Adv. Hort. Sci., 2019 33(3): 441-446 DOI: 10.13128/ahs-23998

Short note

AHS
Advances in Horticultural Science

Pollen viability and *in vitro* germination of six pistachio (*Pistacia vera* L.) cultivars grown in northern Jordan

A. Aldahadha (*), K. Al Sane, A. Bataineh, A. Abu Alloush, Z. Hamouri
National Agricultural Research Center (NARC), Baq'a, Jordan.

Key words: in vitro germination, Jordan, pistachio, pollen storage, pollen viability.

Abstract: This study was conducted on six pistachio cultivars (Lazaourdi, Nab-El Jamal, Boundiki, Batouri, Marawhi and Aschouri) to investigate the percentage of pollen viability and *in vitro* pollen germination under stored and non-stored (fresh) conditions. The results indicated that there was a significant interaction between pollen viability of pistachio cultivars and storage period. This study showed that the non-stored (fresh) pollen of cultivars Batouri and Lazaourdi had significantly the highest viability (87%) and *in vitro* pollen germination (69.7%), respectively; indicating that such cultivars could be used as best pollinators. On the other hand, cultivar Nab-El Jamal had the lowest viability (43.7%) and *in vitro* pollen germination (40.3%). It was found that pollen viability for all stored pollen cultivars were significantly reduced by 30% when compared with non-stored (fresh) pollen. However, stored pollen germination for one month was zero for all pistachio cultivars. In addition, the results of viability for all fresh pollen cultivars were poorly linearly correlated (r^2=0.149) with the results of *in vitro* germination of fresh pollen. Further research is required to examine both pollen viability and *in vitro* pollen germination under different short and long-term storage periods and methods.

(*) **Corresponding author:**
abdallah.aldahadha@narc.gov.jo

Citation:
ALDAHADHA A., AL SANE K., BATAINEH A., ABU ALLOUSH A., HAMOURI Z., 2019 - *Pollen viability and in vitro germination of six pistachio (Pistacia vera L.) cultivars grown in northern Jordan*. - Adv. Hort. Sci., 33(3): 441-446

Data Availability Statement:
All relevant data are within the paper and its Supporting Information files.

Competing Interests:
The authors declare no competing interests.

Received for publication 26 September 2018
Accepted for publication 24 April 2019

1. Introduction

Pistachio (*Pistacia vera* L.) is a member of the family Anacardiaceae (Ak *et al*., 2016). Pistachio trees are dioecious, meaning that the pistillate and staminate flowers are formed on different trees. Pistachio trees are wind pollinated as flowers have no petals to attract insects (Hosseini *et al.,* 2015). One male tree is required for every eight female trees, but this ratio is usually not observed in orchards (Bahramabadi *et al.,* 2018). Therefore, the amount of pollen produced in each cluster and germination rate of pollen must be high in male trees (Ak *et al.*, 2016). Pistachio is mainly cultivated in warm-temperate to subtropical parts of the world for its commercially valuable and edible seeds (Tilkat and Onay, 2009). The center of origin of pistachio species in the Near East includes the Central Asia and Turkey. Jordan ranks number 13 for pistachio production in the world and has a world share of 0.1 %. Production quantity of pistachio in Jordan increased from 10 tons in 1975 to 967 tons in 2016 (FAO, 2016),

with an increase in harvested area up to 301 hectares in 2016.

In vitro pollen germination is a very useful technique because it can unravel the physiological and biochemical conditions required for the successful pollen germination and pollen tube development (Sanjay *et al.*, 2016). In addition, *in vitro* pollen germination is one of the most convenient and reliable methods used to test the viability of fresh or stored pollen. However, pollen grains of *Pistacia vera* L. have been considered to be difficult to germinate *in vitro* (Golan-Goldhirsh *et al.,* 1991). On the other hand, pollen viability usually refers to the ability to distribute functional sperm cells to the embryo sac following compatible pollination (Shivanna and Ram, 1993). The quality of pollen is evaluated on the basis of viability and vigor. Actually, pollen represents a critical stage in the life cycle of plants because viable pollen is essential for effective reproduction of sexual plants.

There has been recurrent interest in developing reliable methods for short and long-term storage of pistachio pollen (Ateyyeh, 2012). Such methods would be useful in storing pollen to be used in pistachio breeding programs (Vithanage and Alexander, 1985) and supplemental pollination programs in pistachio production (Crane and Iwakiri, 1981) which requires collection of sufficient amounts of pollen and its storage for short (hours to weeks) or long (months to years) periods, while maintaining viability (Shivanna and Sawhney, 1997). In addition, storing pollen is very important for cross-pollination, crop breeding, physiology, biotechnology, plant biodiversity and its conservation (Polito and Luza, 1988).

