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SHORT COMMUNICATION :

Effect of Storage Conditions and Methods of Pollen Germination on Viability of Three Date Palm Cultivars.

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ABSTRACT

Date palm pollen grains from Khori, Medjahdil and Bahlani male cultivars were kept in a desiccator which contained either silica gel or calcium chloride, or after drying, in a freeze-dryer. The Pollen was then stored either under room temperature (21 - 25°C), in a refrigerator (4 - 5°C) or in a freeze-dryer (-18°C). After six months, the pollen grains were germinated at 30°C in one of two media, namely modified Brewbaker and Kwack medium and boric acid-sucrose medium. No interaction was observed between the three cultivars and boric acid-sucrose medium. No interaction was observed between the three cultivars and the tested conditions. Generally Bahlani and Medjahdil showed higher germination percentages than Khori. In addition, storage in refrigerator with either silica gel or calcium chloride, using Brewbaker and Kwack medium, were the best storage conditions for retaining pollen viability, with germination percentages of 55.5% and 55.1% respectively. In general, pollen germinated in modified Brewbaker and Kwack medium (30.7%). Accordingly refrigeration and silica gel were recommended for storage and Brewbaker and Kwack was the better medium to determine pollen germination percentages.

Key Words : Pollen, storage, viability, germination.

INTRODUCTION

The scarcity of pollen during the flowering of late and early pistillate date cultivars, and the sift from the traditional to the mechanical pollination, necessitate the storage of pollen. Consequently a reliable, viable germination test has become important, to determine the suitability of pollen before application.

Literature on pollen suitability tests shows that there are significant discrepancies between the results from germination and viability tests of the same pollen samples. Gupta and Thatai (1980) and Basha et al. (1988) reported low fruit set with pollen of high viability tested by acetocarmine. Furr and Enriquez (1966) used tetrazolium and acetocarmine test, but they were unable to differentiate between the viable and the non-viable pollen. This difficulty in discriminating between viable and non-viable pollen may explain the high percentages obtained with staining procedures. On the other hand it seems rather difficult to simulate in vivo germination and growth conditions as in an in vitro system. Available information in the literature tends to indicate that pollen germination tests are more likely.

There are several pollen germination media which have been developed in combination with specific environmental conditions (Brink 1924, Brewbaker and Kwack 1963, Furr and Enriquez 1966). Bursting and irregularities associated with the expanding pollen tube have also received a great deal of attention, which have lead to modifications in media composition and environmental factors to improve the efficiency of the test. Sugar and boron have been commonly applied. Other improvements include changes in their concentration, application of inorganic salts, especially K, Ca, Mg, and Mn. In addition, the media have been employed in either the liquid or gel state. In some cases growth regulators have been added, such as GA (Asif et al. 1983) and IAA, (Loo and Hwang 1944). Environmental factors mainly include temperature and oxygen (Furr and Ream 1968, and Smith 1942).

The experiment reported here was intended to determine the pollen storage abilities of three date cultivars commonly used in the Sultanate of Oman, and to define a reliable germination test which may assist in the mechanical pollination program launched by the Ministry of Agriculture and Fisheries.

MATERIALS AND METHODS

Pollen of three date cultivars commonly used in the Sultanate of Oman, namely Khori, Medjahdil and Bahlani, were collected in the season of 1990. The pollen was dried before separation from the spadix in a heated room, with thermostat controlled electric heater, at 28-300C and a dehumidifier, after which it was divided into three equal portions. Two portions were kept under continuous drying condition in hard paper cups placed in a desiccator containing either silica gel or $CaCl_2$. The third portion was freeze-dried. Each of these portions was stored in a deep freezer (-18°C), refrigerator (4 - 5°C) or at room temperature (21

- 25°C). After six months storage, germination percentages were determined in the following two media composition :

1. Boric acid-sucrose medium containing 300 ppm boric acid and 15% sucrose. A 100 mg pollen was added to 50 ml of the above solution. Of this pollen suspension, 12.5 ml was incubated for 24 hours in Erlenmeyer flask, in a cool water bath adjusted at 30°C as described by Oppenheimer and Reuveni (1965).
2. Modified Brewbaker and Kwack medium containing 300 ppm H_2BO_2, 300 ppm $CaNO_3 4H_2O$, 200 ppm $MgSO_4 7H_2$),100 ppm KNO_3 and 15% sucrose. Of the above solution 12.5 ml was added to 25 mg of pollen as described by Furr and Enriquez (1996). Incubation conditions and periods were as in 1 above.

