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Full Length Research Paper

Study of pollen germination in pome fruit tree of Rosaceae family *in vitro*

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Pollination and fertilization are the basic factors affecting fruit setting volume and pollination is an essential component in fruit tree cultivation. The most important goal for fruit growers is obtaining high quantities and qualities yield in fruit industry which depends on sufficient fruit setting. Commercial fruit trees in Iran belong to stone and pome fruits of Rosaceae family with gametophytic self-incompatibility make necessary the selection of favorable pollinizer in breeding and orchard planning programs. Therefore, knowledge about pollen traits of the species and cultivars in this family is one of the main issues for growers and breeders. In this research, pollen germination and tube growth were studied in some cultivars and selected favorable genotypes of 6 genus including; apple, pear loquat, medlar, red hawthorn and quince. Pollen traits were investigated using *in vitro* medium containing 17% sucrose, 10 ppm acid boric and 1.2% agar. Cultured pollens were incubated in dark condition at 25°C for 24 h and then, pollen germination percentage and pollen tube length evaluated using light-microscope. Results showed significant differences among cultivars and finally favorable cultivars/genotypes of each genus with high pollen germination percentage and tube growth were selected to application in the future breeding and orchard establishment programs.

Key words: Apple, pear, quince, loquat, medlar, red hawthorn, pollen germination, Rosaceae and *in vitro* condition.

INTRODUCTION

Pome fruits including apple (*Mallus pumila* L.), Pear (*Pyrus commonis* L.), Quince (*Cydonia oblonga* L.), medlar (*Mespilus germanica* L.), loquat (*Eriobotria japonica* L.) and red hawthorn (*Cratagus Oxyacantha*) are temperate fruit trees which are grown in many regions of the world with Mediterranean climates and have many roles in the fruit industry of producer countries. Iran is one of the main producers of these pome fruits. Most of the cultivars belong to these geneses have self or cross incompatibility system especially in *Pyrus* (Sharafi 2011; Jackson 2003; Szabo, 2003; Dantas et al., 2005). So, in breeding programs for this species, manual pollination could be carried out in the field or laboratory usually. For successful pollination, the high quantities and qualities pollen must be transferred to the stigma when it is receptive (Dekers and Porreye, 1984; Vasilakakis and Porlingis, 1985). However, sometimes, the pollen is deposited before the receptive period; and the pollen should remain viable for a period long enough to germinate or some of these species have cultivars with parthenocarpic fruits occasionally based on specific physiological-environmental conditions (Stosser et al., 1996). However, synchronized flowering, positive pollination and fertilization are critical for fruit set in the mentioned pome fruits. Furthermore, in breeding programs breeders sometimes should maintain pollens for applying in the controlled artificial pollination methods whereas pollens should protect their viability and germination capacity. The rate of pollen germination of some fruit species and cultivars varies depending on the medium or chemical concentration. For this reason, the suitable pollen germination medium should be obtained for each fruit species and cultivar. Some studies have been carried out on the relationships between viability and pollen germination.

Many researches have been performed to determine quantitatively and qualitatively the components necessary for the best composition of culture medium in pollen grain germination and the best storage conditions for

different species pollens (Dane et al., 2004; Du et al., 2006).

Moreover, temperature is a very basic factor in the control of the environmental conditions and influences pollen grain germination and longevity in stored pollens (Aparecida et al., 2004). Based on this, it could be stated that pollen traits especially germination percentage and tube growth in stored pollens should be carried out for confidence of their viability. Previously in different species; many cultivars and genotypes with unfavorable pollens with sterile pollens, pollens with low germination percentage or low tube growth rate have been reported by breeders and researchers (Sharafi and Bahmani, 2010; Shivanna, 2003; Stosser, 1996; Szabo, 2003).

Zielinski and Thompson (1966) studied pollen germination in *Pyrus* species and species hybrids and reported that pollen germination in most *Pyrus* species was moderately high, usually averaging above 50%. Several researchers previously have studied the pollen viability of some tree fruit species in different storage conditions such as liquid nitrogen (-196°C), refrigerator (+4°C), freezer (different minus temperatures) and freeze dried, organic solvents and etc (Hedhly et al., 2005; Ganeshan and Alexander, 1991; Sharafi and Bahmani., 2010; Shivanna 2003).

