypes from each species were studied after one month maintenance in -20°C using the *in vitro* medium containing 15% sucrose, 1% agar and 5 ppm H3BO3 (boric acid). Pollens were planted in the *in vitro* medium inside the Petri-dishes and incubated at the constant temperature of 24°C for 24 h and then, pollen germination and growth were protected using chloroform. In each Petri-dish six squires were randomly selected for studding Pollen Germination Percentage (PGP) and Pollen Tube Length (PTL) using light-microscope. Data analyzed with SAS software and results showed significant differences in the studied traits among four species and genotypes of each species. However, genotypes of peach showed the highest PGP and PTL proving the high longevity of peach pollens among four studied species.

Key words: Rosaceae family, peach, plum, prune, sour cherry, pollen viablity and *in vitro*.

INTRODUCTION

Peach (*Prunus persica* L.), plum (*Prunus domestica* L.), prune (*Prunus salisina* L.) and sour cherry (*Prunus cerasus* L.), are temperate zone stone fruit trees which are grown in many regions of the world. Currently; in Iran, there are breeding programs to develop superior stone fruit cultivars for several different usages. Flowering and fertilization are critical for fruit set in stone fruits therefore, determining the components of reproduction biology is critical for optimizing yields in orchards and is therefore important for breeding programs. Some of cultivars in mentioned species have self- incompatibility traits although; tetraploid sour cherry (*P. cerasus)* and hexaploid common plum *(P. domestica)* have some self-compatible cultivars (Hegedus and halasz, 2006; Keulemans, 1994; Keulemans and Van lear, 1989; Nikolic and Milatovic, 2010). Stone fruits; peach, plum, prune and sour cherry; need to pollination with high quantity and quality pollens and fertilization are most important factors affecting fruit setting. Some peach, plum, prune and sour cherry cultivars flower early and fruit set can be inhibited by low temperatures at flowering time because of poor pollen germination and tube growth. However, synchronized flowering, positive pollination and fertilization are critical for fruit set in such stated stone fruits (Semenas and Kouhartchik, 2000; Szabo, 2003). Furthermore, in some breeding programs breeders; maintain pollens for applying in the controlled artificial pollination methods (Alburquerque et al., 2007; Parfitt and Almehdia, 1984; Parfitt and Ganeshan, 1989).

Artificial pollination need to using selected pollen from elite cultivars, whereas most of them are self-incompatible or have some week's differences in blooming time and surely do not overlap each other. Due

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to these differences usually pollens could be collected, dried and maintained before controlled pollination programs. Meanwhile; many cultivars and genotypes with unfavorable pollens especially with low germination percentage and tube growth have been reported by breeders and researchers (Du et al., 2006; Koyuncu and Tosun, 2005; Nikolic and Milatovic, 2010; Parfitt and Almehdia, 1984; Parfitt and Ganeshan, 1989). Therefore, study of pollen traits in selected genotypes or cultivars is one of the necessary works which done in such plants. Many studies have investigated pollen viability and germination in peach, plum, prune, sour cherry and other species cultivars and different test methods have been used to determine the pollen viability of fruit trees (Cerovic and Ruzic, 1992, 1994; Herrero and Arbeloa, 1989; Jefferies et al., 1982; Vitagliano and Viti, 1989; Weibaum et al., 2004). For instance, Anjum and Shaukat (2008) studied pollen germination of *Malus pumila* L., up to 48 weeks in a refrigerator (+4C), freezer (-20℃, -30℃) and freeze drier (-60℃) using hanging drop technique in different concentration of sucrose and boric acid solution.

Du et al. (2006), Hedly et al. (2004), Koyuncu and Tosun (2005), and Sharafi and Bahmani (2010), investigated pollen characteristics of different stone fruit cultivars with different objectives and reported various results. The objective of this work was to determine longevity, viability, germination and tube growth capacity of pollens in some favorable selected genotypes of peach, plum, prune and sour cherry genotypes which are grown in different regions of East- Azarbaijan province in Iran, after one month maintenance in -20℃, for using in breeding and orchard establishment programs.

MATERIALS AND METHODS

Experiment region and plant materials

This research was carried out in department of horticulture, University of Maragheh, Maragheh, Iran. Five favorable genotypes with high quality and quantity characteristics from each of the species peach (Pe_1, Pe_2, Pe_3, Pe_4 and Pe_5), plum (Pl_1, Pl_2, Pl_3, Pl_4 and Pl_5), prune (Pr_1, Pr_2, Pr_3, Pr_4 and Pr_5) and sour cherry (Sc_1, Sc_2, Sc_3, Sc_4 and Sc_5) which are grown in different regions of East-Azarbaijan province of Iran were selected.

