Egypt. J. Bot. **Vol. 63**, No. 3, pp. 951-968 (2023)

Egyptian Journal of Botany
http://ejbo.journals.ekb.eg/

Micromorphological Investigation in Leaf Epidermis of Some Monocotyledons Taxa

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TWENTY species that belong to 13 genera under six families, two belonging to order Asparagales and four to order Liliales, are subjected in this recent work. Micro-morphological leaf characters using both Light and Scanning Electron microscopes have been studied. The pavement cells, hairs, prickles and stomatal characters beside stomatal density and index have been calculated. The data obtained has been subjected to clustering analyses using Paleontological Statistics (PAST) program. The results which were obtained from this study do not give clear separations between the two orders or even the families and considered the studied six families have common leaf characters. Meanwhile, characters of epidermal cells, hairs, papillae, prickles, and stomata can give clear features in the identification of some species. Hence, we can conclude that the separation of these orders and families and their taxonomic ranking need further investigation to clarify the perfect relation between them.

Keywords: Asparagaceae, Asphodelaceae, Colchiaceae, Liliaceae, Melanthiaceae, Smilaceae.

Introduction

The monocots are famous as a group that seems extremely stable in its outer borders. This group has one cotyledon in their seeds, and flowers generally have parts in threes or multiply of threes. Recent phylogenetic taxonomic works (APG III, 2009 and APG IV, 2016) recognized this group in a separate clade although that its internal taxonomy is extremely unstable and all the previous taxonomic works did not agree in putting common taxonomic relationships between their taxa (Cronquist, 1981; Dahlgren et al., 1985; Takhtajan, 1997; Tanaka et al., 1997). All the previous classifications (Bentham & Hooker, 1883; Engler, 1888; Melchior, 1964) as well as the recent studies (molecular studies APG I, II, III & IV from 1998 till 2016) did not agree in categorizing the monocotyledons taxa and their taxonomic treatments which are under dispute to date (Reveal, 2012; Soltis et al., 2018). Dahlgren et al. (1985) divided the Liliiflorae into five orders: Asparagales, Burmanniales, Dioscoreales, Liliales and Melanthiales. The separation between the two orders Asparagales and Liliales (sensu Dahlgren et al.,1985; APG III, 2009) is still unclear. Studies are still needed to better understand the relationship between the taxa under these orders. Chang-Gee & Pfosser (2002) reclassified the Korean taxa of the two orders according to molecular data. These confusions in grouping the monocot taxa are still unsolved and need more precise studies using easy tools for the discrimination between them. In this concern, the present study has been carried out as a step to clarify the relation between the two orders using leaf epidermal and stomata characters.

Micro-morphological characters, especially those of leaf surfaces, sometimes appear sufficiently diverse to serve as useful tools for taxonomy and species identification (Haron & Moore, 1996; Mostafavi et al., 2013; Krawczyk & Glowacka, 2015; Barthlott et al., 2017; El-Ghamery et al., 2021 ; Al-Rubai et al., 2022). The Foliar epidermis is one of the most noteworthy taxonomic characters from the biosystematics point of view. Variations and taxonomic opinions have been suggested in the different taxa for a long time ago (Baranova, 1972; Bhatia, 1984; Stace, 1984; Jones, 1986). Some groups of plants seem to be characterized by specific types of epidermal

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Received 20/02/2023; Accepted 24/05/2023
DOI: 10.21608/ejbo.2023.195136.2261
Edited by: Prof. Dr. Wafaa Amer, Faculty of Science, Cairo University, Giza, Egypt.

features, which are the epidermis, stomata, gland and trichomes (Hong & Son, 2000). Recently, Tavakkoli & Assadi (2016), Shah et al. (2019) and Taia & El-Mahdy (2021) have found that leaf and seed macro-and micro-morphological characters proved to be useful in the taxonomy and identification of the *Papaver* and *Bauhinia* species. They found that leaf characters; stomatal type, epidermal cells and hair type and density provide important features that can help in both the taxonomy and phylogeny of the taxa. Shah & Gopal (1971) found many types and abnormalities of stomatal complexes within the studied taxa of Caesalpiniaceae. Stebbins & Khush (1961) grouped the stomatal types of 192 species from 49 monocot families into four groups: 1-with four or more subsidiary cells, 2-complexes with two subsidiary cells, 3-without subsidiary cells, 4-Mature complexes with variable numbers of subsidiary cells; and they also considered the types with more subsidiary cells the more primitive and the other types originated by the reduction in the number of them. Pradham & Bajracharya (2016) found that the stomatal types and epidermal cells are important in the taxonomy and identification of *Dendrobium* species (Orchidaceae).

