African Journal of Biotechnology Vol. 10(41), pp. 8064-8069, 3 August, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.701
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

In vitro pollen germination, pollen tube growth and longevity in some genotypes of loquat (*Eriobotria japonica* Lindl.)

Yavar Sharafi[1]*, Ali-Reza Motallebbi-Azar[2] and Ali Bahmani[3]

[1]Department of Horticultural Science, Islamic Azad University, Maragheh Branch, Maragheh, Iran.
[2]Department of Horticultural Science, Faculty of Agriculture, University of Tabriz, Tabriz, Iran.
[3]University of Maragheh, Department of Horticultural science, Maragheh, Iran.

Accepted 15 June, 2011

Loquat (*Eriobotrya japonica* Lindl.) is one of the most perspective trees with tasty fruits suitable for commercial production in Iran. However, self-incompatibility of most loquat cultivars makes it necessary to select good pollinizers in breeding and orchard establishment programs. Therefore, studies on pollen viability traits of cultivars and genotypes have been one of the main issues for loquat growers and breeders which are investigated in this study. The experiment was conducted in *in vitro* conditions for the identification of the pollen germination and longevity in some loquat genotypes for recognition of the best pollinizers. Pollens of twenty (20) genotypes were gathered and after three weeks storage in 0°C, their pollen germination, pollen tube growth rate and pollen longevity were tested in the *in vitro* medium. The experiment was carried out based on completely randomized design (CRD) and data were analyzed with SAS software. Significant differences were observed among the genotypes in pollen germination, pollen tube growth rate and pollen longevity. Finally, the best genotypes were selected for using in loquat orchard establishment and breeding programs in Iran.

Key words: Loquat, *in vitro*, pollen germination, pollen tube growth, pollen longevity, breeding programs.

INTRODUCTION

Loquat (*E. japonica* Lindl.) from Rosaceae family is a worldwide evergreen fruit tree important for its use as horticultural and ornamental plant, especially in sub-tropical and Mediterranean climatic countries. Most of the loquat cultivars and genotypes have the gametophytic self-incompatibility (GSI) system, such as many other fruit trees of Rosaceae family species (Caballero and Fernandez, 2003; Cuevas, 2003; Freihat et al., 2008). However, when loquat was planted, the low fruit set and small fruit size were often reported because of the unfavorable pollination, which resulted from self (cross) incompatibility relationships among loquat cultivars (Polat, 1996; Polat et al., 2004a, b). Pollination and fertilization are the basic and most important factors which affect fruit setting volume in fruit industry. Therefore, knowledge about pollen traits of the species and cultivars is one of the main issues for all of the fruit tree species growers and breeders (Mesuji et al., 2010; Sharafi, 2010, 2011; Sanzol and Hererro, 2001). For successful pollination, the high quantities and qualities pollen must be transferred to the stigma when it is receptive (Sharafi and Bahmani, 2010, 2011; Wang et al., 2007). However, the pollen is sometimes deposited before the receptive period of ovules and should remain viable for a period that is long enough for it to germinate, although, some of the *Eriobotrya japonica* Lindl cultivars and genotypes set parthenocarpic fruits based on specific physiological-environmental conditions (Mesuji et al., 2010; Polat, 1996; Polat et al., 2004a, b). In breeding programs, breeders should sometimes maintain pollens by applying them in the future controlled artificial pollination methods, whereas pollens should protect their viability and germination capacity. 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 of pollens

*Corresponding author. E-mail: yavarsharafi@iau-maragheh.ac.ir. Tel: +989144200882. Fax: 984213254506.

(Dane et al., 2004; Eti, 1991; Eti et al., 1991; Gozlekci et al., 2010; Guo et al., 2010; Ji et al., 2007, 2008; Polat and Pirlak, 1999).

Moreover, temperature is a very basic factor in the control of the environmental conditions and it influences pollen grain germination and longevity in stored pollens (Sanzol and Hererro, 2001; Sharafi 2010). Pollen traits, especially germination percentage and tube growth rate in stored pollens, should be carried out for confidence of their viability and longevity. Previously in different species, many cultivars and genotypes with unfavorable pollens such as sterile pollens, pollens with low germination percentage or low tube growth rate have been reported by breeders and researchers (Deng et al., 2008, 2010; Sharafi, 2011).

