Journal of Medicinal Plants Research Vol. 5(11), pp. 2232-2236, 4 June, 2011
Available online at http://www.academicjournals.org/JMPR
ISSN 1996-0875 ©2011 Academic Journals

Full Length Research Paper

Investigation on pollen viability and longevity in *Malus pumila* L., *Pyrus commonis* L., and *Cydonia oblonga* L., *in vitro*

Yavar Sharafi

Islamic Azad University, Maragheh Branch, Department of Horticultural Science, Maragheh, Iran. E mail: yavarsharafi@iau-maragheh.ac.ir. Tel: +989144200882. Fax: 984213254506.

Accepted 24 March, 2011

The most important goal of fruit growers is obtaining high quantity and quality yield in fruit industry which depends on sufficient fruit setting. Pollination and fertilization are the basic factors which affect fruit setting volume. Commercial fruit trees in Iran belong to stone and pome fruits of Rosaceae family with gametophytic self-incompatibility, making 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, tube growth and longevity were studied in some cultivars and selected favorable genotypes of three species including; apple, pear and quince. Pollen traits were investigated after 35 days maintenance in 0°C, using *in vitro* medium containing 16% sucrose, 4 ppm acid boric and 1% agar. Cultured pollens were incubated in dark condition at 25°C for 24 h and then, pollen germination percentage and pollen tube length were evaluated using Light-Microscope. Results showed significant differences among cultivars and genotypes of each three studied species for pollen germination, tube growth and longevity characteristics.

Key words: Apple, pear, quince, pollen traits, longevity and *in vitro* condition.

INTRODUCTION

Apple (*Mallus pumila* L.), Pear (*Pyrus commonis* L.) and Quince (*Cydonia oblonga* L.), are temperate fruit trees which are grown in many regions of the world with Mediterranean climates and have essential roles in the fruit industry of producer countries. Iran is one of the main producers of these pome fruits. Most of the cultivars belonging to these species show self or cross incompatibility system especially in *Pyrus.* So, in breeding programs for this species, manual pollination could be carried out in the field or laboratory, usually (Jackson, 2003; Szabo, 2003; Dantas et al., 2005). For successful pollination, the high quantities and qualities pollen must be transferred to the stigma when it is receptive (Deckers 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 should 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 fruit trees. Furthermore, in breeding programs breeders should sometimes maintain pollens for applying in the controlled artificial pollination methods and protect the viability and germination capacity of pollens. Many researchers 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 the above, it could be stated that, pollen traits especially germination percentage and tube growth in stored pollens should be carried out to have confidence of their viability and longevity. Previously in different species, many cultivars and genotypes with unfavorable pollens - 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* and species hybrids and reported that pollen germination in most *Pyrus* species was moderately high, usually average, above 50 percent. Several researchers have previously 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 drier, organic solvents 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 *M. pumila* during storage in some organic solvents; similar results are 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°C, -30°C) and freeze drier (-60°C), using hanging drop technique. 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 (*Malus 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 *Pyrus pyrifolia.* 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 compared the advantages and disadvantages of each method and suggested some practical. The objective of this study was to determine longevity, viability, germination and tube growth capacity of pollens in some cultivars and selected genotypes of apple, pear and quince which are grown in different regions of East-Azarbaijan Province in Iran, after 35 days storage in -0°C, for using in inbreeding and fruit industry programs.

MATERIALS AND METHODS

Plant materials and research area

Totally fifteen cultivars and genotypes from pome fruit tree species (*M. pumila* L., *P. commonis* L. and *C. oblonga* L.) which exist in different regions of East-Azarbaijan Province of Iran were selected. Plants included five cultivars of apple (Noori-Maragheh, Ahar-almasi, Gizil-alma, Gami-almasi and Golab), three cultivars (Sard-roud, Shah-miveh and Sibery) and two favorable genotypes (PC1 and PC2) of pear and three cultivars (Torshe-Azarbaijan, Gortoune-Sfahan and Zarde-nishabour) and two favorable genotypes (CO1 and CO2) of Quince. Research was carried out in Department of Horticultural Sciences, Islamic Azad University of Maragheh, Iran.

Pollen collection and germination test

In the spring of 2008, flower buds in balloon stage were gathered and transmitted to the laboratory. Petals and sepals were separated and anthers placed in Petri dishes for releasing pollens. Pollens gathered and their PGP and PTL were tested immediately and then, stored 35 days in 0^0C. Pollens were planted in the *in vitro* medium containing 1% agar, 16% sucrose and 4 ppm boric acid and incubated in 25°C about 24 h and then tube growth was stopped by 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 scale (µm).

Experimental design and data analysis

Experiments were carried out as in completely randomized design (CRD) with four replications (4 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 variances in Table 1 indicated significant differences among apple, pear and quince studied cultivars and genotypes for pollen germination percentage (PGP) and pollen tube length (PTL) after 35 days storage in 0°C. Among apple cultivars, means of PGP and PTL ranged between 41.3 - 94.1% and 140.2-640.2 µm, respectively; also, in the pear cultivars/genotypes, PGP and PTL were between 28.4-80.8% and 120.7- 320.1 µm; while in quince PGP and PTL were between 25.6- 65.3% and 274.5 - 620.2 µm, respectively (Table 2).