Liu and Lanzhou (1984) determined the germination, respiration and fertility of pollens in *Prunus persica* and *Malus pumila* during storage in some organic solvents; similar results reported by Jain and Shivanna (1988) in other plants. Anjum and Shaukat (2008) studied pollen germination of *M. pumila* L., beyond 48 weeks in the refrigerator (+4°C), freezer (-20 and -30°C) and freeze drier (-60°C) using hanging drop technique. Dafni and Firmage (2000) reviewed the various definitions and terminology of pollen viability and longevity as well as the various tests of its assessment. They compare the advantages and disadvantages of each method and suggest some practical.

Mert (2009), by studying the effects of different temperature on pollen germination in some walnut cultivars reported favorable temperature of walnut pollen germination. Munzuroglu and Gur (2000) investigated the effects of some heavy metals on the pollen germination and pollen tube growth in apples (*M. sylvestris* Miller cv. Golden). Zhang et al. (2010) studied the effects of pollen density and gibberellin on pollen germination, tube growth and fruit set in the *P. pyrifolia.*

Sharafi (2010) reported that the best in-vitro medium for hawthorn pollen germination and tube growth were composed of 15% sucrose with 0.005 to 0.01% boric acid and 1.2% agar and significant differences observed between different media in all of the studied pollen traits. Some favorable genotypes by highest pollen germination percentage (PGP) and pollen tube length (PTL) were recognized for the future breeding and growing programs in Iran which will be introduced for these objectives after some other studies on them. Sharafi and Motallebi-Azar, (2011) in a same research, selected the best genotypes among some loquat genotypes with high PGP and PTL for loquat orchard establishment and breeding program. The objective of this work was to determine longevity, viability, germination and tube growth capacity of pollens in some cultivars and selected genotypes of apple, pear loquat, medlar, red hawthorn and quince which are grown in different regions in Iran, for use in the breeding and fruit industry programs.

MATERIALS AND METHODS

Thirty mature cultivars and genotypes from six pome fruit genus of Rosaceae family (*M. pumila*, *P. commonis*, *C. oblonga*, *M. germanica*, *E. japonica* and *C. oxyacantha*) which exist in different regions of Iran were selected. Plants included were five cultivars of apple ("Morabbaei-Mashhad", "Golden delicious", "Red delicious", "Garah Yaprakh" and "Gandak"), five cultivars of pear ("Dargazie", "Natanzie", "Dom-kaj" "Bartlett" and "Anjo"), five cultivars of quince ("Shams", "Dorosht-e-Lahijan", Dorosht-e-Lavasan", "Champion" and "Pineapple". Five genotypes from loquat were "EJ1a", "EJ2a", "EJ3a", "EJ4a" and "EJ5a", hawthorn genotypes were "CO1a", "CO2a", "CO3a", "CO4a" and "CO5a" while medlar genotypes were "MG1a", "MG2a", "MG3a", "MG4a" and "MG5a" respectively.

In year 2010, flower buds in balloon stage gathered and transmitted to the laboratory. Petals and sepals were separated and anthers isolated and placed in Petri dishes for releasing pollens. Pollens gathered and their pollen germination percentage and pollen tube length were tested immediately in the *in vitro* medium containing 1.2% agar, 17% sucrose and 10 ppm boric acid. PGP and PTL were measured under light- microscope in seven microscopic areas which were counted. Pollen tube, which is at least long 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 five replications (5 Petri dishes for each cultivar/genotypes). 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 in Table 1 indicated significant differences among medlar, loquat and red hawthorn genotypes for pollen germination percentage and pollen tube length. Among medlar genotypes, means of pollen germination percentage and pollen tube length were ranged between 36.4 to 80.1% and 94.8 to 402.4 µm respectively also; in the loquat genotypes, pollen germination percentage and pollen tube length were between 32.7 to 73.1% and 84.9 to 210.8µm while, in red hawthorn genotypes were between 26.4 to 61.2% and 75.6 to 314.2 µm respectively (Table 2). However, analysis of variance in Table 3 indicated significant differences among apple, pear and quince studied cultivars for pollen germination percentage and pollen tube length. Among apple cultivars, means of pollen germination percentage and pollen tube length were

Table 1. Analysis of variances for pollen germination percentage and pollen tube length (based on micrometer) in the studied genotypes of three pome fruits medlar, loquat and red hawthorn.

Species	SOV	DF	Pollen germination percentage	Pollen tube length (µm)
M. germanica	Genotypes	4	1759.3**	2341.6**
	Experimental error	25	83.2	234.5
	CV (%)		9.7	13.8
E. japonica	Genotypes	4	1123.4**	1987.5**
	Error	25	109.2	152.3
	CV (%)		19	11.8
C. Oxyacantha	Genotypes	4	3421.4**	4312.5**
	Error	25	123.1	243
	CV (%)		18.4	16

**: Significant in P<0.01 level.

Table 2. Comparison of means for pollen germination percentage and pollen tube length in the genotypes of three pomefruits medlar, loquat and red hawthorn.

