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Effect of Chemicals and pH on *in Vitro* Germination of Date Palm Pollen

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ABSTRACT. Boron was not essential for initiation of germination of date palm (*Phonex dactylifera* L.) pollen (80% ± 4 of pollen germinated after 4 hours of incubation in medium lacking boron), but was necessary for normal growth of pollen tubes since 76% of germinated pollen grains had burst tubes and the remaining had short tubes (7.5 ± 4 μm).

Calcium was essential for pollen germination and only 2% ± 1 of pollen germinated after 4 hurs in medium lacking calcium. Magnesium or polyamines (spermine diphosphate, spermidine phosphate or putrescine chloride) improved the germination in absence of calcium, but the rate of pollen tube elongation was lower than that obtained for calcium.

The pH optimum for germination and tube growth was 5.5 and the pH of the medium did not change after 3h of incubation.

Sucrose was not important for germination and pollen tube growth.

Date palm is a dioecious plant, artificially pollinated. It has been reported that variation exists in pollen quality in date palm (Furr and Enriquez 1966, Ream and Furr 1970) and in several plant species (Pfahler 1965 and 1967, Mulcahy 1971 and 1974, Ottaviano *et al.* 1980, Currah 1983).

In the date palm it is evident from the work of Nixon (1926) and (1927) that pollen type had a marked influence on time of ripening, production and fruit quality, although the chief differences appeared to be in the seed rather than in the flesh. Also, it has been reported that pollen viability affect fruit set (Ream and Furr 1970). Similarly it has been reported that pollen genotype of maize influence pollen fertilization ability and sporophytic quality which is correlated with the speed of pollen tube growth (Mulcahy 1971 and 1974, Ottavino *et al.* 1980). Therefore, it is beneficial to find a reliable medium for in vitro germination of date palm pollen to aid the selection of highly viable pollen with a high rate of pollen tube growth.

The medium requirements for *in vitro* germination of pollen vary among species (Johri and Vasil 1961). Limited work has been done on in vitro germination of date palm pollen. Furr and Enriquez (1966) and Asif *et al.* (1983) studied the effect of some chemicals on germination. Also, Al-Helal *et al.* (1988) studied the effect of temperature and storage on germination and pollen tube growth. This work was initiated to study the effect of chemicals and pH on in vitro germination of date palm pollen.

Materials and Methods

The pollen grains were collected from a male date palm tree in the university campus and were air dried for 3 days and stored at O°C, which was the best storage temperature for date palm pollen (Al-Helal *et al.* 1988), for no longer than 3 weeks before they were studied.

Pollen grains were suspended in 2 ml of sucrose solution (7.5%) containing 100 ppm H_3BO_3, 10 mM sodium phosphate buffer pH 5.7 and 300 ppm of either $Ca(NO_3)_2$, $Mg(NO_3)_2$, $NaNO_3$, spermine diphosphate (SD), sperimidine phosphate (SP) or 1,4-diamino butane dihydrochloride [putrescine chloride (PC)] in a small sealed glass bottle and incubated at room temperature (25°C) without shaking (2 replicates were used for each treatment). After every hour a drop of pollen suspension was transfered to a slide and examined under a light microscope. The percentage of germination under 12 microscopic fields (for each replicate) was calculated and the length of 15 pollen tubes (for each replicate) was measured using a calibrated eyepiece micrometer.

In another experiment, to test the effect of pH (4-9) on germination and the effect of germination on the pH of the medium, the pH of the germination medium was adjusted by adding 30 mM tris/HCl (sodium phosphate buffer was not added) to give the appropriate pH. The pH of the medium was measured before and after adding 4 mg of pollen and after 3 hours of incubation (three replicates were used). Germination percentage and pollen tube length were measured after 3 hours of incubation.

Results

Under favorable conditions, germination steadily increased with time reaching 87% after 4 hours of incubation and it appeared that most viable pollen germinated within four hours of incubation (Table 1). Also, pollen tube length increased steadily with time under favorable conditions (Fig. 1) and the highest rate of growth was between 2 and 3 hours of incubation.

Table 1. Effect of chemicals on germination percentage of date palm pollen grains

Time in hours	% germination						
	−Ca	Ca	Mg	SP	SD	PC	−B
1	–	62 ± 9	–	–	–	–	–
2	–	77 ± 8	60 ± 9	31 ± 7	62 ± 11	44 ± 6	–
3	–	80 ± 12	–	–	–	–	–
4	2 ± 1	87 ± 11	68 ± 12	49 ± 5	65 ± 3	70 ± 12	80 ± 4

note: Germination was 77% ± 11 after 3½ h of incubation in medium lacking sucrose and containing B, Ca and phosphate buffer.

