

Morphometrics and Scanning Electron Microscopy of Pollen of Date Palm (*Phoenix dactylifera* L.)

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ABSTRACT. Pollen of seven staminate cultivars of date palm were collected from Al-Zafarania Agricultural Experimental Station, Baghdad and studied for pollen germination, pollen tube growth, number of pollen grains per flower and pollen morphometrics. The pollen was also examined by scanning electron microscopy for morphological differences in exine structure.

Significant differences were found among the test cultivars in all the parameters tested. The highest pollen germination and pollen tube growth was observed for cv. Green Ghanami and the lowest was found in cv. Wardi. The greatest number of pollen grains was recorded in Green Ghanami flowers and the lowest in Red Ghanami.

Scanning electron microscope study of pollen of the test cultivars revealed overall similarities in shape and size. The pollen grains are monosulcate, elliptical with one central closed germinal furrow. Exine of the test cultivars showed a tectate perforate pattern with considerable variations in the shape of the sculpturing appearance.

The date palm, *Phoenix dactylifera* L., is the most economically important tree in Iraq. It is considered to be the oldest cultivated tree with a great genetic diversity (Popenoe 1973, Zohary and Spiegel-Roy 1975).

Based on the fruit characters, a vast number of cultivars (over 400) are recognized in Iraq (Al-Beker 1972). However, only few male cultivars with different local names of unknown authorships were known to occur in Iraq. Iraqi farmers prefer to use pollen of certain male cultivars for artificial pollination without any scientific basis for this preference.

Various other studies have focussed on with pollen viability (Al-Taher and Asif 1982, Furr and Enriguez 1966), effects of pollen from different staminate

cultivars on growth and development of fruits (Mathaw *et al.* 1975, Osman *et al.* 1974) and seed formation (Clor *et al.* 1975).

The taxonomy of *P. dactylifera* is difficult due to interbreeding, similarity of vegetative and reproductive morphology and conflicting taxonomic nomenclature (Carpenter and Ream 1976, Nixon 1936). Pollen morphology has been utilized to identify various plant species, clones and varieties (Marcucci *et al.* 1984, Takahashi 1987). Study of the taxonomic characters of pollen from some date palm clones has been reported to be useful in the taxonomic identification of staminate date palm trees (Tisserat and DeMason 1982).

The study of the pollen morphology, sculpturing and viability of staminate cultivars should help in the taxonomic identification of staminate cultivars and elucidate the reason for preference of some cultivars in pollination.

Materials and Methods

Samples of pollen were collected from flowers of date palm cultivars: Red Ghanami, Green Ghanami, Smaismi, Wardi, Adi, Risasi and Gritly growing at Al-Zafarana Agricultural Experimental Station near Baghdad. Sampling was made a few days prior to spadix opening. The spadices were opened and air dried individually to avoid contamination. Pollen grains were collected by shaking the dried inflorescences, and stored at 4°C until use.

Scanning electron microscopy

Pollen samples from each of the test cultivars were dusted onto aluminum specimen holders coated with transparent double-adhesive tape. A gentle air flow was applied to remove excess pollen. The samples were coated with gold using a sputter coater and examined with a JSM-35 CF scanning electron microscope at 15 kV. Mature representative pollen grains of each of the test cultivars (except cv. Adi) were examined and photographed.

Stamen and pollen morphometrics

Ten uniform flowers were taken from each cultivar to measure their stamen lengths. Pollen grains of each cultivars were mounted in glycerol and width and length of 100 grains (uniform of normal appearance) was measured under the light microscope (x600).

Pollen counts

The number of pollen grains per anther was estimated following a standard method (David 1965). Ten flowers from each cultivar were dried on aluminum foil separately and each flower was transferred into test tube containing a solution

composed of lactic acid (85%) and glycerol in a ratio of 3:1 (V:V). The solution was shaken and the suspended pollen grains counted with a haemocytometer. The number of pollen grains per anther was calculated using the following equation:

$$\text{No. of pollen grains/anther} = \frac{\text{No. of pollen grains counted}}{\text{No. of anthers}} \times 111.1$$

Where the number 111.1 is a constant.

Pollen germination and pollen tube length

Modified Brewbaker and Kwack medium (Tisserat *et al.* 1983) was used to germinate pollen grains of the test cultivars. Ten milligrams of each pollen sample were suspended in 5 ml of the liquid medium placed in 250 ml conical flask and incubated at $27 \pm 1^\circ\text{C}$ for 24 h in the dark. The percentage of pollen germination was determined by examining 100 pollen grains for each test cultivar. The length of pollen tubes of the test cultivar was also measured and recorded using a light microscope at $\times 600$ magnification. The samples were replicated 4 times and all data were statically analyzed using ANOVA test.

