Effect of Storage Method on Date Palm and Pistachio Pollen Viability

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ABSTRACT

This study was conducted on date palm and pistachio to establish short-, mid- and long-term pollen storage technique. Pollen was stored in room temperature (24 ^{o}C±2), refrigerator (4^{o}C), freezer (-5^{o}C), and cryogenic storage (immersing 15 minutes in liquid nitrogen then stored in deep freezer -80^{o}C) for up to 52 weeks. New *in vitro* germination medium was modified to test date palm pollen, which consisted of 1% agar, 8% sucrose and 20 ppm citric acid. In addition, pollen viability was tested by using 1% 2, 3, 5–triphenlytetrazolium chloride (TTC) and 30% sucrose for date palm, and 1% TTC and 60% sucrose for pistachio. Cryopreserved pollen of date palm gave the best results; refrigerated pollen was conveniently stored for long period, while frozen pollen was conveniently stored for medium period. On the other hand, cryopreserved pollen of pistachio could be stored for 4 weeks, while in refrigerator and freezer just for 2 weeks.

Keywords: 2, 3, 5–Triphenlytetrazolium Chloride, Cryopreservation, Date Palm, *in vitro* Germination, Pistachio, Viability.

INTRODUCTION

Date palm (*Phoenix dactylifera L)* and pistachio (*Pistacia vera* L.) are *dioecious* species, i.e. male and female flowers being produced on separate trees. Date palm is one of the oldest fruit crops grown in the arid regions of the Arabian Peninsula, North Africa, and the Middle East. Flowers are born on a compound spadix in leaf axils, inflorescences are sheathed in a bract or spathe until just prior to anthesis. Each sex produces thousands of tiny flowers per inflorescence. Male flowers are white, fragrant with six stamens each, and females are more yellowish or creamy colored (Chao and Krueger, 2007). While pistachio is cultivated widely in the Mediterranean regions of Middle East, North Africa, Europe and California. Both staminate and pistillate inflorescences are panicle consisting of up to several hundred flowers; each male flower has five or six anthers (Crane and Iwakiri, 1981).

Storing pollen is very important for cross-pollination, crop breeding, plant biodiversity and its conservation, and for other biological (such as physiology, biochemistry, and biotechnology) and non-biological studies (such as petroleum exploration, archaeology, criminology) (Ganeshan, 1998; Towill and Walters, 1998; Shivanna, 2003). Optimum pollen storage conditions can vary greatly from one species to another (Shivanna and Johri, 1985). Deep freeze of Kiwifruit pollen at -80°C kept them viable for 160 weeks (Bomben *et al*., 1999). Cryopreservation of papaya pollen retained viability as fresh pollen for 485 days (Ganeshan, 1986), and olive pollen for 365 days (Ateyyeh, 2009).

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Pistachio pollen was stored for up to four months at -20 C (Polito and Luza, 1988a). In another study, pollen kept under deep freezer for six days was drastically and irreversibly reduced (Vaknin and Eisikowitch, 2000). On the other hand, date palm pollen stored either at room temperature or in a refrigerator had higher viability than in deep freezer storage (Shaheen *et al*., 1986). Boughediri and Bounanga (1991) stored date palm pollen in desiccators containing anhydrous calcium chloride and placed them in 4ºC for 230 days to maintain pollen viable. Boughediri *et al*. (1995) reported that freeze-drying was the optimal condition for maintaining long term viability of date palm pollen. Recently, Mortazavi *et al*. (2010) have found that, long-term storage of date pollen using an ultra-low temperature (-196°C) can be used without any deteriorating effect on pollen viability. Therefore, this research aims to develop simple reliable methods for short-, mid- and long-term pollen storage techniques for Date palm and pistachio.

MATERIALS AND METHODS

Plant material: date palm pollen was collected from the Agricultural Research Station in the Jordan valley that belongs to I***nstitute of Agricultural Research, Training, Extension and Education,*** The University of Jordan located at latitude 32º 05' N, longitude 35 º 35' E, and altitude -267 m. Pistachio pollen was collected from Mushaqar Agricultural Station that belongs to The National Center for Agricultural Research and Extension, Ministry of Agriculture located at latitude 31º 46' N, longitude 35º 48' E, and altitude 800 m. The experiment was conducted in the laboratories of Department of Horticulture and Crop Science, Faculty of Agriculture, The University of Jordan.