It should be stated that, the means of pollen germination percentage of all plants were higher than 75% immediately after gathering in laboratory (data not shown). Difference in means of PGP and PTL among the studied species showed higher variety in PTL compared with PGP (Table 2). Meanwhile, apple cultivars showed the highest PGP and PTL, proving the high longevity of apple pollens among three species and pear cultivars/genotypes showed the lowest pollen tube length. Based on data which are shown in Table 2, maximum PGP was observed in Gizil- Alma (94.1%), Sard- roud (80.8%) and Gortoune-sfahan (65.3%) respectively in apple, pear and quince cultivars (Table 2). However, high pollen germination percentage of these plants after 35 day maintenance in 0^0C showed their extensive longevity; and they could be selected for orchard establishment and breeding programs as a pollinizer for pollination of other cultivars. Pollen germination and tube growth rate are the most important characteristics related to pollen quality, and successful fertilization needs high germination rates and fast tube growth because, low rates may lead to low fruit set caused by ovule

Table 1. Analysis of variances for pollen germination percentage (PGP) and pollen tube length (PTL based on micrometer) in the studied cultivars and genotypes of three pome fruits (*M. pumila* L., *P. commonis* L., and *C. oblonga* L.,).

Species	SOV	DF	PGP (%)	PTL (µm)
M. pumila L.,	Cultivars	4	1993.4**	4901.4**
	Experimental error	20	142.7	325.7
	CV (%)		16	19.7
P. commonis L.,	Cultivars/Genotypes	4	1251.2**	3004.9**
	Error	20	111.7	346.3
	CV (%)		22	18.9
C. oblonga L.,	Cultivars/Genotypes	4	1121.6**	2718.1**
	Error	20	89.5	356.7
	CV (%)		23.4	25.8

**: Significant in P<0.01% level.

Table 2. Comparison of means for pollen germination percentage (PGP) and pollen tube length (PTL) in the cultivars and genotypes of three pome fruits (*M. pumila* L., *P. commonis* L. and *C. oblonga* L.).

Species	Cultivar/Genotype		PGP (%)	PTL (µm)
M. pumila L.,	Cultivar	Noori-Maragheh	55.6^{bc}	140.2^{d}
		Ahar-almasi	41.3^{c}	230.4^{dc}
		Gizil-alma	94.1^{a}	380.1^{c}
		Gami-almasi	63.6^{b}	640.2^{a}
		Golab	44.7^{c}	460.7^{b}
P. commonis L.,	Cultivar	Sard-roud	80.8^{a}	320.1^{a}
		Shah-miveh	28.4^{d}	271.4^{b}
	Genotype	Sibery	45.6^{dc}	202.8^{c}
		PC1	59.2^{c}	120.7^{d}
		PC2	67.1^{b}	131.2^{d}
C. oblonga L.,	Cultivar	Torshe-Azarbaijan	50.9^{b}	620.2^{a}
		Gortoune-Sfahan	65.3^{a}	595.4^{a}
	Genotype	Zarde-nishabour	41.4^{c}	460.2^{b}
		CO1	25.6^{dc}	356.4^{bc}
		CO2	37.2^{d}	274.5^{c}

Same letters show no difference among cultivars and genotypes of each column.

degradation before the pollen tube reaches the ovary (Cheung, 1996; Sharafi et al., 2010). According to Sharafi and Bahmani (2010), in this research cultivars/genotypes with high PGP were not followed by high PTL. This phenomenon indicates genetically differences among the genotypes which have been reported by many researchers in many 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 pollen germination percentage, 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 are observed in this works. 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 may be sterile or not viable (Nikolic and Milatovic, 2010; Stosser et al., 1996; Vitagliano, 1989; Szabo, 2003). Abreu et al. (2006) investigated the causes of low productivity of *Vitis vinifera* cv. Loureiro pollen by studding pollen viability using fluorochromatic reaction and pollen germinability by *in vitro* assays and reported that the acolporated pollen grains of mentioned cultivar are viable, but no germination was recorded. Anjum and Shaukat (2008) studied pollen germination of *M. pumila* L., beyond 48 weeks in the refrigerator (+4°C), freezer (-20°C, -30°C) and freeze drier (-60°C) in different concentration of sucrose and boric acid solution; results show that pollens stored at low temperature had higher germination percentage compared to pollens stored at +4°C; and in fresh pollen also, freezer dried pollen (-60°C) showed the highest germination percentage. Zielinski and Thompson (1966) studied pollen germination in *pyrus* and hybrids species hybrid and reported that pollen germination in most *Pyrus* species was moderately high, usually average, above 50% and five species were completely or nearly pollen sterile including *Pyrus nivalis* Jacq, *Pyrus ovoideae* Rehd, *Pyrus Decaisne*, *Pyrus salicifolia*, var. pendula and *Pyrus variolosa* Wall, which gave very low germination or none at all. Finally, they attributed this phenomenon to the pollen sterility of these *Pyrus* species.