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), freeze-dried organic solvents, etc (Jain and Shivanna, 1988; Hedhly et al., 2005; Sharafi and Bahmani, 2010). Except for some parthenocarpic fruits of some loquat cultivars, pollination and fertilization are certainly necessary for well and high fruit set in loquat orchards such as many fruit species. Pollen viability levels, environmental conditions and compatibility among cultivars are important for the normal fruit set in most of the loquat cultivars and genotypes (Polat et al., 2004; Sharafi and Bahmani, 2010; Wang et al., 2007). 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 cultivars. Therefore, different nutrition conditions and germination methods for many plant species and varieties were used by researchers (Polat and Pirlak, 1999; Sharafi, 2010).

Deng et al. (2008) used TTC method to determine pollen viability and the *in vitro* culture method to determine pollen germination rate in ‘Longquan No. 5’ lines of loquat. They reported that the pollen viabilities and germination rates in the *in vitro* culture of the lines from the degraded seeds were obviously lower than their maternal plant. Qin et al. (2008) studied the pollen viability, germination percentage and tube growth rate in *in vitro* and self or cross pollinated trees of 'Dawuxing' and ‘Longquan No.1’ and reported that 'Dawuxing' is self-incompatible. Hakan et al. (2007) investigated the effect of natural lipid (lysophosphatidylethanolamine) on pollen germination and tube growth of loquat 'Sayda' cultivar and concluded that application of this material before blooming significantly improved pollen germination and tube growth rate.

In this study, pollen germination, tube growth rate and longevity were studied in some genotypes of loquat using a low cost and easy *in vitro* medium containing 1% agar, 10% sucrose and 0.01% boric acid for selecting favorable Genotypes to be used as polinizer in loquat orchard establishment and breeding programs in Iran.

MATERIALS AND METHODS

Plant materials, pollen collection and germination test

Twenty (20) mature genotypes of loquat (*E. japonica* Lindl.), which exist in different regions of the north province orchards and jungles of Iran were selected, including 'Eb1', 'Eb2', 'Eb3', 'Eb4', 'Eb5', 'Eb6', 'Eb7', 'Eb8', 'Eb9', 'Eb10', 'Eb11', 'Eb12', 'Eb13', 'Eb14', 'Eb15', 'Eb16', 'Eb17', 'Eb18', 'Eb19' and 'Eb20'.

In 2009, for the determination of the pollen germination and pollen tube growth rate of genotypes, the well-grown flower clusters from each genotype were picked in the full flowering season. They were put in paper bags and were transferred to the laboratory in the Department of Horticultural sciences, Islamic Azad University of Maragheh. Petals and sepals were separated and anthers were placed in sterile Petri dishes for 24 h in order to release pollens. The pollens were gathered and their germination was tested immediately and then stored for three weeks in 0°C. After 3 weeks, pollens were planted in the *in vitro* medium containing 1% agar, 10% sucrose and 0.01% boric acid and incubated at 22°C for about 24 h and then the tube growth was stopped with an addition of chlorophorm. Six microscopic areas were counted randomly for evaluation of pollen germination and tube growth rate in each Petri dish. The pollen, whose tube is as long as its diameter, was considered to be germinated and measurements of the pollen tube length were recorded directly by an ocular micrometer fitted to the eyepiece of the microscope based on the micrometer scale (µm).

Experimental design and data analysis

The experiment was carried out in completely randomized design (CRD) in five replications (5 Petri dishes for each genotype) and the data were analyzed using SAS software. 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 twenty studied genotypes of loquat in pollen germination percentage and pollen tube growth rate after three weeks storage in 0°C. Among genotypes, means of pollen germination percentage and pollen tube length ranged between 15.3 and 94.1% and 70.6 and 1021.3 µm, respectively. Means of pollen germination percentage of all genotypes were higher than 85% immediately after gathering them in the laboratory. Difference in the means of pollen germination percentage and pollen tube length showed higher variety in tube length when compared with germination percentage (Table 2 and Figures 1 and 2). Based on the data which are shown in Table 2, the maximum pollen germination was observed in genotype 'Eb17' (94.1%), while the minimum was observed in genotype 'Eb9' (15.3%). Also, the maximum pollen tube length was observed in genotype 'Eb17' (1021.3 µm), while the minimum was observed in genotype 'Eb7' (70.6 µm), respectively. However, high pollen germination percentage of these

Table 1. Analysis of variance for pollen germination percentage and pollen tube length (based on micrometer) in twenty loquat genotypes.

