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An investigation on the pollen germination and tube growth in some *Prunus persica* genotypes and cultivars

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Peaches and nectarines (*Prunus persica*) are stone fruit trees which their pistils needed to be pollinated and fertilized to set fruits therefore; pollen characteristics including pollen viability, longevity, morphological homogeneity, germination and pollen tube growth rate are very important component of fertilization and fruit setting. However, study of main pollen traits is one of the most important approaches for growers and breeders. In this research, main pollen characteristics including germination, tube growth and longevity were investigated in some favorable selected genotypes and cultivars of peach and nectarine. Pollen traits of 5 genotypes and 10 cultivars were studied after two month maintenance in -20°C using the *in vitro* medium containing 14% sucrose and 1.2% agar. Results shows that in all of the studied characteristics, significant differences were observed among cultivars and genotypes. However, genotypes and cultivars with good quantitative and qualitative pollen traits were selected for peach and nectarine orchard establishment and breeding programs in Iran.

Key words: Peach, nectarine, pollen germination, pollen tube growth, viability, *in vitro*.

INTRODUCTION

Peaches and nectarines are temperate zone stone fruit tree 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 usage. Flowering and fertilization are critical for fruit set in stone fruits, Therefore, determining the components of reproduction biologically is critical for optimizing yield in stone fruit orchards and is therefore important for breeding programs. Some of the peaches and nectarines cultivars have self- incompatibility traits although; most of them are self-compatible (Hegedus and halasz, 2006; Keulemans, 1994; Keulemans and Van Lear, 1989; Nikolic and Milatovic, 2010). Stone fruits such as peach, needs pollination with high quantity and quality pollens and fertilization are the most important factors affecting fruit setting although, genetic and ecological conditions affect pollination and fertilization in fruit culture too. Some peaches and nectarines cultivars flower early and the 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 setting in peaches and nectarines trees (Semenas and Kouhartchik, 2000; Szabo, 2003). Furthermore, in breeding programs, breeders sometimes should maintain pollens for applying in the controlled artificial pollination methods in laboratory or in orchards (Albuquerque et al., 2007; Parfitt and Almehdia, 1989; Parfitt and Ganeshan, 1984). Controlled pollination needs the using of selected pollen from elite cultivars, though most of them are self-incompatible or their blooming time often does not overlap between cultivars. 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 to verify their viability. Many cultivars and genotypes with unfavorable pollens have been reported by breeders and researchers previously. For instance, some of the cultivars/genotypes have sterile pollens or pollens with low germination percentage (Du et al., 2006; Koyuncu and Tosun, 2005; Nikolic and Milatovic, 2010; Parfitt and Almehdia, 1989; Parfitt and

Ganeshan, 1984). Therefore, study of pollen traits in selected genotypes or cultivars which was obtained from breeding programs, is one of the necessary works which is done in such plants. Many studies have investigated pollen viability and germination in peach, plum, prune, sour cherry and other species of cultivars and different test methods have been used to determine the pollen viability of fruit trees (Botu et al., 2002; Cerovic and Ruzic, 1992, 1994; Herrero and Arbeloa, 1989; Jefferies et al., 1982; Vitagliano and Viti, 1989; Weibbaum et al., 2004). For instance, Pirlak and Bolat (1999) investigated the viability, germination and tube growth of pollen in some cultivars of apricot, sweet cherry and sour cherry using three stain tests (TTC, IKI and safranin) and two *in vitro* germination tests (hanging drop and agar-plate). Results indicated that viability, germination and tube growth of pollens varied significantly according to species, cultivars and tests. Du et al. (2006), Hedly et al. (2004), Koyuncu and Tosun (2005), and Sharafi et al. (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 and cultivars of peaches and nectarines which are grown in different regions of Iran especially in East-Azerbaijan province, after two month maintenance in -20°C, for use in the future peach breeding and orchard establishment programs in Iran.

MATERIALS AND METHODS

Ten cultivars and five favorable genotypes with high quality and quantity characteristics of peach and nectarine including (cultivars; "Zafarani", "Shaftalou", "Anjiri" ("Donate peach"), "Alamar", "Mikado", "Candoka", "Halberta", "JH Hale", "Yellow Nectarine" and "Red Nectarine" and genotypes; "Pp₁", "Pp₂", "Pp₃", "Pp₄" and "Pp₅") which are grown in different regions of Iran especially in East-Azerbaijan province were selected. In the spring of 2010, flower buds in balloon stage were gathered and their petals and sepals were separated and anthers isolated from flower buds and placed in Petri dishes to release pollens. Pollens were gathered and their pollen germination percentage and pollen tube growth were tested immediately and then, stored for two months in -20°C in the refrigerator. Pollens were planted in the *in vitro* medium containing 1.2% agar, 14% sucrose and maintained at about 24°C for 24 h and then the tube growth was stopped by adding Chlorophorm. Pollen germination percentage and pollen tube length were measured under light- microscope. Seven microscopic areas were counted randomly for evaluation of pollen germination percentage and pollen tube length. Pollen tube length was recorded directly by an ocular micrometer fitted to the eyepiece of microscope in micrometer (µm). Experimental design was completely randomized design (CRD) with 15 treatments (5 genotypes and 10 cultivars) and five replications (5 Petri dishes). Data were analyzed 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 pollen germination percentage (PGP) and pollen tube length among peach and nectarine studied cultivars and genotypes after two months maintenance in -20°C (Table 1). Among cultivars and genotypes, means of pollen germination percentage and pollen tube length ranged between 38.7 to 95.3% and 435.2 to 1102.4 µm respectively.

