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Taxonomic Significance of the Leaf Geometric and Micrometric Attributes in the Discrimination of Some Cultivars of *Mangifera indica* L. (Anacardiaceae)

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THE GENUS *Mangifera* L. belongs to the family Anacardiaceae, order Sapindales with 69 known species. *Mangifera indica* is an essential major tropical crop in the globe economy. This study aims to portray the significance of the usage of geometric and micrometric leaf traits to characterize Mango cultivars. Thirty-three morphological and anatomical leaf traits of 41 Mango accessions belong to six cultivars were investigated. The data were analyzed using statistical packages under R environment. Results showed that geometric and micrometric leaf traits such as the leaf length, width, petiole length, leaf blade shape, the shape of upper and lower epidermal cells, the outline of the vascular cylinder, and the number of phloem resin canals were of significance value in the characterization of Mango cultivars. Taxonomic diagnostic key based on some of those traits was constructed. ANOVAs, MANOVA, correlation, and Principal Component Analysis (PCA) retrieved the significance of applying those leaf traits as cultivar identifiers. The present investigation estimate that the attributes of the Mango leaf could be useful and straightforward cultivar identifiers that could be followed by Mango breeders to save time, efforts and money in terms of being unhindered by long juvenile stage of the tree.

Keywords: Juvenile stage, Leaf anatomy, Leaf morphology, Mango, R environment, Taxonomy.

Introduction

The genus *Mangifera* L. belongs to the family Anacardiaceae, order Sapindales (Litz & Hormaza, 2020). Kostermans & Bompard (1993) recognizes 69 species of *Mangifera* based on flower morphology. Most of these are included in two subgenera *Mangifera* L. and Limus (Marchand) Kosterm. The subgenus *Mangifera* contains most of the species (47) distributed into four sections: section Marchandora Pierre.; section Euantherae Pierre.; section Rawa Kosterm.; and section *Mangifera* Ding Hou. A further 11 species reside in uncertain classification positions (Kostermans & Bompard, 1993). The origin and the center of *Mangifera* diversity has been established as South-East Asia and, from here it has spread and is now cultivated across the world (Bompard, 2009). According to Abdelsalam et al. (2018) *Mangifera indica* L. (Mango), was introduced for cultivation in Egypt at least 200 years ago. In 2015, the total cultivated area of Mango reached ~ 102071.76 hectares, with the main cultivation area concentrated in the Ismailia Governorate.

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Mangifera indica is an essential major tropical crop for the Egyptian economy. It is one of the nation's most noteworthy fruit crops and portrayed as a significant item within the National Food Basket. In 2017, an estimated 1,351,316 metric tons of Mango fruit were harvested in Egypt (Altendorf, 2020). Malshe et al. (2016) was mentioned the yield of the *M. indica* which dependent on a significant criterion is "the number of hermaphrodite flowers" in its panicle inflorescence.

Data on the exact number of Mango cultivars in Egypt is not precisely recorded, leading to an absence of accurately named germplasm and cultivars.

The extensive cultivation of Mango in Egypt and the cultivars high genetic diversity might have resulted in intraspecific variation in Mango (Mansour et al., 2014).

In Egypt six Mango cultivars are favored for breeding systems and are the first choice for Mango producers "Alfons, Balade, Ewias, Fagr Kilane, Sokare, and Zebdah" due to their high yield, high fruit quality, and resistance to diseases (Knight Jr et al., 2009).

Differentiation between Mango cultivars usually depends on fruit macromorphology. The fruit traits will be difficult to determine while trees are still in the juvenile stage of development or the non-fruiting state. Mango trees are characterized by a long juvenile period lasting from 3-5 up to 7-10 years (Aguoru et al., 2016) .

Applying leaf morphological "morphometric" and anatomical "micrometric" techniques to identify plant taxonomic groups such as Mango cultivars doesn't rely only on fruit traits and is therefore unhindered by long juvenile stage of the tree or by fruiting season (Baloch et al., 2019; Igbari et al., 2019; de Oliveira et al., 2020; Ibukun & Yomi, 2020)

Morphology is a straightforward tool used in systematics to differentiate between closely related plant taxa at specific and infraspecific levels (Faried et al., 2018; Ellmouni, 2019). Anatomical evidence has been used in plant systematics and is considered to be consistent and reliable for solving taxonomic difficulties among plants (El-Banhawy et al., 2016a; EL-Banhawy et al., 2016b; El-Banhawy & Al-Juhani, 2019).

Due to limited availability of significant descriptor assessments of Mango cultivars (Litz & Hormaza, 2020), the current study aims to use a reliable and simple descriptor such as leaf shape and structure as primary standards to recognize and discriminate between six Mango cultivars grown in Egypt.

Investigation of the leaf morphological and anatomical attributes will help to differentiate between Mango cultivars at the early growth stage. This is especially beneficial as it is quick, objective, quantitative in approach and simple to complete by breeders to determine which type of Mango tree cultivars they already have before investing in cultivation, therefore saving effort, time, and money.

Materials and Methods

Sampling and study area

Leaf samples of the Mango cultivars under investigation has been collected from the extensive production area at Ismailia governorate, Egypt. Consultation with the local agricultural experts from the Ministry of Agriculture Research Center at Ismailia, Egypt has been established to confirm sample identification and collect information about the studied cultivars regarding the local naming, age and distinct features of the trees. According to Elbous & Abdel-hamid (2018) Ismailia Governorate has been cultivated mango orchards extended to 204694 Feddan in 2018.The geographic location of each of the sampled trees was recorded using a global positioning system (GPS) along with location information and local cultivar names, Table 1.

Leaf descriptors

The qualitative leaf descriptors have been evaluated after (IPGRI, 2006; Sennhenn et al., 2014; Khan et al., 2015; Shalabi, 2016), while quantitative leaf traits have been measured as described by Aguoru et al. (2016), Aykut et al. (2017), Ellmouni (2019), Lo Bianco & Mirabella (2018).

