

Early prediction of sex determination in date palm cultivars using some morphological and scanning electron microscopy studies

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ABSTRACT

Morphological characteristics of the leaves and spathe besides scanning electron microscopy of leaves were studied to show the diversity between two selected date palm females cultivars Zaghoul and Hayani. They were compared with the males ones that might be similar to females to show how far such females are differ with their satellite males. Results showed significant differences in morphological characteristics of the two males and females than each other in leaf base width and thickness leaflets width and length, and number of spines / leaf as well as spathe characters. Results of scanning electron microscopy on the upper leaflets surface showed a big difference between the two male cultivars while differences between the females and their satellite males were observed in number and length of stomata as well as guard cell thickness.

INTRODUCTION

Date palm (*Phoenix dactylifera* L. $2n = 36$) is a monocotyledonous and dioeciously species belonging to arecaceae family that includes 225 genus and 2600 species (Corner, 1966). Since the males of date palm are raised from

seeds of the standard cultivars, then it is expected that the female cultivars and their "satellite" males might be similar in one or more of the

morphological characteristics of the leaves (Al-Baker, 1972). Most of available pollinating date palm males are mainly originated from seed propagation, resulting in many different local males that represent genetic diversity. Characterization and evaluation of available highly potent male palms is the first step to find superior ones to fertile female plants (Rizk *et al.*, 2007). Therefore, it is important to conduct experiments to evaluate male's parents, in terms of vegetative and flower characteristics, determination of biodiversity, opening early and late pollination, and also monitoring the similarities and differences (Elshibli *et al.*, 2007). Most attempts to distinguish date palm males are mainly based on morphological characteristics yet (Shaheen, 1983).The morphology of date palm leaves also was utilized for identification of certain male cultivars in the central region of Saudi Arabia (Shaheen *et al.*, 1986a).

Cultivars of date palm grown in Egypt differ in distribution and frequency in all growing regions. Each date palm cultivar is specific and characterized to a region. Each female cultivar has also appropriate male pollinator. The effect of pollinator source can influence the physical and chemical characteristics of fruit set (Higazy *et al.*, 1983). The available number of date palm males is insufficient for efficient pollinating of increasing number of female plants. Identification of male cultivars mostly depends upon information obtained from local farmers; therefore, the local names of cultivars vary among locations in the country.

The main objectives of this study were to evaluate the mature male and female (Zaghloul and Hayane) date palm cultivars using some morphological and scanning electron microscopy studies to predict sex determination that might be a useful tool in early prediction of sex determination of palm seedlings which produced from seeds.

Materials and Methods

The current study was conducted during the period from 2009 to 2011 on two date palm cultivars Zaghloul and Hayani in the region that are famous for growing these cultivars. Zaghloul farm was located at (59 km) west to the desert road of Alex, Cairo. Alex Governorate while Hayani was from Kalabsho, Bilqas, Dakahlia Governorate. The studies were carried out in the laboratory of Biotechnology, Department of Biotechnology. Faculty of Agriculture, Al Azhar University Cairo.

Plant materials:

The selected trees were 20 years old grown in sandy soil, healthy, uniform in growth, vigor and height, as possible. All cultural practices were carried out according to the normal schedule for experimental palms. Four years old leaves were collected from each of the selected males. Similar leaf samples were also collected from the female palms Zaghloul and Hayani grown together with the selected males in the main directions of each tree.

Morphological characteristics:

The following growth characteristics were studied as suggested by Al-Baker, (1972) as follows.

Leaf: length – leaf base width and thickness

Leaflets: number/leaf – length and width

Spines: number/leaf – length and thickness – length of spine zone.

Length of the leaf was measured below the lowest spine to the end of the top most leaflets (pinnate). Length and width of leaflets were determined as an average of 10 leaflets taken from the middle portion of the rachis, (5 on each side) according to (Shaheen *et al.*, 1986b). Length of the leaflets and

spine zone was also measured. Spathe length, spathe width (cm), spathe thickness (cm), strands number/ spathe, strand length (cm) and flowers number / strand were measured.

Scanning Electron Microscopy (SEM) studies:

Scanning electron microscopy of the upper leaflets surface of male and female of the two date palm (Zaghloul and Hayani) cultivars was done. The samples were coated by gold sputter coater (SPI-Module). Finally the samples were examined by using high vacuum mode at the Regional Center of Mycology and Biotechnology, Cairo, Egypt.

Statistical analysis:

All obtained data were processed by Microsoft Excel (Microsoft, 2000). Data were statistically analyzed by analysis of variance and least significant differences (LSD) according to Steel *et al.* (1997) as well as stepwise multiple regression analysis were conducted using the statistical program (Statistix 8.0).

