



## Leaf Variations within Representative Genera of Tribe Malveae and their Significance in the Taxa Phylogeny

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**L**EAF macro- and micro-characters of seven genera represented by twenty species belonging to tribe Malveae, family Malvaceae were examined, measured and investigated in this study. The obtained results indicated that the petiole length, leaf blade shape, base and apex varied between the studied taxa. Meanwhile the leaf margin, number and depth of teeth/cm in the leaf margin, type of venation and number of lateral veins can be of diagnostic value within the species. Epidermal cell characters are mostly invariable, while the type and density of hairs and presence of cavitated hairs beside the type of stomata and shape of guard cells gave valuable characters within the taxa. The results have been subjected to clustering analyses using PAST program and the resulted dendrogram shows great similarity between the taxa with the separation of both *Malva aegyptia* and *Althaea ludwigii* each in a separate clade. Diagnostic key has been instructed and the phylogenetic thinking has been postulated.

**Keywords:** Leaf, Malvae, Malvaceae, Phylogeny, Taxonomy.

### Introduction

Tribe Malveae is one of the tribes belonging to the subfamily Malvoideae, the family Malvaceae. Based on leaf and floral characters, this subfamily comprises four tribes: Kydieae, Hibisceae, Gossypieae, and Malveae. The most diverse is the Malveae tribe; it comprises about 70 genera and more than a thousand species worldwide. The relation between these genera is still unclear and under dispute. Accordingly, this tribe has been divided into four subtribes: Abutileae, Eumalveae, Malopeae, and Sideae. This classification is based on the carpel arrangement, ovule numbers, epicalyx characters, and the flowers' stigmatic arrangements (Bentham & Hooker, 1862). In Egypt, the genera *Abutilon* Miller, *Alcea* L., *Althaea* L., *Malva* L., *Malvastrum* A.Gray, *Malvasicus* Febr. and *Sida* L. are representatives of three subtribes (Abutileae, Eumalveae, and Sideae), which are the subject of this study. Genus *Abutilon* Miller is considered the largest in this tribe worldwide. It comprises about 200 recognized species distributed in tropical

and subtropical regions of the earth (Sivarajan & Pradeep, 1996). According to the World Plant List, the genus *Sida* (70 species) is the second largest genus, followed by the genus *Malva* (40 species), *Althaea* (17 species), *Malvastrum* (14 species), and at last, *Malvasicus* (one species) (Heywood, 1978; Hill, 1982; Mabberley, 1987). In Egypt, the tribe Malveae is represented by 23 species classified into eight genera. The genera *Abutilon* and *Alcea* are represented by six and four species, respectively. The genera *Althaea*, *Lavatera*, *Malvasicus*, and *Malvastrum* are mono-specific genera. *Malva* and *Sida* have five and four species, respectively. These taxa grow mainly in Elba Mountain, Nile Valley, Sinai, and Mediterranean coastal regions (Tackholm, 1974; Boulos, 2000, 2009). They are distinguished according to epicalyx characters, such as presence, number, and length.

Leaf epidermal traits, namely the epicuticular wax deposition, cuticular ornamentation, epidermal cells, stomata, and trichomes, have proved to be an important tool in taxa

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delimitations in many plant families and in tracing the phylogenetic relations between them (Stace, 1966, 1984; Hickey & Wolfe, 1975; Barthlott, 1981). In addition, Stenglein et al. (2003) found that the epidermal features, stomata, stomatal indices, and trichome characters within the Old-World *Lotus* species are of taxonomic value which used to establish the phenetic relationships between the studied taxa. Further, Uzunhisarcikli & Vural (2012) found a range of characteristic features considered taxonomically important in the investigated *Alcea* and *Althaea* species. These include the indumentum of the whole plant besides division and measurements of the leaf. Moreover, Bhat (1999, 2012) found that the leaf margin serrations type and the number of teeth are important in distinguishing *Hibiscus* and *Abutilon* species. Besides, Bano & Deora (2018) found that the macro-morphological variations within the *Abutilon* species were helpful in the identification and delineation of the studied species. Importantly, Taia & Mahdy (2021a, b) found that the leaf macro- and micro-characters within the *Bauhinia* species are useful in separating the species and clarifying the evolutionary line within the studied species. Accordingly, this study aimed to investigate the macro- and micro-morphological leaf characters of twenty Egyptian species representing seven genera from the tribe Malveae to evaluate the relationship between them and trace the evolutionary trend of these taxa.

### **Materials and Methods**

Besides the freshly collected specimens of the twenty taxa, herbarium sheets from different Egyptian herbaria and Alexandria University Botanical Garden (AUBG) were studied carefully for the macro-morphological characteristics of the leaves. Samples of perfect leaves were taken from herbarium sheets and fresh specimens for micro-morphological investigations (Table 1). The names were confirmed according to the taxonomic authorization of Latin species names, datasets of the Global Biodiversity Information Facility (GBIF). This study examined, measured, and investigated seven genera represented by the twenty species.

The macro-morphological characters of the studied species were carried out on ten collected individuals or herbarium sheets from each taxon at the fourth node from the top of

the branch. The leaf petiole, leaf blade length, width, and their ratio were measured by a ruler. Besides, the number and state of teeth in the leaf margin per unit length were counted under the stereomicroscope in 1cm length of the margins in ten leaves. Description of the leaf blade, bases, margins, apices as well as venation was according to Stearn (1985).

For the micro-morphological investigation of the leaves, the fourth leaf from the top was examined first by a stereomicroscope to estimate the hair density and leaf texture. Then, the middle part from both the abaxial and adaxial surfaces was heated in water with a few drops of both dish-wash solutions (Tepol) and nitric acids to peel the epidermal layers for epidermal cells, stomata, and hair type investigations using Olympus U-CMAD3 light microscope. Dry mid parts were stuck onto Aluminum stubs and coated with 30 nm gold in a polaron JFC-1100E coating unit, then examined and photographed at 25KV with JEOL JSM-5300 SEM in the electron microscopes unit, Faculty of Science, Alexandria University, Egypt. Leaf micro-morphological descriptions followed Barthlott (1981) and Al-Shammary & Gornall (1994).

Investigations and measurements of stomata were tallied in 30 randomly selected microscopic fields. The stomatal size was subsequently measured using 10x10 and 10x40 magnifications equipped with a standardized micrometer. Stomatal density (SD) was calculated as the number of stomata per field. The stomatal size was represented as the guard cell area and calculated by Francos constant method (Guard cells area = length x width x 0.7854). Stomatal length (SL,  $\mu\text{m}$ ) and stomatal width (SW,  $\mu\text{m}$ ) were measured from 30 stomata selected randomly. The stomatal index (SI) was calculated according to Metcalfe & Chalk (1979) using the formula:

$$\text{SI} = \frac{S}{E+S} \times 100$$

where: S = number of stomata per unit area, E = number of epidermal cells in the same area.

Trichome type and density were observed under the light microscope; their length was estimated as short, medium, long, and very long, while their walls were described from the SEM investigations. The terminology of stomata used was according to Van Cotthem (1970).