Species	Genotype	Pollen germination percentage	Pollen tube length (µm)
M. germanica	"MG1a"	36.4^{bc}	94.8^{c}
	"MG2a"	37.3^{c}	230.4^{bc}
	"MG3a"	80.1^{a}	380.1^{a}
	"MG4a"	57.6^{b}	384.2^{a}
	"MG5a"	38^{c}	402.6^{a}
E. japonica	"EJ1a"	64.3^{b}	210.8^{a}
	"EJ2a"	73.1^{a}	171.4^{b}
	"EJ3a"	45.6^{dc}	202.8^{a}
	"EJ4a"	59.2^{c}	84.9^{d}
	"EJ5a"	32.1^{d}	131.2^{d}
C. Oxyacantha	"CO1a"	51.6^{b}	120.2^{c}
	"CO2a"	55.3^{b}	195.4^{b}
	"CO3a"	41.4^{c}	260.2^{b}
	"CO4a"	61.2^{a}	316.2^{a}
	"CO5a"	26.4^{d}	75.6^{c}

Same letters show no difference among genotypes of each column.

ranged between 50.2 to 96% and 181.3 to 721.2 µm respectively also; in the pear cultivars, PGP and pollen tube length were between 43.2 to 87.3% and 163.6 to 375.4 µm while, in quince cultivars were between 45.4 to 82.3% and 314.6 to 674.1 µm, respectively (Table 4).

Based on data shown in Table 2, maximum pollen germination percentage were observed in "MG3a" (80.1%), "EJ2a" (73.1%), and "CO4a" (61.2%) in medlar, loquat and red hawthorn genotypes, respectively (Table 2). While maximum PGP was observed in "Red delicious" (96%), "Shah-miveh" (87.3%), and Dorosht-e-Lahijan (82.3%) respectively in apple, pear and quince cultivars (Table 4). However, high pollen germination percentage of these plants showed that they could be select for orchard establishment and breeding programs as a pollinizer for pollination of other cultivars.

Difference in means of pollen germination percentage and pollen tube length among the studied species showed higher variety in pollen tube length in compared with pollen germination percentage (Tables 2 and 4). Meanwhile, apple cultivars showed the highest pollen germination percentage and pollen tube length proving

Table 3. Analysis of variances for pollen germination percentage and pollen tube length (based on micrometer) in the studied cultivars of three pomefruits (*M. pumila* L., *P. commonis* L. and *C. oblonga* L.).

Species	SOV	DF	Pollen germination percentage	Pollen tube length (µm)
M. pumila	Cultivars	4	2324.1**	3457**
	Experimental error	25	142.7	321.9
	CV (%)		12.3	14.3
P. commonis	Cultivars	4	986.2**	1904.9**
	Error	25	73.9.6	214.3
	CV (%)		11.3	20.6
C. oblonga	Cultivars	4	2132.4**	3451.5**
	Error	25	90.2	243.6
	CV (%)		13.1	15.6

**: Significant in P<0.01% level.

Table 4. Comparison of means for pollen germination percentage and pollen tube length in the cultivars of three pomefruits (*M. pumila* L., *P. commonis* L. and *C. oblonga* L.).

Species	Cultivars	Pollen germination percentage	Pollen tube length (µm)
M. pumila	"Morabbaei-Mashhad"	61.4^{bc}	181.3^{d}
	"Golden delicious"	47.2^{c}	230.4^{dc}
	"Red delicious"	96^{a}	721.2^{a}
	"Garah Yaprakh"	70.6^{b}	640.2^{a}
	"Gandak"	50.2^{c}	460.7^{b}
P. commonis	"Dargazie"	86.8^{a}	370.1^{a}
	"Shah-miveh"	87.3^{a}	375.4^{a}
	"Dom-kaj"	51.6^{dc}	262.8^{c}
	"Bartlett"	64.2^{c}	180.7^{d}
	"Anjo"	43.2^{d}	163.6^{d}
C. oblonga	"Shams"	54.3^{b}	520.6 ^{a}
	"Dorosht-e-Lahijan"	82.3^{a}	674.1^{a}
	"Dorosht-e-Lavasan"	45.4^{d}	314.6^{c}
	"Champion"	55.6^{b}	356.4^{bc}
	"Pineapple"	41.9^{d}	374.5^{c}

Same letters show no difference among genotypes of each column.

the high longevity of apple pollens among six pome fruit geneses and red hawthorn genotypes showed the lowest pollen tube length. PGP increased significantly with the increase in incubation period from 24 to 72 h irrespectively. Variable genetic constitution of different cultivars and genotypes might be the reason for variation in PGP.