Pollen collection and storing: inflorescences of date palm and pistachio (The cultivars used for date palm and pistachio must be specified) were collected during May, 2006 and 2007. The flowers were at balloon stage just before anthesis. The inflorescences were spread over white papers on the bench in the laboratory at room temperature (24 ºC±2) for one day, this procedure allowed anthers to dehisce, and then, pollen of each species was collected in 12 glass tubes. Pollen in open glass tubes was dehydrated for 4 hrs in desiccators, which partially filled with dry silicagel. Dehydrated pollen was stored for twelve months in the following storage conditions (three glass tubes in each storage condition): a- room temperature (24 ºC±2), b- refrigerator (4ºC), c- freezer (-5ºC) and d- cryopreservation under -80 ºC over twelve months. Pollen viability and germinability were tested after 0, 2, 4, 8, 18, 28, 40 and 52 weeks of anthesis.

Cryopreservation: for each species, dehydrated pollen in three glass tubes was transferred into another three cryotubes, which were subsequently sealed and immersed directly in liquid nitrogen for at least 15 minutes. Thereafter, cryotubes were stored in a freezer at –80◦C.

In vitro pollen germination test: pistachio pollen was rehydrated and germinated *in vitro* on medium consisted of only 20% sucrose as described by Vaknin and Eisikowitch (2000). On the other hand, media of previous studies were used to test pollen germination of date palm; unfortunately, these media gave bad results; therefore a preliminary study was conducted to find the suitable germination medium. Many combinations of agar, sucrose and citric acid or boric acid were tested. In all cases, the petridishes were covered with aluminum foil, and then incubated in chamber room at 27ºC for 12 hours. Number of germinated pollen was calculated under light microscope at (10X) magnification power. Fresh pollen of each species was tested *in vitro* as indicator for all treatments.

Pollen viability test: TTC (2, 3, 5–triphenlytetrazolium

chloride) stain test was used in this research study. To find the best TTC-sucrose combination for date palm and pistachio pollen, different concentrations of TTC (0.2, 0.4, 0.6, 0.8, and 1 %) and sucrose (5, 10, 20, 40, 60 and 70%) were tested. One drop of TTC-sucrose solution was placed on a microslide, then a small amount of pollen was suspended in that drop and a coverglass was placed onto the microslide. The covered microslide was wrapped with aluminum foil and then incubated in the chamber room at 30 ± 2 °C for 60 min. Each TTC-sucrose combination was repeated five times, about two hundred pollen of each replicate from four different areas were counted under a light microscope.

Data Analysis: Statistical Analysis System from SAS Institute Corporation (SAS Institute Inc., 1999) was used to analyze the results. Pollen germinability and viability percentages of fresh pollen were analyzed by one way analysis of variance. The comparisons among the treatments were carried out by LSD test. Pollen storage results were analyzed using split-plot in time, least squares means were sorted with the pdmix800 macro (Saxton, 1998).

RESULTS

Preliminary experiments

***In vitro* germination of date palm pollen:** many combinations of agar, sucrose, citric acid and boric acid were discarded because they gave no results or very low germination percentage. Germination medium consists of 1% agar, 8% sucrose and 20ppm citric acid was significantly higher than the others, so, it was used in the rest of the experiment (Table 1).

Table 1: Effect of different combinations of agar, sucrose, and citric or boric acid on *in vitro* pollen germination of date palm.

Agar	Sucrose	Citric acid	Germination %	Agar	Sucrose	Boric acid	Germination %
1%	2%	40 ppm	69.4 B	1%	2%	40 ppm	50.9 C
1%	2%	60 ppm	21.5 G	1%	2%	60 ppm	35.8 E
1%	6%	20 ppm	29.1 F	1%	6%	20 ppm	40.3 D
1%	8%	20 ppm	75.8 A	1%	8%	20 ppm	29.2 F

Means in columns having the same letters are not significantly different at P=0.05

TTC viability test: many combinations of TTC and sucrose in viability test were discarded because they gave no results or very low viability percentage. Viability percentage of the combinations (1% TTC and 30% sucrose) for date palm pollen and (1% TTC and 60% sucrose) for pistachio were significantly higher than the others, so, they were used to test pollen viability in the rest of the experiment (Table 2). For pistachio, germination% of fresh pollen at the first season was significantly higher than the second season (Table 3).