TABLE 1. Studied taxa, confirmation site, collecting site, date of collection and herbarium allocated, F= Fresh, H= Herbarium sheet

No.	Genus	Species	Confirmation	Site	Date	Herbaria
1		<i>A. fruticosum</i> Guill. & Perr.	Fl. Senegamb. Tent. 1:70 (1831).	Sudan, Desert Northeast of Khartoum,(Sudan)	1959	Alexandria (H)
2		<i>A. hirtum</i> (Lam.)Sweet.	Hort. Brit. 53. (1826).	Elba Mountain, (Egypt)	March 1984	Tanta (H)
3	<i>Abutilon</i>	<i>A. longicaepe</i> Hochst.ex A. Rieh	Tent. Fl. Abyss. 1:69 (1847).	Shehab Mazhar botanic garden, Giza, (Egypt) El-Dakhla – El- Kharga road, (Egypt)	June 2018 Feb. 1988 March 1984	Mazhar (H) Tanta (H) Tanta (H)
4		<i>A. pannosum</i> (G. Forst.) Schltld.	Bot. Zeitung (Berlin) 9:828 (1851).	Elba Mountain, (Egypt)	March 1998	Alexandria (H)
5		<i>A. theophrasti</i> Medik.	Malenfam. 28 (1787).	El-Mahala El-Kobra, Canal bank, (Egypt)	May 1998	(F)
6		<i>A. acutis</i> (Cav.) Alef.	Österr. Bot. Zeitschr. 12: 251 (1862).	Tanta – El-Mahala El-Kobra road, Egypt)	June 2004	(F)
7	<i>Alcea</i>	<i>A. rosea</i> L.	Sp. Pl., ed. 1, 687 (1753).	E;-Sheikh mountain, Damascus, (Syria)	Sept. 2001	Boulos (H)
8		<i>A. setosa</i> (Boiss.) Alef.Österr.	Bot. Zeitschr. 12: 255 (1862).	Faculty of science botanical garden, Alex. (Egypt)	May 2018	(F)
9		<i>A. ludwigii</i> L.	Mant. 98 (1767).	Wadi Zarka, (Egypt)	March 1953	Boulos (H)
10	<i>Althaea</i>	<i>A. officinales</i> L.	Sp. Pl.: 686. (1753).	Jebel Hareem, 1900 m, fallow field, (Oman).	MAY 2002	Boulos (H)
11		<i>A. cannabina</i> L.	Sp. Pl.: 686. (1753).	France, Latte Herault, (France)	August 1948	Alexandria (H)
12		<i>M. aegyptia</i> L.	Sp. Pl., ed. 1, 690 (1753).	France, Latte Herault, (France)	August1948	Alexandria (H)
13		<i>M. neglecta</i> Wallr.	Syll. Pl. Nov. 1: 140 (1824).	Burg El-Arab, Alexandria, (Egypt)	March 1992	(F)
14	<i>Mahva</i>	<i>M. nicaeensis</i> All.	Fl. Pedem. 2: 40 (1785).	Burg El-Arab, Alexandria, (Egypt)	April 2005	(F)
15		<i>M. parviflora</i> L.	Sp. Pl., ed. 1, 696 (1753).	Siwa, Med.Ter.Reg., (Egypt) Movenspick Hotel, Quseir, (Egypt)	March 1989 August 1996	(F) (F)
16		<i>M. sylvestris</i> L.	Plant Database (2009)	Faculty of Science, Botanical Garden, (Egypt)	August 2012	(F)
17	<i>Mahvastrum</i>	<i>M. coromandelianum</i> (L.) Garcke.	Bonplandia 5: 295 (1875).	El-Shaer island, (Egypt)	Sept. 2002	(H)
18	<i>Mahviscus</i>	<i>M. arboreus</i> Dill ex Cav.	Plants Database (2009).	Shehab Mazhar Botanic Garden, Giza, (Egypt)	June 2019	(H)
19		<i>S. alba</i> L.	Sp. Pl., ed. 2, 960 (1763).	Fayoum, Hawara, (Egypt)	October 1999	(H)
20	<i>Sida</i>	<i>S. cordata</i> (Burm.fil.)Borss. Waalk.	Blumea 14(1): 182-184. (1966).	Bahala, (Oman)	March 1976	(H)

### Data analyses

All the measured characters are subjected to the SPSS program to calculate the standard deviation. Clustering analysis of the twenty-two characters studied in the different species under investigation (Table 6) with the aid of PAST program v.4 (1999-2018).

## Results

### Leaf macro-morphology

Table 2 and Plate 1 show the leaves' macro-morphological characters. All the studied taxa leaves are petiolate, with lengths varying greatly from less than 1.5cm in *Abutilon fruticosum*, *Althaea officinalis*, *A. cannabina*, *Malva parviflora*, *Malvastrum coromandelianum*, *Sida alba* and *S. cordata*. The petioles exceed 4 cm in *Abutilon theophrasti*, *Alcea acaulis* and *A. rosea*. The rest of the species have petioles varied from 1.5cm to 3.6cm. The leaf blade length and width varied from less than 1.0cm to 2.5cm in *Abutilon fruticosum*, *A. pannosum*, *Alcea acaulis*, *A. rosea*, *Althaea ludwigii*, *Malva aegyptia*, *M. nicaeensis*, *Malva parviflora*, *Sida alba* and *S. cordata*. In *Abutilon hirtum*, *Alcea setosa*, *Althaea officinalis* and *Malva neglecta*, the leaf blade length exceeded 4.0cm. In most studied taxa, leaf blade shapes are ovate, broadly ovate, cordate, subrotund, or orbicular. In *Alcea rosea*, *A. setosa*, *Althaea ludwigii*, and the studied *Malva* species, the leaf blades are dissected or lobated (Photos 15-19). The appearance and texture of the leaf blades are velvety in *Abutilon fruticosum*, *A. hirtum*, *A. theophrasti* and *Althaea cannabina* (Photos 2, 4 & 7). They are coriaceous (leathery), i.e., having a leathery appearance in *Abutilon longicuspe*, *A. pannosum* and *Malvaviscus arboreus* (Photos. 5, 6 & 20), farinaceous (mealy), i.e., having mealy texture in *Alcea acaulis* (Photo 8), papyraceous, i.e., writing papers appearance in *Althaea officinales* and *Sida alba* (Photos 13 & 22). The rest of the studied taxa have scariosus leaf blade texture, i.e., a thin, dry, shriveled appearance.

The leaf blade bases are mostly cordate or truncate, except they are cuneate in *Althaea cannabina* (Photo 12) and breve angustate in *Malvaviscus arboreus* (Photo 11). The leaf blade apices are mostly acute to acuminate, except they are tridentate in *Althaea ludwigii* (Plate 1, Photo 11) and obtuse in *Alcea acaulis*, *Malva aegyptia*, *M. neglecta* and *M. sylvestris* (Photos 8, 15, 16 & 19). In the studied *Abutilon* species, the leaf blade

apices varied from the mucronate and apiculate to the rostrate.

The most important characteristics within the studied species are the leaf blade margin and the number of teeth/cm. The leaf margin is dentate in most of the studied species, except in *Abutilon theophrasti* it is erosus (Photo 7), and in *Alcea acaulis* it is crenate (Photo 8) and entire in both *Althaea ludwigii* and *Malva aegyptia* (Photos 11 & 15). Within the dentate margins, the number of teeth/cm differed greatly. It was from 4 to 5 teeth in *Abutilon fruticosum*, *A. theophrasti*, and *Malvastrum coromandelianum* and 10/cm or over in *Abutilon hirtum*, *A. longicuspe*, *Alcea rosea*, *A. setosa*, *Malva neglecta*, *M. sylvestris* and *Malvaviscus arboreus*. The elevations of the teeth varied from low/shallow to very high. The type of venation is palmate except in *Abutilon fruticosum*, *Althaea officinalis*, *Malvastrum coromandelianum*, *Malvaviscus arboreus*, and the two *Sida* species the type of leaf blade venation is pinnate (Photos 22 & 23). The number of veins varied from one only in *Sida alba* (Photo 22) to over 10 in *Abutilon pannosum*, *Malva aegyptia*, and *Malvastrum coromandelianum* (Photos 15 & 20). The rest of the studied species have from 3 to 9 veins.

