

## THE DISCRIMINATING POTENTIAL OF SOME LEAF MORPHOLOGICAL AND ANATOMICAL CHARACTERS IN CERTAIN DATE PALM CULTIVARS.

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### ABSTRACT

Some morphological, epidermal and anatomical characters of the leaflet were studied in date palm cultivars, Bent-Aisha, Hyeane, Samanie, and Zaghlool. Moreover, The discriminating potential of the chosen characters between studied cultivars were examined. Leaflet blade length / width ratio, number of veins at each side of mid-vein, number of stomata / unit area, number of cell files in the costal region, number of stomatal files within the intercostal region, number of small vascular bundles between two large vascular bundles, and certain characters of the fibrous patches were proved helpful in cultivar - identification. Keys for identifying the studied cultivars were constructed based on characters of high discriminating potential.

**Keywords:** Leaf morphology, stomata, blade anatomy, date palm, *Phoenix dactylifera*, cultivar - identification

**Abbreviations:** B = Bent-Aisha; cv(s) = cultivar(s); H = Hyeane; LVBs = large vascular bundle(s); no. = number; S = Samanie; SVB(s) = small vascular bundles; Z = Zaghlool.

### INTRODUCTION

Date palm is one of the most important pomological crops in Egypt. Different cultivars are cultivated which differ significantly in their commercial value owing mostly to the characters of the fruits. Cv Hyeane is the most commercially valuable cv in Egypt, so it is widely cultivated. When cultivation, the offsets of less valuable cvs may be misinterpreted as Hyeane, which in turn, reduce the outcome of the orchard. So, it is important to develop a reliable methods for cv-identification in date palm.

For the purpose of cv-identification in date palm, different methods may be employed. Some of these methods are complicated and require highly elaborated equipment such as the adoption of Restriction Fragment Length Polymorphism, RFLP, (Corniquel and Mercier, 1994) and RAPD (Saker and Moursy, 1998; Sedra et al, 1998; Ben Abdallah et al, 2000) techniques for cv-identification. In addition, isoenzymes banding pattern was employed (Al-Jibouri and Adham, 1990; Bannaceur et al, 1991; El-Houmaizi et al, 1993; Booij et al, 1995). Cytological delimitation through chromosomal characterization was also employed (Al-Salih and Al-Rawi, 1987; Aly and Bacha, 2000). Nevertheless, these methods are complicated, costly, and out of hand of most growers. Alternatively, morphologically- and anatomically-based cv-discrimination is less costly and could be easily employed. The most important of which are the epidermal features which proved to be useful for cv-delimitation in mango (El-Fiki et al, 1986) and in banana (Salama and Riad, 1991). In this context, cv-identification in date palm has been tried on the basis of leaflet dimensions and no. of stomata / unit area (Al-Salih and Al-

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Sheikh Hussaim, 1980), some morphological characters (Bellabaci, 1988), and the pattern of leaf epicuticular wax (Rahmania and Huan, 1997).

The present investigation was undertaken to study some morphological and anatomical characters of the main date palm cvs grown in Egypt. Another objective is to elucidate the taxonomic usefulness of the studied characters for cv-identification.

## MATERIALS AND METHODS

Some morphological and anatomical characters of the leaflet of certain date palm cvs namely Bent-Aisha, Hyeane, Samanie and Zaghlood were investigated. For standardization, these characters were studied in leaflets situated halfway on the rachis of the 4<sup>th</sup> leaf from the top of about one-year-old offsets. For minimizing the interference of the environmental factors, the leaflets were secured from two locations, Damietta and Rachid regions. From each locality, 50 leaflets were collected from 4 different offsets. The morphological characteristics of the leaflet were recorded from the whole sample i.e., 100 leaflets.

Preliminary examination of leaf surface preparations revealed higher stomatal frequency on the abaxial epidermis, hence only abaxial surface preparations were made and mounted in glycerin-jelly. In these preparations, the following quantitative characters were determined: no. of stomata / mm<sup>2</sup>, dimensions of the stomatal apparatus, no. of cell files in the costal region, no. of stomatal files within each intercostal region, along with some qualitative characters.

