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Factors Regulating *In Vitro* Germination of Date Palm Pollen Grains After Storage

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العوامل التي تنظم إثبات حبوب لقاح نخيل البلح بعد خزنها

الملخص: تم خزن ثلاث من أنواع حبوب اللقاح المشهورة في السلطنة (خوري، متجدل، بهلاني) لمدة ٦ و ١٢ شهراً في الثلاجة (٤- ٥ ^{0}م)، في البراد (- ١٨ ^{0}م) وتحت ٢٣-٢٥ ^{0}م. تم تحديد نسبة الإنبات في وسط سائل تحت ظروف هوائية و لا هوائية. أوضحت النتائج أن الثلاثة أنواع من حبوب اللقاح أعطت نسب إنبات أعلى عند خزنها في الثلاجة عن خزنها في البراد، بينما أدى الخزن في ٢٣٢٣-٢٥ ^{0}م إلى إنخفاض الإنبات وكانت الفروق معنوية أدت عملية التهوية إلى تحسين نسبة الإنبات بعد الخزن في الثلاجة و البراد، وخفضها بعد الخزن في الغرفة لمدة ٦ أشهر. كما أوضحت النتائج أن التخزين في الثلاجة قد يتسبب في تثبيط الإنبات و لكن يمكن إزالة هذا الأثر بإطالة مدة التخزين أو بواسطة التهوية، في حين أن التخزين في ٢٣-٢٥ ^{0}م يؤدي مباشرة إلى خفض حيوية حبوب اللقاح ولم يؤد إلى تثبيط الإنبات.

ABSTRACT: Dried pollen grains of three date palm (male) cultivars grown in Oman were stored either in a freezer (-18°C), a refrigerator (4 to 5°C), or at room temperature (23 to 25°C) for 6 or 12 months. The three cultivars include Khori, Medjahdil, and Bahlani. Germination percentage was determined after 6 hours incubation in aerated and non-aerated liquid media. The results showed that date palm pollen was better adapted to refrigerator storage than freezing for all three cultivars, while room temperature storage significantly reduced pollen germination. Aeration was found to enhance germination of refrigerator- and freezer-stored pollen, but decreased that of pollen stored at room temperature for 6 months. The results also indicated that refrigeration might have caused the inactivation of pollen germination. The latter could either be overcome by aeration or reversed if pollen is refrigerated for a longer period. Room temperature storage directly reduced viability and did not induce temporary inactivation of pollen.

Specific temperature and moisture levels are required for pollen grains to retain viability and germinate through long periods of storage. These requirements may vary according to the adaptation characteristics of the pollen genotype at pre-harvest or during storage conditions. Experiments on date palm pollen in Algeria (Cauvet, 1914) and Saudi Arabia (Abo-Hassan *et al.*, 1982; Bacha *et al.*, 1988) showed that pollen stored at room temperature retained viability and produced fruit-set as effectively as pollen stored in the refrigerator. However, pollen germination percentages and fruit-set of refrigerated- and room-stored pollen were higher than those of pollen stored in the freezer. These results are in conflict with those provided by experiments in California (Stout, 1924; Crawford, 1938; Aldrich and Crawford, 1941), Egypt (Brown and Bahgat, 1938), and the Sultanate of Oman (El Mardi *et al.*, 1996). These studies indicated that pollen stored at room temperature produced poorer fruit-set and germination percentages than refrigerator-stored pollen.

Investigations on fruit set in Algeria (Pereau-Leroy, 1957) revealed that pollination during late morning to early afternoon resulted in higher fruit-set percentages than pollination during late afternoon. However, pollination during the same period in Egypt (Moustafa *et al.*, 1986) resulted in lower fruit-set percentages as compared to late afternoon pollination. These studies indicated that variations in the fruit-set percentages might be attributed to the different ecological features in the areas where date palms are

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grown. On the other hand, experiments on pecan pollen, which is representative of a temperate zone plant, revealed different adaptation characteristics from those of the date palm pollen. Yates and Sparks (1989; 1990) and Yates *et al.* (1991) reported that viability of undried pollen of pecan was higher when stored at −196°C than at −80°C. However, they observed no significant differences in fruit-set percentages of pollen stored under these conditions. For pecan pollen to be stored at higher temperatures, pre-storage oven drying at 35°C was found necessary. Pre-germination conditioning of dry pollen at a relative humidity of 97.2% and a temperature of 25°C resulted in higher germination percentages at −12°C than at either 5°C or 23°C. Thus, freezer-stored pollen grains had a higher germination percentage than refrigerator-stored ones. Room temperature storage was found to be the most unsuitable mode of storage for pecans.