Results and Discussion

Morphological characteristics:

Results in Table (1) revealed significant differences between the two male cultivars in most vegetative characteristics. In this regard, Hayani cultivar showed superiority over Zaghloul cultivar in most traits, however there was no significant difference between them in some characters such as width of leaflets and thickness of leaf base. These differences between cultivars may be due to genotypic variability.

Results also showed that the two cultivars significantly varied in the width and the shape of the leaf bases. Regarding the differences between the male and female palms

concerning this point, it can be concluded that the width of leaf bases of the male palms were bigger than those of the females, being of 27.3 cm, in width in the Hayani cultivar male followed by the Zaghloul date male which was about 21.5 cm versus they were 21.4 cm and 18.5 cm respectively for the female palms.

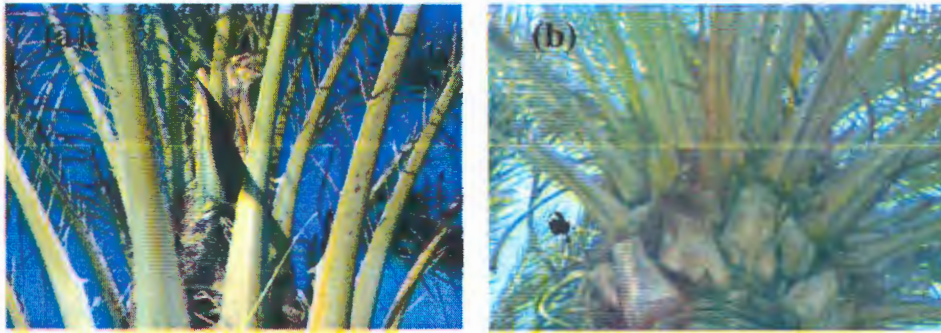


Fig. 1: Shape of leaf base of Hayani female (a) and male (b) cultivar.

Also looking for a morphological parameter to distinguish between male and female palms, results showed that the male palms possessed the greatest values of the leaflets length in comparison with female ones. The leaflets length of the Zaghloul cultivar males were 41.8 cm, versus 38.1 cm of the females. The leaflets length of the Hayani males is 30.4 cm, versus 48.3 cm in the female one. The results are in agreement with Abdalla (1979) and Abdalla (1986) reported that the male palm tree usually have rachises broader and thicker than those of the female ones.

Table (1): Morphological characteristics of males and females of the two data palm cultivars

Characteristics	Male		Female		.L.S.D at 0.05
	Zaghloul	Hayani	Zaghloul	Hayani	
Leaf length (m)	5.36	5.19	4.96	4.99	0.28
Number of leaflets/ leaf	215.8	213.4	220.3	211.6	6.75
Width of leaf base (cm)	21.5	27.3	18.5	21.4	0.39
Thickness of leaf base (cm)	5.4	6.1	4.4	4.2	0.21
Leaflets length (cm)	41.8	30.4	38.1	48.3	2.36
Leaflets width (cm)	2.35	2.55	2.20	2.19	0.13
Spin zone length (cm)	102.8	108.4	126.8	133.4	8.90
Number of spines/ leaf	14.87	18.61	11.73	24.66	1.70
Spine length (cm)	15.37	9.07	19.87	22.25	3.20
Spine thickness (cm)	0.42	0.54	0.33	0.51	0.06

The results in Table (1) also showed clear differences between the two studied cultivars regarding spine lengths. The values were ranged between 15.3 cm in the Zaghloul and 9.07 cm in the Hayani. Regarding the differences between the male and female palms, it can be conducted that the leaves of the female palms have longer spines than those of the males. The females of the Hayani palms possessed longer spines (22.2 cm), followed by the females of the Zaghloul one, which was about 19.8 cm, versus 15.3 cm and 9.07 cm, in the males ones. These results are in agreement with Abdalla (1979); Mousa (1981, 1985); Shaheen et al. (1986b) and Nail (1994)

reported that the length of the date palms spines were between 7.8 and 15.6 cm according to the cultivar.

