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Factors Influencing Date Pollen Viability

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العوامل التي تؤثر على حيوية حبوب لقاح نخيل التمر

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خلاصة : تم حفظ حبوب اللقاح لسبع أصناف من نخيل البلح تحت درجة حرارة ٤,٥ - ٥ مئوية باستخدام مجففة مزودة بهلام السيليكا أو بدونه ومباشره في القناني المخبرية كحاكم. تم تحديد نسبة الإنبات بواسطة المجهر بواسطة الهيماسايتوميتر بعد الحصاد وبعد ثلاثة وستة أشهر من الحفظ على التوالي وذلك باستخدام وسط مكون من حمض البوريك والسكروز. لم يكن هناك تأثير لصنف النخيل على حيوية حبوب اللقاح. أعطت حبوب اللقاح المحفوظة لمدة ستة أشهر نسبة إنبات أعلى (٤٥,٩٪) من تلك المحفوظة لمدة ثلاثة أشهر (٤٠,٩٪) مما يدل على أن حبوب اللقاح تحتاج لأكثر من ثلاثة أشهر تحت درجة حرارة بين ٤,٤ - ٥ مئوية لأعادة تنشيط عوامل نموها. كذلك أدى ارتفاع الرطوبة النسبية داخل المجففة إلى انخفاض نسبة الإنبات. كانت نسب الإنبات ٦٢,٢ و ٤٢,٩ و ٢٢,٧٪ عند درجات رطوبة نسبية قدرها ١٨، ٥٥ وأعلى من ٥٥، على التوالي. أعطت حبوب اللقاح المحفوظة في مجففات مزودة أو غير مزودة بهلام السيليكا لمدة ستة أشهر نسبة إنبات أعلى منها في ذات الثلاثة أشهر بينما كانت نسبة الإنبات أضعف كثيراً في حالة عدم استخدام المجففة. وتم الحصول على أعلى معدل إنبات في حبوب اللقاح المحفوظة في مجففة مزودة بهلام السيليكا لمدة ستة أشهر.

ABSTRACT : Pollen of seven date cultivars was stored at 4.5 - 5.0°C using desiccators with or without silica gel and in vials outside the desicator as a control. Germination percentage was determined microscopically with a hemacytometer after harvest and at three and six months of storage, respectively, using a boric acid-sucrose medium. Cultivar genotype did not influence the viability of pollen. Pollen stored for six months gave a higher germination percentage (45.9%) than three months (40.9%) indicating that pollen requires more than three months at 4.5 - 5°C for the reactivation of its growth factors. An increase in relative humidity in storage containers resulted in reduced germination profiles. Germination percentages were 62.2, 42.9 and 22.7% at 18, 55 and over 55 relative humidity, respectively. Pollen stored in desiccator with and without silica gel gave a higher germination profile after six months than at three months storage whereas the control gave the lowest. The highest germination percentage was observed in pollen kept in a desiccator in the presence of silica gel for six months.

The date palm (*Phoenix dactytifera L.*) is traditionally hand-pollinated and requires climbing the female palm to place male strands inside the spadix. This method has been found tedious, time consuming and costly (Ibrahim, 1988; Shabana *et. al.* 1986). Changing life styles of people in areas where the date palm is grown intensively has resulted in a scarcity of skilled labour. This situation necessitates the introduction of mechanical pollination. The method, however, requires larger amounts of pollen in a shorter period of time than traditional techniques. The problem is aggravated by the fact that the availability of pollen is at its lowest level during the early part of the pollination season, while large amounts are available towards the end of the season when the demand is lowest. Consequently freshly collected pollen needs to be stored under suitable conditions so that it can be used when the demand is high.

The techniques of storing date pollen depend on the provision of low temperature and low relative humidity (Shaheen et al, 1986; Hussain *et al.* 1986; Yates *et al.* 1991). Bukhaev *et al.* (1983) reported that moisture content of fresh date pollen grown in Iraq ranges between 49.2 and 56.3%. The moisture content of pollen prior to storage largely determines its storing ability. Barnabas and Rajki (1981) reported the beneficial effect of reducing moisture content of maize pollen to prolong the storage period. Similar attributes were reported by Hanna (1990) on *pennisetum glaucum*. However, Rodriguez (1986) stressed the importance of a certain level of moisture in the cotton pollen to prolong its life storage. The moisture content of pollen after storage is vital for germination. It must be higher than the moisture content during storage (Stanley and Linkens, 1974). Yales (1991) suggested the possibility of membrane leakage when pollen was stored at -10° C, causing the inability of its protoplasm to regain a turgid state. He also observed loss of turgidity when the pollen, stored at -196° C, was incubated directly in the germination medium without being rehydrated.