Cross sections of the lamina (15 $\mu$  thick), halfway between leaflet tip and base, were made using rotary microtome after proceeding the usual paraffin method (O' Brien and Mc Cully, 1981). Sections were used for elucidating the comparative anatomy of the lamina in the studied cvs. Ten leaflets selected randomly from the whole sample of each cv were cross-sectioned and the following anatomical characters were recorded: no. of hypodermal cell layers above LVBs, no. of fibrous layers of the bundle sheath, no. of SVBs between two LVBs, no. of hypodermal and subhypodermal fiber patches between two LVBs. Some qualitative leaf anatomical characters were also recorded.

## RESULTS

### a) Morphological characters of the leaflet:

The immature leaf's base of cvs B and H is light-green whereas it is yellowish-green in cvs S and Z. The two halves of the leaflet are joined in an acute angle in cv Z and in a divergent angle in cvs B and H whereas in cv S, this angle is either acute or divergent. The leaflets of cvs B and H have higher length/width ratio compared with those of cvs S and Z. The terminal leaflet spine was longer in H and then in Z, B and S in a descending order (table 1). The leaflets of cvs B and Z are characterized by the presence of 5 and 9

small veins, respectively, at each side of the mid-vein. This character is less-specific in cvs H and S where 6, 7 and 5-9 small veins are found at each side of the mid-vein, respectively.

**Table (1): Certain morphological characters of the leaf in date palm cvs B, H, S and Z.**

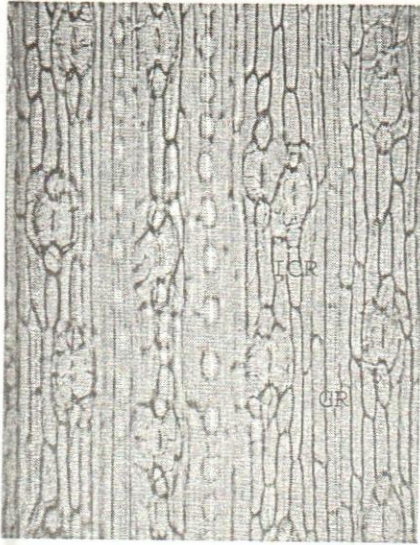
Characters	cvs	B	H	S	Z
		Leaflet length/width	Range	17-30.3	20-32.9
	Mean	23.8	23.9	17.0	14.4
Spine length (mm)	Range	3-12	6-18	2.5-7	4.5-12
	Mean	6.6	12.0	4.2	7.4
No. of veins at each side of mid-vein		Usually 5	Usually 6 and less-frequently 7	5-9	Usually 9
Immature leaf base color		Light green	light green	yellowish green	yellowish green
The attachment angle of the leaflet halves		Divergent	Divergent	Divergent & acute	Acute

**b) Epidermal features of the leaflet:**

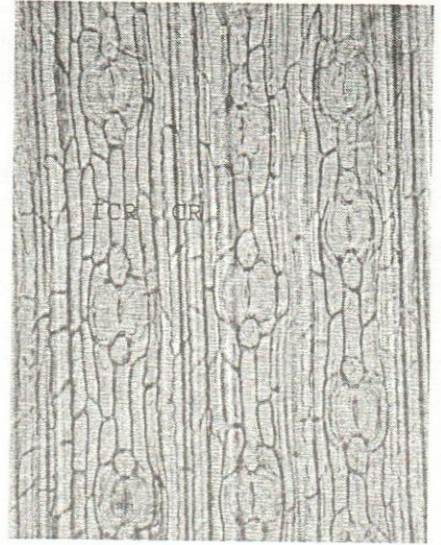
In all cvs, the abaxial epidermis has costal and intercostal bands with stomata restricted to intercostal bands (Fig 1). Stomata are tetracytic with short terminal subsidiary cells. The abaxial epidermis of cvs S and Z contained higher no. of stomata /unit area, 174.0 and 218.0 respectively, compared with 128.0 and 105.0 in cvs B and H respectively (Table 2).