According to all the previous views the stomatal complex, the two guard cells and subsidiary cells, besides the epidermal cells give valuable characters in systematic, taxonomy and phylogeny.

The twenty studied taxa show variety in distribution throughout the world; *Dipterostemon capitatus* ranges from northern Mexico through California, Oregon, Nevada, New Mexico, and possibly north to Idaho and Washington. It is quite distinct with six fertile stamens, and it has unique corm, seed, and ovule characteristics. *Maianthemum racemosum* is a species of flowering plant native to North America. Furthermore, it is a common, widespread plant that known from every US state except Hawaii, and from every Canadian province and territory (except Nunavut and the Yukon), as well as from Mexico. *M. racemosum* is a woodland herbaceous perennial plant, that spreads by cylindrical rhizomes. *Dipcadi erythraeum* is found in Africa (Northern Africa, Egypt) and Asia (Afghanistan; India; Iran; Iraq; Kuwait; Oman; Pakistan; Palestine; Saudi Arabia; Sinai). *Drimia* spp. are widely distributed throughout Africa, Madagascar, the Mediterranean area, southeast Asia, and India. They are widely known for their medicinal properties, as indicated in the oldest written record from 1500 BC. Until now, the species of *Drimia* have been well investigated for bioactive compounds and commercialized (Bozorgi et al., 2017). *Muscari comosum* is a herbaceous plant, widespread in Mediterranean regions. The flowers bloom in spring and are persistent until the summer. *Ornithogalum arabicum* is native to northern Africa and southern Europe, where it grows best in full sun and fertile, well-drained soils. In late spring and early summer, *Ornithogalum arabicum* produces large and fragrant white flowers. *Scilla peruviana* is native to the western Mediterranean. It is a herbaceous perennial geophyte with a spectacular conical raceme of blue flowers borne over several weeks in late spring. The genus *Asphodelus* comprises 16 species distributed in Eurasia and the Mediterranean (Días Lifante & Valdés, 1996). *Colchicum* is mainly found in Central and South Europe, Germany, Greece, Spain, Turkey, and England. The genus *Calochortus* includes approximately 70 species distributed from southwestern British Columbia, through California and Mexico, to northern Guatemala and eastwards to New Mexico, Nebraska, and the Dakotas. *Gagea* is a large genus of spring flowers in the lily family. It is found primarily in Eurasia with a few species extending into North Africa and one species in North America. *Toxicoscordion fremontii* is a west coast native that produces an attractive spike of cream-colored flowers from an underground bulb. It is native to the west coast of North America from southwestern Oregon to northern Baja, California. *Smilax aspera* is native to the tropical and temperate-warm countries and is widespread in Mexico, the Canary Islands, central Africa, Central Asia, and regions of the Mediterranean area.

This study investigated micro-morphological characters of leaf epidermis variations within 20 taxa belonging to six families within Liliales and Asparagales. The goals are (1) to investigate the diversity of leaf epidermis in some monocot taxa including both epidermal cell sculpture, density of papillae, prickles and hair type, position and density, (2) to determine the stomatal type, density and guard cells variations, (3) to analyze the taxonomic significance of characters of leaf epidermal variations by delineating species groups on the basis of these characters, and (4) to evaluate infra-specific variation of these characters.

Materials and Methods

Twenty species that belong to 13 genera under six families were studied. Two families; Asparagaceae and Asphodelaceae belong to the order Asparagales and four families; Colchieaceae, Liliaceae, Melanthiaceae and Smilacaceae belong to order Liliales. Three subfamilies belong to the family Asparagaceae (Brodiaenoideae, Nolinoideae and Scilloideae with one tribe, Ornithogaleae) and one subfamily belongs to family Asphodelaceae (Asphodeloideae). Family Liliaceae has two subfamilies: Calortoideae and Liliodeae, and one tribe (Lilieae). Subfamilies and tribes are classified according to Dahlgren et al. (1985) (Table 1). Herbarium specimens were examined carefully from the following herbaria: Alexandria university (ALEX), Cairo University (CAI), King Saud University (KSU). The nomenclature and the synonyms of each species obtained from the International Plant Names Index IPNI (2022), and World Checklist of Selected Plant Families as listed in Table 2.