The objective of the present investigation was to study the effect of storage conditions on the viability of pollen collected from different cultivars or from the same cultivar as influenced by the environment conditions of growth and germination.

Materials and Methods

Pollen of seven date cultivars namely Naghaili

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(Rumais), Fardh, Suhaili, Gharifi, Naghaili (Nizwa), Nashoe and Khori were collected toward the end of the pollination season. After drying at 28° C ± 2° C, the germination percentage of freshly collected pollen was determined microscopically with a hemacytometer. Pollen was then kept in open vials in desiccators with and without silica gel (desiccant) and in vials outside the desiccator, and stored in a refrigerator at 4.5 - 5.0° C in a factorial experiment with four replications.

Germination percentages of the stored pollen were determined microscopically with a hemacytometer after three and six months in a medium containing 500 ppm boric acid, 15% sucrose with one drop of tween 20 added to disperse the pollen. Then 12.5 ml of each suspension were placed in a 100 ml Erlenmeyer flask and incubated for 24 h using a water bath (Haake - D8, Gallenkamp) adjusted to 30° C. One drop of pollen - medium mixture was placed in each chamber of the hemacytometer. Germination counts were taken from 10 fields. In each field, the total number of pollen (germinated and nongerminated) were counted. The germination percentage was determined based on the average of the 10 fields. Data was statistically analyzed using MSTAT Computer Software and the means were separated using the LSD method.

Results and Discussion

No differences ($P>0.05$) were observed in the fresh pollen germination percentages of the seven tested cultivars at any of the testing periods (Table 1). Fresh Naghaili (Rumais) was the highest (81.3%) and Khori was the lowest (66.5%). On the other hand pollen stored for six months gave a significantly higher germination percentage (45.9%) than pollen stored for three months (40.9%). These results suggest that pollen needed more than three months to adjust at 4.5 - 5.0° C because of physiological changes which may result in a temporary inactivation of the pollen tube nucleus. It is generally agreed upon that such a phenomenon occurs in certain types of pollen. Inactivation could be controlled physically by low moisture content of mature pollen (Stanley and Linkens, 1974). It could also be attributed to certain ripening processes involving biochemical changes as a result of the accumulation of chilling requirement during imbibition (Towill, 1985). The lower germination percentages recorded within the three months period are more likely due to inactivation of growth factors which regulate the process of germination Yates and Sparks; 1989, 1990; Yates *et al.* 1991; Wang and Faust 1990, Jain and Shivana, 1989). These factors seem to require a longer period to be reactivated. Also, no differences ($P>0.05$) were observed in pollen germination of the cultivars at both storage periods. Germination ranged from 38.1 to 47.6%. However, a considerable reduction in pollen germination was observed after three and six months storage when compared with the fresh pollen. The largest drop after six months occurred in the germination of Naghaili (Rumais) (45.0%) followed by Suhaili (37.8%).

TABLE 1

Mean pollen germination percentage as affected by length of storage period

Male Cultivar	Germination (%) Fresh Pollen	3 Months	6 Months	SE
Nashoe	68.1	40.3	43.9	8.7
Naghaili (Nizwa)	67.3	41.0	47.1	8.0
Khori	66.5	38.4	44.0	8.6
Naghaili (Rumais)	81.3	42.8	45.7	12.4
Fard	68.4	41.0	47.4	8.3
Gharifi	67.1	41.8	45.9	7.8
Suhaili	76.5	41.6	47.6	10.8
Average	70.7	40.9[a]	45.9[b]	9.2

[a,b] Means in the same row with different superscripts differ ($P<0.05$).

As shown in Table 2, the average germination percentage of pollen stored in the desiccator containing silica gel was significantly higher than in that without it. A further drop in pollen germination percentage was observed in the control. The recorded germination percentages at or under the three storage conditions were 65. 2, 42.9 and 22.7 respectively. Our results are supported by the early findings of Crawford (1938) and Aldrich and Crawford (1941) which showed that relative humidity (RH) has a direct effect on reducing date pollen viability. The average RH in a desiccator with silica gel it was 18%, whereas in a desiccator in the absence of silica gel it was 55%. It was not possible to measure RH in the control vials. The RH in the vials was assumed to he higher than in the other two treatments because they were not as airtight as the desiccator. Consequently, this method of storage produced the lowest pollen germination percentages in all of the tested cultivars. The average germination percentage of pollen from the seven cultivars were not significantly different. It ranged from 41.2% to 45.8%. The highest germination (72.1%) was obtained with Naghaili (Rumais) pollen stored in a desiccator with silica gel, whereas the lowest (20.4%) was with Khori pollen stored in vials (i.e. in the absence of both silica gel and desiccator). Although Naghaili pollen collected from Rumais gave the highest germination (81.3%)