Leaf descriptors were investigated, described, and measured for 41 representatives from the six cultivars under investigation. Table 2 represents the descriptor type, name, and abbreviation.

TABLE 1. Cultivar name of *Mangifera indica* growing in Egypt, sample size, collection site, and location

Cultivars name	Location		Region
	Latitude	Longitude	
Alfons	30°33′40.4″N	32°13′20.8″E	Ismailia
Balade	30°32′52.6″N	32°16′52.3″E	
Ewias	30°32′39.9″N	32°17′08.3″E	
Fagr Kilane	30°33′39.9″N	32°13′19.2″E	
Sokare	30°32′52.7″N	32°16′54.8″E	
Zebdah	30°33′43.1″N	32°13′19.8″E	

TABLE 2. Quantitative, qualitative, and anatomical leaf descriptors (traits) used for morphological and anatomical analysis

Leaf descriptors	Abbreviation
I- Quantitative traits	
1. Angle of blade tip	A1
2. Angle of blade fitting	A2
3. Lamina Area	LA
4. Lamina Length	LL
5. Lamina Width	LW
6. Laminar Ratio	LR= LL / LW
7. Leaf Base Width	LBW
8. Petiole Length	PL
9. Petiole vein angle (left)	Z1
10. Petiole vein angle (right)	Z2
11. Petiole Width	PW
12. Total Leaf Length	TLL = LL + PL
II- Qualitative traits	
1. Leaf Apex Shape [Obtuse; Acute; Acuminate]	LAS
2. Leaf Base Shape [Acute; Obtuse; Round]	LBS
3. Leaf Lamina Shape [Elliptic; Oblong; Ovate; Obovate; Lanceolate; Oblanceolate]	LLS
4. Leaf Margin [Entire; Wavy]	LM
5. Leaf Venation/Angle of secondary veins to the midrib [Narrow (< 45°); Medium (45 – 60°); Wide (> 60°)]	LVA
6. Leaf Venation/Curvature of secondary veins [Absent; Present]	LVC
7. Thickness of Pulvinus [Thin; Thick and tapering]	TP
III-Anatomical traits	
1. Length of Central Vascular Cylinder	LCVC
2. Lower Epidermis	LE
3. Lower Epidermis at Wing	LEW
4. Lower Hypodermis	LHPY
5. Lumine of Largest Xylem Vessel	LLXV
6. Number of Resin Canals	NRC
7. Palisade Layer	PL
8. Spongy Layer	SL
9. Upper Epidermis	UE
10. Upper Epidermis at Wing	UEW
11. Upper Hypodermis	UHPY
12. Width of Central Vascular Cylinder	WCVC
13. Width of Largest Resin Canal	WLRC
14. Width of Pith Resin Canals	WPRC

Figure 1 represents the descriptor relative position on a mature leaf of *M. indica*. The leaf blade shape descriptor might be a source of ambiguity. To accurately address this descriptor, we calculated the ratio between leaf blade length and leaf blade width as described by Dilcher (1974).

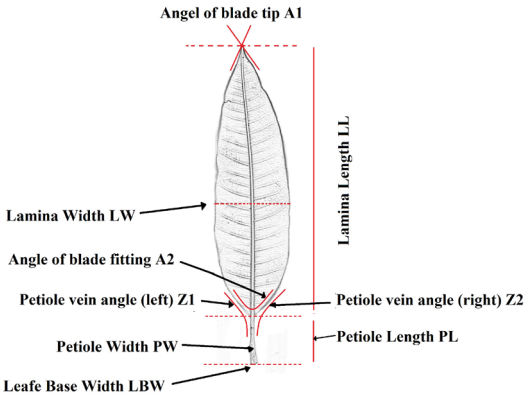

Fig. 1. Relative position of some leaf descriptors

For anatomical investigations, the fresh leaf samples were collected from the field and preserved in Formalin Acetic Acid Alcohol (F.A.A) until transferred to the laboratory. Leaf anatomy was conducted according to the conventional method of Johanson (1940). Ten thin sections of the leaf blade of each cultivar were examined. Leaf micrometric attributes were examined and recorded using Olympus© CHS Binocular Microscope. Photomicrograph were taken at bench level by the aid of Samsung© camera fitted to the microscope. The magnification was calibrated using stage micrometer MA285©. ImageJ Tool software (Rasband, 2011) was used to measure the micrometric traits of the leaves.

Key descriptor (Artificial key)

A dichotomous key has been constructed using morphological and anatomical leaf descriptors of the Mango cultivars under investigation. Delta Editor 1.02 (2088), was used to construct the diagnostic key (Dallwitz et al., 2016).

Statistical analysis

The morphological and anatomical data were analyzed using the R open source software with the required packages installed (R Development Core Team, 2011).

Analysis of Variance (ANOVA), was used to measure and compare the effect of different leaf traits on the variation among the cultivars using (aov) function, followed by Post Hoc Tukey Honestly Significant Difference (HSD).

The significance of variables has been tested using the Multivariate Analysis of Variance (MANOVA) by applying the function (manova) at the R-package "dplyr" (R Development Core Team, 2011).

Using the "cormat" function of the Pearson's correlation coefficients, the morphological and anatomical matrices have been calculated.

Following the correlation test, Principal Component Analysis (PCA) were conducted using the 12 and 14 quantitative morphological and anatomical data respectively.

For agglomerative clustering, all data of morphological and anatomical traits have been scaled and standardized using Euclidian distance (Kassambara & Mundt, 2017). A cluster analysis was directed with the R-package "pvclust" to evaluate the uncertainty in hierarchical cluster analysis (Suzuki & Shimodaira, 2013).

To confirm the consistency and robustness of the key descriptors of the cultivars under investigation; the "pheatmap" and "ggplot2" packages were installed in R environment (Kassambara, 2020). Both packages have previously been used for visualizing the distance matrices by combining all the data for morphological and anatomical traits (Sennhenn et al., 2014).

