

**BIOTECHNOLOGICAL STUDIES ON THE PRODUCTION
OF SOMATIC EMBRYOGENESIS AND SYNTHETIC SEEDS
OF DATE PALM
PART I : FEATURES OF LEAVES AND THEIR
ULTRASTRUCTURAL SURFACE VARIATIONS**

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By

**I. A. Ibrahim, A. A. Gargas , M.E.Nasr, A.A.Elbanna* and
M.A.Abd El-Baky***

*Genetic Engineering and Biotechnology Research Institute, Menofyia
University. * Central Laboratory of Date Palm Research and
Development , Agricultural Research Center.*

ABSTRACT

Morphological leaf characters and its structural surface variations using the Scanning Electron Microscope (SEM) on some cultivars of date palm were studied. SEM was mainly used to clarify the stomata variation patterns among the studied cultivars. These cultivars are ; Gondeila, Sewi and Zaghloul. The study carried out on leaves obtained from *in vitro* plants that represent these cultivars.

The morphological and SEM results indicated that Gondeila cultivar is more close to Sewi than to Zaghloul. Both earlier cultivars are similar to each other in most of the studied morphological charactrs. In addition, the similarity between both cultivars had been also observed in most SEM features; e.g. stomatal pore length, stomatal system length, subsidiary (adjacent) cell length and width, distance between stomata at each row, distance between rows, number of cells between rows and epidermal cell length. The only obvious exception of SEM results was the stomata type, which was anisocytic in Gondeila and Sewi, while it was paracytic in Zaghloul .

Keywords: *date palm,SEM.*

1. INTRODUCTION

Date palm, *Phoenix dactylifera* L. belongs to family Palmaceae (Arecaceae). The family contains over 200 genera and 2500 species (Comer, 1966 and Murnier, 1973). The approximated number of date palm plants is about 100 million worldwide, where 62% of this number is found in the Arabian areas and Middle East (FAO and Arab Countries League, 2000 and Moursy and Sakr, 1996).

Date palms are classified according to fleshiness into; soft date (e.g. Zaghoul, Samani and Barhee), semi-dry date (e.g. Sewi, Aglany and Amry) and dry date (e.g. Sakkoty, Gondeila and Malakaby).

Conventional vegetative propagation made through offshoots is very slow and laborious. The number of offshoots produced by individual tree is highly variable (from zero to 30); it is affected by cultivar and the possibility of inducing *in vivo* plants is highly appreciated (Murnier, 1973). Therefore, the development of micropropagation technique is very important in this concept.

The recent investigation was conducted to clarify the relationships among some cultivars of date palm by studying the leaf morphological features, in addition to the stomata variation patterns by using Scanning Electron Microscope (SEM).

2. MATERIALS AND METHODS

The current investigation was performed at the tissue culture laboratory, Technological Institution for Genetic and Molecular Engineering, Sadat branch, Menofya University and at the Central Laboratory, Faculty of Agriculture, Ain Shams University, Cairo.

Offshoots of the three cultivars, namely; Gondeila, Sewi and Zaghoul were secured from different farms and leaves of offshoots were completely removed with bases to expose the shoot tip of each offshoot.

Shoot tips (5-7 x 3-4 cm) washed with running tap water for 30 minutes were sterilized in 95% ethanol for 5 min. Thereafter, they were dipped in 20% (v/v) commercial bleach (2.25% sodium hypochlorite) for 30 min, and finally in 0.1% mercuric chloride (MC) for 5 min. The explants were rinsed 3 times with sterile distilled water. Under aseptic conditions the outer 3-4 leaf primordia (LP), about 3 cm in length, were dissected from the apical shoot tips (ST);

the later were longitudinally cut into 3-4 pieces. The explants were cultured on MS medium (Murashige and Skoog, 1962) supplemented with 0.5 mg/L nicotinic acid, 0.5 mg/L pyridoxine – HCl, 1.0 mg/L thiamin HCl, 100 mg/L myoinositol, 2.0 mg/L glycine, 3000 mg/L agar, 3000 mg/L activated charcoal and 30000 mg/L sucrose . The pH media was adjusted to 5.7 before autoclaving at 121°C. For callus initiation and proliferation, the medium was supplemented with 2,4-D, 2-ip, ansemydol and pacloprazol. Such media proved the most suitable for callus growth. The cultures were incubated at 25-27°C in darkness, at 6 weeks intervals.