### Epidermal cells

The outline of the epidermal cells (Table 3, Plate 2) is elongated in all the studied taxa, except in the *Abutilon* species, it is isodiametric (Photo 1). The shape of the anticlinal wall is either straight or undulate, except in *Malvaviscus arboreus* it is sinuate with sunken or elevated relieves (Photo 31). The periclinal wall is flat, concave, or convex with different ornamentations, from smooth, pitted, or even striate, except *Alcea acaulis*, which is folded (Photo 11). Wax deposition on the periclinal walls is either absent or globular particles with different densities.

### Stomatal characters and indices

Table 4 and Plate 2 show the stomatal characters and indices of the studied species. The type of stomata in most species is anomocytic, except in the taxa of the genera *Alcea* and *Althaea*, it is paracytic (Photo 19). Diacytic stomata were recorded only in *Abutilon fruticosum*, *A. theophrasti*, and *Sida alba* (Photo 31). The stomata are superficial in all the species except in *Alcea acaulis*; they are sunken (Photo 11) and raised in *Abutilon hirtum*, *Alcea setosa*, *Malva*

*aegyptiaca* and *M. nicaeensis* (Photos 3, 15, 20 & 24). The guard cells are kidney-shaped, except in the two studied *Sida* species are elliptic (Photos 31 & 34). The area of the guard cells considered from the medium sizes, from 17.0 to 25.0 $\mu$ m, except in the two *Sida* species, they are from 11.0-14.0 $\mu$ m. The stomatal density range from 4 to 8 in all the

studied species, except in *Alcea setosa*, *Althaea ludwigii*, and *Althaea officinalis*, reaching ten or eleven. The stomatal index exceeds 20 in most studied species, except in *Abutilon longicuspe*, the three *Althaea* sp., *Malvastrum coromandelianum*, *Malvaviscus arboreus*, and the two *Sida* species, it was less than twenty.

**TABLE 2. Macro-morphological characters of the studied species, DE=Dental elevatioin, H=High, L=Low, M=Moderate, ND=Number of teeth, VH=Very high, VL=Very low**

No.	Species	Petiole L.	Leaf blade				
			Length (L, cm)	Width (W, cm)	L/W	Shape	Texture
1	<i>Abutilon fruticosum</i>	0.6-1.1 0.8 $\pm$ 0.40	1.5-2.2 1.9 $\pm$ 0.35	1.6-2.3 1.97 $\pm$ 0.3	0.96	Cordate	Velvety
2	<i>A. hirtum</i>	3.5-3.6 3.5 $\pm$ 0.05	4.0-5.0 4.5 $\pm$ 0.5	4.1-6.1 4.90 $\pm$ 1.0	0.92	Cordate	Velvety
3	<i>A. longicuspe</i>	1-1.5 1.27 $\pm$ 0.25	1.8-3.0 2.23 $\pm$ 0.6	1.8-3.0 2.40 $\pm$ 0.6	0.93	Broadly ovate	Coriaceous
4	<i>A. pannosum</i>	1.5-1.7 1.6 $\pm$ 0.10	2.0-2.5 2.4 $\pm$ 0.11	1.50-2.0 1.75 $\pm$ 0.25	1.37	Broadly ovate	Coriaceous
5	<i>A. theophrasti</i>	4.3-5.0 4.65 $\pm$ 0.35	5.4-6.0 5.7 $\pm$ 0.3	5.0-5.0 5.0 $\pm$ 0	1.14	Broadly ovate	Velvety
6	<i>Alcea acaulis</i>	4.0-4.5 4.2 $\pm$ 0.25	1.6-1.9 1.77 $\pm$ 0.15	1.9-2.5 2.23 $\pm$ 0.3	0.79	Orbicular	Farinaceous
7	<i>A. rosea</i>	4.0-4.3 4.1 $\pm$ 0.15	1.6-1.9 1.85 $\pm$ 0.05	2.1-2.4 2.29 $\pm$ 0.15	0.8	Trilobate	Scariosus
8	<i>A. setosa</i>	1.7-2.0 1.85 $\pm$ 0.15	4.2-4.6 4.25 $\pm$ 0.05	2.8-2.9 2.85 $\pm$ 0.05	1.49	Pentalobate	Scariosus
9	<i>Althaea ludwigii</i>	2.1-2.4 2.25 $\pm$ 0.15	1.0-1.3 1.15 $\pm$ 0.15	1.3-1.5 1.4 $\pm$ 0.1	0.82	Pentalobate	Scariosus
10	<i>A. officinales</i>	0.6-0.9 0.8 $\pm$ 0.05	4.5-4.9 4.3 $\pm$ 0.6	3.7-3.8 3.7 $\pm$ 0.05	1.21	Hastate	Papyraceus
11	<i>A. cannabina</i>	0.4-0.4 0.4 $\pm$ 0.0	2.7-3.2 3.0 $\pm$ 0.03	1.4-1.4 1.4 $\pm$ 0.0	2.14	Ovate	Velvety
12	<i>Malva aegyptia</i>	1.1-1.9 1.43 $\pm$ 0.4	1.0-2.0 1.57 $\pm$ 0.5	0.9-2.5 1.6 $\pm$ 0.8	0.98	Pentalobate	Scariosus
13	<i>M. neglecta</i>	4.0-4.1 4.03 $\pm$ 0.05	4.0-4.2 4.1 $\pm$ 0.1	6.6-6.7 6.63 $\pm$ 0.05	0.62	Pentalobate	Scariosus
14	<i>M. nicaeensis</i>	1.5-1.7 1.6 $\pm$ 0.1	1.3-1.4 1.35 $\pm$ 0.05	2.0-2.0 2.0 $\pm$ 0.0	0.68	Pentalobate	Scariosus
15	<i>M. parviflora</i>	0.8-1.5 1.03 $\pm$ 0.4	0.8-1.2 0.97 $\pm$ 0.2	1.0-1.8 1.33 $\pm$ 0.9	0.73	Pentalobate	Scariosus
16	<i>M. sylvestris</i>	2.1-2.9 2.35 $\pm$ 0.15	2.5-3.7 3.2 $\pm$ 0.03	3.9-4.2 3.7 $\pm$ 0.05	0.87	Pentalobate	Scariosus
17	<i>Malvastrum coromandelianum</i>	0.6-0.75 0.7 $\pm$ 0.0	3.4-3.4 3.4 $\pm$ 0.0	1.1-1.1 1.1 $\pm$ 0.0	3.1	Subrotund	Scariosus
18	<i>Malvaviscus arboreus</i>	1.0-1.5 1.3 $\pm$ 0.25	2.9-3.6 3.3 $\pm$ 0.35	1.0-1.5 1.3 $\pm$ 0.25	2.54	Ovate	Coriaceous
19	<i>Sida alba</i>	0.6-1.3 0.85 $\pm$ 0.05	1.4-1.6 1.5 $\pm$ 0.1	0.7-1.0 0.85 $\pm$ 0.15	1.76	Ovate	Papyraceus
20	<i>S. cordata</i>	0.6-0.8 0.65 $\pm$ 0.05	1.3-1.9 1.6 $\pm$ 0.11	0.7-0.8 0.75 $\pm$ 0.05	2.13	Ovate	Scariosus



TABLE 2. Cont.