Experiment stages

In the spring of 2009, flower buds in balloon stage gathered and transmitted to the laboratory. Petals and sepals were separated and anthers isolated from flower buds and placed in Petri dishes for releasing pollens. Pollens gathered and their PGP and PTL were tested immediately and then, stored one month in -20℃. Pollens planted in the *in vitro* medium containing 1% agar, 15% sucrose and 5 ppm boric acid; maintained about 24 h in 24℃ and then tube growth was stopped with adding chlorophorm. Pollen Germination Percentage (PGP) and Pollen Tube Length (PTL) were measured under light- microscope. Seven microscopic areas were counted randomly for evaluation of PGP and PTL. Pollen tube long at least as its diameter was considered to be 'germinated and measurements of pollen tube length were recorded based on micrometer (µm); directly using an ocular micrometer fitted to the eyepiece on microscope.

Experimental design and data analysis

Experimental design was Completely Randomized Design (CRD) with four replications (4 Petri dishes). Data were analyzed separately for genotypes in each of the species, using SAS software and comparison of means was carried out with Duncan's multiple range tests.

RESULTS AND DISCUSSION

Analysis of variance indicated significant differences for PGP) and pollen tube length PTL among peach, plum, prune and sour cherry studied genotypes after one month maintenance in -20℃ (Tables 1). Among peach genotypes, means of PGP and PTL were ranged between 54.5 to 88.1% and 840.2 to 1012.5 µm, respectively also; in the plum studied genotypes, PGP and PTL were ranged between 48.9 to 96.3% and 148.5 to 687.8 µm, respectively (Table 2). However, data in (Table 3) shows that in genotypes of prune; PGP and PTL were ranged between 35.8 to 63.2% and 253.6 to 671.2 µm while, in genotypes of sour cherry; were 2500.7 to 68.4% and 254.2 to 785.1 µm, respectively. Difference in means of PGP and PTL among four stone fruit tree species and genotypes showed higher variety in PTL in compared with PGP. Meanwhile, genotypes of peach showed the highest PGP and PTL proving the high longevity of peach pollens among four species. It should be stated that in this research; the means of pollen germination percentage in four species genotypes were higher than 80% in all of the genotypes immediately after gathering in laboratory (data not shown).

Maximum PGP were observed in genotypes Pe_3 (88.1%), Pl_3 (96.3%), Pr_2 (63.2%) and Sc_5 respectively (Tables 2 and 3). Therefore, all of the genotypes especially Pe_3 (peach), Pl_3 (plum), Pr_2 (prune) and Sc_5 (sour cherry) with highest PGP and longevity could be select for orchard establishment and breeding programs as a pollinizer for pollination of commercially growing cultivars.

In fruit trees pollen germination and tube growth rate are the most important characteristics related to pollen quality and effective fertilization requires the high rates of germination and fast tube growth. Excessively low rates may lead to low fruit set because of ovule degradation before the pollen tube reaches the ovary (Cheung, 1996; Sharafi and Bahmani, 2010). In this research genotypes with high PGP have not shown high PTL necessarily too. This phenomenon indicates genetically differences among the genotypes which reported by many researchers in almond, apricot, sweet cherry, sour cherry, apple, pear and other fruit trees (Pirlak and bolat, 1999; Sharafi and Bahmani, 2010; Stosser et al., 1996). Sometimes, cultivars produce high quantity of pollens but not with high quality such as low pollen germination

Table 1. Analysis of variances of the pollen germination percentage (PGP) and pollen tube length (PTL based on micrometer) in studied genotypes of peach, plum, prune and sour cherry tested in the *in vitro* medium.

Species	SOV	DF	PGP (%)	PTL (µm)
	Genotypes	4	987.4**	1114.2**
P. persica	Error	20	34.7	401.3
	CV (%)		10	12.3
	Genotypes	4	1314.5**	4025**
P. domestica	Error	20	306.8	218.4
	CV (%)		9.5	14.1
	Genotypes	4	3023.7**	1045.1**
P. salisina	Error	20	401.1	203.6
	CV (%)		11.4	13,6
	Genotypes	4	997.3**	1098.4**
P. cerasus	Error	20	101	146.1
	CV (%)		9.8	17.4

**: Significant in P<0.01% level

Table 2. Comparison of means for pollen germination percentage (PGP) and pollen tube length (PTL based on micrometer) in the genotypes of peach and plum.