Results

Morphological and anatomical characterization

Table 3 and Fig. 2 represent the variation in leaf shapes of six Mango cultivars under investigation. The full list of other morphological traits presented in the supplementary material (Table S1).

The lamina length averages were: 17.5cm, Balade; 18cm, Fagr Kilane; 20cm, Ewias, and Alfons; 23cm, Sokare; and 24cm, Zebdah. The lamina width averages were: 4.4cm, Alfons; 5.1cm, Ewias; 5.3cm Sokare; 5.4cm in Balade; and 6cm, in Fagr Kilane and Zebdah). Average leaf petiole lengths were: 1.5cm, Balade; 2.5cm, Fagr Kilane and Sokare; 3.5cm, Alfons and Zebdah; and 4cm, Ewias).

TABLE 3. Quantitative and qualitative morphological leaf blade traits

Cultivar	LL (cm)		LW (cm)		PL (cm)		LLS	LAS	LBS
	Range	Average	Range	Average	Range	Average			
Alfons	16-22	20	3.9-4.9	4.4	2.5-4.7	3.5	Narrow oblong	Acuminate	Acute
Balade	15-19	17.5	4.9-6.4	5.4	0.8-2.1	1.5	Lanceolate	Acute	Obtuse
Ewias	16-23	20	4.1-5.7	5.1	2.9-4.7	4	Lanceolate	Acuminate	Acute
Fagr Kilane	17-19	18	5.4-6.7	6	2.8-3.9	2.5	Narrow elliptic	Acute	Obtuse
Sokare	17-25	23	4.2-6.1	5.3	1.7-4.9	2.5	Narrow oblong	Acuminate	Acute
Zebdah	20-27	24	5.4-7	6	3-3.75	3.5	Narrow oblong	Acute	Acute

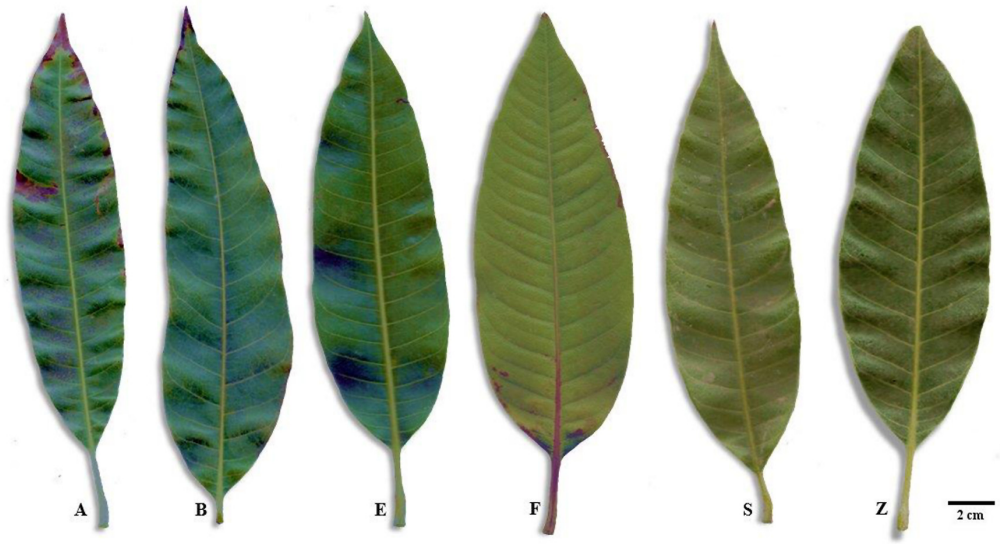

Fig. 2. Variation of leaf shape recorded in six Mango cultivars; (A) Alfons, (B) Balade, (E) Ewias, (F) Fagr Kilane, (S) Sokare, (Z) Zebdah.

The lamina shapes were narrow elliptic in Fagr Kilane, lanceolate in Balade and Ewias, whilst narrow oblong was found in Alfons, Sokare, and Zebdah. Acute leaf apexes were recorded in Balade, Fagr Kilane, and Zebdah, whilst acuminate leaf apexes was documented in Alfons, Ewias, and Sokare. The leaf base shape was acute in Alfons, Ewias, Sokare, and Zebdah, whilst it was observed to be obtuse in Balade, and Fagr Kilane (Fig. 2).

Tables 4 and 5 represent the qualitative and quantitative leaf traits. Figure 3 represent transvers sections of six Mango leaf showing the variations in anatomical traits. The complete list of anatomical traits is presented in supplementary material (Table S2).

The epidermis was composed of one layer of rectangular (cuboidal-like cells) in Alfons, Ewias, and Sokare, whilst it was composed of columnar cells in Balade, Fagr Kilane and, Zebdah. The thicknesses of both upper and lower epidermis layers were recorded respectively: Alfons, 5.079µm, and 4.73µm; Balade, 6.00µm, and 6.335µm; Ewias, 5.695µm, and 3.602µm; Fagr Kilane, 11.18µm, and 6.325µm; Sokare, 6.289µm, and 5.207µm; Zebdah, 13.601µm, and 7.333µm.

The hypodermis was present in adaxial and abaxial leaf surfaces of all cultivars under investigation. It was composed of sclerenchyma cells in Alfons, Balade, Ewias, and Sokare, whilst in Fagr Kilane, Zebdah it was composed of collenchyma tissue. The thicknesses of upper hypodermis were: 19.84µm, Alfons; 26.926µm, Fagr Kilane; 32.757µm, Sokare; 36.021µm, Ewias; 40.552µm, Zebdah; and 42.463µm, Balade. Thicknesses of the lower hypodermis were: 15.315µm, Alfons; 17.123µm, Ewias; 24.021µm, Fagr Kilane; 33.947µm, Sokare; 38.601µm, Balade; and 52.017µm, Zebdah.