No.	Species	Leaf blade		Margin			Venation	
		Base	Apex	Type	ND / cm	DE	Type	No. of main veins
1	<i>Abutilon fruticosum</i>	Cordate	Mucronate	Dentate	3	L	Pinnate	9
2	<i>A. hirtum</i>	Cordate	Apiculate	Dentate	12	M	Palmate	9
3	<i>A. longicuspe</i>	Cordate	Rostrate	Dentate	12	H	Palmate	7
4	<i>A. pannosum</i>	Truncate	Rostrate	Dentate	7	VH	Palmate	11
5	<i>A. theophrasti</i>	Cordate	Rostrate	Erosus	4	VL	Palmate	7
6	<i>Alcea acaulis</i>	Cordate	Obtuse	Crenate	6	M	Palmate	9
7	<i>A. rosea</i>	Truncate	Acute	Dentate	10	L	Palmate	3
8	<i>A. setosa</i>	Cordate	Acute	Dentate	11	VH	Palmate	3
9	<i>Althaea ludwigii</i>	Truncate	Tridentate	Entire	0	0	Palmate	5
10	<i>A. officinales</i>	Truncate	Acuminate	Dentate	8	VH	Pinnate	3
11	<i>A. cannabina</i>	Cuneate	Acute	Dentate	7	L	Palmate	5
12	<i>Malva aegyptia</i>	Truncate	Obtuse	Entire	0	0	Palmate	13
13	<i>M. neglecta</i>	Truncate	Obtuse	Dentate	10	VH	Palmate	5
14	<i>M. nicaeensis</i>	Truncate	Acute	Dentate	9	H	Palmate	5
15	<i>M. parviflora</i>	Cordate	Obtuse	Dentate	9	H	Palmate	5
16	<i>M. sylvestris</i>	Truncate	Acute	Dentate	11	M	Palmate	5
17	<i>Malvastrum coromandelianum</i>	Truncate	Obtuse	Dentate	5	VH	Pinnate	13
18	<i>Malvaviscus arboreus</i>	Breve angustate	Acuminate	Dentate	10	H	Pinnate	3
19	<i>S. alba</i>	Truncate	Acute	Dentate	8	M	Pinnate	1
20	<i>S. cordata</i>	Truncate	Acuminate	Dentate	8	H	Pinnate	3

### Trichomes

The trichomes within the twenty studied taxa (Table 5, Plate 2) are either of one type on both the abaxial and axial surfaces or two or three types on both the leaf surfaces. The most recorded types are stellate (Photos 4, 7, 9, 16, 29 & 32) and multicellular uniseriate pointed or tabulated (Photos 1, 5, 6, 10, 12 & 27). Cavitated hairs were recorded in *Abutilon fruticosum* and *Sida aba* (Photos. 3 & 33). The hairs differ in lengths and densities within the taxa, and their walls are ornamented by either spines or echinae (Protrusions with a wide base and pointed tips). Echinae are types of protrusion on hair wall.

### Clustering analysis (Table 6, Fig.1)

All the obtained results for clustering analyses are summarized in Table 6 and Fig. 1. The twenty studied species were used as Operational Taxonomic Units (OUTs). The twenty-two employed characters are of four types: multistate qualitative unordered (MQU), multistate quantitative (MQ), continuous and

binary. Continuous characters are those measured or calculated, binary characters (i.e., presence or absence) or even two-state characters, multistate qualitative unordered are unrelated states. The quantitative characters are those having many states depending on the quantity. The most important characters have been coded and subjected to the analyses (Appendix S1). The resulting clustering figure separates *Althaea ludwigii* very early at a similarity index of 14.25, while the rest of the taxa are grouped in two categories, 1 and 2. The first category has six species in two subgroups (I & II); *Malva aegyptia*, *Abutilon fruticosum*, *A. pannosum*, *A. theophrasti*, *Malvastrum coromandelianum*, and *Alcea acaulis*; with the separation of *Malva aegyptia* in a separate subgroup (I) at similarity index of 10.75 with gradual separation of the rest five species in the subgroup (II). The remaining 13 species in group 2 are subdivided into three subgroups (A, B & C). Subgroup A has six taxa, divided into two categories at similarity index 8, each with three taxa: category 1 includes *Alcea*

*rosea*, *Malva nicaeensis*, and *M. parviflora*, while category 2 gathers *Alcea setosa*, *Althaea officinalis* and *Malva neglecta*. Subgroup B has three taxa, which separates *Abutilon longicuspe* alone into a separate category at a similarity

index of 7.5 from the two taxa, *Abutilon hirtum* and *Malva sylvestris*. Subgroup C has four taxa, separating *Athaea cannabina* and *Malvasicus arboreus* from the two *Sida* species at a similarity index of 8.0.