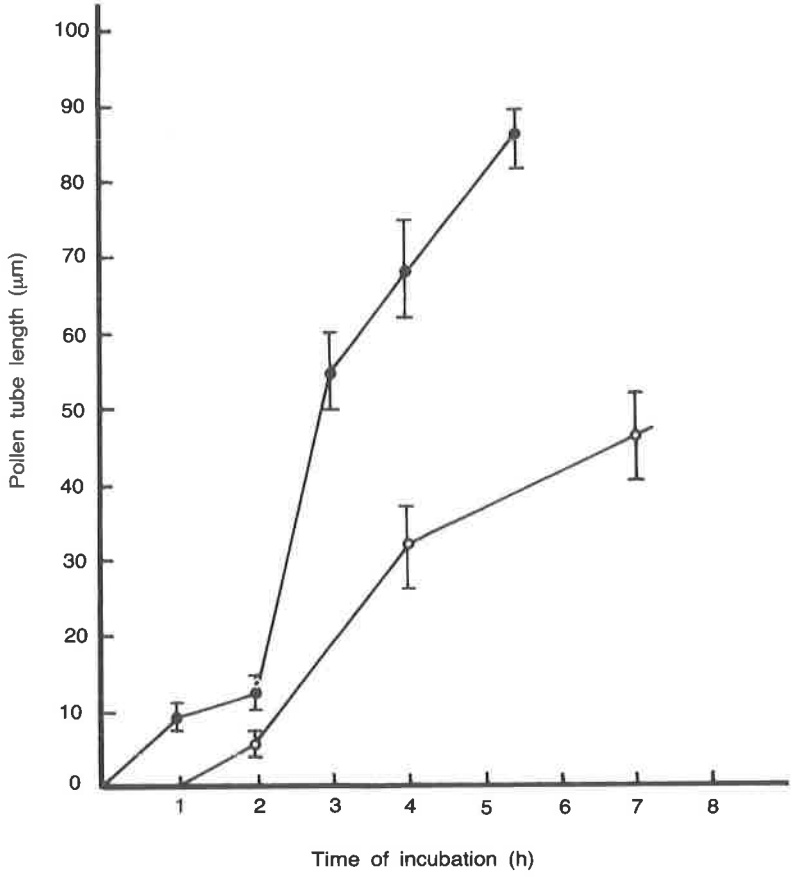

Fig. 1. Effect of calcium (●———●) and magnesium (○———○) on growth rate of date palm pollen.

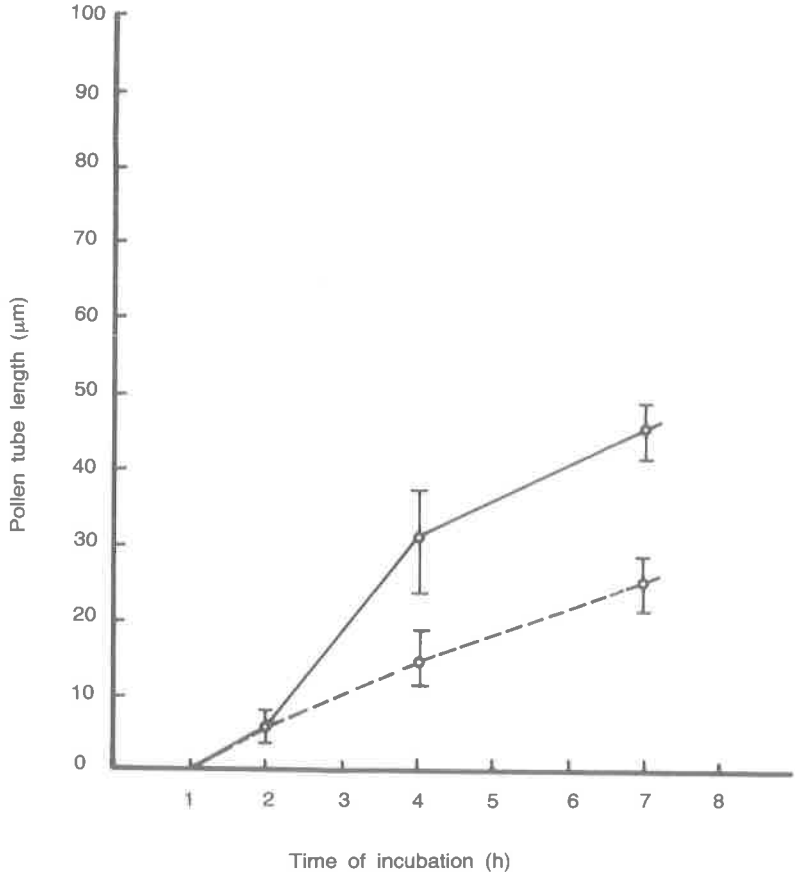

Fig. 2. Effect of polyamines calcium (o——o SP,o– – –o SD) on growth rate of date palm pollen. *Note:* Average pollen tube length in medium containing PC was 42±9.6 µm after 7 hours.