A clear discussion of the differences in results of the two seasons (2006/2007 and 2007/2008) is lacking. In other words, the reason(s) of the variation between the two seasons should be explained in Discussion section.

Table 2: Effect of different combinations of 2, 3, 5–triphenlytetrazolium chloride (TTC) and sucrose on viability of fresh pollen of date palm and pistachio.

Date palm			Pistachio		
TTC	Sucrose	Viability %	TTC%	Sucrose %	Viability %
1%	10%	47.9 B	1%	50%	63.6 C
1%	30%	79.3 A	1%	60%	87.4 A
1%	60%	78.7 A	1%	70%	79.1 B

Means in the same column having the same letters are not significantly different at P=0.05

Date Palm.

First season: germination % of pollen was significantly reduced in room temperature after 2 weeks, while viability % was significantly reduced in room temperature, freezer and refrigerator (Table 4). Meanwhile, cryopreserved, refrigerated and frozen pollen had significantly the highest germination%; viability % of cryopreserved pollen was the highest but not significantly higher than frozen pollen. After 4 weeks, there was significant reduction in germination and viability % in all storage conditions in comparison to fresh pollen. Cryopreserved pollen had significantly the highest germination% but not significantly higher than frozen and refrigerated pollen, also the highest viability% but not significantly higher than frozen pollen. After 8 weeks, cryopreserved pollen had significantly the highest germination and viability % but not significantly higher than frozen and refrigerated pollen. After 18 and 28weeks, cryopreserved pollen had significantly the highest germination and viability %; besides, room-stored pollen didn't germinate after 28 weeks. After 40 weeks, room-stored pollen lost its viability, and germination% of frozen pollen reduced dramatically. After 52 weeks, Cryopreserved pollen still had significantly the highest germination and viability % (Table 4).

Table 3: Germination and viability% of fresh pollen of date palm and pistachio for both seasons.

Species	Date Palm		Pistachio	
	Germination %	Viability %	Germination %	Viability %
2006/2007	77.6 A	86.5 A	74.4 A	87.4 A
2007/2008	76.8 A	83.1 A	67.5 B	85.8 A

Means in the same column having the same letters are not significantly different at P=0.05.

Table 4. Effect of storage conditions on pollen germination and viability of date palm during 2006/2007.

Storage period	Germination %				Viability %			
	Room temp.	Refrig.	Freezer	Cryo.	Room temp.	Refrig.	Freezer	Cryo.
0 weeks	77.6 a	77.6 a	77.6 a	77.6 a	86.5 a	86.5 a	86.5 a	86.5 a
2 weeks	68.2 def	76.7 a	75.6 ab	74.5 ab	67.7 k	79.5 cde	82.6 bc	85.4 ab
4 weeks	45.9 k	71.1 cd	70.8 cd	73.2 bc	55.4 l	78.1 ef	80.7 cde	82.1 bcd

Storage period	Germination %				Viability %			
	Room temp.	**Refrig.**	**Freezer**	**Cryo.**	**Room temp.**	**Refrig.**	**Freezer**	**Cryo.**
8 weeks	28.7 l	66.8 ef	66.9 ef	68.8 de	43.9 m	77.2 efg	78.4 def	80.6 cde
18 weeks	16.1 o	60.1 hi	63.6 gh	67.6 ef	38.0 n	74.8 fghi	75.1 fgh	79.7 cde
28 weeks	0.0 p	59.3 i	59.5 i	67.5 ef	29.6 o	73.6 ghij	72.5 hij	78.1 ef
40 weeks	0.0 p	57.6 ij	23.2 m	65.8 efg	0.0 p	71.3 ijk	70.7 jk	75.6 fgh
52 weeks	0.0 p	55.4 j	19.7 n	65.2 fg	0.0 p	67.9 k	69.9 jk	75.3 fgh
	Means in columns and rows having the same letters are not significantly different at P=0.05.				Means in columns and rows having the same letters are not significantly different at P=0.05			