TABLE 4. Quantitative anatomical leaf traits, all measurements in micrometer (µm)

Cultivar	UE	LE	UHPY	LHPY	PL	SL	LCVC	NRC	WPRC
Alfons	5.079	4.73	19.84	15.315	13.18	31.448	219.634	12-14	18.024
Balade	6.00	6.335	42.463	38.601	60.299	150.053	361.867	8	-
Ewias	5.695	3.602	36.021	17.123	14.957	57.84	238.549	4-7	10.336
Fagr Kilane	11.18	6.325	26.926	24.021	59.703	131.183	386	8	-
Sokare	6.289	5.207	32.757	33.947	7.28	24.459	314.916	5	-
Zebdah	13.601	7.333	40.552	52.017	61.741	89.968	337.092	9-13	36.497

TABLE 5. Qualitative anatomical traits of six Mango cultivars

Cultivar	UE/LE	UHPY/LHPY	PL	Vascular cylinder	Bundle sheath	Crystal
Alfons	Rectangular	Sclerenchyma	Double	Zigzag	Parenchyma, collenchyma and sclerenchyma	Druses
Balade	Columnar	Sclerenchyma	Single	Triangle	Parenchyma, collenchyma and sclerenchyma	Absent
Ewias	Rectangular	Sclerenchyma	Double	Triangle	Parenchyma, collenchyma and sclerenchyma	Solitary
Fagr Kilane	Columnar	Collenchyma	Single	Triangle	Absent	Solitary
Sokare	Rectangular	Sclerenchyma	Single	Triangle	Absent	Solitary
Zebdah	Columnar	Collenchyma	Single	Zigzag	Absent	Absent

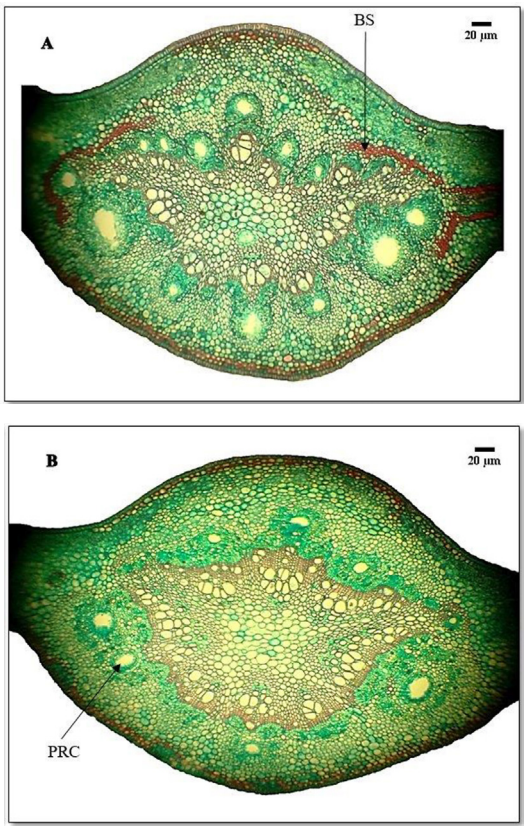

Fig. 3. Leaf transverse sections of Mango cultivars: (A) Alfons, (B) Balade [(BS) Bundle Sheath, (PRC) Phloem Resin Canal].

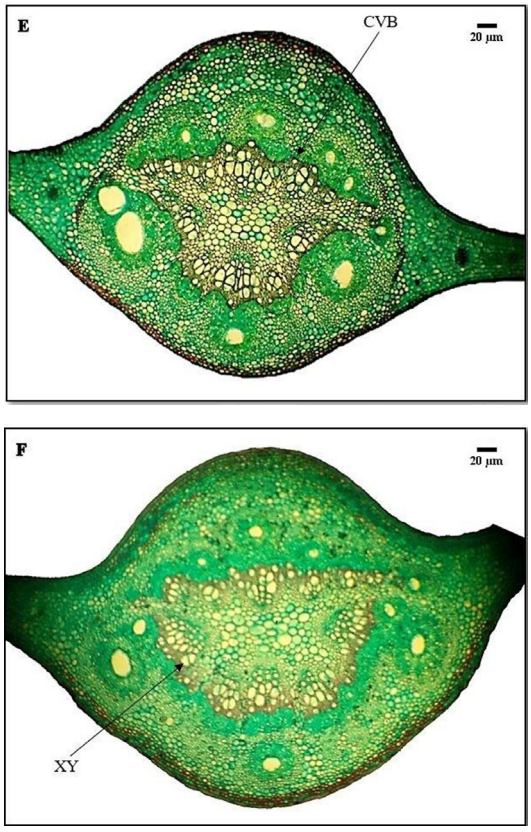

Fig. 3. (Cont.). Leaf transverse sections of Mango cultivars: (E) Ewias, (F) Fagr Kilane [(CVB) Central Vascular Bundle, (XY) Xylem].

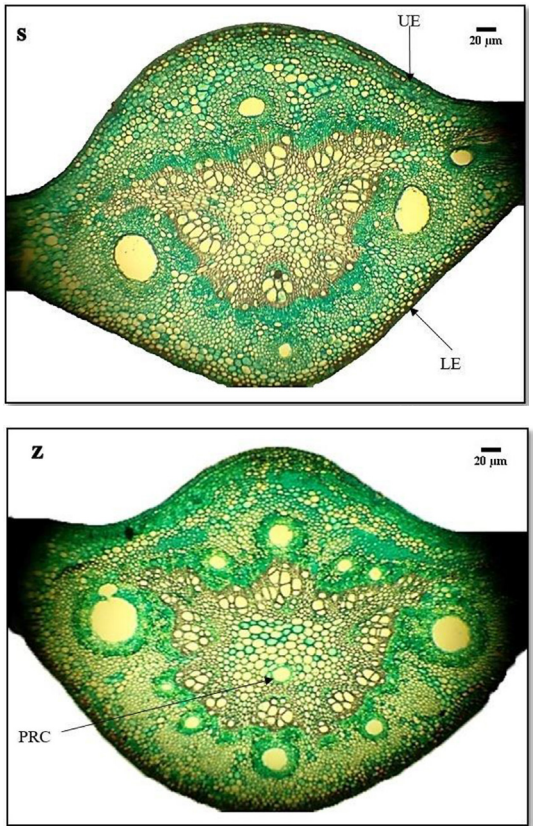

Fig. 3 (Cont.). Leaf transverse sections of Mango cultivars: (S) Sokare, (Z) Zebdah [(UE) Upper Epidermis, (LE) Lower Epidermis, (PRC) Pith Resin Canal].