Results

Scanning electron microscopic examination revealed an overall similarities in shape and size (Figs. 1-12) of all the test staminate cultivars. The grains were monosulcate, elliptical with one central closed germinal furrow extending from pole to pole. The ends of the grains were similar; however, in certain cultivars such as cv. Green Ghanami and cv. Smaismi the ends tend to be more rounded. Wax like deposite of unknown nature was observed in cv. Gritly only (Figs. 1,2).

Exine of the test cultivars showed a tectate perforate topography pattern. These preforations showed a considerable variation in shape, size and sculpturing appearance among the test cultivars and within single grain. They are either more rounded in cv. Red Ghanami (Figs. 3,4) and to less extent in cv. Green Ghanami, or angular in the rest of the test cultivars (Figs. 5,6). The largest perforations were found in cv. Adi (Figs. 7,8) and the smallest in cv. Red Ghanami. Exine structure appeared smoother in cv. Red Ghanami and Green Ghanami than in the others. The roughest exine structure was observed in cv. Wardi (Figs. 9,10) and to a lesser extent in cv. Smaismi (Figs. 11,12). In such cases, the exine sculpture appeared with great depth of field.

Significant differences ($P=0.05$) were found among the anther lengths and length and width of pollen grain (Table 1). The highest anther length was observed in cv. Green Ghanami and cv. Risasi while the lowest was observed in cv. Red Ghanami and cv. Wardi. Cultivar Wardi showed the greatest length of pollen and

the lowest pollen length was recorded in Green Ghanami. All the test cultivars except cv. Adi showed similar pollen grain width. Green Ghanami showed the highest number of pollen per anther while cultivar Red Ghanami showed the lowest number of pollen per anther (Table 2).

Pollen germination and pollen tube lengths were found to be significantly different ($P=0.05$) among the test staminate cultivars (Table 2). The cultivar Green Ghanami showed the highest pollen viability and pollen tube growth compared with the other test cultivars. The lowest germination and tube length was recorded for cv. Wardi.

Table 1. Anther and pollen morphology characteristics in six male cultivars of date palm, *Phoenix dactylifera* L.

Cultivar	Anther Length (mm)	Pollen grains per Anther	Pollen grains per Flower	Pollen Length (μm)	Pollen Width (μm)	Length: Width ratio
Green Ghanami	5.45	3439	20637	22.77	13.59	1.7:1
Risasi	5.28	3014	18082	24.69	13.35	1.9:1
Smaismi	5.03	2490	14937	24.15	13.10	1.8:1
Adi	4.61	2162	12971	24.70	11.85	2.1:1
Wardi	4.02	2051	12304	25.80	13.25	2.0:1
Red Ghanami	4.54	1574	9444	23.25	13.35	1.7:1
LSD=P(0.05)	0.19	121	728	1.54	0.93	

Table 2. Comparison of *in vitro* pollen germination and pollen tube length of six male cultivars of date palm, *Phoenix dactylifera* L.

