African Journal of Agricultural Research Vol. 6(28), pp. 6021-6026, 26 November, 2011
Available online at http://www.academicjournals.org/AJAR
DOI: 10.5897/AJAR11.938
ISSN 1991-637X ©2011 Academic Journals

Full Length Research Paper

In vitro pollen germination in stone fruit tree of Rosaceae family

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Accepted 18 August, 2011

In the tone fruit trees including almond, apricot, plum, prune, sour cherry and sweet 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 pollen traits is one of the most important approaches for growers and breeders. In this research, main pollen traits including germination and tube growth were investigated in some selected cultivars of *Prunus* species including *P. dulcis, P. armeniaca, P. domestica, P. ceracus, P. salicina* and *P. avium*. Pollen traits of 5 cultivars from each species were studied using the *in vitro* medium containing 15% sucrose, 1% agar 5 ppm H_3BO_3 (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 by adding chloroform. Data analysis showed that in all of the studied traits significant differences were observed among six species and cultivars of each species.

Key words: Almond, apricot, plum, prune, sour cherry, sweet cherry, Rosaceae, pollen viability, *in vitro*.

INTRODUCTION

Almond (*Prunus dulcis* Batch), apricot (*P. armeniaca* L.), plum (*P. domestica*), prune (*P. salisina* L.), sour cherry (*P. cerasus* L) and sweet cherry (*P. avium*) 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 stone fruit tree orchards and is therefore important for breeding programs. Some of cultivars in these species have self- incompatibility traits although; tetraploid sour cherry (*P. cerasus)* and hexaploid common plum *(P. domestica)* have some self-compatible cultivars (Botu et al., 2002; Hegedus and Halasz, 2006; Keulemans, 1994; Keulemans and Van lear, 1989; Nikolic and Milatovic, 2010). Stone fruits: almond, apicot, plum, prune, sour cherry and sweet cherry; need to pollination with high quantity and quality pollens and fertilization are most important factors affecting fruit setting although, genetic and ecological conditions affect pollination and fertilization in fruit culture too. Some almond, apricot, plum, prune, sour cherry and sweet cherry 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 set in mentioned stone fruits (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 (Alburquerque et al., 2007; Parfitt et al., 1984, 1989). Because; controlled pollination need to using selected pollen from elite cultivars, whereas most of them are self-incompatible or their blooming time often do not overlap between cultivars. Meanwhile, pollen traits especially germination percentage and tube growth rate in stored pollens should be carried out for confidence their viability. Many cultivars and genotypes with unfavorable pollens

Table 1. List of the studied cultivars from different six species of genus *Prunus* (stone fruit trees).

Species	Cultivars
P. dulcis	"Azar", "Sahand", "Shokofeh", "Franies" and "Yalda"
P. armeniaca	"Ordobad", "Nasiri", "Shahroudi", "Nouri" and "Kaisi"
P. salisina	"Gatre-tala", "Santarosa", "Victoria", "Methley" and "Siah"
P. domestica	"Atabaki", "Robab", "Shirin", "Keshavarzi" and "Talaei"
P. cerasus	"Albalou-gilas", "Champaye Mashhad", "Erdi jubileum", "Montmorency" and "Siahe zoudras"
P. avium	"Siahe Mashhad", "Sefide rezaeieh", "Meshkin shahr", "Germeze rezaeieh" and "Siahe shabestar"

have been reported by breeders and researchers previously. For instance, some of the cultivars/genotypes have sterile pollens or pollens with low germination percentage (Botu et al., 2002; Du et al., 2006; Koyuncu and Tosun, 2005; Nikolic and Milatovic, 2010; Parfitt et al., 1984, 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 stone fruit tree 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, Pırlak 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 germination and tube growth capacity of pollens in some selected cultivars of almond, apricot, plum, prune, sour cherry and sweet cherry cultivars which are grown in different regions of Iran for using in breeding and orchard establishment programs.