Source of variation	DF	Pollen germination percentage (%)	Pollen tube length (µm)
Cultivars	19	984.1**	2534.3**
Experimental error	80	53.9	231.2
Coefficient value (%)		9.6	11.7

**: Significant in P<0.01% level.

Table 2. Comparison of means for pollen germination percentage and pollen tube length (based on micrometer) in twenty loquat genotypes.

Cultivar	Pollen germination percentage (%)	Pollen tube length (µm)
'Eb1'	18.5^{g}	125.8^{cd}
'Eb2'	62.3^{c}	953.7^{a}
'Eb3'	24^{f}	321.9^{cd}
'Eb4'	76.3^{b}	893.7^{a}
'Eb5'	46.2^{d}	278^{cd}
'Eb6'	35.2^{de}	637.1^{ab}
'Eb7'	17.6^{g}	70.6^{d}
'Eb8'	82.4^{a}	416.2^{c}
'Eb9'	15.3^{g}	86.2^{d}
'Eb10'	58.1^{e}	650^{ab}
'Eb11'	82.3^{a}	979.3^{a}
'Eb12'	43.4^{e}	282.5^{cd}
'Eb13'	59.1^{e}	761.3^{ab}
'Eb14'	34.5^{de}	461.2^{c}
'Eb15'	33d^{e}	137.8^{cd}
'Eb16'	78.1^{b}	632^{b}
'Eb17'	94.1^{a}	1021.3^{a}
'Eb18'	48^{e}	446^{c}
'Eb19'	25.1^{f}	87^{d}
'Eb20'	67.2^{c}	545.4^{b}

Same letters show no difference among cultivars in each column.

genotypes after three weeks maintenance in 0°C showed their extensive longevity; and they could be selected for orchards establishment and breeding programs as a pollinizer for pollination of other cultivars and genotypes.

Pollen germination and tube growth rate are the most important characteristics related to pollen quality and successful fertilization of high germination rates and fast tube growth, because low pollen tube growth rates may lead to low fruit set caused by ovule degradation before the pollen tube reaches the ovary (Sharafi, 2010, 2011).

According to the report of Sharafi and Bahmani (2011), in some species of genus *prunus*, genotypes with high pollen germination was not followed by high pollen tube growth in this study; although, in some genotypes with high pollen germination, high pollen tube growth rate was shown too (Table 2 and Figures 1 and 2). This phenolmenon indicated genetic differences among the cultivars and genotypes which were reported by many researchers in numerous fruit tree species and cultivars (Polat and Pirlak, 1999; Sharafi et al., 2010). Moreover, Sharafi (2011) and Sharafi and Bahmani (2010) investigated the pollen germination percentage, longevity and pollen tube growth rate after different short storage times in low temperature in some almond, apricot, hawthorn, peach, plume, prune, sour cherry and sweet cherry geno-types and reported similar results to those observed in this work.

Sometimes, cultivars and genotypes 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 (Deng et al., 2008; Sharafi et al., 2010, 2011). Gozlekci et al. (2010) studied pollen viability in four cultivars of loquat using TTC staining methods (1% 2,3,5-triphenyl tetrazolium chloride), I_2KI (iodine/potassium iodide solution) and Safranin staining. However, in their study, *in vitro*