TABLE 1. Taxonomic status of the studied taxa according to APG IV family list (2016) subfamilies and tribes are according to Dahlgren et al. (1985)

Order	Family	Subfamily	Tribe	Genus	Species
Asparagales	Asparagaceae	Brodiaeoideae		*Dipterostemon*	*D. capitatus* (Benth.) Rydb.
		Nolinoideae		*Maianthemum*	*M. racemosum* (L.) Link
		Scilloideae		*Dipcadi*	*D. erythraeum* Webb & Berthel.
				Drimia	*D. purpurascens* J.Jacq.
				Muscari	*M. comosum* (L.) Mill.
				Scilla	*S. peruviana* L.
			Ornithogaleae	*Ornithogalum*	*O. arabicum* L.
	Asphodelaceae	Asphodeloideae	---	*Asphodelus*	*A. fistulosus* L.
					A. ramosus L.
					A. tenuifolius Cav.
Liliales	Colchicaceae			*Colchicum*	*C. cornigerum* (Schweinf. ex Sickenb.) Tackh. & Drar
					C. ritchii R.Br.
	Liliaceae	Calochortoideae		*Calochortus*	*C. amabilis* Purdy
					C. monophyllus (Lindl.) Lem
					C. pulchellus (Doug. ex Benth.) Alph. Wood
					C. uniflorus Hook. & Arn.
		Lilioideae	Lilieae	*Gagea*	*G. dayana* Chodat & Beauverd.
					G. fibrosa (Desf.) Schult. & Schult.f.
	Melanthiaceae			*Toxicoscordion*	*T. fremontii* (Torr.) Rydb.
	Smilacaceae			*Smilax*	*S. aspera* L.

TABLE 2. Collection data, localities, source of the specimens, synonyms, and confirmation of nomenclature, ALEX=Alexandria University Herbarium, CAI=Cairo University Herbarium, KSU=King Saud University Herbarium