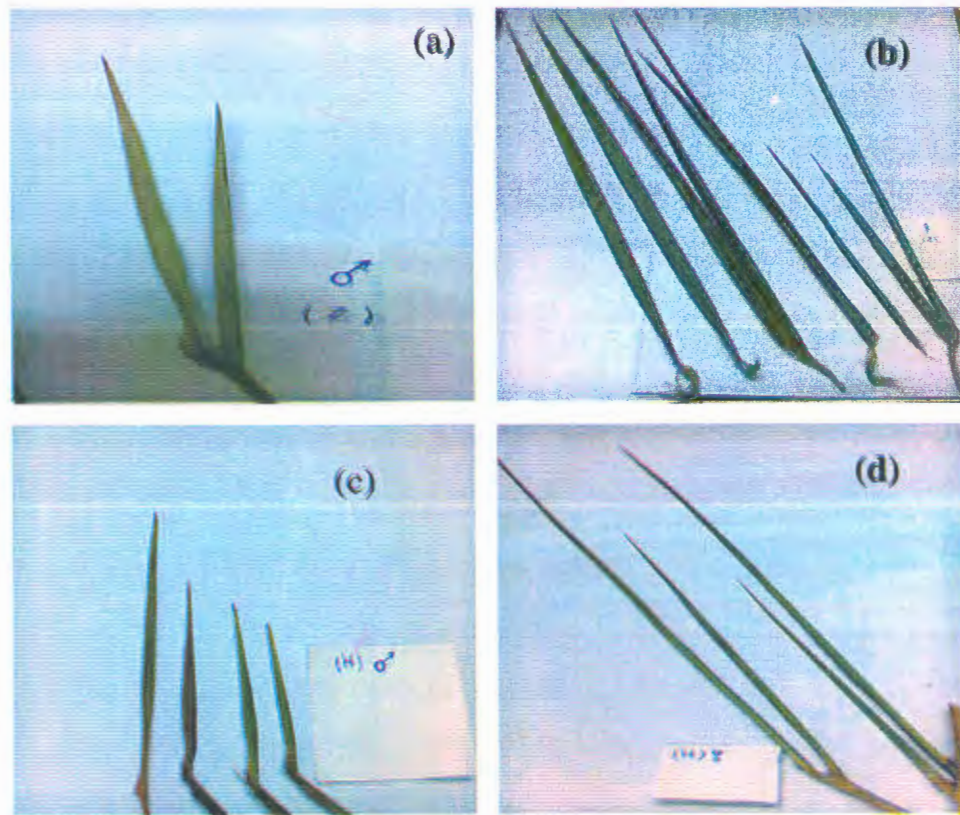


Fig. 2: Spines morphology of Zaghloul, Hayani male and female cultivars.
(a) Zaghloul male, (b) Zaghloul female, (c) Hayani male and (d)
Hayani female.

Spathe characteristics:

Tables (2 and 3) comprise the data of the length, width and thickness of the male and female spathes, the number of stalks per spathe, the length of the stalks and the number of flowers per stalk. Concerning the differences between the male and female palms, data showed that the male palms produced longer spathes than those of the female ones. The males of the Zaghloul produced longer spathes, 83.1 and 82.3 cm, in the first and second

years 2009 and 2010 respectively, than the Hayani one which were about 64.2 and 63.4 cm in the two seasons, respectively. Our results are in agreement with those obtained by (Abdalla, 1979; Mousa, 1985; Shaheen *et al.*, 1986b; Abdalla, 1986 and Makki and Osman, 1991) reported that the length of the male spathes differs according to cultivar and lies between 60 to 125 cm in length.

Tables (2 and 3) showed that the spathes of the male palms have more stalks than those present in the female ones. In the Hayani date palm, the number of stalks per spathe was 208.07 and 210.8 in the males versus 90.30 and 81.87 in the females in the two years of study. The number of stalks in the spathes of the Zaghoul date ranked second having 120.7 and 117.6 male stalks versus 66.83 and 74.80 female stalks per spathes in 2009 and 2010. These results agreed with those of Abdalla (1979)

; Mousa (1985) and Shaheen *et al.* (1986b) stated that the number of the stalks in the male palms ranges between 160 and 285 stalks/spathe according to the cultivar.

Results in Tables (2 and 3) revealed significant differences between the two male cultivars in most of the spathe characteristics. Zaghoul cultivar showed superiority over Hayani cultivar in all spathe characters, however there were no significant differences between them in spathe thickness. These differences between cultivars could be attributed to the antic differences.

Table 2: Spathe characters of two male and female date palm cultivars in 2009.

Cultivars Characteristics	Male		Female		L.S.D 0.05%
	Zaghloul	Hayani	Zaghloul	Hayani	
Spathe length (cm)	83.07	64.20	65.80	57.10	4.92
Spathe width (cm)	22.40	17.46	7.53	8.30	1.26
Spathe thickness (cm)	2.83	3.92	3.90	4.27	0.20
Number of stalks / spathe	120.2	208.0	66.83	90.30	18.4
Stalk length (cm)	29.5	26.22	58.53	50.83	2.40
Number of flowers /stalk	63.7	58.27	38.50	69.10	2.90

Table 3: Spathe characters of two male and female date palm cultivars in 2010.