In this study, six pistachio cultivars were grown in Maru Agricultural Research Station, Irbid, Jordan since 1984 (all cultivars were introduced from Syria). The percentages of pollen viability and *in vitro* pollen germination have never been tested in our orchard and these percentages may be varied among these tested cultivars. Thus, the overall aim of this experiment was to investigate *in vitro* germination and pollen viability in six pistachio cultivars under non-stored (fresh) and stored pollen in refrigerator for one month. In addition, this experiment was performed to find a correlation between *in vitro* pollen germination and viability, to check efficiency of stored pollen and to determine which pistachio cultivar could be recommended as best pollinator.

2. Materials and Methods

Plant material and location

This research was carried out on six pistachio cultivars: Lazaourdi, Nab-El Jamal, Boundiki, Batouri, Marawhi and Aschouri from Pistachio orchard at Maru Agricultural Research Station. This station is located in Irbid governorate at 32° 33' mN latitude, 35° 51' E longitude and 589 m above mean sea level (Al-Ghzawi *et al.,* 2018). Maru has typical Mediterranean climate conditions with hot and dry summer and an average annual precipitation of about 380 mm and represents an intermediate drought area. *In vitro* pollen germination and viability tests were carried out at the biotechnology laboratory of the National Agricultural Research Center (NARC), Baqa'a, Jordan.

Pollen collection

Pollen collection took place during the flowering period from 3rd to 6th of April, 2018. Pollen was collected from six pistachio cultivars. Upon anthesis, pistachio clusters of each cultivar were detached and shacked to a glass square. Care was taken through this process to prevent contamination. Afterwards, pollen of each cultivar was placed in a closed vial to be used later on.

Pollen storage

For each pistachio cultivar, samples of pollen were placed in small glass vials and these samples of non-stored (fresh) pollen were immediately taken to laboratory for *in vitro* germination and viability tests. Other samples were stored in refrigerator at 4°C for 1 month to compare *in vitro* pollen germination and viability with those of non-stored pollen.

In vitro *pollen germination test*

The medium used for *in vitro* germination testing as recently described by Ateyyeh (2012) contained 1% agar, 15% sucrose and 100 ppm boric acid (H_3BO_3). Pollen grains were placed on medium and incubated at 24°C for 24 hours. After this time, 100 pollens from each cultivar were counted using light microscope to estimate the percentage of *in vitro* pollen germination. For each cultivar, 3 replicates (petri-dishes) were used. Pollen is considered to be germinated if the developed pollen tube is exceed (2-3 times) of its diameter.

Pollen viability test

Pollen viability was estimated as described by Ateyyeh (2012) by using 1% TTC (2, 3, 5-triphenyl

tetrazolium chloride) and 60% sucrose. TTC-sucrose solution was stored in brown glass bottle in a refrigerator. One drop of solution was placed onto microslide then a small amount of pollen was suspended in the drop and cover glass was placed onto the microslide, wrapped with aluminum foil and incubated in chamber room at 28°C for 60 minutes. After incubation, 100 pollens from each cultivar were counted using light microscope to estimate the percentage of pollen viability. For each cultivar, 3 replicates (microslides) were used. Pollen grains stained orange or bright red color were considered viable.

Experimental design and statistical analysis

The experiment was performed in a factorial design with six pistachio cultivars and two storage treatments (non-stored and stored pollen for 1 month) to investigate pollen viability and *in vitro* pollen germination separately. There were three replicates for each cultivar and storage treatment.

Data were analyzed by factorial ANOVA. When there were significant interactions, one-way ANOVA was used and means were separated using least significant difference (LSD).

3. Results and Discussion

In vitro *pollen germination*

Both proper pollination and pollen vigor are essential for pistachio productivity since the marketable portion is the seed. To obtain a good fruit set, pollination and fertilization are required. Previous studies reported that the yield and quality of nuts were influenced by pollen performance in pistachio (Acar and Kakani, 2010). It has been indicated that the validity of the *in vitro* evaluation of pollen germination is a predictor of *in vivo* behavior (Acar and Kakani, 2010).