Mesophyll is differentiated into two layers (i.e.) palisade and spongy. The palisade layer is composed of columnar cells, it is a single layer of cells in Balade, Fagr Kilane, Sokare, and Zebdah. It is double layer in Alfons, and Ewias, the thicknesses found were: 7.28µm, Sokare; 13.18µm, Alfons; 14.957µm, Ewias; 59.703µm, Fagr Kilane; 60.299µm, Balade; and 61.741µm, Zebdah. The spongy layer is composed of irregular chlorenchyma cells, it is thicker than the palisade layer. The thicknesses of spongy layers were: 24.459µm, Sokare; 31.488µm, Alfons; 57.54µm, Ewias; 89.968µm, Zebdah; 131.181µm, Fagr Kilane; and 150.053µm, Balade.

The general outline of the vascular cylinder, and the arrangement of vascular bundles was a zigzag shape in Alfons, and Zebdah. However, it was arranged in a central triangle in Balade, Ewias, Fagr Kilane, and Sokare. The sizes of the vascular cylinders were: 219.634µm, Alfons; 238.549µm, Ewias; 314.916µm, Sokare; 33.092µm, Zebdah; 361.867µm, Balade and 386µm, Fagr Kilane. Bundle sheaths were recorded in Alfons, Balade, and Ewias and were composed of parenchyma, collenchyma and sclerenchyma cells, they were absent from Fagr Kilane, Sokare and Zebdah. The numbers of phloem resin canals were: 4-7, Ewias; 5, Sokare; 8, Balade and Fagr Kilane; 9-13, Zebdah; and 12-14, Alfons. The resin canal was recorded in the pith region of Alfons, Ewias, and Zebdah. The sizes of the pith resin canals were: 10.336µm, Ewias; 18.024µm, Alfons; and 36.497µm, Zebdah. The pith resin canal was absent in Balade, Fagr Kilane, and Sokare. Solitary crystal of calcium oxalate was found in Ewias, Fagr Kilane, and Sokare, whilst druses crystal was found in Alfons. Neither type of crystal was found in Balade or Zebdah, Figure 3 (A, B, E, F, S, and Z).

Artificial key based on morphological and anatomical traits

1.a) Leaf with acute apex, the adaxial epidermis composed of one layer of columna……....**2**

1.b) Leaf with acuminate apex, the adaxial epidermis composed of one layer of rectangular cells …..……...**4**

2.a) lanceolate leaf shape, petiole ≤ 2 cm length, composed of sclerenchyma cells, bundle sheath present, resin canals absent in the pith region ……**Balade**

2.b) Elliptic to oblong leaf shape, petiole > 2cm length, composed of collenchyma cells, bundle sheath absent, resin canals present in pith region ……**3**

3.a) Leaf length < 20cm, with elliptic form and obtuse base, the central vascular cylinder of triangular shape, crystal present of solitary type ….…….…… **Fagr Kilane**

3.b) Leaf length ≥ 20cm, with oblong form and acute base, the central vascular cylinder of zigzag shape, crystal absent .....…....**Zebdah**

4.a) Leaf with petiole length ±1.7(-4.9)cm, mesophyll consists of a single layer of palisade cells, compact spongy tissue of irregular shape chlorenchyma cells, bundle sheath absent, resin canals absent in pith region.....……...**Sokare**

4.b) Leaf with petiole length ±2.5(-4.7)cm, mesophyll consists of double layers of palisade cells, lose spongy tissue of irregular shape chlorenchyma cells, bundle sheath present, resin canals present in pith region ...**5**

5.a) Narrow Oblong leaf shape, the central vascular cylinder of zigzag shape, number of phloem resin canals >10, Druses crystals……**Alfons**

5.b) Lanceolate leaf shape, the central vascular cylinder of triangular shape, number of phloem resin canals <10, solitary crystals……..……..…..**Ewias**

Data analysis

Tables 6 and 7, represent the results of the univariate analysis of variance ANOVAs and multivariate analysis of variance MANOVA for all recorded quantitative morphological and anatomical leaf traits of the six Mango cultivars under investigation. Both tests revealed significant differences among the studied cultivars, with ($P< 0.05$).

The morphological quantitative traits exhibited a degree of affinity < |0.95| indicating that the correlation between pairs of characters were not high provided as Supplementary material (Tables S3 and S4, respectively), so all leaf descriptors were considered "non-redundant characters" and could be used in the Principle Component Analysis (PCA).

Principal Component Analysis (PCA) for morphological and anatomical quantitative traits showed that the first four components with Eigenvalues greater than one explained 94% of the total variation (Table 8a and 8b).

In the morphological quantitative traits PCA, the first principal component (PC1) explained 49.13% of the total variation and was related to Lamina length (LL), Lamina width (LW), Area of leaf (LR), laminar ratio (LR), Petiole width (PW), Angle of blade tip (A1) and Leaf petiole vein angle (Z1). The second component (PC2) described 23.47% of the total variation and was related to Angle of blade tip and fitting A1, A2. The third component (PC3) represented 14.7% of the total variation and was mainly associated with Petiole vein angle (right) Z2; the fourth component (PC4) accounted for 7% of the total variation (Table 8a).

In the anatomical quantitative traits PCA, the first principal component (PC1) represented 62.73% of the total variation, and covered the most of features except upper hypodermis (UHPY), width of largest resin canal (WLRC), width of pith resin canal (WPRC) and (NRC), the two lateral traits beside lower hypodermis (LHPY) were related to the second component (PC2) which described 14.52% of the total variation. The third component (PC3) explained 10.89% of the total variation being mainly linked with width of pith resin canal (WPRC); and the fourth component (PC4) accounted for 9 % of the total variation and was correlated with upper hypodermis (UHPY) (Table 8b).