**Table (2): Certain epidermal features of the leaflet in date palm cvs B, H, S, and Z.**

Characters	cvs	B	H	S	Z
Mean no. of stomata/mm <sup>2</sup>		128.0	105.0	174.0	218.0
Dimensions of the stomatal apparatus (μ)	Range	38.2-47.3	47.3-61.9	36.4-47.3	34.6-43.7
		x	x	x	x
	Mean	23.7-29.1	34.6-45.5	25.5-34.6	27.3-36.4
No. of cell files/costal region	Range	4-6	3-7	4-6	5-9
	Mean	4	5	6	7
No. of stomatal files/intercostal region		Predominantly 1	Predominantly 1	1 or 2	1 or 2



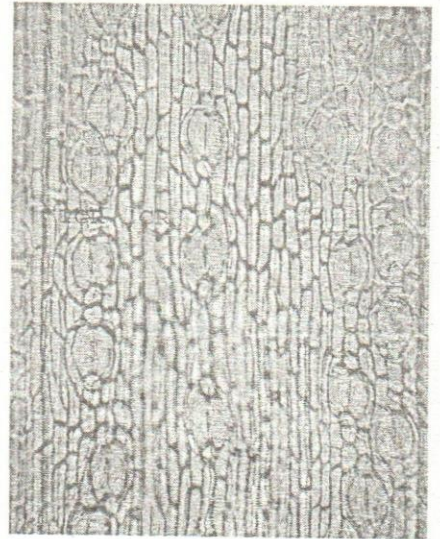
(a)



(b)



(c)



(d)

Fig (1): Images of the abaxial epidermis of date palm cvs B (a), H (b), S (c) and Z (d). CR, costal region; ICR, intercostal region.

On the other hand, it seems that no. of stomata / unit area is negatively correlated with the size of the stomatal apparatus where cv H, in which the lowest no. of stomata was recorded, was characterized by the highest dimensions of the stomatal apparatus. On the other hand, the lowest length of the stomatal apparatus was recorded in cv Z, which was characterized by the highest no. of stomata / unit area. Mean no. of cell files in the costal region was a cv-specific where 4, 5, 6, and 7 cell files were recorded in cvs B, H, S, and Z respectively. The intercostal regions contain one or two stomatal files in cvs S and Z whereas predominantly one in cvs B and H (Fig.1). It is worth mentioning that the microscopical examination of the abaxial epidermis revealed presence of contiguous stomata in all examined cvs, so it is of no discriminating potential.

### **c) Comparative Leaflet cross-sectional characters:**

In all investigated cvs, the leaf is isolaral and covered by uni-layered adaxial and abaxial epidermis. There is a hypodermal layer underneath each epidermis except above LVBs where two layers exist adaxially in all cvs whereas, abaxially, there are either two layers (cvs H and S) or 2-3 layers (cvs B and Z). The mesophyll consists mostly of compact isodiametric chlorenchyma, but its outermost layers are more palisade-like. Vascular bundles are equidistant from both surfaces and of two alternating types, SVBs and LVBs with a higher no. of the first type. Small vascular bundles are embedded in the mesophyll and enclosed in a sheath consisting of outer parenchymatous layer and a fibrous layer mostly 2-celled thick. LVBs are usually separated from the hypodermal layer by a layer of large colorless cells. However, in cvs B and Z, LVBs are sometimes separated from the hypodermis by two layers.