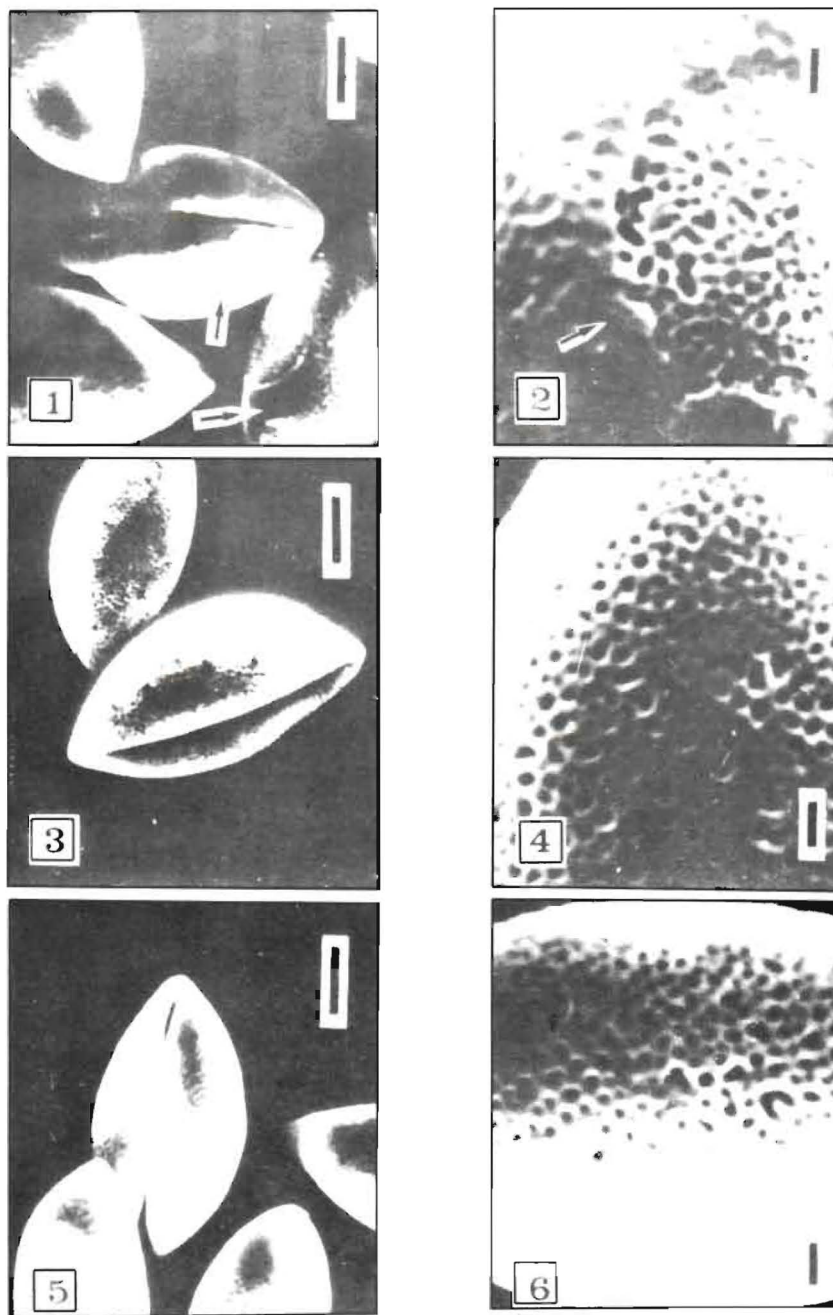
Cultivar	Germination (%)	Pollen Tube Length (μm)
Green Ghanami	87	323
Risasi	79	305
Smaismi	81	298
Adi	74	298
Red Ghanami	66	293
Wardi	63	288
LSD P = (0.05)	3	25

Discussion

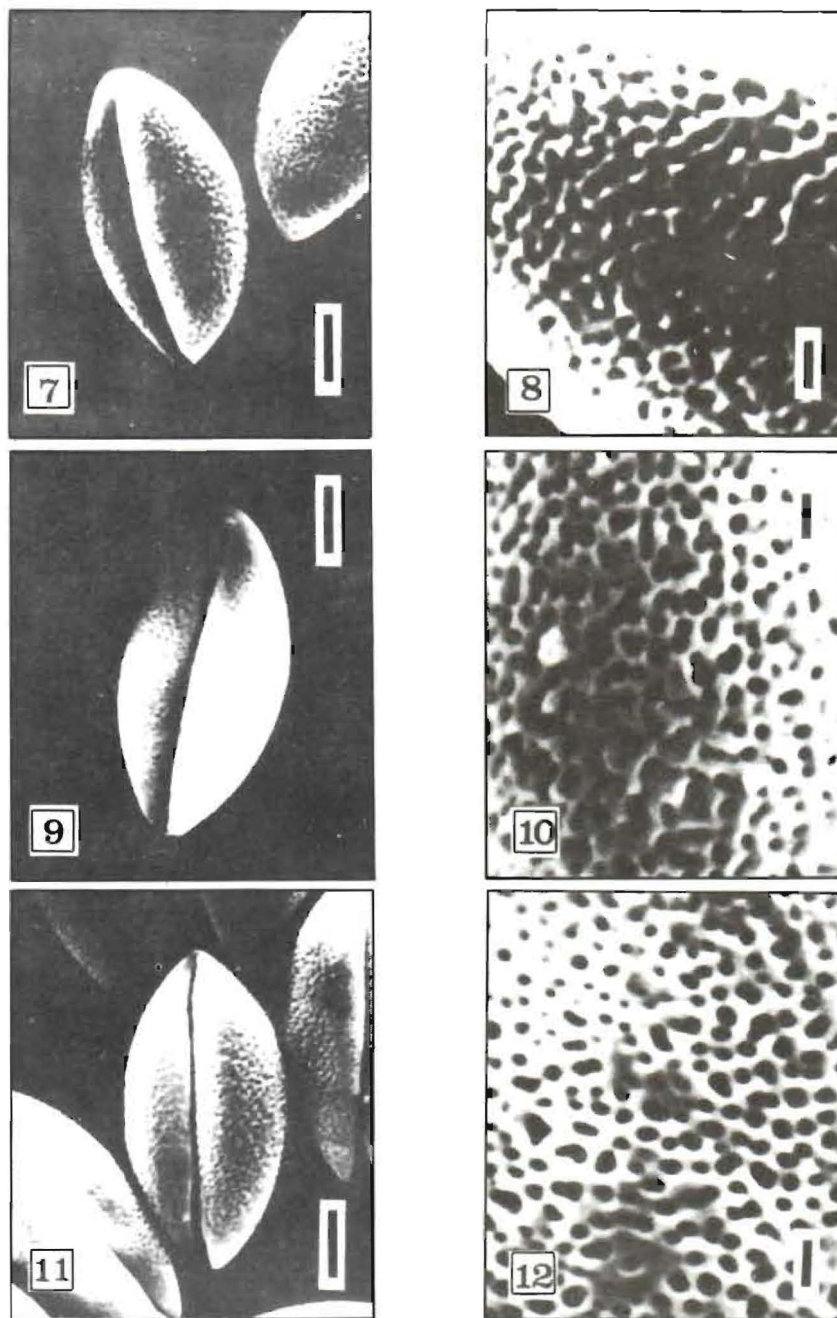
Tisserat and DeMason (1982) found that pollen grains of date palm species are unique and can be used for cultivar identification. The present study further supports Tisserat and DeMason's findings in that pollen structure and morphology can be used in identification of the common staminate cultivars of date palm in Iraq. Moreover, our study is the first to utilize scanning electron microscopy of pollen in identification of male date palm cultivars in Iraq. It appears feasible to use this technique in identification of other male cultivars of unknown origin. Some of these cultivars were grouped according to their similarities of some morphological features, such as spadix shape, size and color. This study may furnish alternative, reliable characters such as pollen morphology that can be utilized separately or in combination with other characters in grouping closely related cultivars of date palm.