The present study was aimed at identifying the post-harvest factors that influence *in vitro* germination of date palm pollen grains.

Material and Methods

Spadices of three date palm male cultivars commonly used in Oman, namely Khori, Medjahdil, and Bahlani, were harvested and dried for 72 hours at 28°C. A machine designed by the Agricultural and Water Resources Center, Iraq, for extracting pollen grains from the spadices was used. The pollen was stored under the temperature conditions of deep freeze (-18°C), refrigerator (3 to 4°C), and room temperature (23 to 25°C). The relative humidity was controlled using either $CaCl_2$ or silica gel contained in a desiccator. To account for a variety of environmental conditions under which pollen grains grow, the experiment was conducted, independently, for pollen grains of each variety produced in 1990, 1991, and 1992.

Pollen germination was tested in a modified Brewbaker and Kwack liquid medium as described by Furr and Enriquez (1966), at a concentration of 100-mg pollen per 50-ml solution, using 12.5 ml of the medium in 100-ml Erlenmeyer flasks. Half of the cultures were aerated while the other half were non-aerated. Every six months, germination percentages were determined after 6 hours of incubation at 25°C in a water bath on hemacytometer fields using a light microscope (100x).

For each variety, a full factorial design model with three replications was fitted to the data. The analysis was carried-out using the SAS statistical software (release 6.12). The Duncan's multiple ratio test (DMRT) was used to compare means, wherever appropriate.

Results

The results of the analysis of variance of the data for the three varieties are summarized in Table 1. The data were first analyzed with the year of pollen as a factor. However, the effect was negligible in each case, justifying treating the three years as replications for the analysis to provide more precise error estimations. About 87% of the variability in the data were explained by the models that included up to four levels of interaction. Checks on the residuals from each model indicated that they were approximately normal with constant variances. Although, a few observations had unusually large residuals in each case, the exclusion of these observations did not significantly change the results.

KHORI VARIETY: There were strong three-way interactions among storage type, aeration, and storage period ($p < 0.05$) (Table 1). The four-way interactions were also statistically significant ($p < 0.02$). Other two-way interactions and main effects were significant. However, their interpretation would be misleading due to the presence of the higher order interactions.

Table 2 shows the mean germination percentages of pollen based on the four-way interactions. Cold-stored

TABLE 1

Analysis of variance of germination percentages of date palm pollen grains from 3x2x2x2 factorial design experiments, with three replications for the three varieties.

Source of Variation	Degrees of Freedom	Khori	Medjahdil	Bahlani
Storage (A)	2	4712.9**	6695.7**	4528.9**
Dessicant (B)	1	94.5^{NS}	43.4^{NS}	0.1^{NS}
Aeration (C)	1	404.3**	870.1**	634.9**
Period (D)	1	386.9**	239.4*	627.8**
AxB	2	112.5*	36.2^{NS}	283.0**
AxC	2	293.6**	548.2**	555.1**
AxD	2	877.0**	812.9**	1058.4**
BxC	1	5.6^{NS}	77.9^{NS}	699.4**
BxD	1	243.1**	21.2^{NS}	10.3^{NS}
CxD	1	28.8^{NS}	123.5^{NS}	0.0^{NS}
AxBxC	2	68.3^{NS}	76.4^{NS}	63.9^{NS}
AxBxD	2	58.5^{NS}	87.5^{NS}	378.3**
AxCxD	2	154.9*	194.2*	792.1**
BxCxD	1	83.0^{NS}	104.9^{NS}	4.9^{NS}
AxBxCxD	2	194.7*	49.7^{NS}	343.8**
Error	48	44.0	55.8	55.8
R^2		0.871	0.873	0.871
Root MSE		6.63	7.47	7.47

F = (14.03, 14.39, and 14.02, respectively, with $p < 0.001$).
*Significant at the 5% level.
**Significant at the 1% level.
^{NS}Not significant.