LVBs are enclosed only in fibrous bundle sheath, two-layered at both sides but multi-layered at the poles. In cvs S and Z, there were 3-5 and 4-6 fibrous layers at adaxial and abaxial poles, respectively. In cv B there are 4-6 fibrous layers at both poles whereas in cv H there are 3-6 and 4-5 at adaxial and abaxial poles, respectively. It is worth mentioning that sclerotic cells were detected in the phloem of the LVBs in all cvs (Fig.2). The no. of SVBs between two LVBs is a cv-specific where mostly 3, 6 or 7, 5-7, 7 were recorded in cvs B, H, S, and Z, respectively (Table3). The mechanical tissue of the blade is in the form of mostly hypodermal or subhypodermal fibrous patches. Between each two LVBs, there are 7-11, 9-18, 7-15, and 9-18 fibrous patches in cvs B, H, S, and Z, respectively. Within the investigated cvs, cv Z was unique in that its fibrous patches are absolutely subhypodermal and it was specified also by the presence of deeply-situated fibrous patches (Fig.2, d).

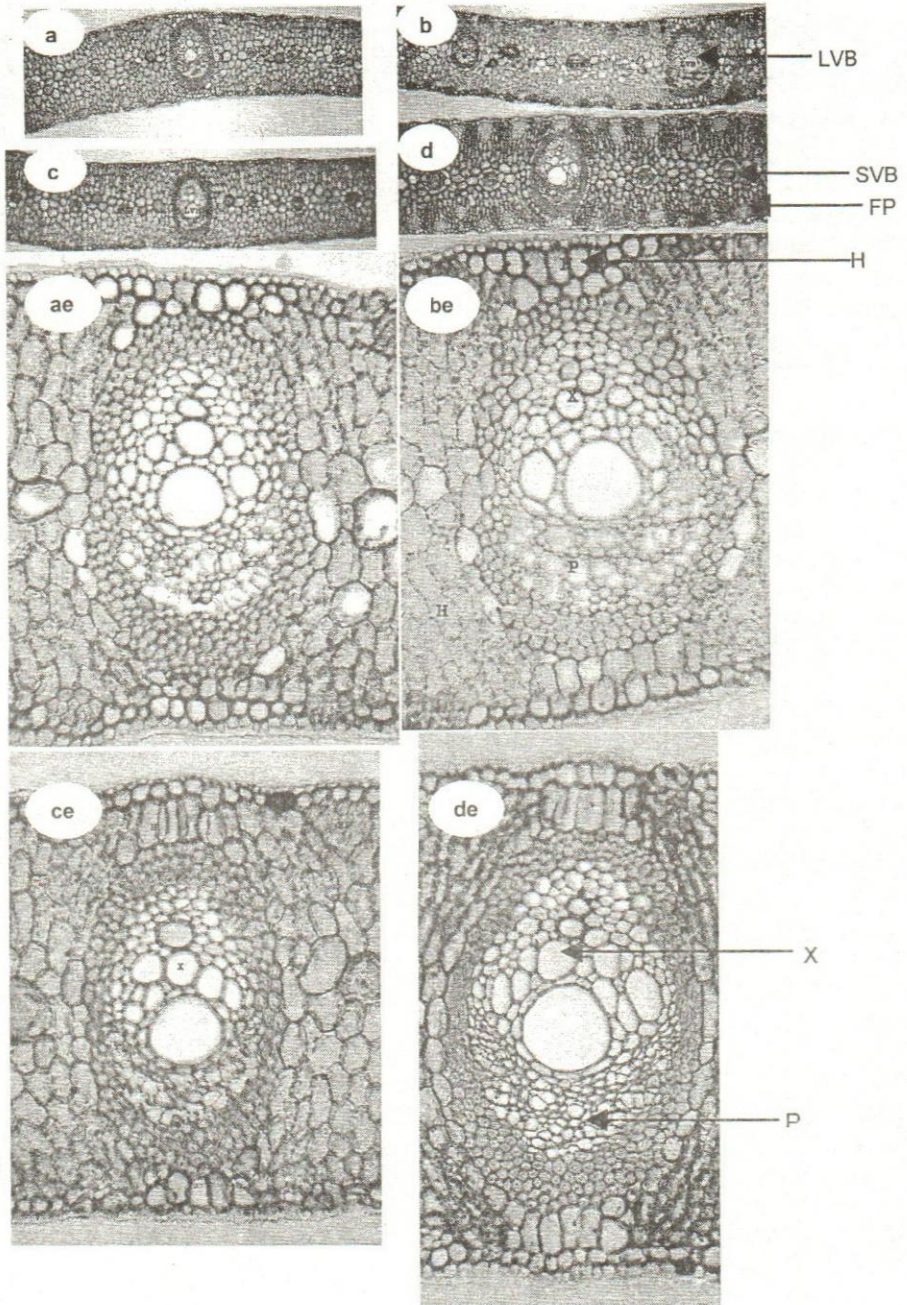


Fig (2): Blade C.S in date palm cvs B (a, ae; enlarged portion of a), H (b, be; enlarged portion of b), S (c, ce; enlarged portion of c), and Z (d, de; enlarged portion of d). FP, fiber patch; H, hypodermis; LVB, large vascular bundle; P, phloem; SVB, small vascular bundle; X, xylem.

**Table (3): Certain anatomical characters of the leaflet in date palm cvs B, H, S and Z.**

Characters	Cvs	B	H	S	Z
No. of hypodermal cell layers between epidermis and LVBs	Adaxial	2	2	2	2
	Abaxial	2,3	2	2	2,3
No. of fibrous layers of LVB's sheath (at poles)	Adaxial	4-6	3-6	3-5	3-5
	Abaxial	4-6	4-5	4-6	4-6
No. of SVBs between two LVBs		Usually 3	6 or 7	5 - 7	Mostly 7
No. of hypodermal/subhypodermal fibrous patches between two LVBs	Range	7-11	9-18	7-15	9-18
	Mean	8.8	13.8	10.0	14.3
Fibrous patches within the mesophyll		-	-	-	+
Location of external fibrous patches		h or sh	h or sh	h or sh	sh