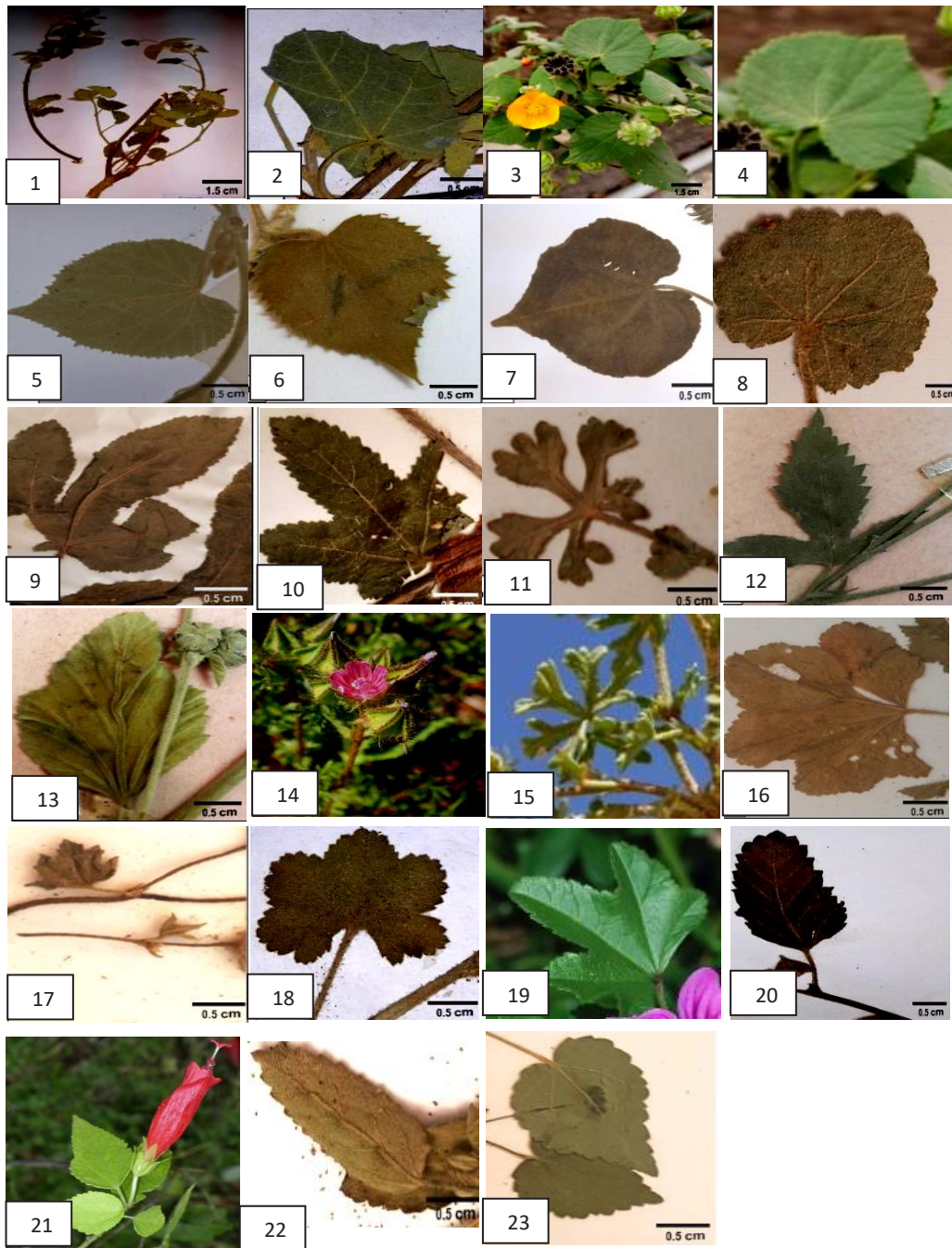


Plate 1. Photos in the leaves of the studied taxa showing Macro-morphological features. 1, 2 *Abutilon fruticosum*, 3, 4 *Abutilon hirtum*, 5 *Abutilon longicuspe*, 6 *Abutilon pannosum*, 7 *Abutilon theophrasti*, 8 *Alcea acualis*, 9 *Alcea rosea*, 10 *Alcea setosa*, 11 *Althaea ludwigii*, 12 *Althaea cannabina*, 13 *Althaea officinalis*, 14, 15 *Malva aegyptia*, 16 *Malva neglecta*, 17 *Malva nicaeensis*, 18 *Malva parviflora*, 19 *Malva sylvestris*, 20 *Malvastrum coromandelianum*, 21 *Malvaviscus arboreus*, 22 *Sida alba*, 23 *Sida cordata*

**TABLE 3. Epidermal cell characters in the studied species as shown by SEM**

No	Species	Outline	Anticlinal wall		Periclinal wall		Wax depositions	
			Shape	Relief	State	Ornam	Type	Density
1	<i>Abutilon fruticosum</i>	Iso.	Straight	Elevate	Concave	Striate	Globular	Low
2	<i>A. hirtum</i>	Iso	Straight	Elevate	Flat	Striate	Globular	Medium
3	<i>A. longicuspe</i>	Iso	Straight	Elevate	Flat	Smooth	Absent	--
4	<i>A. pannosum</i>	Iso	Straight	Elevate	Flat	Smooth	Absent	--
5	<i>A. theophrasti</i>	Iso	Straight	Sunken	Convex	Smooth	Globule	Dense
6	<i>Alcea acaulis</i>	Elong.	Undulate	Elevate	Flat	Folded	Globule	Few
7	<i>A. rosea</i>	Elong.	Undulate	Sunken	Convex	Pitted	Globule	Few
8	<i>A. setosa</i>	Elong.	Undulate	Elevate	Convex	Pitted	Globule	Medium
9	<i>Althaea ludwigii</i>	Elong.	Straight	Elevate	Concave	Striate	Absent	--
10	<i>A. officinales</i>	Elong.	Straight	Elevate	Concave	Striate	Absent	--
11	<i>A. cannabina</i>	Elong.	Straight	Elevate	Concave	Striate	Absent	--
12	<i>Malva aegyptia</i>	Elong.	Straight	Elevate	Flat	Striate	Globule	Dense
13	<i>M. neglecta</i>	Elong.	Straight	Elevate	Concave	Striate	Absent	--
14	<i>M. nicaeensis</i>	Elong.	Undulate	Sunken	Convex	Striate	Globule	Dense
15	<i>M. parviflora</i>	Elong.	Straight	Elevate	Flat	Striate	Globule	Dense
16	<i>M. sylvestris</i>	Elong.	Straight	Elevate	Flat	Striate	Globule	Dense
17	<i>Malvastrum coromandelianum</i>	Elong.	Undulate	Sunken	Convex	Smooth	Globule	Few
18	<i>Malvaviscus arboreus</i>	Elong.	Sinuate	Sunken	Convex	Striate	Globule	Few
19	<i>S. alba</i>	Elong.	Undulate	Sunken	Convex	Striate	Globule	Few
20	<i>S. cordata</i>	Elong.	Undulate	Sunken	Convex	Smooth	Globule	Few