Second season: after 2 weeks, germination and viability % of room-stored and refrigerated pollen were reduced significantly (Table 5). Meanwhile, cryopreserved pollen had significantly the highest germination and viability % but not significantly higher than frozen pollen. After 4 weeks, there was a significant reduction in germination and viability % in all storage conditions in comparison to fresh pollen except viability% in cryogenic storage. Cryopreserved pollen had significantly the highest germination% but not significantly higher than frozen and refrigerated pollen; also the highest viability %, but not significantly higher than frozen pollen. After 8 weeks, viability % of cryopreserved pollen was significantly reduced in comparison to fresh pollen. Cryopreserved pollen had significantly the highest germination and viability %, but germination % was not significantly higher than frozen and refrigerated pollen.

Table 5. Effect of storage conditions on pollen germination and viability of date palm during 2007/2008.

Storage period	Germination %				Viability %			
	Room temp.	**Refrig.**	**Freezer**	**Cryo.**	**Room temp.**	**Refrig.**	**Freezer**	**Cryo.**
0 weeks	76.8 a	76.8 a	76.8 a	76.8 a	83.1 a	83.1 a	83.1 a	83.1 a
2 weeks	60.2 f	72.9 b	74.3 ab	76.6 a	58.4 j	76.8 def	82.0 ab	82.3 a
4 weeks	55.7 hi	69.4 cd	69.2 cd	72.5 bc	50.2 k	76.0 efg	78.2 cde	80.7 abc
8 weeks	18.7 j	66.5 de	67.7 d	69.5 cd	39.0 l	75.6 efg	75.8 efg	79.1 bcd
18 weeks	6.1 k	59.3 fg	59.4 fg	68.6 d	30.0 m	75.2 efg	73.1 g	76.7 def
28 weeks	0.0 l	57.7 fgh	57.0 fghi	68.1 d	26.0 n	74.4 fg	69.2 h	75.4 efg
40 weeks	0.0 l	55.7 hi	18.4 j	67.3 de	0.0 o	73.2 g	68.3 hi	74.6 fg
52 weeks	0.0 l	53.9 i	16.1 j	67.2 de	0.0 o	65.4 i	67.9 hi	74.1 fg
	Means in columns and rows having the same letters are not significantly different at P=0.05.				Means in columns and rows having the same letters are not significantly different at P=0.05			

After 18 weeks, cryopreserved pollen had significantly the highest germination and viability %, but viability % was not significantly higher than refrigerated pollen. After 28 weeks, room-stored pollen didn't germinate. Cryopreserved pollen had significantly the highest germination and viability %, but viability % was not significantly higher than refrigerated pollen. After 40 weeks, room-stored pollen lost its viability, in addition, germination% of frozen pollen reduced dramatically. Cryopreserved pollen had significantly the highest germination and viability %, but viability % was not significantly higher than refrigerated pollen. After 52 weeks, cryopreserved pollen had significantly the highest germination and viability%.

Pistachio.

First season: after 2 weeks, germination and viability% was reduced significantly in all storage conditions except germination% in refrigerator and cryogenic storage (Table 6). Meanwhile, cryopreserved pollen had significantly the highest germination and viability %, but germination % was not significantly higher than refrigerated pollen. After 4 weeks, room-stored, refrigerated and frozen pollen didn't germinate and lost its viability. Germination and viability % of cryopreserved pollen was significantly reduced. After that, germination and viability % of cryopreserved pollen were significantly reduced. Finally, cryopreserved pollen didn't germinate after 28 weeks and lost its viability after 40 weeks.

Table 6 Effect of storage conditions on pollen germination and viability of pistachio during 2006/2007.