No	Species	Localities	Source of Sp.	Synonyms	Confirmation of nomenclature
1	*Dipterostemon capitatus* (Benth.) Rydb.	California, USA.	KSU	*Brodiaea capitata* Benth.	IPNI (2022)
2	*Maianthemum racemosum* (L.) Link	North Mexico	KSU	*Smilacina racemose* (L.) Desf. *Convallaria racemose* L.	IPNI (2022)
3	*Dipcadi erythraeum* Webb & Berthel.	El-Omayed, Coastal region	ALEX	-*Dipcadi unicolor* (Stocks) Baker -*Ornithogalum erythraeum* (Webb & Berthel.) J.C. Manning & Goldblatt -*Uropetalon erythraeum* (Webb & Berthel.) Boiss.	IPNI (2022)
4	*Drimia purpurascens* J. Jacq.	El-Arish, Sinai	CAI	*Urginea undulata* (Desf.) Steinh. *Drimia undulate* Stearn	IPNI (2022)
5	*Muscari comosum* (L.) Mill.	El-Omayed, Coastal region	ALEX	*Leopoldia comosa* (L.) Parl	IPNI (2022)
6	*Ornithogalum arabicum* L.	El-Omayed, Coastal region	ALEX	-*Caruelia arabica* (L.) Parl. -*Eustachys latifolia* (L.) Salisb. -*Melomphis arabica* (L.) Raf. -*Myanthe arabica* (L.) Salisb.	IPNI (2022)
7	*Scilla peruviana* L.	El-Omayed, Coastal region	ALEX	-*Melomphis peruviana* (L.) Raf. -*Scilla hemisphaerica* (L.) Boiss.	IPNI (2022)
8	*Asphodelus fistulosus* L.	North Sinai	ALEX	-*Asphodeloides ramosa* Moench -*Ophioprason fistulosum* (L.) Salisb. -*Verinea fistulosa* (L.) Pomel	IPNI (2022)
9	*Asphodelus ramosus* L.	El-Arish, Sinai	CAI	-*Asphodelus affinis* Parl. -*Asphodelus africanus* Jord. -*Asphodelus albus* Boiss.	IPNI (2022)
10	*Asphodelus tenuifolius* Cav.	Baheig Burg El Arab Road	ALEX	-*Ornithogalum flavum* Forssk. - *Verinea tenuifolia* (Cav.) Pomel	IPNI (2022)
11	*Colchicum cornigerum* (Schweinf. ex Sickenb.) Tackh. & Drar	Mersa-Matruh- El-Dabaa	ALEX	No synonyms	IPNI (2022)
12	*Colchicum ritchii* R.Br.	Baheig Burg El Arab Road	ALEX	-*Colchicum aegyptiacum* Boiss. -*Colchicum stenopetalum* Boiss. & Buhse ex Stef.	IPNI (2022)
13	*Calochortus amabilis* Purdy	California, USA.	KSU	*Calochortus pulchellus var. amabilis* (Purdy) Jeps.	IPNI (2022)
14	*Calochortus monophyllus* (Lindl.) Lem	California, USA.	KSU	-*Cyclobothra monophylla* Lindl. -*Calochortus pulchellus* **var**. *parviflorus* Regel	IPNI (2022)
15	*Calochortus pulchellus* (Doug. ex Benth.) Alph. Wood	California, USA.	KSU	-*Cyclobothra pulchella* Benth.	IPNI (2022)
16	*Calochortus uniflorus* Hook. & Arn.	California, USA.	KSU	-*Calochortus lilacinus* Kellogg -*Cyclobothra uniflora* (Hook. & Arn.) Kunth	IPNI (2022)
17	*Gagea dayana* Chodat & Beauverd.	Baheig Burg El Arab Road	ALEX	No synonyms	IPNI (2022)
18	*Gagea fibrosa* (Desf.) Schult. &Schult.f.	El-Omayed, Coastal region	ALEX	-*Ornithogalum fibrosum* Desf. - *Gagea articulata* subsp. *fibrosa* Schult. & Schult.f.) Maire & Weiller	IPNI (2022)
19	*Toxicoscordion fremontii* (Torr.) Rydb.	California, USA.	KSU	-*Anticlea fremontii* Torr. -*Toxicoscordion fremontii* var. minor (Baker) R.R. Gates -*Zigadenus fremontii* (Torr.) Torr. ex S. Wat. -*Zigadenus glaberrimus* Hook. & Arn.	IPNI (2022)
20	*Smilax aspera* L.	Siwa	ALEX	-*Smilax aspera* subsp. *mauritanica* (Poir.) Arcang. -*Smilax balearica* (Willk. ex A.DC.) Burnat & Barbey	IPNI (2022)

Leaf peels preparation for light microscope investigation (LMI)

Proper leaves have been chosen to make the peels from both the abaxial and adaxial surfaces, by warming in 3 drops of HCl in 5ml water until the peels separated. Then, the peels were stained with safranin, mounted in few drops of diluted glycerol, examined, and photographed using 40X10x magnification lens in OPTICA (B-150D) light microscope fitted with USB digital-Video Camera and Computer Software. Meanwhile, the adaxial leaf surfaces have been examined to recognize the presence of any differences between the two surfaces. Type of stomata, shape of epidermal cells and both trichome density and type were recorded after examining about 5 microscopic fields with diameter of 0.45 mm (magnification 40X10). Stomatal index (SI) and stomatal density (SD) were calculated as mentioned by Kadiri & Olowokudejo (2008) in five fields in each species:

%SI= SN/SN+EC X 100, %SD= SN/A X 100

where SN= number of stomata in unit area (A), EC= number of epidermal cells in this area. The terminology used here is that of Dilcher (1974) and Barthlott (1981).

Leaf epidermal surface for scanning electron microscope investigation (SEMI)

Central parts of dry leaves 5X5 mm^2 were cut using razor, stuck onto Aluminum stubs using double cello tape, then coated with 30nm Gold in a polaron JFC-1100E coating unit, and then examined and photographed under 15Kev, with JEOL JSM-IT200 SEM in the electron microscopes unit, Faculty of Science, Alexandria University, Egypt, for more detailed structures of both the stomata and hairs. Hair terminology followed Mannethody & Purayidathkandy (2018) and Shah et al. (2019).