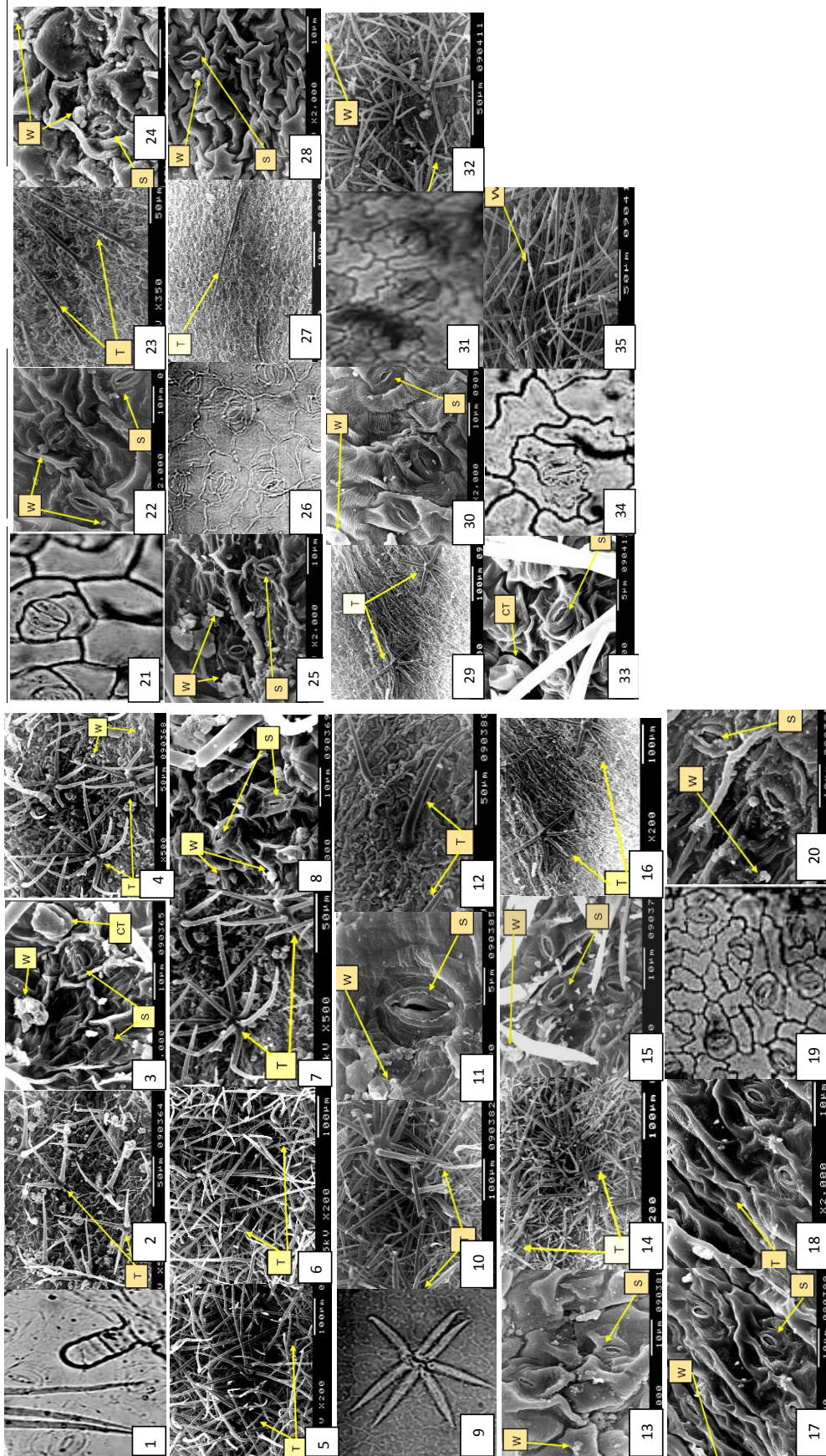


Plate 2. LM and SEM photos in the leaves of the studied taxa showing micro-morphological features. 1, 2, 3 *Abutilon fruticosum*, 4 *Abutilon hirtum*, 5 *Abutilon longicuspe*, *Abutilon pannosum*, 7, 8, 9 *Abutilon theophrasti*, 10, 11 *Alcea acutalis*, 12, 13 *Alcea rosea*, 14, 15 *Alcea setosa*, 16, 17 *Althaea ludwigii*, 18, 19 *Althaea cannabina*, 20, 21 *Malva aegyptia*, 22 *Malva neglecta*, 23, 24 *Malva nicaeensis*, 25 *Malva parviflora*, 26 *Malva sylvestris*, 26, 27 *Malvastrum coromandelianum*, 28, 29 *Malva viscus arboreus*, 30, 31, 32, 33 *Sida alba*, 34, 35 *Sida cordata* (CT=cavitated trichomes, S=stomata, T=trichome, W=wax)

**TABLE 4. Stomatal characters within the studied species as shown by both LM & SEM, SD= Stomatal density, SI= Stomatal index**

No	Species	Type	Position	Guard cell		SD	SI%
				Shape	Size ( $\mu\text{m}$ )		
1	<i>Abutilon fruticosum</i>	Diacytic	Surface	Kidney	20.0-24.2 23.2 $\pm$ 1.4	6-8	22.9-24.6 23.3 $\pm$ 2.8
2	<i>A. hirtum</i>	Anisocytic	Raised	Kidney	19.0-22.2 21.4 $\pm$ 0.9	6-8	19.8-22.4 21.1 $\pm$ 3.5
3	<i>A. longicuspe</i>	Anisocytic	Surface	Kidney	20.0-24.4 23.2 $\pm$ 1.4	5-7	16.8-18.4 17.8 $\pm$ 1.4
4	<i>A. pannosum</i>	Anomocytic	Surface	Kidney	18.5-22.0 19.4 $\pm$ 1.9	6-8	20.8-24.3 21.0 $\pm$ 3.9
5	<i>A. theophrasti</i>	Diacytic	Surface	Kidney	21.4-23.8 22.4 $\pm$ 1.6	4-6	19.8-25.3 22.0 $\pm$ 3.6
6	<i>Alcea acaulis</i>	Paracytic	Sunken	Kidney	20.8-23.4 21.2 $\pm$ 2.1	4-6	21.0-23.4 22.4 $\pm$ 1.5
7	<i>A. rosea</i>	Paracytic	Surface	Kidney	17.5-20.8 18.2 $\pm$ 1.8	3-5	19.8-24.8 20.4 $\pm$ 2.9
8	<i>A. setosa</i>	Paracytic	Raised	Kidney	18.0-22.2 20.8 $\pm$ 0.9	8-10	21.0-27.3 22.9 $\pm$ 2.3
9	<i>Althaea ludwigii</i>	Paracytic	Surface	Kidney	17.8 -20.4 19.1 $\pm$ 1.2	8-11	13.9-15.7 14.8 $\pm$ 0.8
10	<i>A. officinales</i>	Paracytic	Surface	Kidney	18.6-20.2 19.2 $\pm$ 1.8	9-11	17.5-21.8 20.2 $\pm$ 2.3
11	<i>A. cannabina</i>	Paracytic	Surface	Kidney	19.5-21.2 20.2 $\pm$ 2.1	6-8	16.4-21.2 18.5 $\pm$ 2.1
12	<i>Malva aegyptia</i>	Anisocytic	Raised	Kidney	22.2-24.8 22.8 $\pm$ 1.4	6-8	20.3-22.4 21.3 $\pm$ 0.02
13	<i>M. neglecta</i>	Anisocytic	Surface	Kidney	22.4-25.2 23.8 $\pm$ 1.8	6-8	20.7-21.9 21.6 $\pm$ 0.04
14	<i>M. nicaeensis</i>	Anisocytic	Raised	Kidney	19.6-23.6 20.2 $\pm$ 1.3	4-6	18.4-21.2 19.5 $\pm$ 2.1
15	<i>M. parviflora</i>	Anisocytic	Surface	Kidney	18.6-21.0 19.2 $\pm$ 1.8	4-6	17.8-20.2 18.5 $\pm$ 2.1
16	<i>M. sylvestris</i>	Anisocytic	Surface	Kidney	18.8-20.2 19.8 $\pm$ 1.8	4-6	19.4-21.2 20.5 $\pm$ 2.1
17	<i>Malvastrum coromandelianum</i>	Anisocytic	Surface	Kidney	17.8-20.2 18.5 $\pm$ 2.1	4-6	17.4-22.2 19.5 $\pm$ 1.8
18	<i>Malvaviscus arboreus</i>	Anisocytic	Surface	Kidney	19.8-22.6 20.4 $\pm$ 1.3	6-9	16.8-21.2 18.5 $\pm$ 2.1
19	<i>S. alba</i>	Diacytic	Surface	Elliptic	12.4-13.8 12.9 $\pm$ 2.1	4-6	15.4-18.2 17.5 $\pm$ 1.8
20	<i>S. cordata</i>	Anisocytic	Surface	Elliptic	11.2-14.0 12.5 $\pm$ 2.1	4-6	14.8-17.5 15.4 $\pm$ 2.1

**TABLE 5. Trichome characters within the studied species as shown by both LM & SEM, H= Hair, Gl.Un= Glandular unicellular, Mult.unp= Multicellular uniserriate pointed, Mult.unT= Multiserriate uniserriate tabular, Vlong= Very long, VDense=Very dense**

No	Species	Homogeneity	Type	Length	Density	Wall
1	<i>Abutilon fruticosum</i>	3 types	Stellate+ Gl.Un+ Cavitated H	Long	Dense	Echinate
2	<i>A. hirtum</i>	1 type	Stellate	Vlong	Dense	Spinulose
3	<i>A. longicuspe</i>	1 type	Mult.UnP	Vlong	Woolly	Spinulose
4	<i>A. pannosum</i>	1 type	Mult.UnP	Vlong	Woolly	Spinulose
5	<i>A. theophrasti</i>	1 type	Stellate	Vlong	Dense	Spinulose
6	<i>Alcea acaulis</i>	2 type	Stellate+ Mult.UnT	Vlong	VDense	Spinulose
7	<i>A. rosea</i>	1 type	Mult.UnP	Long	Medium	Spinulose
8	<i>A. setosa</i>	2 type	Stellate+ Mult.UnP	Vlong	VDense	Spinulose
9	<i>Althaea ludwigii</i>	1 type	Stellate	Medium	Few	Echinate
10	<i>A. officinales</i>	1 type	Stellate	Medium	Few	Echinate
11	<i>A. cannabina</i>	---	---	---	---	---
12	<i>Malva aegyptia</i>	---	---	---	---	---
13	<i>M. neglecta</i>	---	---	---	---	---
14	<i>M. nicaeensis</i>	1 type	Mult.UnP	Long	Medium	Spinulose
15	<i>M. parviflora</i>	1 type	Stellate	Medium	Few	Echinate
16	<i>M. sylvestris</i>	1 type	Stellate	Medium	Few	Echinate
17	<i>Malvastrum coromandelianum</i>	1 type	Mult.UnP	Long	Medium	Spinulose
18	<i>Malvaviscus arboreus</i>	1 type	Stellate	Medium	Few	Echinate
19	<i>S. alba</i>	3 type	Stellate+ Mult.UnP+ Cavitated H	Vlong	VDense	Spinulose
20	<i>S. cordata</i>	2 type	Mult.UnP+ Stellate	Vlong	Woolly	Spinulose