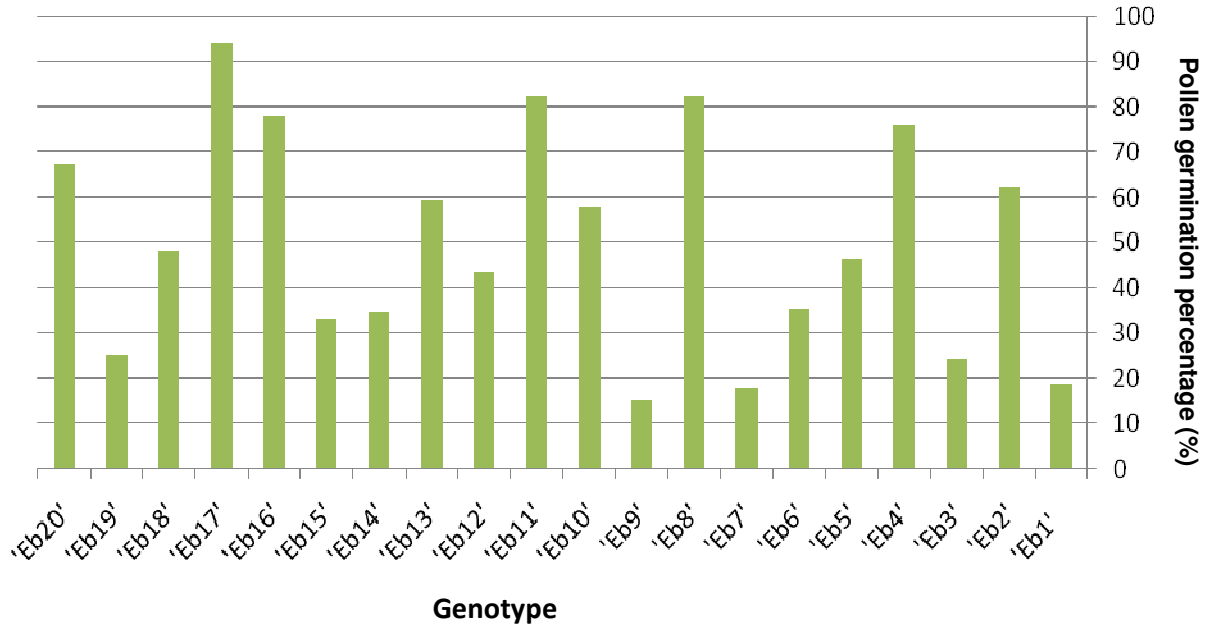

Figure 1. Comparison of means for pollen germination percentage in twenty loquat genotypes.

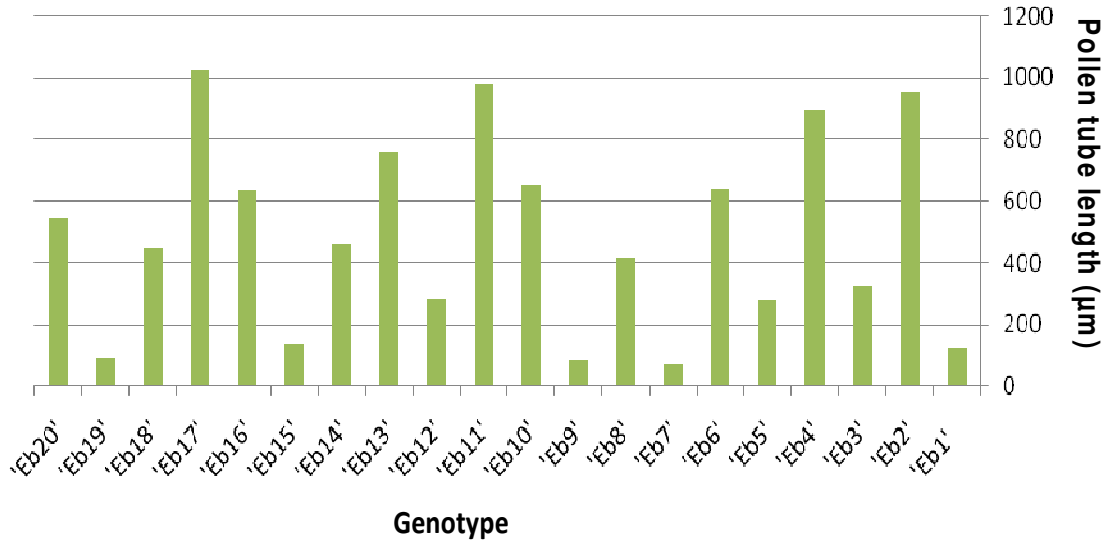

Figure 2. Comparison of means for pollen tube length (based on micrometer) in twenty loquat genotypes

germination tests were carried out with the Agar-Plate method in a medium consisting of 0.5% agar, 10% sucrose and 0.01% boric acid. The results showed that pollen viability estimation varied with tests and cultivars. Using TTC test, the highest pollen viability was found in 'Gold Nugget' and the lowest in 'KKTC4'. In the I_2KI test, 'Gold Nugget' (98.6%) had the highest pollen viability value, while 'KKTC4' had the lowest. With Safranin staining, 'Gold Nugget' (75.6%) had the highest pollen viability (19.7%, 28.2 kg/tree), while the 'G6' type (29.8%) had the lowest (8.0%, 6.0 kg/tree).