## DISCUSSION

For cv-identification in date palm, although some cytological characters (Al-Salih and Al-Rawi, 1987; Aly and Bacha, 2000), flavonoids distribution (Ouafi et al, 1988) and some biochemical characteristics (Al-Whaibi, 1988) were evaluated, the most frequently adopted protocol is the enzymatic polymorphism (Al-Jibouri and Adham, 1990; Bannaceur et al 1991; Boojj et al, 1995; Bendiab et al, 1998). Recently, more elaborated techniques were employed such as RFLP (Corniquel and Mercier, 1994) and RAPD (Saker and Moursy, 1998; Sedra et al, 1998 a,b; Ben Abdallah et al, 2000).

Apart from the aforementioned costly, highly-elaborated methods which are out of hands of most growers, the morphological and anatomical characters provide more easily and less-costly means for cv-identification in date palm and proved dependable for cv-identification in other plants as well. Stomatal no./unit area and the pattern of stomatal files were useful for differentiating between varieties of banana (Salama and Riad, 1991), and a range of macro- and micro-morphological characters were considered for diagnosing six cvs of mango (El-Fiki et al, 1986).

In the case of date palm, morphological characters were useful for cv-identification (Al-Salih and Al-Sheikh Hussaim, 1980; Bellabaci, 1988; El-Houmaizi et al, 1993). According to Al-Salih and Al-Sheikh Hussaim (1980), three date palm cvs were differed in leaflet dimensions. Based on the results of the present investigation, though some characters such as the no. of veins at each side of the mid-vein was sometimes a cv-specific, it is advisable that these characters are used in combination for cv-identification.

The blade epidermal and anatomical features recorded here are comparable to those reported elsewhere (Tomlinson, 1961; Lötschert, 1995). In his anatomical characterization of the Genus *Phoenix*, Tomlinson (1961) reported the isolateral condition of the blade, with stomata on both sides, presence of one-layered hypodermis which sometimes replaced by fibers, and the abundance of small or large strands of fibers either adjacent to hypodermis or sometimes adjacent to epidermis. He also reported the presence of mesophyll-embedded fiber strands and the presence of

sclerenchyma in the phloem of LVBs. On the other hand, vascular tissues in the bundles were found to be normally-oriented in contrast to the frequent inverse orientation according to his statement.