TABLE 6. Hierarchical sum of squares ANOVA of six examined Mango cultivars

	SS	MS	df	F value	P value
Population	11040829	1840138	6	894.3	<2e-16***
Residuals	72018	2058	36		
Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1					

SS: Sum of Squares, MS: Mean Sum of Squares (i.e., SS divided by df), df: Degrees of freedom.

TABLE 7. MANOVA test of six examined cultivars

	Df	Pillai's Trace	F value	Num DF	Den DF	Pr > F
Population	6	4.109	5.2512	72	174	<2.2e-16***
Residuals	35					
Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1						

Df: degrees of freedom, Pillai's Trace: A multivariate test applied in MANOVA, F Value: F statistic for the given predictor, Num DF: The number of degrees of freedom in the model, Den DF: The number of degrees of freedom associated with the model errors, Pr > F: The P-value associated with the F statistic of a given effect and test statistic.

TABLE 8a. Principle Component loading of 12 quantitative morphological traits

Character	PC1	PC2	PC3	PC4
LL	0.4456427	-0.88160172	0.07587614	0.09224713
LW	0.9150063	0.27258058	-0.10964116	-0.27255423
LR	0.4578229	0.19119135	-0.80625581	0.22948738
LA	0.8912117	-0.40529997	0.08708554	-0.02514174
PL	-0.8551486	-0.16828113	0.02807352	-0.39835538
PW	0.8153713	-0.23675859	0.41996605	-0.31100529
LBW	-0.9221029	0.05207238	-0.25643334	-0.25440531
TLL	-0.883382	-0.1041069	-0.22837728	-0.26775215
A1	0.5657826	0.72101569	0.27743159	-0.28393342
A2	0.2023496	0.9359421	0.04934207	-0.09693769
Z1	0.5034663	-0.49503393	-0.49264092	-0.49161501
Z2	-0.4626514	-0.16046781	0.68631876	0.01035441
Standard deviation	2.428	1.6784	1.1328	0.92721
Proportion of Variance	0.4913	0.2347	0.147	0.07164
Cumulative Proportion	0.4913	0.726	0.873	0.94461

TABLE 8b. Principle component loading of 14 quantitative anatomical traits

Character	PC1	PC2	PC3	PC4
UE	0.8085111	0.3383037	0.31546728	-0.26321638
LLE	0.9349698	0.1533703	0.12664481	-0.01061688
UHPY	0.4660814	0.3260106	-0.3703986	0.70319981
LHPY	0.7324197	0.5644097	-0.11504519	0.29046029
WCVC	0.8883282	0.0175785	0.32512594	-0.32197582
LCVC	0.9143473	-0.1420235	-0.35792258	-0.1190735
WLRC	0.1519638	0.6593223	-0.51882852	-0.4765567
WPRC	0.1134768	0.6152866	0.75967136	0.1728383
LLXV	0.9023564	-0.1814745	-0.13839998	-0.36428323
UEW	0.9137208	-0.2771549	0.21559986	0.01943223
LEW	0.9419159	-0.3293076	-0.04398639	-0.03693267
PL	0.9392081	-0.2066092	0.17922747	0.19943219
SL	0.7899336	-0.5180525	-0.04857419	0.30128477
NRC	-0.8926375	-0.3276038	0.27573065	-0.0564568
Standard deviation	2.9634	1.4259	1.2346	1.13
Proportion of Variance	0.6273	0.1452	0.1089	0.09223
Cumulative Proportion	0.6273	0.7725	0.8814	0.97361

Furthermore, the distribution of the studied cultivars based on the first two components showed that the phenotypic variation among the six cultivars under study and how widely discrete they are along the two axes (Fig. 4).

The first principal component axis (PC1), for both the morphological and anatomical quantitative trait PCAs, separated Fagr Kilane and Zebdah from Ewias and Alfons. The second principal component axis (PC2) confirmed the grouping of Ewias and Alfons whilst pairing together Fagr Kilane and Balade, and also grouping Zebdah and Sokare (Fig. 4).

In the hierarchical cluster analysis for morphological and anatomical traits (pvclust; Fi. 5) the six Mango cultivars have been divided into two main clusters at an approximately unbiased (AU) P value of 100. The morphological and

anatomical clusters gathered Ewias, Alfons and Sokare in the same cluster and Fagr Kilane and Balade in the other cluster, only Zebdah switched between clusters.

Pheatmap (Fig. 6), succeeded in clustering the six Mango cultivars under investigation into two main clusters. The first cluster includes Alfons, Ewias, and Sokare whilst the second cluster included (Balade, Fagr Kilane and Zebdah). Heatmap clustering supports the anatomical pvclust and the diagnostic key.

Discussion

This study explores the usefulness of 33 leaf attributes, using geometric and micrometric approaches for discrimination among six commercially significant Mango cultivars growing in Egypt.

Rajwana et al. (2011) had previously noted the differences in the shape and size of the Mango leaf and found them to be a good basis for differentiating varieties. The studied cultivars showed three leaf blade shapes "elliptic, lanceolate, and oblong" that allowed rapid and efficient characterization of Mango cultivars. Leaf length, width and petiole length represented another good leaf descriptor that could be used to distinguish between Mango cultivars (Vieccelli et al., 2016; Kanchan et al., 2018; Igbari et al., 2019). Other leaf descriptors such as leaf blade apex, blade base, and leaf color overlapped and couldn't be used to discriminate between studied cultivars.