Difference in means of pollen germination percentage and pollen tube length among the studied cultivars/genotypes showed higher variety in pollen tube length in compared with pollen germination percentage (Table 2). Meanwhile, genotypes of peach showed the highest pollen germination percentage and pollen tube length proving the high longevity of peach pollens (Table 2). It should be stated that in this research; the mean of pollen germination percentage in the genotypes were higher than 90% in all of the cultivars and genotypes immediately after gathering in the laboratory (data not shown).

Maximum PGP was observed in "Anjiri" cultivar (95.3%) and genotype "Pp₂" about 93.2% (Tables 2). Therefore, cultivars and genotypes 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 required for high rates of germination and fast tube growth. Excessively low rates of germination and slow tube growth rate may lead to low fruit set because of ovule degradation before the pollen tube reaches the ovary (Cheung, 1996; Sharafi, 2011; Sharafi et al., 2010). In this research cultivars and genotypes with high pollen germination percentage have not shown necessarily high pollen tube growth rate too. This phenomenon indicates genetical differences among the genotypes which was reported by many researchers in almond, apricot, sweet cherry, sour cherry, apple, pear and other fruit trees (Sharafi, 2011; Pirlak and bolat, 1999; Sharafi et al., 2010; Stosser et al., 1996). Sometimes, cultivars produce high quantity of pollens but not with high quality such as low pollen germination percentage or low tube growth rate 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, Pirlak 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

Table 1. Analysis of variances of the pollen germination percentage and pollen tube length (based on micrometer) in studied cultivars and genotypes of peaches and nectarines tested in the *in vitro* medium.

Peaches and nectarines (<i>Prunus persica</i> L.)			
SOV	DF	Pollen germination percentage (%)	Pollen tube length (µm)
Genotypes	14	1098.7**	1206.8**
Error	70	54.3	324.3
CV (%)		13	11.6

** : Significant in P<0.01% level.

Table 2. Comparison of means for pollen germination percentage and pollen tube length (based on micrometer) in the studied cultivars and genotypes of peaches and nectarines carried out based on Duncan's multiple range tests.

Peaches and nectarines cultivars		
Cultivars	Pollen germination percentage (%)	Pollen tube length (µm)
"Zafarani"	88.5 ^a	1102.4 ^a
"Shaftalou"	71.4 ^b	840.2 ^b
"Anjiri" ("Donate peach")	95.3 ^a	1012.5 ^a
"Alamar"	39.7 ^e	987.1 ^a
"Mikado"	67.2 ^c	856.3 ^{ab}
"Candoka"	68.4 ^{ab}	213.4 ^b
"Halberta"	90.2 ^a	148.5 ^c
"JH Hale"	76.3 ^b	720.7 ^a
"Yellow Nectarine"	48.9 ^d	567.6 ^{ab}
"Red Nectarine"	68.7 ^c	687.8 ^a

Peaches and nectarines genotypes		
Genotype	Pollen germination percentage (%)	Pollen tube length (µm)
"P _{p1} "	78.3 ^{ab}	513.4 ^b
"P _{p2} "	93.2 ^a	435.2 ^c
"P _{p3} "	79.3 ^b	720.7 ^a
"P _{p4} "	38.7 ^d	567.6 ^{ab}
"P _{l5} "	73.2 ^{ab}	687.8 ^a

Same letters show no difference among genotypes of each column.

whereas; pollen tube length was very high when compared with their results, because, in this research when compared with their incubation temperature was higher (24 °C) and high temperature induces pollen tube growth rate. 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 cultivars and genotypes of other *Prunus* 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 and Almehdia (1989) and Parfitt and Ganeshan (1984) 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, Albuquerque 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 result showed that pollen viability could be maintained at reasonably high percentages after storage at -20°C for one year for all studied cultivars. Also, pollen viability decreased after 15 or 30 days of storage at 4°C . In their study, 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 that this affect 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, to our knowledge, cultivars and genotypes which was 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 et al. (2010c), investigated that pollen traits consisted of pollen germination percentage and pollen tube growth rate after short storage (6 weeks in 4°C) in some almond, apricot, peach and sweet cherry genotypes and reported similar result.

Also, Sharafi (2011b) studied pollen viability and longevity of some peach, plum, prune and sour cherry favorable genotypes and reported that in the 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 pollen germination percentage, pollen tube length and longevity among four species and genotypes with high pollen germination percentage have not shown high pollen tube length necessarily. However, genotypes with highest pollen germination percentage selected for orchard establishment and breeding programs as a pollinizer, for pollination of commercially growing cultivars.

Same results reported by him in some of the apple, pear, and quince, cultivars and genotypes (Sharafi, 2011a).

Conclusion

In this research pollen germination and pollen tube length in studied cultivars and genotypes of peaches and nectarines were normal after two months maintenance in -20°C although, some decrease was observed. However, cultivars and genotypes with highest pollen germination percentage and pollen tube length PGP for example

"Anjiri" and "Pp2" select for peaches and nectarines orchard establishment and breeding programs as a pollinizer, for pollination of commercially growing cultivars.

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