Storage period	Germination %				Viability %			
	Room temp.	Refrig.	Freezer	Cryo.	Room temp.	Refrig	Freezer	Cryo.
0 weeks	74.4 a	74.4 a	74.4 a	74.4 a	87.4 a	87.4 a	87.4 a	87.4 a
2 weeks	18.1 e	72.1 a	65.1 b	71.7 a	22.6 f	42.4 d	75.2 c	80.2 b
4 weeks	0.0 g	0.0 g	0.0 g	58.2 c	0,0 g	0,0 g	0,0 g	73.1 c
8 weeks	0.0 g	0.0 g	0.0 g	22.6 d	0,0 g	0,0 g	0,0 g	35.9 e
18 weeks	0.0 g	0.0 g	0.0 g	3.0 f	0,0 g	0,0 g	0,0 g	21.2 f
28 weeks	0.0 g	0.0 g	0.0 g	0.0 g	0,0 g	0,0 g	0,0 g	19.7 f
	Means in columns and rows having the same letters are not significantly different at P=0.05.				Means in columns and rows having the same letters are not significantly different at P=0.05			

Second season: after 2 weeks, germination and viability% was reduced significantly in all storage conditions except viability% in liquid N (Table 7). Meanwhile, cryopreserved pollen had significantly the highest germination and viability % but not significantly higher than frozen pollen. After 4 weeks, room-stored and refrigerated pollen didn't germinate. Cryopreserved pollen had significantly the highest germination and viability %. After 8 and 18 weeks, room-stored, refrigerated and frozen pollen didn't germinate and lost its viability, in addition, germination and viability % of cryopreserved pollen were significantly reduced. Finally, cryopreserved pollen didn't germinate and lost its viability after 40 weeks.

Table 7. Effect of storage conditions on pollen germination and viability of pistachio during 2007/2008.

Storage period	Germination %				Viability %			
	Room temp.	**Refrig.**	**Freezer**	**Cryo.**	**Room temp.**	**Refrig**	**Freezer**	**Cryo.**
0 weeks	67.5 a	67.5 a	67.5 a	67.5 a	85.8 a	85.8 a	85.8 a	85.8 a
2 weeks	20.6 f	55.8 c	58.5 bc	61.3 b	43.7 e	71.4 c	79.0 b	82.5 ab
4 weeks	0.0 i	0.0 i	18.2 f	49.4 d	18.2 h	38.7 f	65.3 d	77.4 b
8 weeks	0.0 i	0.0 i	0.0 i	29.4 e	0.0 i	0.0 i	0.0 i	69.1 c
18 weeks	0.0 i	0.0 i	0.0 i	8.0 g	0.0 i	0.0 i	0.0 i	36.6 f
28 weeks	0.0 i	0.0 i	0.0 i	2.2 h	0.0 i	0.0 i	0.0 i	22.3 g
	Means in columns and rows having the same letters are not significantly different at P=0.05.				Means in columns and rows having the same letters are not significantly different at P=0.05			

Discussion

Tetrazolium used to test pollen viability, but it has many limitations. *In vitro* germination test is the most commonly used and acceptable test, but this decision is restricted by the optimum germination media (Shivanna, 2003). Mortazavi *et al*. (2010) have found that maximum germination was achieved on medium containing 50 mg/l boric acid, 200 mg/l calcium nitrate and 15% (w/v) sucrose. In the current study, the germination medium improved to achieve the highest germination%, and it consisted of 1% agar, 8% sucrose and 20 ppm citric acid. On the other hand, medium consisted of 20% sucrose was satisfied for pistachio (Vaknin and Eisikowitch, 2000), whereas Polito and Luza (1988a) used medium consisted of 20% sucrose and1% agar, and AK *et al*. (1995) used two media, one consisted of 5% sucrose and 1% agar, and the other consisted of 15% sucrose, 1% agar, 0.03% $CaNO_3$ and 20 ppm boric acid.

Under room conditions, date palm pollen had lost their longevity gradually by the time; after 28 weeks the pollen didn't germinate, and after 40 weeks the pollen was nonviable in both seasons. While the pollen of pistachio had lost their longevity very quickly; in the first season, the pollen didn't germinate and was nonviable 4 weeks after anthesis, in the second seasons, pollen lost its viability after 8 weeks. Many factors affect pollen longevity such as genetic variation between species, abiotic environmental conditions, nutritional and physiological conditions under which the plants are grown, and the methods of pollen collection and storage (Baja, 1987; Barnabas and Kovacs, 1997). Longevity has been found to be greatest at relative humidity between 6 and 60% for most species, with optimum conditions typically being considerably below 60% {Stanley and Linskens, 1974). Therefore, pollen of the two species was dehydrated in a desiccator. Concerning pistachio, rehydration after storage was very critical for pollen germination (Polito and Luza, 1988b).