Pollen germination percentages were determined under the microscope using a hemacytometer. The results were analyzed statistically in a factorial design with three replications, each replication consisting of ten hemacytometric fields.

RESULTS AND DISCUSSION

Main effects

There was a significant difference in the mean germination percentages in the three cultivars due largely to Khori ($p<0.0001$). While the germination percentages of Medjahdil and Bahlani were on par (33.5% and 34.8% respectively with S.E. = 0.86), for Khori the mean percentage was significantly lower (29.3%) Table 1.

The differences in storage conditions were equally significant. Refrigeration storage was best of all (39.9%, S.E. = 0.86), followed by freezer storage (37.2%). Room temperature storage had the lowest germination percentage of 21.2% (table 2).

The two desiccants also resulted in significant difference in mean germination percentages. Silica gel gave the highest figure (40.5%, S.E. = 0.86), closely followed by $CaCl_2$ (37.1%). Freeze-drying had the poorest performance with 20.8% germination (Table 2).

The mean germination percentage obtained using Brewbaker and Kwack medium was significantly higher (34.4%, S.E. = 0.70) than that of boric acid-sucrose medium (30.7%) Table 3.

Table 1. Mean pollen germination percentages of date cultivars by storage conditions.

	Cultivars			
Storage	**Khori**	**Medjahdil**	**Bahlani**	**Mean %**
Freezer	32.3_{c}	38.7_{ab}	37.8_{ab}	36.3
Refrigeration	37.0_{b}	41.0_{a}	41.8_{a}	39.9
Room temp.	18.7_{e}	20.9_{e}	25.0_{d}	21.5
Mean %	29.3	33.5	34.8	

* Number subscripted by the same letter are not significantly different at the 0.05% significance level.

Table 2. Mean pollen germination percentages of the date cultivars based on the interaction between storage conditions and desiccants.

	Storage conditions			
Desiccant	**Freezer**	**Refrigerator**	**Room Temp.**	**Mean %**
$CaCl_2$	37.6_{c}	49.7_{a}	24.1_{d}	37.1
Silica gel	44.0_{b}	50.6_{a}	26.8_{d}	40.5
Freeze-dry	30.0_{e}	19.5_{e}	12.8_{f}	20.8
Mean %	37.2	39.9	21.2	

* Numbers subscripted by the same letter are not significantly different at the 0.05% significance level.

Interactions

Of more importance however were the highly significant interactions among the different factors. Interaction between storage conditions and the three desiccants was highly significant (Table 2). The combinations that gave the highest mean germination percentages were refrigeration with either silica gel (50.6%, S.E. = 1.48) or $CaCl_2$ (49.7%). The next best combinations were freezer storage with either silica gel (44.0%) or $CaCl_2$ (37.6%). The combinations of freeze-drying with either refrigeration (19.5%) or room temperature storage (12.8%) were considerably unsuitable for storage.

Storage conditions also interacted with the germination media (Table 3). The highest germination percentage (48.4%) was obtained when refrigerated pollen was tested in the Brewbaker and Kwack medium. This was followed by pollen stored in the freezer and tested in Brewbaker and Kwack medium (37.5%) or boric acid-sucrose medium which reacted similarly. Room-stored pollen gave the lowest percentages in either of the germination media.

Table 3. Mean pollen germination percentages of the date cultivars resulting from interaction between germination media and storage conditions.

	Storage conditions			
Medium	**Freezer**	**Refrigerator**	**Room Temp.**	**Mean %**
Boric acid-sucrose	35.0_b	31.4_c	25.7_d	30.7
Brewbaker & Kwack	37.5_b	48.4_a	17.3_e	34.4
Mean %	36.3	39.9	21.5	32.6
	S.E. = 1.21	F = 44.43	p = 0.0001	

* Numbers subscripted by the same letter are not significantly different at the 0.05 significance level.

Except for freeze-dry, the germination performances of the other two desiccants were about the same when either medium was used. The germination percentages obtained with $CaCl_2$ were 37.0%, and 37.7% for boric acid-sucrose and Brewbaker and Kwack media, respectively. With silica gel, the percentages were 41.8% and 39.4% respectively. Very low mean germination percentages were recorded for free-drying with either medium (Table 4).