**TABLE 6. Basic data matrix (BDM) of the leaf macro-and micro-morphological characters and their coding in the studied taxa, 20 operational taxonomic units (OTUs) × 22 characters**

No.	↓OUT's/Char.↓	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1	<i>Ab. fruticosum</i>	0.8	1.9	2.0	5	4	1	7	3	1	9	1	1	1	3	4	2	2	1	7	23.3	4	4
2	<i>Ab. hirtum</i>	3.5	4.5	4.9	5	4	1	3	12	2	9	1	1	1	1	4	2	3	2	7	21.1	2	4
3	<i>Ab. longicuspe</i>	1.3	2.2	2.4	2	3	1	4	12	2	7	1	1	1	1	1	1	1	2	6	17.8	2	6
4	<i>Ab. pannosum</i>	1.6	1.6	2.4	2	3	2	4	7	2	11	1	1	1	1	1	1	1	3	7	21.0	2	6
5	<i>Ab. theophrasti</i>	4.7	5.2	5.0	2	4	1	4	4	2	7	1	1	2	2	1	2	4	1	5	22.0	2	4
6	<i>Al. acaulis</i>	4.2	1.8	2.2	4	5	1	5	6	2	9	2	2	1	1	2	2	2	4	5	22.4	3	5
7	<i>Al. rosea</i>	4.1	1.9	2.3	7	2	2	2	10	2	3	2	2	2	2	3	2	2	4	4	20.4	2	3
8	<i>Al. setosa</i>	1.9	4.3	2.9	8	2	1	2	11	2	3	2	2	1	2	3	2	3	4	9	22.9	3	5
9	<i>Alth. ludwigii</i>	2.3	1.2	1.4	8	2	2	6	0	2	5	2	1	1	3	4	1	1	4	10	14.8	2	2
10	<i>Alth. officinales</i>	0.8	4.3	3.7	6	1	2	1	8	1	3	2	1	1	3	4	1	1	4	10	20.2	2	2
11	<i>Alth. cannabina</i>	0.4	3.0	1.4	1	4	3	2	7	2	5	2	1	1	3	4	1	1	4	7	18.5	1	1
12	<i>Mal. aegyptia</i>	1.4	1.6	1.6	8	2	2	5	0	2	13	2	1	1	1	4	2	4	2	7	21.3	1	1
13	<i>Mal. neglecta</i>	4.0	4.1	6.6	8	2	2	5	10	2	5	2	1	1	3	4	1	1	2	7	21.6	1	1
14	<i>Mal. nicaeensis</i>	1.6	1.4	2.0	8	2	2	2	9	2	5	2	2	2	2	4	2	4	2	5	19.5	2	3
15	<i>Mal. parviflora</i>	1.0	1.0	1.3	8	2	1	5	9	2	5	2	1	1	1	4	2	4	2	5	18.5	2	2
16	<i>Mal. sylvestris</i>	2.4	3.2	3.7	2	2	2	2	11	2	5	2	1	1	1	4	2	4	2	5	20.5	2	2
17	<i>Malvas. coromandelianum</i>	0.7	3.4	1.1	3	2	3	5	5	1	13	2	2	2	2	1	2	2	2	5	19.5	2	3
18	<i>Malvav. arboreus</i>	1.3	3.3	1.3	1	3	4	1	10	1	3	2	3	2	2	4	2	2	2	8	19.5	2	2
19	<i>Sid. alba</i>	0.9	1.5	0.9	1	1	2	2	8	1	1	2	2	2	2	4	2	2	1	5	18.5	4	5
20	<i>Sid. cordata</i>	0.7	1.6	0.8	1	2	2	1	8		3		2	2	2	1	2	2	2	5	17.5	3	6



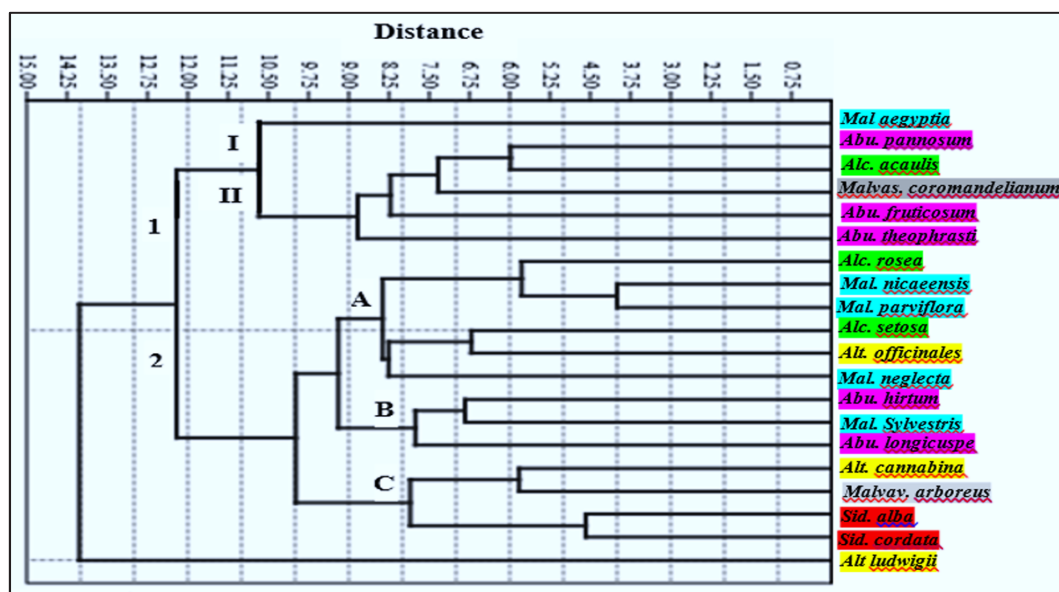


Fig. 1. Dendrogram based on the taxonomical characters of the twenty species belonging to the tribe Malveae