TABLE 2

Effects of storage type, aeration, dessicants, and storage period on the mean germination percentages of pollen grains from Khori variety of date palm male cultivar [a].

Period (months)	Dessicant	Storage Type					
		Freezer		Refrigeration		Room Temperature	
		Aerated	Non-Aerated	Aerated	Non-Aerated	Aerated	Non-Aerated
6	$CaCl_2$	34.3aAB	25.4aB	43.7aA	25.1bB	11.4abC	29.0aB
	Silica Gel	42.4aA	28.7aBC	38.9aAB	28.9bBC	20.3aC	17.9aC
12	$CaCl_2$	41.2aA	27.1aB	44.4aA	46.1aA	3.5bC	0.8bC
	Silica Gel	31.0aA	31.9aA	35.5aA	28.1bA	0.0bB	0.8bB

[a]Averages of three replications (S.E. = 3.83).
Means followed by the same capital (small) letter in each row (column) are not significantly different by DMRT at the 5% level.

aerated pollen grains had the highest germination with the results from refrigerated pollen stored in $CaCl_2$ desiccant slightly better than those pollen grains desiccated in silica gel. The results for freezer storage were only slightly lower, and not significantly different. The performance of non-aerated pollen grains in cold storage was generally low, with the exception of those stored in $CaCl_2$ desiccant in the refrigerator for 12 months. These pollen grains performed significantly better than either of those stored for six months in cold storage. The results were equivalent to the best results obtained by aerated pollen.

Room-temperature storage gave the lowest germination percentages. The only exception was obtained from non-aerated pollen stored for six months, which was equivalent to the results from non-aerated cold-storage conditions. Long-term storage at room temperature appeared to be the most unsuitable for pollen storage.

MEDJAHDIL VARIETY: There were two strong two-way interactions, one between storage type and aeration ($p < 0.001$) and the other between storage type and storage period ($p < 0.001$) (Table 1). The only higher order statistically-significant interaction was the one among storage type, aeration, and storage period ($p < 0.05$).

Based on the three-way interactions, the mean germination yields for Medjahdil pollen grains are shown in Table 3. The results indicate that the best storage conditions were obtained when pollen grains were stored either in the freezer or refrigerator and aerated during germination. Non-aerated pollen grains gave significantly lower results. The exception was the result of refrigeration storage for 12 months. As in the case of the variety Khori, the mean germination percentage was comparable with that obtained for cold-stored aerated pollen. Again, room temperature storage was the least suitable, especially when storage was for a 12-month period.

BAHLANI VARIETY: The analysis of variance of Bahlani variety had a number of statistically-significant main effects and two-way interactions (Table 1). There was also highly significant four-way interactions among storage type, desiccant, aeration, and storage period ($p < 0.01$). The Bahlani variety produced the highest overall mean germination percentage (59.7 %) when aerated pollen grains were freezer-stored in silica gel desiccant for a 6-month period. Comparably good results were obtained with both $CaCl_2$ for a 6-month period and silica gel for a 12-month period, under refrigeration storage. Similar germination percentages were obtained from non-aerated, refrigeration-stored pollen grains in $CaCl_2$ desiccant for a 12-month period. Generally, non-aerated cold-storage pollen grains yielded lower mean percentage of germination than aerated ones.

TABLE 3

Mean germination percentages of pollen grains from Medjahdil variety as affected by storage types, aeration, and storage period [a].

Period (months)	Storage Type					
	Freezer		Refrigeration		Room Temperature	
	Aerated	Non-Aerated	Aerated	Non-Aerated	Aerated	Non-Aerated
6	45.32aA	31.8aB	53.5aA	32.0bB	18.7aC	25.0aBC
12	48.4aA	37.1aB	46.4aAB	42.8aAB	3.9bC	5.8bC

[a]Averages of two dessicant types and three replications (S.E. = 3.05).
Means followed by the same capital (small) letter in each row (column) are not significantly different by DMRT at the 5% significance level.

TABLE 4

Mean germination percentages of date pollen grains from Bahlani variety as affected by storage type, dessicants, aeration, and storage period.