The percentages of *in vitro* pollen germination for six pistachio cultivars are summarized in Table 1. The results indicated that the germinability of pistachio pollen varies according to the cultivar under non-stored (fresh) condition (Table 1). In particular, fresh pollen of cultivar Lazaourdi had significantly the highest *in vitro* germination percentage (69.7%), followed by cultivars Aschouri and Boundiki, then cultivars Batouri and Marawhi. However, fresh pollen of cultivar Nab-El Jamal had significantly ($P < 0.01$) the lowest germination percentage (40.3%). In addition, Acar *et al.* (2010) found that under *in vitro* conditions, pollen germination showed that Atli, Uygur and Kaska male pistachio cvs were generally better than their F1 hybrids.

Contrary to the reports of Polito and Luza (1988) who found that pistachio pollen lost its germinability after several days, preliminary tests of Vaknin and Eisikowitch (2000) revealed that fresh pollen lost most of its germinability within several hours. Results of Vaknin and Eisikowitch (2000) indicated that freshly collected pollen showed the highest germination rate (76.7%). In addition, Günver-Dalkılıç and Dayı-Doğru (2011) found that pollen grain germination ratio for pistachio was changed between 78.22% and 63.29% under room conditions at initial day (day 1) in pistachio. However, pollen grain germination ratios were found between 55.83% and 43.26% in pistachio at the 2nd day storage in refrigerator.

After one month of pollen storage in refrigerator, *in vitro* pollen germination was zero for all pollen pistachio cultivars (Table 1). This is supported precisely with findings of Ateyyeh (2012), suggesting that the

Table 1 - *In vitro* pollen germination and pollen viability percentage of six pistachio cultivars grown in Maru Agricultural Research Station under non-stored (fresh) and stored pollen for one month at 4°C

Cultivars	*In vitro* pollen germination (%)		Pollen viability (%)	
	Non-stored pollen (fresh)	Stored pollen (1 month)	Non-stored pollen (fresh)	Stored pollen (1 month)
Lazaourdi	69.7 a	0	66.7 b	43.0 d
Nab-El Jamal	40.3 d	0	43.7 d	34.3 e
Boundiki	60.0 b	0	55.0 c	35.7 e
Batouri	51.0 c	0	87.0 a	53.7 c
Marawhi	47.3 c	0	65.0 b	46.0 d
Aschouri	62.3 b	0	85.0 a	67.0 b
Mean	55	0	67	47
Standard error	1.64	0	1.64	3.24
LSD (0.05)	5.142	0	7.547	7.547

Data with the same letter in each column are not significantly different (least significant difference at P<0.05).

stored pollen in refrigerator for one month is not effective method for *in vitro* pollen germination. Furthermore, the pollen grain germination ratios for pistachio were dramatically decreased and reached to about zero starting from the 4th day of storage under room conditions and at 10th day of storage under refrigerated conditions (Günver-Dalkılıç and Dayı-Doğru, 2011). Pistachio pollen could be stored in refrigerator just for two weeks, which is enough for artificial cross pollination purpose, if the difference in flowering period between males and females didn't exceed two weeks. Vaknin and Eisikowitch (2000) found that germinability of pollen kept in the refrigerator for six days was reduced but it retained about 60%.

Pollen viability

The results showed that the percentages of pollen viability are significantly different in regard to pistachio cultivar under non-stored (fresh) and stored conditions (Table 1). Specifically, non-stored pollen of cultivars Batouri (87%) and cv. Aschouri (85%) had significantly the highest pollen viability percentage, followed by cultivars (Lazaourdi and Marawhi), and then cultivar Boundiki. Nevertheless, cultivar Nab-El Jamal had significantly (P<0.01) the lowest pollen viability percentage (43.7%). On the other hand, stored pollen for one month significantly (P<0.01) reduced pollen viability percentage in all pistachio cultivars when compared with non-stored pollen. For example, the highest and lowest pollen viability were in cultivars Aschouri (67%) and Nab-El Jamal (34.3%); respectively under stored conditions for one month (Table 1). The mean percentage value for fresh pollen viability for all pistachio cultivars was 67%, while for those of stored pollen viability was 47%. Thus, all pistachio cultivars lost approximately 30% of their pollen viability when pollen stored at 4°C in refrigerator. However, the mean value for fresh *in vitro* pollen germination for all pistachio cultivars was 55%. Therefore, the percentage of pollen germination was 18% less than those for pollen viability under non-stored conditions.