MATERIALS AND METHODS

Five cultivars from six species of genus *Prunus* including; almond, apricot, plum, prune, sour cherry and sweet cherry which are grown in different regions of Iran were selected (Table 1). In the spring of 2011, 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 pollen germination percentage and pollen tube growth were tested immediately. 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 gro wth 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 directly by an ocular micrometer fitted to the eyepiece on microscope based on micrometer (µm). Experimental design was completely randomized design (CRD) with six replications (6 Petri dishes). Data were analyzed separately for cultivars 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 pollen germination percentage and pollen tube length among almond, apricot, plum, prune, sour cherry and sweet cherry studied cultivars (Tables 2 and 4). Among almond cultivars, means of pollen germination percentage and pollen tube length were ranged between 43.4 to 94.2% and 653 to 1123.3 µm respectively also; in the apricot studied cultivars, were ranged between 26.7 to 81.6% and 324.1 to 879.8 µm respectively (Table 3) while in sour cherry were 31.2 to 69.7% and 423.1 to 571.2 µm. However, data in Table 5 shows that in cultivars of prune; pollen germination percentage and pollen tube length were ranged between 54.3 to 76.4% and 217.1 to 623.2 µm also, in cultivars of plume; were 46.2 to 71.6% and 346.7 to 1011.4µm respectively while in sweet cherry were 37.9 to 85.6% and 327 to 956.1 µm (Table 5). Difference in means of pollen germination percentage and pollen tube length among the studied species and cultivars showed higher variety in pollen tube length in compared with pollen germination percentage. Meanwhile, cultivars of almond showed the highest pollen germination percentage and pollen tube length (Tables 3 and 5).

Maximum pollen germination percentage was observed in cultivars "Sahand" (94.2%), "Kaisi" (81.6%), "Siah" (69.7%), "Keshavarzi" (71.6), "Siahe zoudras" (76.4%) and "Siahe Mashhad" (85.6%) respectively (Tables 3 and 5). Therefore, all of the cultivars especially "Sahand" (almond), "Kaisi" (apricot), "Siah" (sour cherry), "Keshavarzi" (plume), "Siahe zoudras" (prune) and "Siahe Mashhad" (sweet cherry) with highest pollen germination percentage 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

Table 2. Analysis of variances of the pollen germination percentage and pollen tube length in studied cultivars of stone fruits; almond, apricot and prune.

Species	Source of variation	DF	Pollen germination percentage	Pollen tube length (µm)
	Cultivars	4	1234.5**	2346.4**
P. dulcis	Experimental error	30	324.7	479.8
	CV (%)		11	13.1
	Cultivars	4	1678.8**	1567.9**
P. armeniaca	Experimental error	30	213.4	209.3
	CV (%)		14	26.7
	Cultivars	4	2971**	3011.2**
P. salisina	Experimental error	30	407.6	57.3
	CV (%)		21	22.7

**: Significant in P<0.01% level.

Table 3. Comparison of means for pollen germination percentage and pollen tube length in the studied cultivars of stone fruits; almond apricot and prune.

Species	Cultivars	Pollen germination percentage	Pollen tube length (µm)
	"Azar"	86.4^{ab}	1075.6^{a}
	"Sahand"	94.2^{a}	1123.4^{a}
P. dulcis	"Shokofeh"	53.8^{d}	653^{c}
	"Franies"	78.9^{c}	785.8^{b}
	"Yalda"	43.4^{e}	679.4^{bc}
	"Ordobad"	52.7^{c}	879.8^{a}
	"Nasiri"	26.7^{d}	462.9^{c}
P. armeniaca	"Shahroudi"	45.8^{c}	324.1^{d}
	"Nouri"	73.4^{b}	347.8^{d}
	"Kaisi"	81.6^{a}	568.3^{b}
	"Gatre-tala"	27.6^{d}	489.7^{b}
	"Santarosa"	31.2^{d}	543.7^{ab}
P. salisina	"Victoria"	47.3^{c}	423.1^{bc}
	"Methley"	56.4^{b}	467.4^{c}
	"Siah"	69.7^{a}	571.2^{a}

Same letters show no difference among genotypes of each column.

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 et al., 2010). In this research cultivars 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 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 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

Table 4. Analysis of variances of the pollen germination percentage and pollen tube length in studied cultivars of stone fruits; plum, sour cherry and sweet cherry.

Species	Source of variation	DF	Pollen germination percentage	Pollen tube length (µm)
	Cultivars	4	1253.3**	2314.2**
P. domestica	Experimental error	30	49.8	581
	CV (%)		13.3	24.1
	Cultivars	4	2151.4**	1414.7**
P. cerasus	Experimental error	30	38.6	346.3
	CV (%)		9.8	14.7
	Cultivars	4	1679.4**	2007.5**
P. avium	Experimental error	30	523	671.3
	CV (%)		15.4	17.6

Table 5. Comparison of means for pollen germination percentage and pollen tube length in the studied cultivars of stone fruits; plum, sour cherry and sweet cherry.