As shown in Table 1, relatively large number of pollen grains (80% ± 4 after 4h) germinated in medium lacking boron, but most of germinated pollen grains had short pollen tubes (the average length of pollen tubes was 7.5 ± 5.4 µm) and 76% of germinated pollen had burst pollen tubes.

The data in Table 1 show that calcium is essential for pollen germination and only 2% ± 1 of pollen germinated, after 4h, in medium lacking $Ca(NO_3)_2$ [replaced by $NaNO_3$ as a source for nitrogen], and the germinated pollen had short tubes (the average length of pollen tubes was 5.71 ± 0.94 µm).

Replacement of calcium with either magnesium or polyamines (SD, SP or PC) resulted in relatively high percentage of germination (Table 1). The highest was obtained for PC (70% ± 12 after 4 h) and the lowest was obtained for SP (49

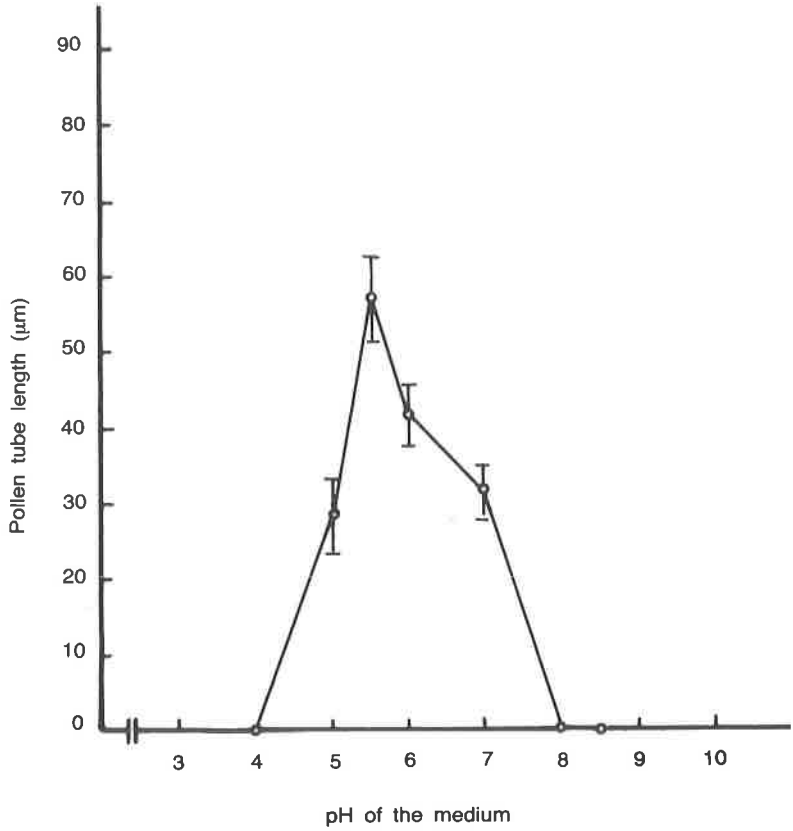

Fig. 3. Effect of pH on growth of date palm pollen tube.

± 5 after 4 h). The growth rate of pollen tube was better in medium containing calcium compared to the medium containing either Mg or polyamines (Figs. 1 and 2).

Adding pollen (10 mg/10 ml) to unbuffered medium caused an increase in pH of the medium from 5.4 to 6.3 but the pH of buffered medium (pH 5.8) was not affected by adding pollen and the pH of both medium change very slightly with time (pH was 6.1 after 4 hours of incubation).

The data presented in Table 2 and Fig. 3 show that pH of the medium affected germination and pollen tube growth. No germination occured at pH 4 and at pH 8 and above. The optimum pH for germination and tube growth was 5.5. The pH of the medium did not change after 3 hours of incubation.