Cultivars Characteristics	Male		Female		L.S.D 0.05%
	Zaghloul	Hayani	Zaghloul	Hayani	
Spathe length (cm)	82.33	63.46	66.33	53.80	4.75
Spathe width (cm)	22.73	17.13	7.10	8.60	1.46
Spathe thickness (cm)	3.87	3.90	4.10	4.0	0.24
Number of stalks / spathe	117.6	210.0	74.80	81.87	5.09
Stalk length (cm)	28.50	28.9	60.90	49.50	2.76
Number of flowers /stalk	57.43	55.43	41.47	66.67	3.81

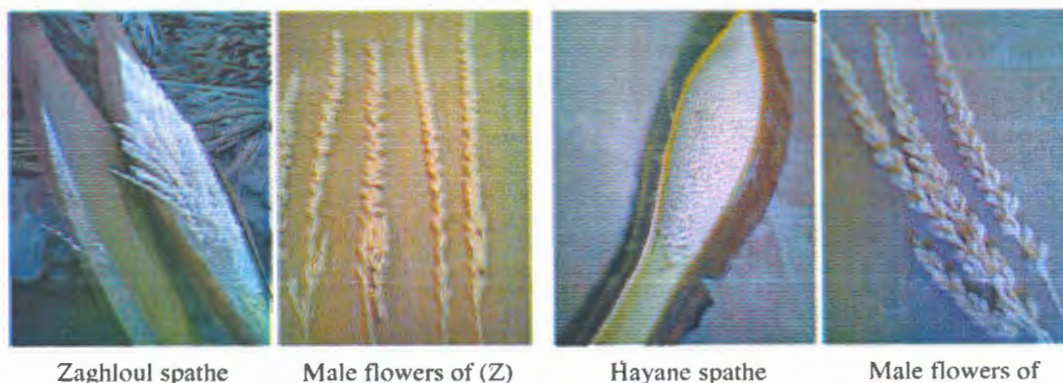


Fig. 3: Spathe and flowers of Hayani and Zaghoul male cultivar.

Scanning electron microscopy (SEM) study:

Results of scanning electron microscopy study were analyzed using Photoshop version 8.0. Results showed that the Zaghoul male and female variety has 78 and 48 stomata/ mm² respectively, versus 52 and 40 stomata/ mm² in female of Hayani, ones (Figs. 4a, b, c and d).

The results in Table (4) showed that the length of stomata (μ) the male palms than those present in the female ones. In the Hayani date palm, the length of stomata was 16.6 μ m in the male versus 13.84 μ m in the female. The stomata length of the Zaghoul date ranked second having 15.33 μ m male versus 11.11 μ m female.

The average distance between stomata in the same row (Horizontally) proved that, the Zaghoul male and female variety possessed has 66.03 and 51.85 μ m respectively, versus 70.66 and 81.88 μ m in the Hayani palm male and female, ones (Figs. 4a, b, c and d).

Table (4) Morphometric characteristics of leaflets using SEM from male and female date palm cultivars.

Cultivars Parameters		Male		Female	
		Zaghloul	Hayani	Zaghloul	Hayani
Number of stomata / mm ²		78	52	48	40
Length of stomata (μm)		15.33	16.6	11.11	13.84
Stomata width (μm)	Right	4.0	6.78	5.73	6.15
	Middle	6.0	9.99	9.62	10
	Left	4.3	6.42	5.36	6.5
Guard cell thickness (μm)		1.3	3.45	2.40	2.01
Horizontally (μm)	Middle	66.03	70.66	51.85	81.88
	Longest distance	100	100	75	95.65
	Less distance	45.45	54.34	36.36	67.39
Vertically (μm)	Middle	49.34	50.72	72.43	61.87
	Longest distance	59.69	58.69	86.36	69.56
	Less distance	29.54	45.65	56.80	54.34