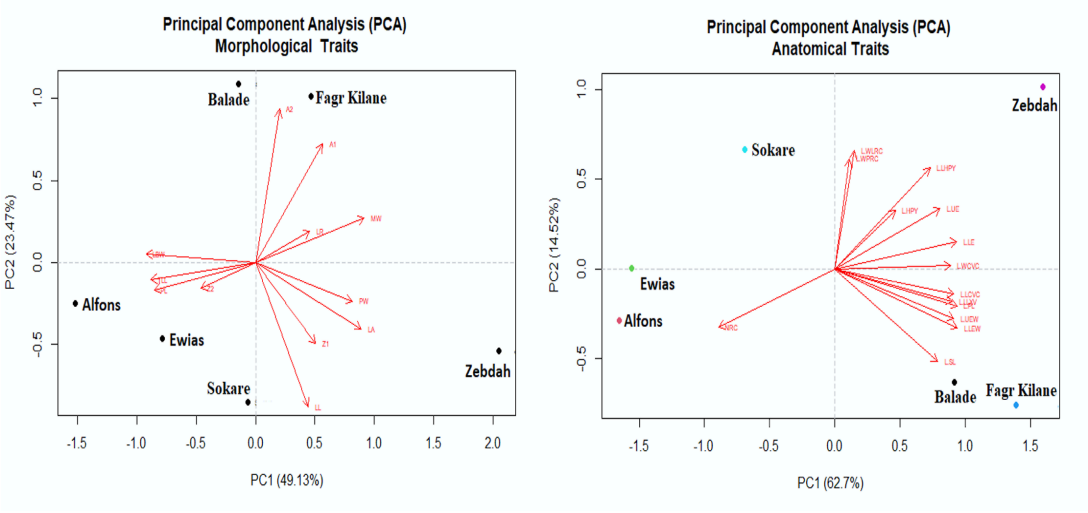

Fig. 4. Principal component analysis (PCA) of morphological and anatomical traits

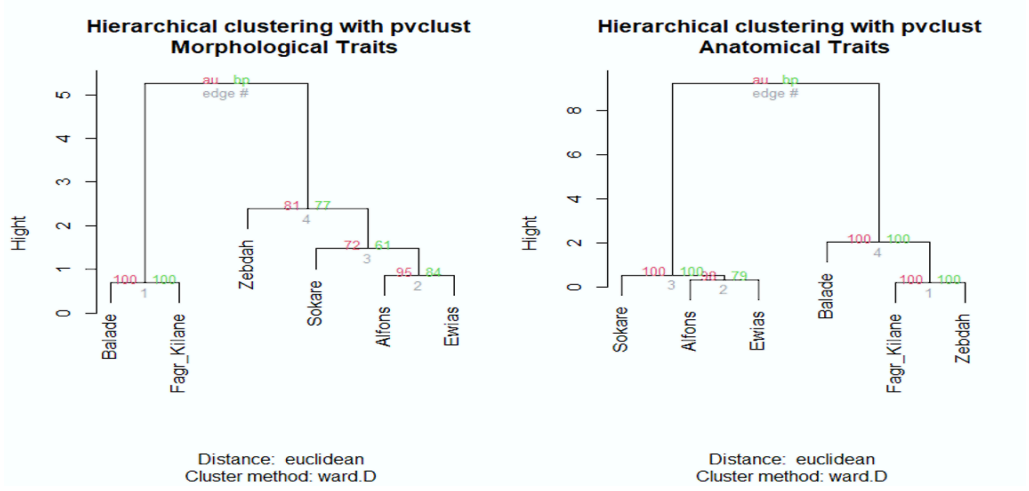

Fig. 5. Dendrograms for similarity percentage of six Mango cultivars based on morphological and anatomical traits

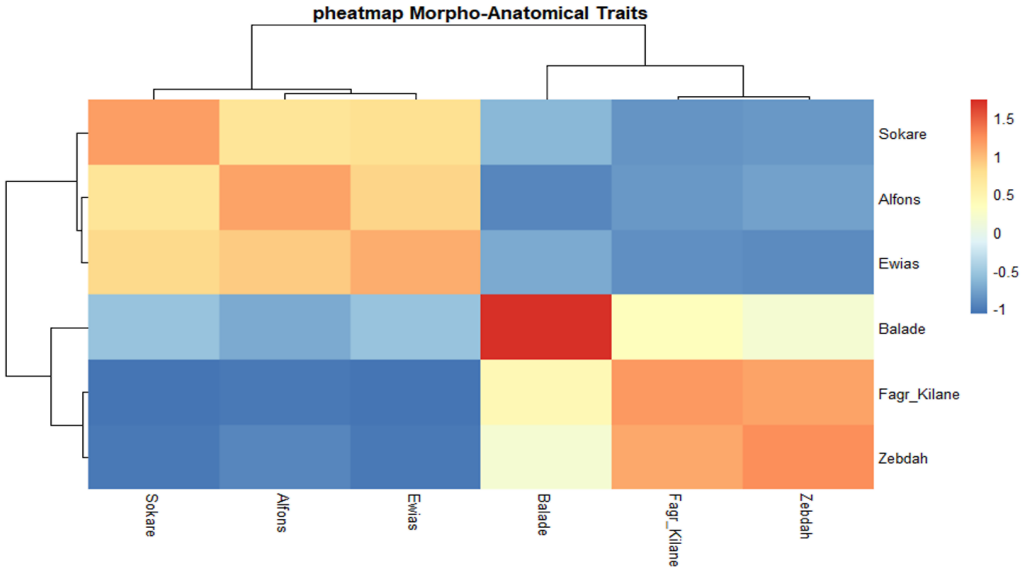

Fig. 6. Heatmap representing the value of the divergence between investigated cultivars based on morphological and anatomical traits [The scale of color is relative to the value of the divergence between investigated cultivars]

Anatomical traits have been widely used to distinguish closely related Mango cultivars (Norfaizal & Latiff, 2013; Cahyanto et al., 2017). In the present study, the anatomical traits explained the total variation of the cultivars with fluctuating degrees of influence. The divergence between the six studied Mango cultivars was related to share anatomical features of the leaf. The features such as epidermis layer varied; the cells were columnar or rectangular, the hypodermis layer could be absent, and if present it was composed of either sclerenchyma or collenchyma, the palisade layer was found to be either a double or single layer, the vascular cylinder appeared as a zigzag or a triangular shape, the bundle sheath was either present or absent, the calcium oxalate crystals appeared as druses, were solitary or were even absent, and the number of the phloem resin canals was considered as a good discriminating feature. All the aforementioned traits were of valuable discriminating value between the studied cultivars (Fig. 3 and Tables 4, 5).