Günver-Dalkılıç and Dayı-Doğru (2011) found that the highest and lowest pollen grain viability ratios were obtained as 88.24% (in safranin test) and 70.18% (in TTC test), respectively, in pistachio types (4 male pistachios (*Pistacia vera* L.) grafted on terebinth). Ateyyeh (2012) found that fresh pollen viability of pistachio was 87.4% during 2006/2007 season. Ateyyeh (2012) found that pollen viability of pistachio under refrigerated conditions for 4 weeks was 0% and 38.7% for seasons 2006/2007 and 2007/2008; respectively. In fact, many factors affect pollen viability and longevity such as genetic variation between species, abiotic environmental conditions, temperature, moisture content, oxygen pressure, nutritional and physiological conditions under which the plants are grown, and the methods of pollen collection and storage (Barnabas and Kovacs, 1997). It was suggested that the loss of pollen viability in the course of short-term storage is directly related to changes in the water content of the pollen grains, rather than to a deficiency of essential metabolites (Barnabas and Kovacs, 1997).

Correlation between tests

There was a significant (P<0.05) regression of *in vitro* pollen germination on viability (Fig. 1), meaning that there is a relationship (linear correlation) between *in vitro* germination and viability for the fresh or non-stored pollen but with poor fit (r^2=0.149). Particularly, *in vitro* pollen germination and viability percentages were similar for cultivars (Lazaourdi, Nab-El Jamal and Boundiki), However, for cultivars (Batouri, Marawhi and Aschouri), *in vitro* pollen germination was less than viability percentages. This is why the overall correlation between pollen viability and *in vitro* germination is weak under non-stored pollen condition. On the other hand, there was no correlation between *in vitro* pollen germination and viability when pollen stored in refrigerator at 4°C as pollen germination was zero for all tested pistachio cultivars. Pollen viability has been correlated with *in vitro* pollen germination in Banksia and some other *Proteaceae* Plants (Schori *et al*., 1992). A positive and highly significance correlation between different pollen viability stains and pollen germination test in *Momordica* species

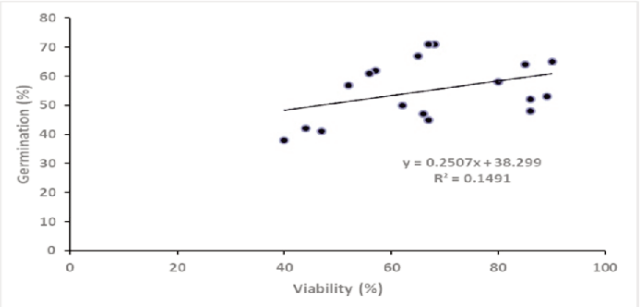

Fig. 1 - Correlation between viability and *in vitro* germination of fresh pollen in six pistachio cultivars grown in Maru Agricultural Research Station. Significant regression at P<0.05. n=3.

(Rathod *et al*., 2018), Even though, very high percent pollen viability was reported but when the germination test was performed the pollen germination was not showed more than 70 %. It has been pointed out that *in vitro* pollen germination rates are considered the best indicator of pollen viability (Shivanna *et al*., 1991). Furthermore, a good correlation was revealed between *in vitro* pollen germination with fruit and seed setting in three ornamental tropical tree species (Sanjay *et al*., 2016).

4. Conclusions

A significant interaction between pollen viability of pistachio cultivars and storage period was found in this experiment. Pollen viability in all pistachio cultivars was reduced under storage conditions. Pistachio cvs. Batouri and Lazaourdi might be used as best pollinators. Based on our results, it is not recommended to store pollen in refrigerator for one month for germination purposes. Further research is necessary to test pollen germination under different conditions and periods of storage.

Acknowledgements

Authors would like to warmly thank the Director General of the National Agricultural Research Center (NARC), Jordan for supporting and facilitating this study. Thanks are also extended to the staff of Maru Agricultural Research Station /Irbid.

References

ACAR I., AK B.E., SARPKAYA K., 2010 - *Effects of boron and gibberellic acid on in vitro pollen germination of pistachio (*Pistacia vera *L.)*. - Afr. J. Biotechnol., 9: 5126-5130.

ACAR I., KAKANI V.G., 2010 - *The effects of temperature on* in vitro *pollen germination and pollen tube growth of* Pistacia *spp*. - Sci. Hort., 125(4): 569-572.

AK B.E., ACAR I., SAKAR E., GOURSOZ S., 2016 - *The importance of* Pistacia *species for pistachio production in Turkey*. - Acta Horticulturae, 1139: 183-188.