The formed embryos on all media were systematically isolated and cultured on free hormone medium and incubated at 16 hrs. light photoperiod regime with 3000 Lux light intensity to stimulate embryo germination into normal plantlets. The normal plantlets were transferred and planted in container filled with peatmoss and sand at a ratio of 3: 1, irrigated every 2 days for about 1.5 months under green house conditions, which was 27° C with 60% humidity and 4000 – 6000 Lux. After 6 months leaves from each cultivar were chosen to carry out the SEM study.

The mother stock of the three studied cultivars was used for studying the leaves morphological features. Data were calculated on average of 10 readings of each cultivar.

The detailed leaf surface scan features were examined by using Scanning Electron Microscope with different magnifications at 15 kv. The SEM micrographs were taken after mounting of the completely mature leaf with SPI supplies on copper stubs and coated with a thin layer of gold palladium in Edwards Sputter Coater Unit, S 150 B. Scanning was carried out by JEOL-JSMT 100 Model Scanning Electron Microscope. It should be mentioned that the magnification power was $x = 1000$.

Numerical analysis in this study was concentrated on the infraspecific level, the rank of Operational Taxonomic Unit (OTU) was the individual specimens representing each cultivar. The equal numbers of mother stock specimens (10 specimens) were studied for leaf morphological characters . The chosen characters in this study was 10, which have an equal weight (importance). After choosing the characters and OTU's the resemblance between OTU's was calculated using the cluster method; the technique was Single Likage Clusterig (Sneath and Sokal, 1973) .

3.RESULTS AND DISCUSSION

3.1. Leaf morphological characters

Leaf morphological characters are represented in Table (1) and illustrated in Figure (1 a,b,c). The tabulated measurements, which are the average of 10 specimens of each cultivar, were as follows :

3.1.1.Leaf length and width

In this study, the average measurements of these characters were quite similar in both cultivars Gondeila and Sewi, (352 & 57 cm) and (345 & 60 cm) ; respectively. While leaf length and width of Zaghoul were slightly higher (385 & 70 cm.) (Table 1).

According to Ahmed *et al.* (1979) the date palm leaves could be divided into 3 groups : short less than 325 cm, medium from 325-425 and long more than 425 cm. Abdalla (1986) mentioned that mature leaves of palms varied according to cultivars.

3.1.2.Leaflet region and petiole lengths

It is worthy to mention that the sum of these characters is the leaf length. The leaflet area and petiole length of Gondeila and Sewi had the same trend, where these lengths were (193 & 159 cm) and (189 & 156 cm); respectively. The length of leaflet area in Zaghoul (312 cm) was nearly the fold of that of both cultivars, which reflect on petiole length to make it the lowest (73 cm) .

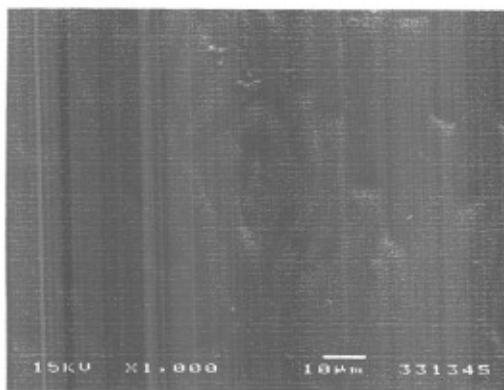
3.1.3.Spine region and spine region percentage

Nixon (1945) suggested three groups of spine region in relation to the total leaf length; short (less than 15%) , medium (from 15-25%) and long (more than 25%). Accordingly, the spine region percentages of the three studied cultivars; Gondeila , Sewi and Zaghoul (5.9, 6.7 and 9.9%) were ranking as short group .