Pollen germination and tube growth rate are the most important characteristics related to pollen quality and successful fertilization needs to high germination rates and fast tube growth because, low rates may lead to low fruit set caused by ovule degradation before the pollen tube reaches the ovary (Cheung, 1996; Sharafi et al., 2010).

According to Sharafi and Bahmani (2010), in these research cultivars/genotypes with high pollen germination percentage was not followed by high pollen tube length. This phenomenon indicates genetically differences among the genotypes which reported by many researchers in numerous of the fruit tree species and cultivars (Alburquerque et al., 2007; Pirlak and Bolat, 1999; Sharafi et al., 2010; Stosser et al., 1996; Visser

and Oost, 1981). Meanwhile; except for some special conditions, there is a linear relation between pollen viability and germination capability in many fruit species reported. Moreover, germination capability of pollen is related to varieties, nutrition conditions, and environmental factors and there is a big variation in optimum germination conditions of pollen among plant species and varieties. For instance, Sharafi and Bahmani (2010) investigated the PGP, longevity and pollen tube growth rate after different storage times in low temperature in some almond, apricot, hawthorn, loquat, peach, plume, prune, sour cherry and sweet cherry genotypes and reported similar results which observed in this works.

The viability, attainability, vigor, germinability, fertility, and fertilization ability indicate different aspects of pollen potential. The terms germinability, fertility, and fertilization ability are more restrictive than viability. A pollen grain may have the capacity to germinate, but does not due to improper conditions. It may even germinate, but not have the ability to fertilize an ovule because of some form of incompatibility. Sometimes, cultivars produce high quantity of pollens but not with high quality such as low PGP or low tube growth also; some of the pollens may be sterile or not viable (Nikolic and Milatovic, 2010; Stosser et al., 1996; Vitagliano, 1989; Szabo, 2003).

Anjum and Shaukat (2008) with studding pollen germination of *M. pumila* L., beyond 48 weeks in the refrigerator (+4°C), freezer (-20 and -30°C) and freeze drier (-60°C) in different concentration of sucrose and boric acid solution; resulted that pollens which stored at low temperature had higher germination percentage in comparing with pollens stored at +4°C and in fresh pollen also; freeze dried pollen (-60°C) showed the highest germination percentage. Zielinski and Thompson (1966) studied pollen germination in *Pyrus* species and species hybrids and reported that pollen germination in most *Pyrus* species was moderately high, usually averaging above 50% and five species were completely or nearly pollen sterile including; *P. nivalis* Jacq, *P. ovoideae* Rehd, *P. Decaisne*, *P. salicifolia*, var. pendula and *P. variolosa* Wall, which gave very low germination or none at all. Finally, they attributed this phenomenon to the pollen sterility of these *Pyrus* species. In the same study, Sharafi (2011) studied PGP and pollen tube length investigation on pollen viability-longevity in *M. pumila*, *P. commonis* and *C. oblonga in vitro* and reported that PGP and PTL were nearly standard in cultivars/genotypes of three pome fruit species after 35 days storage in 0°C, although, some decrease was observed. However, cultivars with high PGP including "Gizil-Alma" (apple), "Sard-roud" (pear), and "Dorosht-e-Lahijan" (quince) were selected for orchard establishment and breeding programs as a pollinizer, for pollination of commercially growing cultivars. Sharafi et al. (2010) and Sharafi and Bahmani (2011a, b) reported same results in different hawthorn specie, loquat, almond, apricot, peach, plume, prune, sweet cherry and sour cherry cultivars/genotypes too.

Conclusion

It was concluded that pollen germination percentage and pollen tube length were high in six geneses of pome fruits in the same *in vitro* condition. Cultivars of apple showed the highest range of pollen germination percentage and pollen tube length among six geneses cultivars/genotypes. However, cultivars/genotypes with high pollen germination percentages including; "Red delicious", "Shah-miveh", Dorosht-e-Lahijan, "MG3a", "EJ2a" and "CO4a" were selected respectively for apple, pear, quince, medlar, loquat and red hawthorn orchard establishment and breeding programs.

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