Period (months)	Dessicant	Storage Type					
		Freezer		Refrigeration		Room Temperature	
		Aerated	Non-Aerated	Aerated	Non-Aerated	Aerated	Non-Aerated
6	$CaCl_2$	37.9bBC	25.7aD	51.9aA	33.9bCD	11.4aE	44.1aAB
	Silica Gel	59.7aA	29.1aBC	38.9bB	28.9bBC	20.3aC	23.0bC
12	$CaCl_2$	32.0bB	30.5aB	48.4abA	51.6aA	2.4bC	0.0cC
	Silica Gel	29.6bBC	28.6aBC	54.2aA	32.5bB	18.3aC	4.3cD

Means followed by the same capital (small) letter in each row (column) are not significantly different by DMRT at the 5% level (S.E. = 3.05).

Room-temperature storage consistently gave the lowest mean germination percentages for all combinations of conditions with the surprising exception of those pollen grains stored for a 6-month period in $CaCl_2$ desiccant. These produced mean percentages comparable with those stored in cold storage. Similar to the other two varieties, storing pollen at room temperature for a 12-month period resulted in poor germination.

Discussion

The results of this study agree with those obtained by Stout (1924), Brown and Bahgat (1938), Crawford (1938), and Aldrich and Crawford (1941). Evidently, the ambient air temperature and relative humidity in pollen-collection areas may have made the pollen more adapted to refrigeration than to freezing and, in particular, to room-temperature storage. Comparing the results of the present study with those of Yates *et al.* (1991) on pecan indicates that pollen retains better viability after freezing than after refrigeration in temperate-zone plants, whereas pollen does better after refrigerator storage in tropical-zone plants. Adaptation of date pollen to storage at room temperature (Cauvet 1914; Abo-Hassan *et al.*, 1982; Bacha *et al.*, 1988) provides some evidence that date palm pollen grains produced in either Algeria or Central Saudi Arabia might contain less free water, compared to those produced in Egypt, California, or the Sultanate of Oman. This could be attributed to dryer conditions in the former locations.

It was of particular interest to observe that, for all three varieties, non-aerated refrigerator-stored pollen tended to yield significantly higher germination percentages when stored for a 12-month period rather than six months. Non-aerated freezer-stored pollen did not, however, demonstrate the same effect. These results indicate that non-aerated germination following refrigeration may have induced some type of inactivation resembling "dormancy" which has been overcome by aeration, and suggests that it is largely refrigeration that induces the inactivation process.

Membrane permeability plays an important role in the induction of dormancy as it affects the activity of membrane-associated enzymes. This permeability is maintained by several factors, including the ratio of sterols to phospholipids (Wang and Faust, 1990). Sterols and lipids in date palm pollen have been reported to change with varieties (Bukhaev *et al.*, 1983). Loss of viability during storage is associated with changes that have occurred in phospholipids (Jain and Shivanna, 1989). Further evidence supporting inactivation of pollen is that not all viable pollen, as tested by acetocarmine or tetrazolium, is able to germinate (Pereau-Leroy, 1957; Furr and Enriquez, 1966) or to set fruit (Shaheen *et al.*, 1986).

Conclusions

Our study was conducted to determine the specific factors for date palm pollen grains to retain the ability to germinate following storage. The best germination percentages for the three date palm male varieties were achieved when pollen was cold-stored. For Khori variety, freezer storage in silica gel desiccant for 6 months gave the best result. Good results were also obtained with aerated germination of refrigeration-stored pollen using $CaCl_2$ desiccant, regardless of the storage period. For Medjahdil variety, the best results were obtained from pollen grains stored in either the freezer or the refrigerator and aerated during germination. Comparable results were also obtained from Bahlani variety, using either medium with refrigeration storage for 12 months or freezer storage in silica gel desiccant for six months (in both cases with aeration during germination). Overall, aeration was found to enhance germination of pollen after cold storage.

Room-temperature storage consistently gave the lowest mean germination percentages for all combinations of factors. This storage type tends to significantly reduce the ability of pollen to germinate.

Non-aerated refrigerator-stored pollen yielded significantly higher germination percentages when stored for a period of 12 rather than six months. These results indicate that refrigeration may induce dormancy that is reversed or mitigated when refrigeration continues for a longer period. This phenomenon was not observed in freezer storage. Further investigations are needed to better understand the process of the temporary loss of ability of date palm pollen to germinate following storage.

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