Theoretically, storing pollen in liquid nitrogen for many years is possible, because biological activity is stopped (Withers 1991). Cryopreserved pollen of date palm had the best results in short-, mid- and long-term storage in both growing seasons, the same results found by Mortazavi *et al*. (2010). On the other hand, cryopreserved pollen of pistachio had the best results in short storage, but unfortunately, the results in mid-term storage was very poor and in long-term storage was failed, the same results found by Polito and Luza,

(1988b). Other species were successfully cryopreserved for long period such as walnut (Juvenal and Polito, 1985), mango (Custodio *et al*., 2006), *oil palm* (Tandon *et al*., 2007) *and* Pecan (Conner, 2011).

Refrigerated pollen of date palm was conveniently stored for short-, mid- and long-periods, while frozen pollen was conveniently stored for short-, and mid-periods, these results agreed with Shaheen *et al*. (1986), but disagreed with Al-Helal (1995) who found that freezing pollen of date palm was more convenient than refrigeration for long storage periods.

Pistachio pollen could be stored in refrigerator and freezer just for 2 weeks, which is enough for artificial cross pollination purpose, if the difference in flowering period between males and females didn't exceed 2 weeks. Vaknin and Eisikowitch (2000) found that refrigerated pollen of pistachio retained their germinability for at least a week, while the germinability of pollen kept under deep freezer storage for 6 days was drastically and irreversibly reduced.

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REFERENCES

Ak, B., A. Ozguven and Y. Nikpayam, 1995. An investigation on determining the ability of some *Pistacia spp*. pollen germination. Acta Horticulturae. 419: 43-48.

Al-Helal, A. 1995. Response of date palm pollen tube growth to storage period and conditions. Qatar University Science Journal. 14:71-75.

Ateyyeh, A. 2009. Storing pollen of two olive varieties 'Rasie' and 'Nabali Baladi'. International Journal of Fruit Science. 74:255-270.

Baja, Y. 1987. Cryopreservation of pollen and pollen embryos, and the establishment of pollen banks. In: Pollen cytology and development. K. Giles and J. Pracash (ed.). International Review of Cytology. 107: 397–418.

Barnabas, B. and G. Kovacs. 1997. Storage of pollen, In: Pollen biotechnology for crop production and improvement. K. Shivanna and V. Sawhney (ed.). Cambridge University press, UK. p. 293-314.

Bomben, C., C. Malossini, G. Cipriani, and R. Testolin. 1999. Long term storage of kiwifruit pollen. Acta Horticulturae. 498:105-110.

Boughediri, L. and N. Bounanga. 1991. Storage of date palm pollen (*Phoenix dactylifera* L.), I-Preliminary results. Annales des Sciences Naturelles. 11:119-124.

Boughediri, L., M. Cerceau-Larrival and J. Dore. 1995. Significance of freeze- drying in long term storage of date palm pollen. Grana Palynologica. 34:408-412.

Chao, C. and R. Krueger. 2007. The date palm *(Phoenix dactylifera L.):* Overview of biology, uses, and cultivation. HortScience. 42:1077-1082.

Conner, P. 2011. Optimization of *in vitro* Pecan Pollen Germination. HortScience. 46:571-576.

Crane, J. and Iwakiri B. 1981. Morphology and reproduction of pistachio. Horticultural Reviews. 3:376-393.

Custodio, L., N. Fernandes, M. Carneiro and A. Romano. 2006. Cryopreservation of pollen of carob tree. Acta Horticulturae. 725:863-868.

Ganeshan, S. 1986. Cryogenic preservation of papaya pollen. Scientia Horticulturae. 28:65-70.

Ganeshan, S. 1998. Pollen storage in tropical fruits, In: Tropical fruits in Asia diversity, maintenance, conservation and use. R. Arora and R. Ramanatha (ed.). IPGRI. p. 120-125.

