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In vitro study of pollen traits after short storage in some almond, apricot and sweet cherry favorable genotypes

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Iran is one of the most important producers of almond, apricot and sweet cherry known as stone fruits belong to genus *Prunus* of Rosaceae family. Most of the stone fruit trees are gametophytically self-incompatible which makes necessary the selection of favorable pollinizer in orchard planning programs for producing positive crop. However, study of main pollen traits is one of the most important issues for growers and breeders. In this research, main pollen traits including germination, tube growth and longevity were investigated in some favorable selected genotypes of three *Prunus* species including almond, apricot and sweet cherry. Pollen traits of five genotypes from each species were studied after six weeks storage in 4°C using the *in vitro* medium containing 10% sucrose and 1.2% agar. Pollen Germination Percentage (PGP) and Pollen Tube Length (PTL) were studied by Light-Microscope. Analysis of data was showed significant differences among the species and genotypes. PGP and PTL were normal after six weeks storage in 4°C especially; in almond and sweet cherry genotypes which showed high PGP and PTL in compared with apricot genotypes. Finally, the best genotypes of each species were selected for orchard establishment and breeding programs.

Key words: Rosaceae family, almond, apricot, cherry, pollen traits and *in vitro*.

INTRODUCTION

Pollination is a very important and inseparable component in respect of regular and consistent production in a number of fruit crops particularly in the stone fruits almonds, apricots and cherries. Pollination with high quantity and quality pollens and fertilization are most important factors affecting fruit setting although, genetic and ecological conditions also affect pollination and fertilization in fruit culture. Almond (*Prunus amygdalus* L.,), apricot (*Prunus armeniaca* L.,) and sweet cherry (*Prunus avium* L.,) are temperate zone fruit trees which are grown in many regions of the world. All of these species have self- incompatibility traits in the most of their cultivars and genotypes also; flowering and fertilization are critical for fruit set in stone fruits (Alonos and Socias, 2005). However, in breeding programs breeders sometimes should maintain pollens for applying in the controlled pollination methods in laboratory or in orchards (Parfitt et al., 1984, 1989). Because, controlled pollination issues need to use selected pollen from elite cultivars, whereas most of them are self-incompatible and their blooming time often do not overlap between cultivars and genotypes. Due to these differences usually pollens could be collected, dried and maintained before controlled pollination programs. Meanwhile, pollen traits especially; germination percentage and tube growth in stored pollens should be carried out for confidence their viability in controlled pollinations. Many cultivars and genotypes with unfavorable pollens have been reported by breeders and researchers previously. Some of the cultivars/genotypes have sterile pollens or pollens with low germination percentage (Ortega et al., 2002; Oukabli et al., 2002; Parfitt et al., 1984, 1989). Therefore, study of pollen traits in selected genotypes or cultivars which obtained from breeding programs is one of the necessary issues should be done in such plants. Because of its importance in fertilization and therefore production, many studies have investigated pollen viability and germination

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in almond, apricot and cherry cultivars and many different tests have been used to determine the pollen viability of fruit trees (Alonos and Socias, 2005; Ortega et al., 2002; Oukabli et al., 2002). Pırlak and Bolat (1999) investigated the viability, germination and tube growth of pollen in five cultivars of apricot (*P. armeniaca* L.), four cultivars of sweet cherry (*P. avium* L) and one cultivar of sour cherry (*P. cerasus* L.). In their study, three stain tests (TTC, IKI and safranin) and two *in vitro* germination tests (hanging drop and agar–plate) were used to estimate pollen viability and germination. Results indicated that viability, germination and tube growth of pollens varied significantly according to species, cultivars and tests. However, pollen viability in the safranin test was generally higher than in the others. The highest pollen germination was obtained with the 15% sucrose solution in the hanging drop and agar plate tests in all cultivars. Moreover, Pirlak and Bolat (2001, 2002) studied the effects of different temperature on pollen germination of apricot and cherry cultivars. Du et al. (2006), Hedly et al. (2004), Kamali et al. (2009), Kenji and Ikuo (1999), Koyuncu and Tosun (2005). Martin et al. (2009), Sharafi et al. (2010), Socias (1987) investigated pollen characteristics of almond, apricot and cherry cultivars with different objectives and reported various results. The objective of this work was to determine the germination capacity *in vitro* of the pollen of some favorable selected genotypes of almond, apricot and sweet cherry genotypes which are grown in East- Azarbaijan Iran, after six weeks storage in 4°C, for using in breeding and orchard establishment programs.

MATERIALS AND METHODS

Plant materials

Five favorable genotypes with high quality and quantity characteristics from each of the species almond (AL_1, AL_2, AL_3, AL_4 and AL_5), apricot (AP_1, AP_2, AP_3, AP_4 and AP_5) and sweet cherry (SC_1, SC_2, SC_3, SC_4 and SC_5) which are grown in East-Azarbaijan, Iran were selected.

Experiments stages

In the spring of 2010, flower buds in balloon stage gathered and transmitted to the laboratory. Research was carried out in department of horticulture, University of Maragheh, Iran. Petals and sepals were separated and anthers isolated from flower buds and placed in Petri dishes for releasing pollens. Pollens gathered and their Pollen germination percentage (PGP) and Pollen Tube Length (PTL) were tested immediately and then, stored six weeks storage in 4°C in refrigerator. Pollens cultured in the *in vitro* medium containing 1.2% agar and 10% sucrose and maintained about 24 h in 22°C and then tube growth was stopped with adding chlorophorm. PGP and pollen tube length (PTL) was measured using light- microscope in seven microscopic areas which were selected and counted randomly. A pollen grain was considered germinated when PTL was at least equal to or greater than the grain diameter. Measurements of pollen tube length were recorded directly by an ocular micrometer fitted to the eyepiece on micro-scope based on micrometer (µm).