Identification key to the studied taxa

- |   |                                   |
|---|-----------------------------------|
| 1-Leaf blade hastate                    | <i>Althaea officinales</i>        |
| 1-Leaf blade trilobate                  | <i>Alcea rosea</i>                |
| 1-Leaf blade subrotund                  | <i>Malvastrum coromandelianum</i> |
| 1-Leaf blade orbicular                  | <i>Alcea acaulis</i>              |
| 1-Leaf blade cordate                    |                                   |
| 2-Leaf blade venation palmate           | <i>Abutilon hirtum</i>            |
| 2-Leaf blade venation pinnate           | <i>Abutilon fruticosum</i>        |
| 3-Leaf blade shape narrow ovate         |                                   |
| 3.i-Leaf blade venation palmate         | <i>Althaea cannabina</i>          |
| 3.ii-Leaf blade venation pinnate        |                                   |
| a - No. of veins 8                      | <i>Sida alba</i>                  |
| b -No. of veins 3                       |                                   |
| bi-Woolly, very long hairs              | <i>Sida cordata</i>               |
| bii-Sparsely hairy, medium length hairs | <i>Malvastrum arboreus</i>        |
| 4-Leaf blade broadly ovate              |                                   |
| a-Leaf blade venation palmate           |                                   |
| ai. -No. of veins 11                    | <i>Abutilon pannosum</i>          |
| aii.-No. of veins 7                     |                                   |
| aii.i-dense or woolly surface           |                                   |
| aii.i.i -No. of teeth/cm 12             | <i>Abutilon longicuspe</i>        |
| aii.i.ii -No. of teeth/cm 4             | <i>Abutilon theophrasti</i>       |
| 5-Leaf blade pentalobate                |                                   |
| a-Leaf blade venation palmate           |                                   |
| a.i.-No. of veins 3                     | <i>Alcea setosa</i>               |
| a.ii-No. of veins 13                    | <i>Malva aegyptia</i>             |
| a.iii- No. of veins 5                   |                                   |
| a.iiiix- Glabrous leaf surface          | <i>Malva neglecta</i>             |
| a.iiiixx- Hairy leaf surface            |                                   |
| a.iii.xx.i -No. of teeth/cm 0           | <i>Althaea ludwigii</i>           |
| a.iii.xx.ii -No. of teeth/cm 9          |                                   |
| -Hairs multicellular uniseriate         | <i>Malva nicaensis</i>            |
| a.iii.xx.iii-No. of teeth/cm 11         | <i>Malva parviflora</i>           |
|   | <i>Malva sylvestris</i>           |

## Discussion

Family Malvaceae *s.s.* is from the important families that face a lot of taxonomical arguments concerning their belonging taxa. According to the new classification systems, Sterculaceae and Bombacaceae have been included within Malvaceae's taxa to become Malvaceae *s.l.* (APG I, 1998; APG III, 2009). These taxa are characterized, mainly by their leaf characters, despite the variations in their life forms, floral and mericarp characters. Leaf macro- and micro-morphological features gained great attention in the discrimination and recognition of taxa within the family, as Hickey (1973) described the leaves of Malvaceae by being simple with actinodromous venation. Afterward, attention has been given to the leaf characters and features in the taxonomy of the family by Bhat (1995), Essiet & Iwok (2001), Adedeji & Illoh (2004), and Celka et al. (2006). Larano & Buot (2010) described the Malvaceae leaves as simple with entire or serrate margin, pinnately venate with secondary craspedodromous veins, which tend to form incomplete or looped marginal veins.

The present investigations were carried out to assess the importance of the leaf characters in the discrimination of the studied taxa. The results obtained assess the importance and variation in the leaf characters within the studied Malveae tribe in identifying the related species. The petiole lengths, leaf shape, size, blade base shape, texture, apex, margin, and venation varied greatly within the studied taxa. The most important macro-morphological leaf characters are those related to the leaf margin number of dental teeth and their depths, besides the type of venation and the number of lateral veins. The number of dental teeth/cm has been mentioned before by Bhat (1999 & 2012) in some genera of the Malvaceae. In this study, the depth of the teeth can also be of taxonomical use. The type of venation is either pinnate or palmate with different numbers of lateral reticulodromous or incomplete craspidodromous veins. The number of lateral veins is a good diagnostic character within the studied species. It was one of three in the two *Sida* species, *Alcea rosea*, *Alcea setosa*, and *Malva viscus arboreus*, to 13 lateral veins in *Malva aegyptia* and *Malvastrum coromandelianum*. These features have been discussed by Taia & Mahdy (2021b) within the *Bauhinia* species and Larano & Buot (2010) and are considered useful

characters in recognizing some Malvaceous taxa. From the results obtained, the leaf macro-morphological characters gave a clear and easy way to recognize some taxa. *Sida* species have leaf characters that can be used for their identification. *Malva aegyptia* and *Althaea ludwigii* are the only taxa with smooth leaf margins; accordingly, they are separated into separate clades in the dendrogram. The leaves' shape, size, and texture can easily recognize some taxa, as shown in the identification key. The clustering dendrogram did not give a clear separation between the genera and species, and this can be attributed to the great similarity between their leaf characters. Despite the homogeneity in the clustering dendrogram, the two *Sida* species came together for their clear leaf morphological difference from the rest of the studied taxa. This result agrees with that of Bassey et al. (2016).

Several studies (e.g., Aworinde et al., 2012; Okoli & Ajuru, 2014; Green, 2015) have clarified the importance of the stomata and micro-morphological features in plant classification. On the other side, Stace (1965) stated that the variation in the epidermal characters besides stomatal density, index, and size of the guard cells and trichomes density is due to the leaf ages, position, or other characteristics of environmental conditions. In addition, other researchers, such as Metcalfe & Chalk (1979) and Ozgur & Tugha (2006), mentioned that the epidermis has many important characteristics that can be used as clues in taxonomic identification, such as their sizes, shape, stomata, guard and subsidiary cells besides the type and length of trichomes. The results obtained from this study revealed that the most important leaf microcharacters are those concerning the type of stomata, hair type, and density. These characters can identify certain taxa, while the stomatal density and index can be attributed to habitat variations and ecological conditions.

### Phylogenetic aspect

Many previous works correlate the morphological features of plants with their evolutionary steps. Several studies (e.g., Beerling & Kelly, 1996; Willson et al., 2008; Brodribb & Field, 2010; El Kholy et al., 2023; Ibrahim et al., 2023). *Alcea* have correlated the development of the epidermis, cuticle, stomatal type, and density with the type of venation and the number of lateral veins as ways of succession in the adaptation



to terrestrial habitats. The increase in the lateral veins could be attributed to more adaptation to the arid environment. Thus, we can consider the type of venation beside the number of lateral veins as steps in the evolution within the taxa as indicated by Uhl & Osbruggen (1999) and Walls (2011). The palmate venation with the increase in the lateral veins is more advanced than the pinnate venation with one or few lateral veins.

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*Authors' contributions:* Taia WK; Collection, measurements, description and writing the paper, El-Badan GE; SEM, Clustering analyses, revising the measurements, revising of the manuscript for submission, Hassan AM; Editing of the paper and made the photographs, All authors have read and agreed to the published version of the manuscript.

*Ethics approval:* Not applicable

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### اختلافات الأوراق ضمن الأجناس الممثلة لقبيلة Malveae وأهميتها في نشأة الأصناف

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تم فحص وقياس الصفات الدقيقة للأوراق من سبعة أجناس ممثلة في عشرين نوعًا تنتمي إلى قبيلة Malveae، العائلة الخبازية. أوضحت النتائج أن طول الأعناق وشكل نصل الورقة والقاعدة والقمة تفاوتت بين الأصناف المدروسة. وفي الوقت نفسه يمكن أن يكون حرف الورقة وعدد الأسنان وعمقها / سم في حرف الورقة ونوع الانقفاخ وعدد التفرعات الجانبية ذات قيمة تشخيصية داخل الأنواع. غالبًا ما تكون خصائص خلايا البشرة ثابتة، في حين أن نوع الشعر وكثافته ووجود الشعر المجوف بجانب نوع الثغور وشكل الخلايا الحارسة أعطت خصائص قيمة داخل الأصناف. خضعت النتائج لتحليلات عنقودية باستخدام برنامج PAST وأظهر مخطط الشجرة الناتج تشابهًا كبيرًا بين الأصناف مع فصل كل من *Malva aegyptia* و *Althaea ludwigii* في كليل منفصل. تم تصميم مفتاح تشخيصي وافترض التفكير النشئي للأصناف.