Farmers in Iraq prefer to use certain cultivars such as Green Ghanami and Red Ghanami for pollination (Rahim 1975). The preference of Green Ghanami appeared to be related to the production of abundant pollen, higher germination ability and fast pollen tube growth (Tables 1,2), in addition to its higher number of flower per spadix and large spadix (Rahim 1975). Red Ghanami, however, appeared to be preferred because of the greatest number of flowers per spadix and large spadix but not for pollen germination, pollen tube growth and pollen per anther.

Thus it appears that further pollen morphometric and patterning studies may be useful in the identification of more different male cultivars and in the selection of potential pollinators.



Figs. 1-6. Scanning electron micrographs of pollen grains of different *Phoenix dactylifera* L. cultivars. 1,2) cultivar Gritly. 3,4) cultivar Red Ghanami. 5,6) cultivar Green Ghanami. Scale line = 5 µm (Figs. 1,2,3) and 1 µm (Figs. 2,4,6). Arrows = Wax-like deposits.



Figs. 7-12. Scanning electron micrographs of pollen grains of different *Phoenix dactylifera* L. cultivars. 7,8) cultivar Adi. 9,10) cultivar Wardi. 11,12) cultivar Smaismi. Scale line = 5 μ m (Figs. 7,9,11) and 1 μ m (Figs. 8,10,12).

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القياسات المورفولوجية والفحص بالمجهر الالكتروني الماسح لحبوب لقاح أفحل نخيل التمر

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مُجمعت حبوب لقاح سبعة أصناف من أفحل نخيل التمر من محطة البستنة في الزعفرانية - بغداد، لدراسة إنبات حبوب اللقاح وطول الأنبوب اللقاحي وعدد حبوب اللقاح في الزهرة الواحدة اضافة إلى القياسات المظهرية الأخرى. استخدم المجهر الالكتروني الماسح لتحديد الاختلافات المظهرية للسطح الخارجي لحبوب اللقاح.

دلت النتائج على وجود اختلافات معنوية بين الأصناف في جميع الصفات المدروسة، حيث أظهر الصنف غنامي أخضر أعلى نسبة إنبات وأعلى معدل لنمو الأنبوب اللقاحي في حين أعطى الصنف وردي أقل نسبة إنبات وأقل معدل لنمو الأنبوب اللقاحي. وكان أكبر عدد لحبوب اللقاح في الزهرة الواحدة في الصنف غنامي أخضر وأقله في الصنف غنامي أحمر.

وبين الفحص بالمجهر الالكتروني الماسح تشابهاً عاماً في حبوب اللقاح للأصناف المدروسة من حيث الحجم والمظهر. وكانت حبوب لقاح هذه الأصناف أحادية الحز وبيضوية الشكل مع وجود اخدود وسطي واحد. وتميز السطح الخارجي لحبة اللقاح بطرازه الشبكي المنقر، ولوحظت اختلافات واضحة في حجم وشكل النقر لحبوب لقاح الأصناف المدروسة.