Qin et al. (2008) studied the pollen viability, germination percentage and tube growth of self or cross pollinated trees of 'Dawuxing' and 'Longquan No.1' with each other's pollen in the open and controlled pollination conditions and reported that 'Dawuxing' was self-incompatible. Hakan et al. (2007) investigated the effect of Lysophosphatidylethanolamine lipid (with 100 ppm concentration) in two different times (1: before blooming and 2: two weeks after first application) on pollen germination and tube growth on loquat cultivar 'Sayda' and concluded that application of lysophosphatidylethanolamine

mine lipid before blooming significantly improved pollen germination and tube growth of the 'Sayda' loquat cultivar. Also, they tested different sucrose concentrations and different incubation temperatures on pollen germination and tube growth rate and concluded that 'Sayda' cultivar showed the highest pollen germination and tube growth in 20°C and 20 to 15% sucrose concentrations, respectively. Sharafi and Bahmani (2010) investigated pollen germination percentage, longevity and pollen tube growth rate after different storage times in low temperature in some loquat cultivars and reported similar results. Also, Deng et al. (2008) used five methods to identify the male fertility. They used the paraffin section to observe anther and pollen structure during balloon stage, stereomicroscope to observe anther configuration and pollen dispersal situation, scanning electron microscope to observe pollen submicroscopic morphology, TTC method to determine pollen viability, and *in vitro* culture method to determine pollen germination rate. The results showed that there were great differences in pollen quantity and morphology between 'Longquan No. 5' loquat and its offspring lines obtained from the moderately degenerated seeds. While 'Longquan No. 5' was fertile with male and its offspring lines from the degraded-seeds had poor pollens, most anthers dehisced abnormally or delayed to dehisce after blossoming. Consequently, there were many abnormal and poorly developed pollens and the pollen exine ornamentation was very different from their maternal plant. Thus, the pollen viabilities and germination rates in the *in vitro* culture of the lines from the degraded seeds were obviously lower than their maternal plant (3.67 to 16.83 and 0.89 to 15.67%, respectively). The lines from the degraded seeds showed pollen abortion, belonging to sporogenesis male sterility, although, there were some differences in the male sterility degree among the materials.

In another study, Deng et al. (2010) investigated the style characteristics, stigma receptivity, *in situ* pollen germination and pollen tube growth from styles with different length in 'Longquan No.5' and 'Chuannong No.1' of loquat (a line derived from small seeds of 'Longquan No.5'). They concluded that the number of styles varied with cultivar. In 'Chuannong No.1', the style number varied from 4 to 10, while the long and middle sized styles of 'Chuannong No.1' had stigma receptivity from the balloon stage to 9 days after anthesis. Stigma receptivity was more extended in long styles than in short and ultra short-styles. In 'Chuannong No.1', the long and medium sized styles were more receptive to pollen germination and pollen tube growth than short and ultra short styles.

Conclusion

We conclude that pollen germination and pollen tube growth rate were standard in all twenty genotypes of loquat after three weeks storage in 0°C, although, several decreases were observed in some of them. Five genotypes including 'Eb2', 'Eb4', 'Eb11', 'Eb13' and 'Eb17' showed the highest range of pollen germination, tube growth rate and longevity among the twenty genotypes, but the genotypes with high pollen germination did not show high pollen tube growth necessarily. However, the mentioned genotypes with high pollen germination percentages and high pollen tube growth rate were selected for loquat orchards establishment and breeding programs in Iran.

ACKNOWLEDGEMENT

The authors would like to thank the research section of Islamic Azad University, Maragheh Branch, for their cooperation.

REFERENCES

Caballero P Fernandez MA (2003). Loquat production and market. First international symposium on loquat. Zaragoza, Ciheam-Iamz., pp. 11-20.