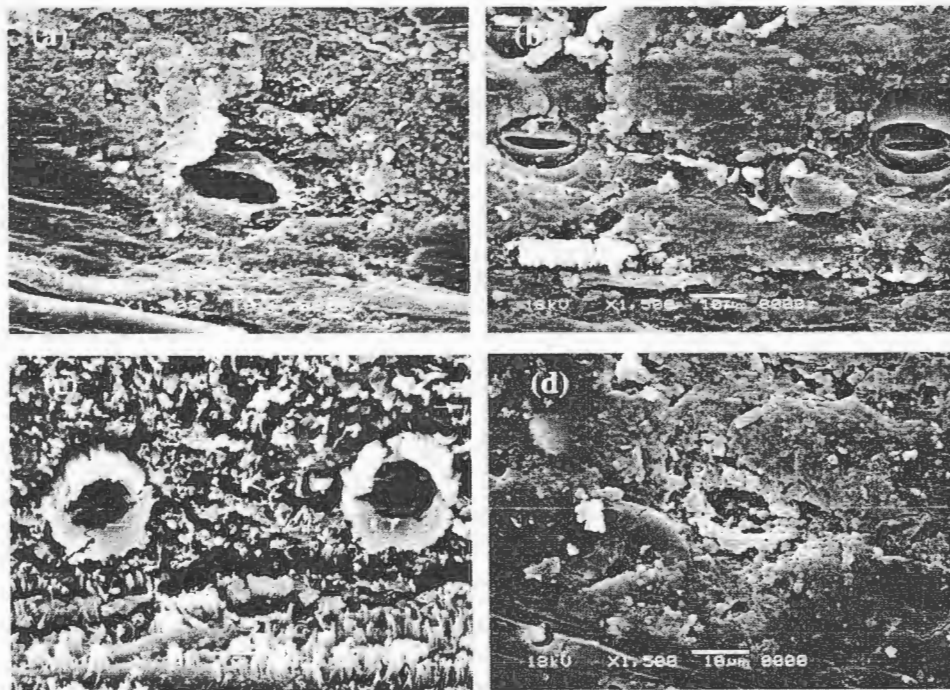


Fig. 4: Scanning

electron microscopy (SEM) images of upper leaflets surface showing stomata of date palm (*Phoenix dactylifera* L.). (a) Zaghoul male, (b) Hayani male, (c) Zaghoul female and (d) Hayani female.

Conclusion

We can come to a conclusion that, morphological and scanning electron microscopy studies can be used to differentiate between date palm cultivars as well as possibly early prediction of sex determination, which can be used to distinguish between male and female transplants produced from seeds.

It was clear that there was positive similarity build up morphological finger print based on morphometric character on SEM for stomata of upper

leaf for males, which help us to early determination to differentiate between female and male and within satellite males.

Morphological characteristics in addition to scanning electron microscopy studies were useful to differentiate between mature male and female in palm tree for early prediction of sex determination palm seedlings which produced from seeds germination

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التنبؤ المبكر لتحديد الجنس في اصناف نخيل البلح باستخدام بعض الدراسات المورفولوجيه
والميكروسكوب الالكتروني الماسح

سامى حامد الغياتى¹ و فوزى على الفقى² و عبدالمنعم عبدالودود البنا³ و احمد البغدادى احمد
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تمت هذه الدراسه فى مواسم ٢٠٠٩ - ٢٠١٠ - ٢٠١١ على صنفين من نخيل البلح (زغلول)
بمزرعه خاصه الكيلو ٥٩ طريق مصر اسكندريه الصحراوى والمزرعه الثانيه (الحيانى) فى
قلايشو مركز بلقاس محافظه الدقهليه وتهدف الدراسه الى التمييز بين ذكور نخيل البلح
مورفولوجيا بصفات ظاهريه واضحه يمكن استخدامها فى التنبؤ المبكر بتحديد جنس البادرات
الناتجه من زراعه البذور وتعتمد القياسات فى تحديد الجنس على المقارنه بين اناث وذكور النخيل
المنتشر زراعتها فى نفس المنطقه الجغرافيه.

واظهرت النتائج وجود اختلافات بين ذكور النخيل فى الصفات المورفولوجيه منها عرض وسمك
قاعده الاوراق ، طول وعرض الوريقات ، عدد الاشواك على الورقه وكذلك طول وعرض
الاغريض.

كما تم ايضا استخدام تحليل صور الميكروسكوب الالكتروني الماسح فى توصيف وتمييز ذكور
واناث النخيل عن طريق قياسات عدد الثغور فى السطح العلوى للورقه حيث اظهرت النتائج وجود
اختلافات فى شكل وعدد الثغور وكذلك ترتيبها وانتشارها على الوريقات. وبالتالي يمكن استخدام
نتائج الدراسه المورفولوجيه وصور الميكروسكوب الماسح للثغور فى التمييز بين اناث وذكور

النخيل وتحديد درجات التشابه بينهما واستخدامها كبصمه مورفولوجيه وعمل دليل انتخابى يعتمد على الصفات الظاهرية التى يمكن بها المحافظه على الاصناف المحليه بعمل سجلات اصول النخيل وهذا ايضا يحتاج الى مزيد من الدراسات.