TABLE 2

Effect of storage condition and cultivar on pollen germination

Cultivars	Germination (%) Dessicator with silica gel	Dessicator without silica gel	Control	SE
Nashoe	59.1[a]	42.4[b]	24.8[c]	9.9
Naghaili (Nizwa)	65.9[a]	44.0[b]	22.7[c]	12.5
Khori	72.1[a]	41.1[b]	20.4[c]	15.0
Naghaili (Rumais)	72.1[a]	43.6[b]	21.8[c]	14.6
Fard	65.6[a]	45.1[b]	22.0[c]	12.6
Gharifi	67.3[a]	41.3[b]	23.0[c]	12.9
Suhaili	69.1[a]	43.1[b]	21.6[c]	13.7

[abc] Means in the same row with different superscripts differ ($P<0.05$).

when fresh, it showed the highest reduction in viability (45%) during prolonged storage. Furthermore, pollen of the same cultivar grown at Nizwa gave lower germination (67.3%) than that grown at Rumais, but showed better storage ability as indicated by a lower reduction in its germination (30%). These results indicated that male date cultivars grown under humid conditions (Rumais) produce pollen of higher viability than cultivars grown under drier conditions in Nizwa. However, their higher viability could easily be lost during storage. It can also be pointed out that the contrasting effect of humidity in storage and on the tree is probably a result of environmental modification provided by the spathe which forms a good protection against humidity.

The interaction of storage conditions and storage periods on the viability of pollen is shown in Table 3. It can be seen that pollen germination developed a similar trend in that a desiccator with silica gel always gave higher percentages while the absence of a desiccator always gave the lowest percentages. Pollen germination was significantly higher after six months than after three months in the desiccator with or without silica gel. In the case of the vials, pollen germination was reduced during prolonged storage. This effect could be related to the high humidity which might induce pollen to germinate earlier or activate fungal infection. The highest germination was obtained after six months in a desiccator with silica gel (69.5%) and the lowest was after six months in the vials (21.5%).

Table 4 shows the effect of cultivar genotype, storage condition and length of storage period on the germination of pollen. The cultivar genotypes did not

TABLE 3

Effect of storage condition and storage period on pollen germination

Storage Period	Germination (%) Dessicator with silica gel	Dessicator without silica gel	Control	SE
3 months	61.0[b]	38.8[a]	23.2[c]	11.0
6 months	69.5	47.1	21.5	13.9

[ab] Means in the same row with different superscripts differ ($P<0.05$).

show any significant effect on the germination of pollen under each of storage conditions during the two periods. Furthermore, the desiccant (silica gel in a desiccator) provided the best storage conditions while the vial in the absence of desiccator and silica gel provided the worst. Previous reports Abo-Hassan *et al.*, (1982) and Basha *et al.* (1988) showed that low in vitro pollen viability can produce a high percentage of fruit set at a time when pollen was unavailable and pistillate flowers were ready for pollination. Substantial yield and good quality fruit were also produced.

Conclusions

Results of pollen germination studies of seven date cultivars revealed that up to 75% of pollen viability was retained when the pollen was stored in a desiccator containing silica gel under refrigeration. Relative humidity is thus an important factor regulating stored pollen viability. Due to the close relationship between in vitro and in vivo pollen germination, results of the former can be employed safely in selecting viable pollen for mechanical pollination.

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TABLE 4

Effect of storage condition, length of storage period on pollen germination of the seven cultivars

Male Cultivar	Germination (%)						
	3 Months			6 Months			
	Dessicator with silica gel	Dessicator without silica gel	Control	Dessicator with silica gel	Dessicator without silica gel	Control	SE
Nashoe	58.3^{a}	38.2^{b}	24.4^{c}	60.0^{a}	46.6^{b}	25.2^{c}	6.4
Naghaili (Nizwa)	62.2^{a}	36.9^{b}	23.5^{c}	69.3^{a}	51.1^{b}	21.9^{c}	7.7
Khor	56.8^{a}	37.2^{b}	21.3^{c}	67.6^{a}	45.0^{b}	19.6^{c}	7.8
Naghaili (Rumais)	62.9^{a}	42.7^{b}	22.8^{c}	71.7^{a}	44.5^{b}	20.9^{c}	8.4
Fard	60.1^{a}	40.0^{b}	23.1^{c}	71.1^{a}	50.3^{b}	21.0^{c}	8.2
Gharifi	62.8^{a}	38.1^{b}	24.6^{c}	71.9^{a}	44.6^{b}	21.4^{c}	8.3
Suhaili	63.5^{a}	38.4^{b}	22.9^{c}	74.8^{a}	47.8^{b}	20.4^{c}	8.9

^{abc} Means in the same row (within the same storage time) with different superscripts differ ($P < 0.05$).

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