Cuevas J, Hueso JJ, Puertas M (2003). Pollination requirements of loquat (*Eriobotrya japonica* Lindl.), cv. `Algerie'. Fruits, 58: 157-165.

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.

Deng QX, Yang Q, Wang YQ, Liu L, Lv XL, Tao L, Luo SF, Fu Y (2010). Style morphology, in situ pollen germination and pollen tube growth of loquat. 3th International Symposium on Loquat, 22-25 May. Antakya. Turkey.

Deng QX, Wang YQ, Lu L, Yan F, Li YH, Tao, L, Shi-Feng L (2008). Identification of male fertility of longquan No.5 lines in loquats (*Eriobotria japonica* Lidl.). Plant Sci., 1(3): 61- 67.

Eti S (1991). Determination of pollen viability and germination capability of some fruit species and varieties *in vitro* conditions. J. Agric. Fac. Cukorova Univ. 6: 69-80.

Eti S, Kaskas N, Kurnaz S, Kilavuz M (1991). The relationships among pollen production, pollen viability rate, pollen germination ability and fruit set in some loquat (*Eriobotria japonica* Lidl.) varieties (in Turkish with English summary), Trend J. Agric. For., 14: 421- 430.

Freihat NM, Al-Majeed Al-Ghzawi A, Zaiton S, Alqudah A (2008). Fruit set and quality of loquats (*Eriobotria japonica* Lidl.) as affected by pollinations under sub- humid Mediterranean. Sci. Hortic., 117 (1): 1258- 1262.

Gozlekci S, Uzun HI, Tepe S (2010). Determination of pollen viability, germination and quality in loquat cultivars. 3th International Symposium on Loquat, 22-25 May. Antakya. Turkey.

Guo QG, Wu Q, Zhang C, Ji K, Li XL, He Q, Wang WX, Xiang SQ, Liang GL (2010). Effects of PH on pollen germination, behavior of Ca^{2+} and diversion of generative nucleus in loquat. 3th International Symposium on Loquat, 22-25 May. Antakya. Turkey.

Hakan Demirkeser T, Caliscan OA, Polat AA, Ozgan M, Serce S (2007). Effect of natural lipid on pollen germination and tube growth on loquat. Asian J. Plant Sci. 6(2): 304- 307.

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

Mesuji C, Reiga C, Martínez-Fuentesa A, Agustí M (2010). Parthenocarpic fruit production in loquat (*Eriobotrya japonica* Lindl.) by using gibberellic acid. Sci. Hortic., 126: 37-41.

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.

Ji K, Wu YK, Zhu HX, Liang GL Li XL, Xiang SQ, Guo QG (2007). Pollen viability determination on loquat polyploidy with FDA. 3th National loquat Academy Conference (in Chinese). 4: 62-67.

Ji K, Wu YK, Zhu HX, Liang GL Li XL, Xiang SQ, Guo QG (2008). Embryogenesis and plant regeneration from anther culture in loquats (*Eriobotria japonica* Lidl.). Sci. Hortic., 115 (4): 329- 336.

Polat AA (1996). An important fruit species for Mediterranean region: Loquat (in Turkish with English summary). J. Agric. Faculty. MKU, 1: 39-46.

Polat AA, Durgac C, Kailoglu O, Caliskan O (2004a). Effects of different planting densities on the vegetative growth, yield and fruit quality of loquat. Acta. Hortic., 632: 189-195.

Polat AA, Durgac C, Kailoglu O, Caliskan O (2004b). Effects of protected cultivation on the precocity, yield and fruit quality in loquat. Sci. Hortic., 104: 189- 198.

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

Qin Y, Deng QX, Wang YQ, Lu L, Yan F, Li YH, Tao L, Shi-Feng L (2008). Study on characteristics of *in situ* pollen germination and tube growth of loquat. Plant Sci., 1(3): 50-55.

Sanzol J, Herrero M (2001). The effective pollination period in fruit trees. Sci. Hortic., 90: 1-17.

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

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 (2011). *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.

Wang WX, Xiang SQ, Guo QG, Liand Xl, Liang GL (2007). Observation on situation of pollen germination in vitro of natural triploids of loquat. 3th National loquat Academy Conference (in Chinese). 4: 42-47.