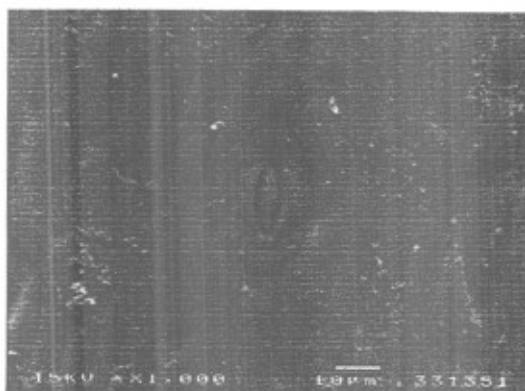
The rest of leaf morphological character measurements from no. 7 to 11 had no difference among the studied cultivars. Where, Gondeila and Sewi were quite similar to each other with low measurements than that of the highest of Zaghoul (Table 1) .

From the above mentioned results, it could be stated that Gondeila and Sewi, as dry and semi-dry cultivars are similar to each other compared to the soft date Zaghoul. These results are in harmony with those of Ahmed *et al.* (1979), Amer (2000) and Rizk *et al.* (2004) .

a- Anisocytic



b- Anisocytic



c- Paracytic

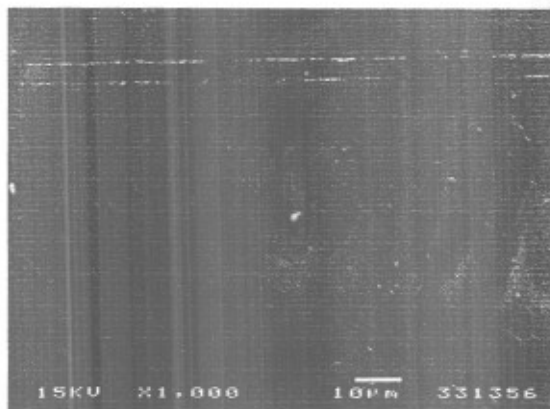


Fig.(1): leaf stomatal shape as shown by SEM.
A-Gondeila B-Sewi C- Zaghlou

Table (1) : Leaf morphological features of some cultivars of date palm (average of 10 readings of each cultivar cv.).

Features	Cultivars	Gondeila	Sewi	Zaghloul
1. Leaf length (cm)		352	345	385
2. Leaf width (cm)		57	60	70
3. Leaflet region length(cm)		193	189	312
4. Petiole length (cm)		159	156	73
5. Spine region length		21	23	38
6. Spine region percentage		5.9%	6.7%	9.9%
7. Spine length (cm)		5.3	5.4	6.3
8. No. of spines / leaf		23	25	20
9. Petiole base width		7	8	8
10. Leaflet length (cm)		29	33	39
11. No. of leaflet / leaf		210	209	200

3.2. SEM results

The Scanning Electron Microscope (SEM) measurements on the leaf of the three cultivars are shown in Table (2). It is worthy to notice that all the measurements recorded on the stomatal characters; stomatal pore length, stomatal system length and with, subsidiary cell width, distance between stomata per row and distance between rows were lower in both Gondeila and Sewi cultivars than those of Zaghloul. The same trend has also observed with both epidermal characters of the studied cultivars. The stomata type of the earlier cultivars was anisocytic, while it was paracytic in Zaghloul.

Table (2) : Scanning Electron Microscope measurements (μ) and counts on leaf of some cultivars of date palm. (average of 2 readings of each cv.).

Characters cultivars	Stomatal pore length (μ)	Stomatal system length (μ)	Stomatal system width (μ)	Stomatal system diameter (μ)	Subsidiary cell length (μ)	Subsidiary cell width (μ)
Gondeila	14.4	34.6	16.8	498.9	17.6	4.4
Sewi	13.5	39.0	21.8	669.8	16.8	4.4
Zaghloul	18.8	52.5	31.7	1263.5	17.9	7.1
characters cultivars	Distance between stomata/row	Distance between rows (μ)	No.of cells between rows	Epidermal cell length (μ)	Epidermal cell width (μ)	Stomata type
Gondeila	73.7	58.1	6.0	50.7	8.2	anisocytic
Sewi	61.2	57.2	6.0	50.3	9.7	anisocytic
Zaghloul	91.6	60.5	4.0	53.8	9.8	paracytic

Most of the recorded measurement values of both cultivars, Gondeila and Sewi are lower than those recorded for Zaghoul. The only two exceptions were ; the number of cells between stomatal rows and types. This number was 6 in Gondeila and Sewi compared with 4 in Zaghoul. This may be due to the fact that these two cultivars are adapted to their dry habitat, while the third cultivar is common in the moderate regions. The stomatal types were anisocytic in Gondeila and Sewi and paracytic in Zaghoul .