AL-GHZAWI A.A., BANI KHALAF Y., AL-AJLOUNI Z.I., AL-QURAAN N.A., MUSALLAM I., BANI HANI N., 2018 - *The effect of supplemental irrigation on canopy temperature depression, chlorophyll content, and water use efficiency* in *three wheat (*Triticum aestivum *L. and* T. durum *Desf.) varieties grown in dry regions of Jordan*. - Agriculture, 8(5): 1-23.

ATEYYEH A.F., 2012 - *Effect of storage method on date palm and pistachio pollen viability*. - Jor. J. Agric. Sci., 8: 573-582.

BAHRAMABADI E.Z., JONOUBI P., REZANEJAD F., 2018 - *Some cytological and physiochemical features relating to non-storability of pistachio (*Pistacia vera *L.) pollen*. - Grana, 57(6): 1-8.

BARNABAS B., KOVACS G., 1997 - *Storage of pollen*, pp. 293-314. - In: SHIVANNA K.R., and V.K. SAWHNEY (eds.). *Pollen biotechnology for crop production and improvement.* Cambridge University Press, UK, pp. 448.

CRANE J.C., IWAKIRI B.T., 1981 - *Morphology and reproduction of pistachio.* - Hort. Rev., 3: 376-393.

FAO, 2016 - *FAOSTAT. Agricultural Statistics Database*. - FAO. Food and Agriculture Organization of the United Nations, Rome, Italy.

GOLAN-GOLDHIRSH A., SCHMIDHALTER U., MÜLLER M., OERTLI J.J., 1991 - *Germination of* Pistacia vera *L. pollen in liquid medium.* - Sex. Plant Reprod., 4(3): 182-187.

GÜNVER-DALKILIÇ G., DAYI-DOGRU Ö., 2011 - *Determination of pollen grain viability and germination levels for pistachio and terebinth in Aydin/Turkey ecology*. - Pak. J. Bot., 43(2): 841-848.

HOSSEINI N., BAHRAMABADI E.Z., REZANEJAD F., 2015 - *Study of morphological and anatomical traits of male flower, developmental stages of anther and pollen grain of pistachio (*Pistacia vera *L.)*. - Iran. J. Biol., 28(1): 116-125.

POLITO V.S., LUZA J.G., 1988 - *Low temperature storage of pistachio pollen*. - Euphytica, 39: 265-269.

RATHOD V., BEHERA T.K., MUNSHI A.D., DURGESH K., JAT G.S., KRISHNAN B.G., SHARMA N., 2018 - *Pollen viability and* in vitro *pollen germination studies in* Momordica *species and their intra and interspecific hybrids.* - Inter. J. Chem. Studies, 6(6): 32-40.

SANJAY K.K., KHANDURI V.P., KAR K., MOHAN SHARMA Ch., RIYAL M.K., 2016 - *Effect of growth regulators and time on* in vitro *pollen germination in three ornamental tropical tree species*. - J. Agr. Sci. Tech., 18(5): 1247-1255.

SCHORI Y., GOREN T., BEN-JACOV J., 1992 - *Pollen germination and storage in banksia and some other* Proteaceae *plants*. - Acta Horticulturae, 316: 19-20.

SHIVANNA K.R., LINSKENS H.F., CRESTI M., 1991 - *Pollen viability and pollen vigor*. - Theor. Appl. Genet., 81(1): 38-42.

SHIVANNA K.R., RAM H.Y.M., 1993 - *Pollen biology: Contribution to fundamental and applied aspects*. - Current Sci., 65(3): 226-233.

SHIVANNA K.R., SAWHNEY V.K., 1997 - *Pollen biology and pollen biotechnology: an introduction*, pp. 1-12. - In: SHIVANNA K.R., and V.K. SAWHNEY (eds.) *Pollen biotechnology for crop production and improvement.* Cambridge University Press, UK, pp. 448.

TILKAT E., ONAY A., 2009 - *Direct shoot organogenesis from* in vitro *derived mature leaf explants of pistachio*. -

In Vitro Cell. Dev. Biol. - Plant, 45: 92-98.
VAKNIN Y., EISIKOWITCH D., 2000 - *Effects of short term storage on germinability of pistachio pollen*. - Plant Breed., 119: 347-350.
VITHANAGE H.I.M.V., ALEXANDER D.Mc.E., 1985 - *Synchronous flowering and pollen storage techniques as aids to artificial hybridization in pistachio (*Pistacia *spp.).* - J. Hort. Sci., 60: 107-113.