The only significant three-factor effect was that of storage conditions, germination media and desiccants (Table 5). The combinations that gave the highest mean germination percentages were those of refrigeration storage tested in Brewbaker and Kwack medium with either silica gel (55.5%, S.E. = 2.09) or $CaCl_2$ (55.1%). The most unsuitable conditions were when either refrigerated or room stored pollen grains were freeze-dried and tested in boric acid-sucrose medium (4.4% and 7.4% respectively).

Table 4. Mean pollen germination percentages of the date cultivars resulting from interaction between germination media and desiccants.

	Desiccants			
Medium	**$CaCl_2$**	**Silica gel**	**Freeze-dry**	**Mean %**
Boric acid-sucrose	37.0_b	41.8_a	13.2_d	30.7
Brewbaker & Kwack	37.7_b	39.4_{ab}	26.2_c	34.4
Mean %	37.4	40.6	19.7	32.6
	S.E. = 1.21	F = 22.58	p = 0.0001	

* Numbers subscripted by the same letters are not significantly different at the 0.05 significant level.

CONCLUSION

Pollen of all three cultivars reacted similarly in the tested storage conditions and germination methods. However, Medjahdil and Bahlani cultivars out-performed Khori cultivar. The study also demonstrated that the combined treatment of refrigeration storage and air-tight container, including silica gel, tested in Brewbaker and Kwack medium provides the best results in terms of mean pollen germination percentages, and can thus be recommended for date pollen storage and testing. While the results with calcium chloride are equally good, silica gel is preferable because it has the added advantage of colour indication which allows it to be changed whenever the colour turns from blue to white. It is clear from these results that room temperature storage, combined with freeze-drying, is the most unsuitable combination, irrespective of either media of germination.

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Table 5. Mean pollen germination percentages of date cultivars as affected by the interaction of storage conditions, desiccants and germination media.

	Germination Media					
	Boric acid-sucrose			Brewbaker & Kwack		
Desiccant	Freezer	Refrigerator	Room Temp.	Freezer	Refrigerator	Room Temp
$CaCl_2$	35.0_{de}	44.3_{b}	31.0_{ef}	40.1_{b}	55.1_{a}	55.1_{a}
Silica gel	41.9_{bc}	45.7_{b}	37.0_{cde}	46.1_{b}	55.5_{a}	16.6_{g}
Freeze-dry	28.1_{f}	04.4_{h}	07.4_{h}	25.8_{f}	34.6_{e}	18.1_{g}
	S.E. = 2.09		F = 13.72		p = 0.0001	

* Numbers subscripted by the same letter are not significantly different at the 0.05 significance level.

مجلة الإمارات للعلوم الزراعية (١٩٩٦) ٨ : ١٤٦ - ١٥٤

تأثير التخزين وطرق الإنبات على ثلاثة أصناف في حبوب لقاح النخيل

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ملخص :

تم تخزين حبوب اللقاح لكل من الخوري والمتجحدل والبهلاني من أصناف النخيل الذكرية تحت درجة حرارة الغرفة أو داخل مبردة او مجمدة حيث حفظت داخل مجففة (Desiccator) احتوت على السلكاجل أو كلوريد الكالسيوم أو بعد تجفيفها بواسطة التجفيف المجمد .نبتت حبوب اللقاح بعد ستة أشهر أما في وسط بروبيكر وكواك المعدل أو في وسط مكون من السكر وحامض البوريك تحت ٣٠ درجة مئوية .

بينت النتائج عدم وجود فروقات معنوية في نسبة انبات الاصناف الثلاثة تحت ظروف التجربة وعموما فان البهلاني والمتجحدل اعطيا أعلى نسبا للإنبات عن الخوري. كما وأن التخزين داخل المبردة مع استخدام السلكاجل او كلوريد الكالسيوم هي الأفضل لحفظ حيوية حبوب اللقاح حيث بلغت النسبة المئوية للانبات ٥٠.٦ و ٤٩.٧ على التوالي. كذلك فان الوسط المكون من حامض البوريك والسكر أعطى نسبا أعلى للإنبات (٣٤.٤٪) عن وسط بروبيكر وكواك المعدل (٣٠.٧٪) . وبناء على هذه النتائج فقد جاءت التوصية باستخدام المبردة والسلكاجل للتخزين وتحديد نسبة الإنبات باستخدامم حامض البوريك والسكر.

كلمات مفتاحية : حبوب اللقاح ، التخزين الحيوية ، نسبة الإنبات .