Species	Cultivars	Pollen germination percentage	Pollen tube length (µm)
	"Atabaki"	46.2^{c}	346.7^{d}
	"Robab"	63.8^{b}	594.1^{c}
P. domestica	"Shirin"	49.9^{c}	1011.4^{a}
	"Keshavarzi"	71.6^{a}	768.9^{b}
	"Talaei"	57.8^{bc}	496.7^{dc}
	"Albalou-gilas"	50.3^{c}	328.4^{d}
	"Champaye Mashhad"	54.3^{bc}	217.1^{e}
P. cerasus	"Erdi jubileum"	69.3^{a}	489.4^{c}
	"Montmorency"	59.5^{b}	623.2^{a}
	"Siahe zoudras"	76.4^{a}	547^{b}
	"Siahe Mashhad"	85.6^{a}	956.1^{a}
	"Sefide rezaeieh"	48^{bc}	812^{b}
P. avium	"Meshkin shahr"	37.9^{c}	673.4^{c}
	"Germeze rezaeieh"	75.6^{b}	327.3^{e}
	"Siahe shabestar"	80.9^{a}	543.8^{d}

Same letters show no difference among genotypes of each column.

results. Because, in this research in compared with them incubation temperature was higher (24℃) and high temperature induces pollen tube growth. Hedhly et al. (2004), studied pollen germination of nine sweet cherry cultivars using *in vitro* pollen performance under two temperatures regimes (15 and 30℃). They found a hi ghly 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℃).

Parfitt et al. (1984, 1989) found that storage conditions below 0℃ (-20 and -80℃) did not affect pollen ger mination 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℃ during one year for all studied cultivars. Also, pollen viability decreased after 15 or 30 days of storage at 4℃. In their study, for most cultivars pollen completely lost viability after only 60 days of storage at 4℃. 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℃, 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, to our knowledge, 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 (2011) using *in vitro* study of pollen traits after short storage in some almond, apricot and sweet cherry favorable genotypes and reported that in the studied genotypes of three species, were normal after six weeks storage in 4℃ 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 AL5, AP4 and SC4 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.

Also, Sharafi (2011) 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℃ 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, genotypes with highest PGP selected for orchard establishment and breeding programs as a pollinizer, for pollination of commercially growing cultivars. Same results reported by them in some of the apple, pear, quince, hawthorn and loquat cultivars and genotypes (Sharafi, 2011).

Conclusion

However; cultivars with high pollen germination percentages including "Sahand" (almond), "Kaisi" (apricot), "Siah" (sour cherry), "Keshavarzi" (plume), "Siahe zoudras" (prune) and "Siahe Mashhad" (sweet cherry) were selected for orchard establishment and breeding programs as a pollinizer, for pollination of commercially growing cultivars.

ACKNOWLEDGEMENT

Authors would like to thank the research section of Islamic Azad University of Maragheh Branch and Shahed University helps and cooperation.

REFERENCES

Alburquerque N, García MF, Burgos L (2007). Influence of storage temperature on the viability of sweet cherry pollen. Spanish J. Agric. Res., 5(1): 86-90.

Botu M, Sarpe C, Cosmulescus S, Botu I (2002). The genetic control of pollen fertility, pollinating and fruit set for the *Prunus domestica* L. plum cultivars. Acta Hort., 577: 139-145.

Cerovic R (1992). Pollen tube growth in sour cherry pistils in relation to fruit set. Adv. Hort. Sci., 6: 107-111.

Cerovic R (1994). Histocytological aspects of fertilization dynamics of sour cherry (*Prunus cerasus* L.). PhD thesis, Faculty of Biology, Belgrade.

Cerovic R, Ruzic D (1992). Pollen tube growth in sour cherry (*Prunus cerasus*) at different temperatures. J. Hortic. Sci., 67: 333-340.

Cheung AY (1996). Pollen–pistil interactions during pollen tube growth. Trends Plant Sci., 1: 45-51.

Du YH, Zhang SL, Jiang T, Wu J (2006). Characteristics of pollen germination and pollen tube growth of *Prunus mume* in vitro. Acta Bot., 26: 1846-1852.

Hedhly A, Hormoza JI, Herrero M (2005). Influence of genotype-temperature interaction on pollen performance. J. Evol. Biol., 18: 1494-1502.

Hedly YA, Hormaza JI, Herrero M (2004). Effect of temperature on pollen tube kinetics and dynamics in sweet cherry, Prunus avium (Rosaceae). Am. J. Bot., 91: 558-564.