Juvenal, G. and V. Polito. 1985. *In vitro* germination and storage of english walnut pollen. Scientia Horticulturae. 27:303-316

Mortazavi, S., K. Arzani and A. Moieni.2010. Optimizing storage and in vitro germination of date palm (*Phoenix dactylifera*) pollen. Journal of Agricultural Science and Technology. 12:181-189

Polito, V. and J. Luza. 1988a. Longevity of pistachio pollen determined by in vitro germination. Journal of the American Society for Horticultural Science. 113:214-217.

Polito, V. and J. Luza. 1988b. Low temperature storage of pistachio pollen. Euphytica 39:265-269.

SAS Institute Inc. 2008. SAS/STAT software, Version 9.2, Cary, North Carolina, USA.

Saxton, A. 1998. A macro for converting mean separation output to letter groupings in proc mixed. Proceedings of the twenty-third SAS users group international conference. Cary, NC, SAS Institute Inc. pp 1243-1246.

Shaheen, M., T. Nasr and M. Bacha. 1986. Date palm pollen viability in relation to storage conditions. Second symposium on date palm, King Faisal Univ., Al-Hasa, Saudi Arabia. 331-336.

Shivanna, K. 2003. Pollen biology and biotechnology. Science Publishers, USA. P. 316.

Shivanna, K. and B. Johri. 1985. The Angiosperm pollen, structure and function. Wiley, New Delhi, India. P. 374.

Stanley, R. and H. Linskens. 1974. Pollen: Biology, biochemistry and management. Springer-Verlag, New York, USA. P. 307.

Tandon, R., R. Chaudhury and K. Shivanna. 2007. *Cryopreservation of oil palm pollen*. Current Science. 92:182-183

Towill, L. and C. Walters. 1998. Cryopreservation of pollen, In: Cryopreservation of tropical plant germplasm. Current research progress and application. F. Engelmann and H. Takagi. (ed.). JIRCAS, the Japan International Research Center for Agricultural Sciences. Japan. p. 115-129.

Vaknin, Y and D. Eisikowitch. 2000. Effects of short term storage on germinability of pistachio pollen. Plant Breeding. 119:347-350.

Withers, L. 1991. Biotechnology and plant genetic resources conservation, In: Plant genetic resources-conservation and management. Concepts and Approaches. R. Paroda and R. Arora (ed.). New Delhi, India. p. 274-297.

تأثير طريقة التخزين في حيوية حبوب لقاح نخيل التمر والفستق الحلبي

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ملخـص

أجريت هذه الدراسة على نخيل التمر والفستق الحلبي لإيجاد تقنية لتخزين حبوب اللقاح على المدى القصير والمتوسط والطويل. تم تخزين حبوب اللقاح في درجة حرارة الغرفة (24 °م ±2) وفي الثلاجة (4 °م) وفي المجمد (5- °م) وفي فائق التجمد (تخزينها في مجمد 80- °م بعد غمسها بالسائل النيتروجيني 196- °م لمدة ربع ساعة) لمدة تصل الى 52 اسبوعا. تم تطوير وسط انبات جديد لفحص قدرة حبوب لقاح النخيل على الانبات مخبريا، يتكون هذا الوسط من 1% اجار و 8% سكروز و 20 جزءا من المليون حمض الليمون. بالاضافة الى ذلك تم اختبار حيوية حبوب اللقاح باستخدام 1% كلوريد التترازوليوم و 30% سكروز لنخيل التمر واستخدام 1% كلوريد التترازوليوم و 60% سكروز للفستق الحلبي. بينت الدراسة أن تخزين حبوب لقاح النخيل في فائق التجمد اعطى افضل النتائج، وكان التخزين المبرد مقنعا لحفظ حبوب اللقاح لفترة طويلة، بينما كان التخزين المجمد مقنعا لحفظ حبوب اللقاح لفترة متوسطة. من ناحية اخرى، يمكن تخزين حبوب لقاح الفستق الحلبي في فائق التجمد لمدة 4 اسابيع، بينما في الثلاجة والمجمد لمدة اسبوعين.

الكلمات الدالة: كلوريد التترازوليوم، فائق التجمد، نخيل التمر، الانبات مخبريا، الفستق الحلبي، الحيوية.

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