Some epidermal and anatomical features of certain date palm cvs were previously studied and it was reported that cvs studied could be identified on the basis of stomatal no./unit area (Al-Salih and Al-Sheikh Hussaim, 1980), the pattern of leaf epicuticular wax deposition (Rahmania and Huan, 1997), and fiber characteristics (Al-Attar and Zahroon, 1980). Within the investigated sample, some blade epidermal and anatomical characters proved useful for cv-identification. From the epidermal features, no. of stomata/mm<sup>2</sup>, no. of cell files in the costal region, and no. of stomatal files/intercostal region proved useful. From anatomical characters, no. of SVBs between two LVBs and the spatial distribution of fibrous patches could be used for ascertain cv-identity.

Based on the obtained results, the following keys could be constructed for the purpose of identifying the investigated cvs:

**1) A key based on leaflet morphological characters**

Blade L/W > 20, immature leaf's base light-green, leaflet halves meet in a divergent angle

5 veins usually present at each side of mid-vein cv B

6 veins usually present at each side of mid-vein cv H

Blade L/W < 20, immature leaf's base yellowish-green, leaflet halves meet in an acute or a divergent angle

9 veins usually present at each side of mid-vein cv Z

5-9 veins usually present at each side of mid-vein cv S

**2) A key based on leaflet epidermal features**

1 or 2 stomatal files present in each intercostal region

Mean no. of cell files/costal region 6, mean no. of stomata/mm<sup>2</sup> 174.0 cv S

Mean no. of cell files/costal region 7, mean no. of stomata/mm<sup>2</sup> 218.0 cv Z

1 stomatal file predominantly present in each intercostal region

Mean no. of cell files/costal region 4, mean no. of stomata/mm<sup>2</sup> 128.0 cv B

Mean no. of cell files/costal region 5, mean no. of stomata/mm<sup>2</sup> 105.0 cv H

**3) A key based on leaflet anatomical features:**

Fibrous patches always subhypodermal, mesophyll-embedded patches present cv Z

Fibrous patches either hypodermal or subhypodermal, mesophyll-embedded patches absent

No. of SVBs between two LVBs usually 3, mean no. of fibrous patches between two LVBs < 10 cv B

No. of SVBs between two LVBs > 3, mean no. of fibrous patches between two LVBs 10 or more

No. of SVBs between two LVBs usually 6 or 7 cv H

No. of SVBs between two LVBs usually 5 - 7 cv S



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### اختبار قدرة بعض الصفات المورفولوجية والتشريحية للورقة للتمييز بين بعض اصناف نخيل البلح

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درست بعض الصفات المورفولوجية وبعض الصفات المتعلقة بالبشرة وكذا التركيب التشريحي للنصل في اصناف نخيل البلح بنت عيشة، حياني، سماني وزغلول. ولقد اختبرت هذه الصفات من حيث قدرتها على التمييز بين الاصناف محل الدراسة.

ولقد اوضحت الدراسة ان بعض الصفات التي درست مثل: نسبة طول/ عرض النصل، عدد العروق على جانبي العرق الوسطي للنصل، عدد الثغور/ وحدة المساحة، عدد صفوف خلايا البشرة فوق مناطق العروق، عدد صفوف الثغور في المناطق فيما بين العروق، عدد الحزم الوعائية الصغيرة بين كل حزمتين كبيرتين، وكذا بعض الصفات المتعلقة بمجموعات الالياف في النصل، يمكن استخدامها للمساعدة في التمييز بين الاصناف محل الدراسة. ولقد استخدمت الصفات التي كان لها قدرة تصنيفية مرتفعة في عمل مفاتيح للتمييز بين الاصناف محل الدراسة.