Hegedus A, Halasz J (2006). Self-incompatibility in plums (*Prunus salicina* Lindl., *Prunus cerasifera* Ehrh. and *Prunus domestica* L.). A minireview. Int. J. Hort. Sci., 12(2): 137-140.

Herrero M, Arbeloa A (1989). Influence of the pistil on pollen tube kinetics in peach (*Prunus persica*). Am. J. Bot., 76: 1441-1447.

Jefferies CJ, Brain P, Stott KG, Belcher AR (1982). Experimental systems and mathematical models for studying temperature effects on pollen-tube growth and fertilization in plum. Plant, Cell Environ., 5: 231-236.

Keulemans J (1994). Pollination and fruit set in self-incompatible plum cultivars. Acta Hort., 359: 260-268.

Keulemans J, Van Laer H (1989). Effective pollination period of plums: the influence of temperature on pollen germination and pollen tube growth. In Manipulation of fruiting (Wright, C. J., ed.), London, UK: Butterworth, pp. 159-171.

Koyuncu F, Tosun F (2005). Evaluation of pollen viability and germinating capacity of some sweet cherry cultivars grown in Sparta, Turkey. 5th International Cherry Symposium, 6–10 June, Bursa.

Nikolic D, Milatovic D (2010). Examining self-compatibility in plum (*Prunus domestica* L.) by fluorescence microscopy. Genetika, 42(2): 387-396.

Parfitt DE, Almehdia E (1984). Liquid nitrogen storage of pollen from five cultivated *Prunus* species. HortSci. 19: 69-70.

Parfitt DE, Ganeshan S (1989). Comparison of procedures for estimating viability of *Prunus* pollen. Hortic. Sci., 24: 354-356.

Pırlak L, Bolat I (1999). An investigation on pollen viability, germination and tube growth in some stone fruits. Turk. J. Agric. For., 23: 383-388.

Semenas S, Koukhartchik NV (2000): Investigations in pollination and fertilization in *Prunus* L. breeding via fluorescence microscopy. Acta Hort., 538: 371-374.

Sharafi Y (2011). Study of pollen germination in pome fruit tree of Rosaceae family in vitro. Afr. J. Plant Sci., 5(9):5-8. On Publication.

Sharafi Y (2010). Suitable *In vitro* medium for studding pollen viability in some of the Iranian hawthorn genotypes. J. Med. Plant Res., 4(19): 1967-1970.

Sharafi Y, Bahmani A (2010). Study of pollen germination and tube growth in some Iranian Loquat cultivars and genotypes. 3th International Symposium on Loquat, May. Antakya. Turkey, pp. 22-25.

Sharafi Y (2011). Investigation on pollen viability-longevity in *Malus pumila* L., *Pyrus commonis* L., and *Cydonia oblonga* L., *in vitro*. J. Med. Plant Res., 5(11): 2232-2236.

Sharafi Y Motallebi-Azar AR (2011). *In vitro* pollen germination and pollen tube growth and longevity in some genotypes of loquat (*Eriobotria japonica* Lindl.). Afr. J. Biotechnol., 4(5): 135-137.

Sharafi Y, Bahmani A (2011 A). *In vitro* study of pollen traits after short storage in some almond, apricot and sweet cherry favorable genotypes. J. Med., Plant Res., 5(2): 266-269.

Sharafi Y, Bahmani A (2011 B). Pollen viability and longevity of some peach, plum, prune and sour cherry favorable genotypes. J. Med. Plant Res., 5(2): 275-279.

Stosser R, Hartman W, Anvari SF (1996). General aspects of pollination and fertilization of pome and stone fruits. Acta Hort., 423: 15-21.

Stosser R (1982). The pollen tube growth *in vitro* and in vivo in *Prunus domestica* L. Z. Pflanzenzüchtung, 88: 261-264.

Szabo Z (2003). Plum (*Prunus domestica* L.). In: „Floral biology, pollination and fertilisation in temperate zone fruit species and grape“. Akadémiai Kiado, Budapest, pp. 383-410.

Vitagliano C, Viti R (1989). Effects of some growth substances on pollen germination and tube growth in different stone fruits. Acta Hort., 239: 379-381.

Weinbaum SA, Polito VS, Kester DE (2004). Pollen retention following natural self pollination in peach, almond, and peach × almond hybrids. Euphytica, 4: 193-200.