Species	Genotype	PGP (%)	PTL(µm)
	Pe_1	79.8^{a}	907.2^{a}
	Pe_2	71.4^{ab}	840.2^{b}
P. persica L.	Pe_3	88.1^{a}	1012.5^{a}
	Pe_4	54.5^{b}	987.1^{a}
	Pe_5	67.2^{ab}	856.3^{ab}
	Pl_1	68.4^{ab}	213.4^{b}
	Pl_2	90.2^{d}	148.5^{c}
P. domestica L.	Pl_3	96.3^{a}	720.7^{a}
	Pl_4	48.9^{b}	567.6^{ab}
	Pl_5	70.2^{ab}	687.8^{a}

Same letters show no difference among genotypes of each column.

percentage or low tube growth also; some of the pollens produced by one cultivar may be sterile or not viable (Nikolic and Milatovic, 2010; Stosser et al., 1996; Vitagliano, 1989; Weinbaum et al., 2004). Moreover, Pırlak and Bolat (1999) by investigation on the pollen germination and pollen tube length in apricot cultivars, recorded pollen tube length as 295 µm in Hasanbey, 306 µm in Salak, 251 µm in Karacabey and 268 µm in Sekerpare with 10% sucrose concentration. These results had significant difference with our results whereas, PTL was very high in compared with their results. Because, in this research in compared with them incubation temperature was higher (24°C) and high temperature induces pollen tube growth. It is widely acknowledged that temperature and relative humidity of stored environment are the two important factors that profoundly influence the viability of stored pollen (Anjum and Shaukat, 2008).

Hedhly et al. (2004) studied pollen germination of nine sweet cherry cultivars using *in vitro* pollen performance under two temperatures regimes (15 and 30°C) and found a highly significant effect of pollen genotype and temperature. Higher temperature reduced pollen germination, which maximum values were between approximately 40% in 'Talaguera Brillante' and 'Ambrunés' cultivars and 70% in 'Van' and 'Bing' cultivars also, differences in pollen performance have been found in different cultivars and genotypes of other *Prunus*

Table 3. Comparison of means for pollen germination percentage (PGP) and pollen tube length (PTL based on micrometer) in the genotypes of prune and sour cherry.

Species	Genotype	PGP (%)	PTL(µm)
	Pr_1	35.8^c	490.1^{ab}
	Pr_2	63.2^a	544.2^a
P. salisina L.	Pr_3	48.7^b	580.7^a
	Pr_4	39.5^{bc}	253.6^b
	Pr_5	59.8^a	671.2^a
	Sc_1	29.9^c	252.4^c
	Sc_2	25.7^c	267.8^c
P. cerasus L.	Sc_3	52.9^b	438.5^b
	Sc_4	47.3^{bc}	649.7^a
	Sc_5	68.4^a	785.1^a

Same letters show no difference among genotypes of each column.

species (Cerovic and Ruzic*,* 1992, 1994). However, results from this work are in agreement with those found by Hedhly et al. (2005), who studied pollen germination of nine sweet cherry cultivars testing *in vitro* pollen performance under two temperatures regimes (15 and 30°C).

Parfitt et al. (1984, 1989) found that storage conditions below 0°C (–20 and –80°C) did not affect pollen germination after one year in some species of stone fruits. Furthermore, Alburquerque et al. (2007) studied the influence of storage temperature on the viability of pollen in seven sweet cherry cultivars ('Brooks', 'Cristobalina', 'Marvin', 'New Star', 'Ruby' and 'Somerset') and resulted that pollen viability could be maintained at reasonably high percentages after storage at –20°C during one year for all studied cultivars. Also, pollen viability decreased after 15 or 30 days of storage at 4°C. In their study, for most cultivars pollen completely lost viability after only 60 days of storage at 4°C. Remarkably, 'Cristobalina' and 'New Star' maintained viable pollen in relatively high percentages up to one more month at this temperature. Finally they reported that, pollen viability can be affected by long periods of storage at approximately –20°C, being this effect genotype dependent.

Although, there are some previous studies on the storage of pollen in some stone fruit cultivars for short or long periods of time at different temperatures, genotypes which studied here have been tested for the first time since they are relatively new selections. A procedure to appropriately conserve pollen, maintaining a good viability, may allow a better planning of controlled crosses and also provide a way of exchanging pollen between breeders in different regions. Moreover, according to these results, Sharafi and Bahmani (2010), investigated the pollen traits consisted of pollen germination percentage and pollen tube growth rate after short storage (six weeks in 4°C) in some *Prunus amygdalus* L.*,* *Prunus armeniaca* L., and *Prunus avium* L., genotypes and reported similar conclusions about pollen germination and tube growth.

Conclusion

In this research PGP and PTL in studied genotypes of four stone fruit species, were normal after one month maintenance in -20°C although, some decrease was observed. Genotypes of peach showed the highest range of PGP, PTL and longevity among four species and genotypes with high PGP have not shown high PTL necessarily. However, all of the genotypes especially Pe_3 (peach), Pl_3 (plum), Pr_2 (prune) and Sc_5 (sour cherry) with highest PGP selected for orchard establishment and breeding programs as a pollinizer, for pollination of commercially growing cultivars.

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