Statistical analysis of morphological and anatomical attributes can be used to distinguish the main components accounting for the total variation of leaf descriptors, identifying the trends in leaf shape, and representing the relationships between cultivars and phenetic similarities (Vieira et al., 2014; Igbari et al., 2019).

ANOVA and MANOVA of the Mango leaf attributes, revealed significant differences of all traits. The Pearson's correlation coefficients matrix based on quantitative traits of morphological and anatomical data of the six studied cultivars (Table 8a and 8b), revealed significant similarity of cultivars could be observed using morphological and anatomical quantitative data.

PCA and pvclust of morphological attributes clustered the studied cultivars into separate groups based on morphological traits such as leaf length, leaf width, and leaf base shape (Figs. 4 and 5).

Similarly, PCA and pvclust of anatomical attributes segregated the studied cultivars into distinct groups based on the thickness and the shape of the epidermal cells as well as the width of largest resin canal and the width of pith resin canal (Figs. 4 and 5).

Figure 6 represents the heatmap which allows simultaneous visualization of clusters of samples and features. The heatmap confirmed that both morphological and anatomical clustering were congruent. Moreover, the cluster analysis and the diagnostic key reconfirmed that good key descriptors should lead to the same cluster analysis result (Sennhenn et al., 2014). As a final point, the cluster analysis (pvclust) divided the six Mango cultivars into two clusters. The morphological and

anatomical clusters similarities gathered "Ewias, Alfons and Sokare" in the one cluster and "Fagr Kilane and Balade" in another cluster, only one cultivar (Zebdah), spread among both clusters. Heatmap clustering confirms the anatomical (pvclust) and the diagnostic key.

Throughout the current study, most of morphological and anatomical traits demonstrated homogeneity among Mango cultivars e.g. angle of secondary veins to the midrib, leaf venation, sunken stomata, irregular spongy layer, and the presence of phloem resin canal of all the different cultivars of Mango examined. On the contrary, few leaf attributes showed moderate to low variation within and among the Mango cultivars under investigation. This might be due to phenotypic plasticity detected in the leaves of *M. indica*, cultural practices, climatic conditions, genetic variations and growth stages (Khan et al., 2015; Abderabbi et al., 2018; de Azeredo et al., 2018).

Conclusions

Mango breeders might have to wait years to tell if they have chosen the correct cultivar to tend. Some Mango cultivars might offer a poor crop or yield fruit of an inadequate quality, reducing the breeder's profit and affecting the economy. Due to the difficulty of identifying the cultivar and fruit type until the Mango tree has reached the maturity stage (3-10 years) there has long been a need to assign alternative criteria, rather than fruit descriptors, to select the desired Mango to cultivate. The current study presents data for the leaf geometric and micrometric traits of six economically important Mango cultivars as an alternative cultivar descriptor. Leaf attributes could be followed during the early stage of plant development "beginning from seedling" and could give extraordinary assistance to farmers determining their cultivar type. The current study proposes that leaf shape, leaf length, leaf width, and petiole length are among the most taxonomically valuable features of Mango cultivar. Leaf epidermal cell type, hypodermis tissue composition, vascular cylinder outline, number of resin canal, and calcium oxalate crystal types represent another excellent trait that can be used to distinguish Mango cultivar. Statistical and cluster analyses confirmed the reliability of the geometric and micrometric usage of Mango leaf as an easily applicable alternative identifier for closely related Mango cultivars.

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الأهمية التصنيفية للسمات المورفولوجية والتشريحية للورقة في التمييز بين بعض أصناف *Mangifera indica* L. (Anacardiaceae)

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يقدم هذا البحث استقصاءً دقيقًا للسمات المورفولوجية والتشريحية لأوراق ستة أصناف من اشجار نبات المانجو ذات الأهمية الاقتصادية التي تزرع في مصر وهي (الفونس- بلدي- فجر كيلان - سكري -عويس- زبده)، عن طريق اختيار استخدام 33 سمة من سمات الأوراق المورفولوجية والتشريحية ل 41 عينه تنتمي إلى الانواع الست للتفريق بين تلك السلالات الست.

وقد اظهرت النتائج أن الصفات المورفولوجيه والتشريحيه الدقيقه للورقة مثل طول الورقه وعرضها وطول عنق الورقه و شكل النصل بجانب شكل خلايا البشرة العلوية والسفلية والاطار الخاص بالأسطوانة الوعائية وعدد قنوات الراتنج الموجودة في اللحاء كانت ذات قيمة تصنيفية هامة في توصيف سلالات المانجو. وقد استخدمت هذة الصفات في إنشاء مفتاح للتمييز بين السلالات.

كما اوضحت الاختبارات الإحصائية المختلفة ان إستخدام البيانات الشكلية والتشريحية ذو اهمية لبيان العلاقات التصنيفيه بين السلالات تحت الدراسة حيث انقسمت السلالات الي مجموعتين، المجموعة الأولى تضم الفونس- سكري- عويس والمجموعة الثانية تضم بلدي- فجر كيلان - زبده.

وقد أكدت الدراسة الحالية ان الاستعانه بالصفات الشكليه والتشريحية في التفرقه بين سلالات المانجو يساعد بشكل واضح على التفريق بينهم في مرحلة النمو المبكر. وهذا مفيد بشكل خاص لأنه سريع التطبيق وموضوعي وكمي في النهج، وبسيط في الاستخدام من قبل المربين والمزارعين لتحديد سلالات أشجار المانجو التي لديهم بالفعل مسبقًا دون الحاجة للإنتظار حتى مرحلة التزهير والتي تستغرق من 3 الي 7 سنوات وبالتالي توفير الجهد والوقت والمال.