Table 2. Effect of pH on germination percentage of date palm pollen after 3 hours of incubation. The medium was containing 30 mM tris/HCl bufer, boron and calcium.

pH of the medium	pH of the medium after adding 4 mg pollen / 10 ml	pH of the medium after 3 h of incubation	% germination
4.0	4.2	4.2	0
5.0	5.1	5.1	32 ± 7
5.5	5.5	5.5	59 ± 5
6.0	6.0	6.0	50 ± 6
7.0	7.0	7.0	48 ± 8
8.0	8.0	8.0	0
8.5	8.5	8.5	0

High germination percentage (77% ± 11 after $3\frac{1}{2}$ h of incubation) occured in medium lacking sucrose and containing B, Ca and phosphate buffer and the growth of pollen was normal (the average pollen tubes length was 65.7 ± 3.45 μm after 3 h of incubation).

Discussion

It is evident from the results that boron is not essential for the initiation of germination of date palm pollen but essential for the subsequent normal growth of pollen tube. Furr and Enriquez (1966) pointed out that 40% of date palm pollen germinated in the absence of boron and adding boron to the growth medium increased germination and reduced bursting of pollen tubes. Similarly it has been reported that adding boron to the growth medium improved germination and tube growth of several kinds of pollen grains, but its effect on tube growth is more pronounced than on the germination percentage (Johri and Vasil 1961, Vasil 1964).

The excessive bursting of pollen in the absence of boron might be due to a rapid water uptake or to the weakness of the wall as has been suggested by Schumucker (taken from Vasil 1964). It has been suggested that boron is directly involved in pectin synthesis (Stanley and Loewus 1964).

The data presented show that calcium is essential for germination of date palm pollen since only a small percent germinated in its absence. Similarly, it has been shown that Ca is required for germination of corn pollen and only trace of germination occured in medium laking calcium (Cook and Walden 1965 and 1967). Also, Pfahler (1967) pointed out that adding Ca to the medium increased

germination of maize pollen in the presence of boron and decreased it in its absence. In contrast, Ca appears to be not essential for germination of *Lilium longiflorum* (Southworth 1983).

It appears from the results that magnesium and polymines can substitute, within certain limits, the requirement of calcium for germination and pollen tube growth of date palm pollen. Similar results have been reported for apple pollen (Speranza *et al.* 1983) and the authors suggested three possible roles for polyamines: 1) as osmoregulators 2) involve in lipid biosynthesis 3) bound to pollen membrane constituents and to form the action bridges necessary for membrane integrity.

The data obtained for the effect of pH on date palm pollen germination is in a good agreement with that reported for *Nicotiana tabacum* (Tupy and Rihovo 1984).

The results show that the growth of date palm pollen did not affect the pH of growth medium and this is in disagreement which results reported for *Nicotiana tabacum* which cause a progressive medium acidification (Tupy and Rihovo 1984).

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تأثير المواد الكيميائية والرقم الهيدروجيني على الانبات المختبري لحبوب لقاح النخيل

علي عبدالمحسن الهلال

قسم النبات ـ كلية العلوم ـ جامعة الملك سعود ـ ص. ب ٢٤٥٥ الرياض ١١٤٥١
المملكة العربية السعودية

لقد تم دراسة تأثير الكالسيوم والبورون والمغنيسوم والسكروز وعديدات الأمين والرقم الهيدروجيني على الإنبات المختبري واستطالة أنبوبة تلقيح حبوب لقاح النخيل وقد أوضحت النتائج أن لهذه المعاملات تأثير على نسبة الإنبات ونمو أنبوبة التلقيح.

نسبة عالية (٨٠ ٪ ± ٤ بعد أربع ساعات) من حبوب لقاح النخيل نبتت في بيئة لا تحتوي على بورون ولكن ٧٦ ٪ من الحبوب النابتة كانت أنابيب التلقيح فيها ممزقة والبقية كانت أنابيب التلقيح فيها قصيرة.

عدد قليل جداً من حبوب اللقاح (٢ ٪ ± ١ بعد أربع ساعات) قد نبتت في بيئة لا تحتوي على كالسيوم. إضافة المغنسيوم أو عديد الأمين تحسن الإنبات في غياب الكالسيوم ولكن معدل نمو أنبوبة التلقيح أفضل في وجود الكالسيوم.

الرقم الهيدروجيني الأمثل للإنبات ونمو أنبوبة التلقيح هو ٥ $\frac{١}{٢}$ كذلك لم يلاحظ تغير واضح في الرقم الهيدروجيني للمحلول بعد ثلاث ساعات من الإنبات.

كذلك أوضحت النتائج أن إضافة السكروز لبيئة النمو غير مهم للإنبات والنمو الطبيعي لأنبوبة التلقيح.