3.3. Cluster analysis

The dendrogram (Fig.2) produced by the specimens and showed the highest average taxonomic similarity value of 1.09. At this level, the studied specimens were divided into two clusters. The first, which was distinguished at level 1.06, included all the specimens representing Zaghoul . The second, is divided into two sub-clusters at level 0.92. One of these sub-clusters was distinguished at level 0.84 and included the Sewi specimens. The other sub-cluster which was recognized at level 0.83 included the specimens of Gondeila.

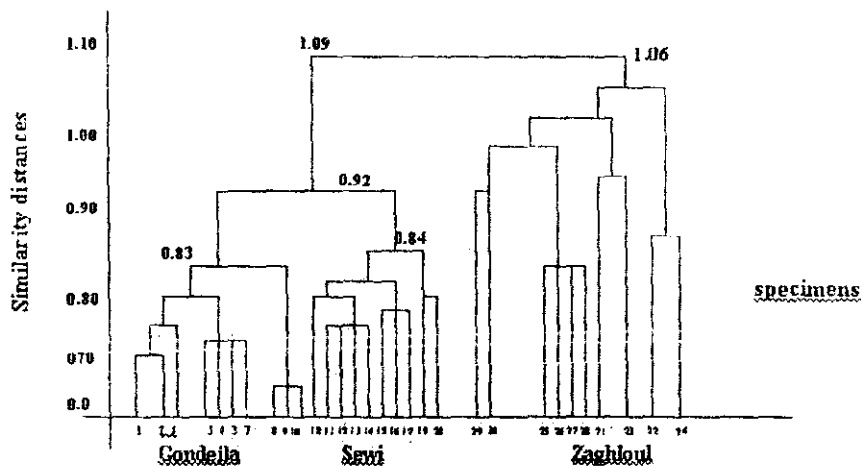


Fig (2). Dendrogram showing the relationships between 30 specimens representing 3 cultivars of date palm.

The results obtained by cluster analysis confirmed those gained from morphological and SEM investigations , where Gondeila and Sewi are more close to each other than to Zaghoul .

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دراسات تكنولوجية حيوية على إنتاج الأجنة الجسمية والبذور الإصطناعية لنخيل البلح

الجزء الأول : صفات الأوراق والتركيب المجهرى الإلكتروني لتباين أسطحها .

إبراهيم عبد المقصود إبراهيم - عادل أبسخرن جرجس- محمود إمام نصر
عبد المنعم عبد الودود البنا* - محمد عبد الرسول عبد الباقي*

معهد بحوث الهندسة الوراثية والتكنولوجيا الحيوية -مدينة السادات - جامعة المنوفية
*المعمل المركزي للأبحاث وتطوير نخيل البلح - مركز البحوث الزراعية

ملخص

تم دراسة الصفات المورفولوجية للأوراق وكذلك تباين تركيبها السطحي باستخدام الميكروسكوب الإلكتروني الماسح لبعض أصناف نخيل البلح . وذلك لتوضيح أنماط تباين الثغور للأصناف تحت الدراسة وهي جنديلة ، وسيوي وزغلول. تمت الدراسة على أوراق نباتات ناتجة من زراعة الأنسجة تمثل تلك الأصناف .

وقد دلت النتائج المورفولوجية وفوق المجهرية بأن الصنف جنديلة أكثر قرابة إلى الصنف سيوي عنه للصنف زغلول . كلا الصنفين الأولين متشابهين لبعضهما في معظم الصفات المورفولوجية المدروسة . بالإضافة إلى التشابه الذي لوحظ بينهما أيضا في معظم الصفات فوق المجهرية لسطح الأوراق على سبيل المثال طول فتحة الثغر ، طول الجهاز الثغري ، وطول وعرض الخلايا المجاورة، والمسافة بين الثغور في كل صف ، والمسافة بين الصفوف ، وعدد الخلايا بين الصفوف وطول خلايا البشرة . الاستثناء لبعض النتائج فوق المجهرية كان نمط الثغر حيث كان أنيزوستيك في الصنفين جنديلة وسيوي بينما كان باراسيتك في الصنف زغلول .

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(يناير ٢٠٠٦): ٩٧-١٠٦ .