Experimental design and data analysis

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

RESULTS AND DISCUSSION

In this research; the means of PGP in three species genotypes (almond, apricot and sweet cherry) were higher than 85% in all of the genotypes immediately after gathering in laboratory (data not shown). Analysis of variance indicated significant differences for PGP and PTL among almond, apricot and sweet cherry studied genotypes after six weeks storage in 4°C (Table 1). Means of PGP were ranged among 58.5 to 90.2%, 48.7 to 81.3% and 50.3 to 92.1% in almond, apricot and sweet cherry genotypes respectively (Table 2). However, the means of PTL was ranged among 497.1 to 863.7 µm, 467.2 to 804.9 µm and 634.2 to 907.4 µm in three species genotypes respectively (Table 2). The variance variety in PGP and PTL among three studied species and genotypes of showed that difference of PTL was higher than PGP. Data of Tables 1 and 2 shows that PGP and PTL in apricot genotypes were low in compared with almond and sweet cherry genotypes. Maximum and minimum PGP were observed in genotypes AL_5 and AL_3, AP_1 and AP_4 and SC_4 and SC_3 among the almond, apricot and cherry studied genotypes respectively (Table 2). Therefore, all of the genotypes especially; AL_5 (almond), AP_4 (apricot) and SC_4 (cherry) with highest PGP could be select for orchard establishment and breeding programs as a pollinizer for pollination of commercially growing cultivars.

Pollen germination and tube growth in fruit trees are the most important characteristics related to pollen quality. An effective fertilization requires the high rates of germination and fast tube growth. For an economical fruit set a higher rate of pollen germination is needed. Excessively low rates may lead to low fruit set because of ovule degradation before the pollen tube reaches the ovary (Cheung, 1996). In this research; genotypes with high PGP have not shown high PTL necessarily too, especially; in almond and apricot genotypes. 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 (Stosser et al., 1996). Sometimes, cultivars produce high quantity pollens but not with high quality such as low pollen germination percentage or low tube growth also; some of the pollens produced by one cultivar may be not viable or sterile (Stosser et al., 1996; Vitagliano, 1989; Weinbaum et al., 2004). For instance; Pırlak and Bolat (1999) by investigation on the pollen

Table 1. Analysis of variances of the pollen germination percentage (PGP) and pollen tube length (PTL based on micrometer) in studied genotypes of almond, apricot and sweet cherry tested in the in vitro medium.

Species	SOV	DF	PGP (%)	PTL (µm)
P. amygdalus	Genotypes	4	1123.2**	12146.4**
	Error	20	48.6	385
	CV (%)		12.2	13.1
P. armeniaca	Genotypes	4	3460.2**	2310**
	Error	20	204.2	302.1
	CV (%)		11.4	15
P. avium	Genotypes	4	2987.5**	1137.7**
	Error	20	348.3	195.2
	CV (%)		13.8	12.7

**: Significant in P<0.01% level.

Table 2. Comparison of means for PGP and PTL based on micrometer in the studied genotypes of almond, apricot and sweet cherry.

Species	Genotype	PGP (%)	PTL (µm)
P. amygdalus	AL_1	87.4^{b}	745.4^{b}
	AL_2	65.3^{c}	863.7^{a}
	AL_3	58.5^{d}	497.1^{c}
	AL_4	69.1^{c}	582.8^{bc}
	AL_5	90.2^{a}	756.2^{b}
	AP_1	81.3^{a}	721.3^{b}
P. armeniaca	AP_2	43.6^{d}	467.2^{d}
	AP_3	55.2^{c}	804.9^{a}
	AP_4	48.7^{d}	574.7^{bc}
	AP_5	68.4^{b}	612.2^{c}
P. avium	C_1	61.2^{dc}	839.2^{b}
	SC_2	68.7^{c}	647.1^{c}
	SC_3	50.3^{d}	728.7^{bc}
	SC_4	92.1^{a}	907.4^{a}
	SC_5	81.2^{b}	634.2^{c}

Same letters show no difference among genotypes of each column.

germination and PTL in apricot cultivars, determined pollen germination as 45.6% in Hasanbey, 41.8% in Salak, 39.6% in Karacabey and 35.9% in Sekerpare. They recorded PTL as 295 µm in Hasanbey, 306 µm in Salak, 251 µm in Karacabey and 268 µm in Sekerpare with 10% sucrose concentration using same method. In addition, they also stated that 20 to 25% sucrose concentrations have an inhibitory effect on pollen germination and PTL. These results had significant difference with our results whereas, PTL was very high in compared with their results. 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). They 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 genotypes of sweet cherry by Hormaza and Herrero (1999) or in other *Prunus* species such as apricot (Egea et al., 1992) and almond

(Martínez-Gómez et al., 2002). According to our results, Martínez-Gómez et al. (2000) indicated that pollen of two almond cultivars was viable during eight weeks when was stored at 4°C. This results were confirmed later with four different almond cultivars (Martínez-Gómez et al., 2002) and also the authors found that storage conditions below 0°C (–20 and –80°C) did not affect pollen germination after one year (Parfitt et al., 1984, 1989). For instance, 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.

Conclusion

In this research; PGP and PTL in studied genotypes of three species, were normal after six weeks storage in 4°C especially, in almond and sweet cherry genotypes which showed high PGP and PTL in compared with apricot genotypes. Genotypes with high PGP have not shown high PTL necessarily too, especially; in almond and apricot genotypes. However, genotypes AL_5, AP_4 and SC_4 among the almond, apricot and sweet cherry studied genotypes with highest PGP selected for orchard establishment and breeding programs as a pollinizer for pollination of commercially growing cultivars.

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