Conclusion

Finally we concluded that PGP and PTL were nearly standard in cultivars/genotypes of three pome fruit species after 35 days storage in 0^0C, although, some decrease was observed. Cultivars of apple showed the highest range of PGP, PTL and longevity among three species and cultivars/genotypes with high PGP have not shown high PTL, necessarily.

However, cultivars with high pollen germination percentages including Gizil- Alma (apple), Sard- roud (pear), and Gortoune-sfahan (quince) were selected for orchard establishment and breeding programs as a pollinizer, for pollination of commercially growing cultivars.

ACKNOWLEDGMENTS

Author would like to thank the Research Section of Islamic Azad University of Maragheh Branch, Research Section of University of Maragheh, Dr Ali Bahmani, Mr Mehdi Karimi and Mr Mojtaba Gorbanifar for their help.

REFERENCES

Abreu I, Costa L, Oliveira M, Cunha M, Castro RD (2006). Ultrastructure and germination of *vitis vinifera* cv. Loureiro pollen. Protoplasma, 228: 131-135.

Aparecida SPL, Darlan RJ, Pasqual M, Carvalho SF, Pereira JK (2004). Receptiveness of the stigma and in vitro germination of orange pollen, submitted to different temperature. Ciênc. agrotec. Lavras, 28(5): 1087-1091.

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

Anjum P, Shaukat A (2008). Maintenance of pollen germination capacity of *Malus Pumila* L., (Rosaceae). Pak. J. Bot., 40(3): 963-966.

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

Dafni A, Firmage D (2000). Pollen viability and longevity: practical, ecological and evolutionary implications. Plant Syst. Aevol., 222: 113-132.

Dantas AC, De M, Peixoto ML, Nodari RO, Guerra MP (2005). Germination of pollen and the development of pollen tubes in apple (*Malus* spp.). Rev. Bras. Frutic., 27(3): 356-359.

Deckers T, Porreye W (1984). Influence of the temperature on pollen germination of different cultivars of apple and pear: Trials *In vitro*. Acta Hortic., 149: 123-130.

Dane F, Olgun G, Dalgic O (2004). *In vitro* pollen germination of some plant species in basic culture medium. J. Cell Mol. Biol., 3: 71–76.

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

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

Ganeshan S, Alexander MP (1991). Cryogenic preservation of lemon (*Citrus lemon* Burm.) pollen. Garten-bauwissenschaft, 56: 228-230.

Jackson JE (2003). Biology of apples and pears. Cambridge, UK: Cambridge University Press.

Jain A, Shivanna KR (1988). Storage of pollen grains in organic solvents: effect of organic solvents on leaching of phospholipids and its relationship to pollen viability. Ann. Bot., 61: 325-330.

Liu W, Lanzhou L, Cao ZX (1984). Germination, respiration and fertility of pollen of *Prunus persica* and *Malus pumila* during storage in some organic solvents. Acta Phytophysiol. Sin., 10(3): 277-284.

Mert C (2009). Temperature response of pollen germination in walnut (*Juglans regia* L.). J. Biol. Eviron. Sci., 3(8): 37-43.

Munzuroglu O, Gur N (2000). The effects of heavy metals on the pollen germination and pollen tube growth of apples (*Malus sylvestris* Miller cv. Golden). Turk. J. Biol., 24: 677-684.

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

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

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, 22-25 May. Antakya. Turkey.

Sharafi Y, Bahmani A (2010). *In vitro* study of pollen traits after short storage in some almond, apricot and sweet cherry favorable genotypes. J. Med., Plant Res., (Unpublished).

Sharafi Y, Bahmani A (2010). Pollen viability and longevity of some peach, plum, prune and sour cherry favorable genotypes. J. Med., Plant Res., (Unpublished).

Shivanna KR (2003). Pollen biology and biotechnology. Science publishers, Enfield.

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

Szabo Z (2003). Apple, Pear and Quince. In: Floral biology, pollination and fertilisation in temperate zone fruit species and grape. Akadémiai Kiado, Budapest, pp. 383-410.

Vasilakakis M, Porlingis IC (1985). Effect of temperature on pollen germination, pollen tube growth, effective pollination period, fruit set of pear. Hortic. Sci., 20: 733–735.
Visser T, Oost EH (1981). Pollen and pollination experiments. III, the viability of apple and pear pollens affected by irradiation and storage. Euphytica, 30: 65-70.
Vitagliano C, Viti R (1989). Effects of some growth substances on pollen germination and tube growth in different stone fruits. Acta Hortic., 239: 379-381.
Zhang C, Tateish N, Tanabe K (2010). Pollen density on the stigma affects endogenous gibberellins metabolism, seed and fruit set, and fruit quality in *Pyrus pyrifolia*. J. Exp. Bot., 61(15): 4291-4302.
Zielinski BO, Thompson MM (1966). Pollen germination in *Pyrus* species and species hybrids. Euphytica, 15: 195-198.