Studied morphological traits

The following traits of leaf epidermis were investigated: (1) shape and thickness of pavement cells (LM), (2) shape and relief of anticlinal walls (SEM) (3) fine relief of the outer periclinal cell wall: micro-sculpture of surface and epicuticular secretions, (4) presence and density of papillae, prickles and hairs on the surface of the epidermis, and (5) stomata type, density, guard cell size and shape as well as stomatal index and density were calculated. Density of stomata was measured in ten fields for each specimen.

Data analyses

All the measured characters are subjected to SPSS program to calculate the standard deviation. Clustering analysis of the twenty characters studied in the different species under investigation (Table 5, 6) by the aid of PAST program v.3 (1999-2013).

Results

The results obtained of the abaxial leaf epidermis of the studied species exhibit several micromorphological structures (Tables 3, 4) as described below:

Pavement cells (Table 3)

Pavement cells or the outermost layer of the epidermis, are the basic unspecialized morphologically protected cells, with no protrusions or other functional abilities. They fulfill the basic role of protecting the tissue layers underneath, for that they are compact cells without intercellular spaces. As shown from light microscope examination, the pavement cells, appendages, epicuticular secretions and stomatal apparatus characters occurred consistently on both adaxial and abaxial leaf blade surfaces from the center section of each leaf of the studied taxa. That is, within a species, the shape of the epidermal cells was the same on the adaxial and abaxial side of the leaf. Thus, only characteristics of the adaxial leaf epidermal cells for the 20 species are summarized in Table 3 and Plates 1& 2. These cells are either elongated or isodiametric, with different outlines and thickness. The outlines of the pavement cells are rectangular, hexagonal, or irregular except in *Muscari comosum* which are longitudinally elongated in parallel position with the veins (Plate 1, photos. 2A, 5B, 6A, 9, 11, 16A, 19A). The thickness of the cell walls varied between the taxa between thin, moderate to even thick. The anticlinal walls are straight in fourteen taxa, while they are slightly wavy in *Drimia purpurascens*, wavy in *Maianthemum racemosum* (Plate 1, photos. 2A, 4) slightly undulate in *Colchicum ritchii, Calochortus monophyllus* and *Gagea fibrosa* (Plate 1, Photos. 12, 14, 18A) or even undulate in *Smilax aspera* (Plate 1, Photo. 20) The reliefs of the anticlinal cell walls are either elevated in nine taxa or sunken in eleven taxa (Plate 2).

The periclinal cell walls are mostly flat in eleven taxa, slightly convex in *Asphodelus fistulosus* and *A. ramosus* or convex in the rest seven species (Table 3). The surfaces of the periclinal walls are smooth in thirteen species, granulate in *Dipterostemon capitatus, Calochortus amabilis* and *C. monophyllus* (Plate 2, Photos. 1, 16, 17), and striate in *Maianthemum racemosum, Dipcadi erythraeum* and *Ornithogalum arabicum* (Plate 2, photos. 3, 4, 8) or papillate in *Asphodelus ramosus* (Plate 2, Photo. 12). Epicuticular secretions in the form of either granules or flakes are present in all the studied species with different densities, except *Dipterostemon capitatus, Maianthemum racemosum* and *Calochortus uniflorus* (Plate 2, Photos.1, 3, 20).

TABLE 3. Summary of the pavement cell characters as seen by both LM & SEM

	Cell shape	Cell wall		Anticlinal		Periclinal		Epicuticular secretions		
		Out.	Th.	Shape	Relief	Level	Sculp.	Presence	Density	Shape
Dipterostemon capitatus	El	Rec	Thn	St	Sn	Cov	Gr	--	--	--
Maianthemum racemosum	Iso	Irg	M	W	Elv	Flat	Str	--	--	--
Dipcadi erythraeum	Iso	Irg	Thn	St	Elv	Flat	Str	+	+	Gr
Drimia purpurascens	El	Rec	M	Slw	Elv	Flat	Sm	+	+++	Fl
Muscari comosum	El	El	Thc	St	Sn	Cov	Sm	+	+++	Gr
Ornithogalum arabicum	El	Rec	Thn	St	Sn	Cov	Str	+	+	Gr
Scilla peruviana	El	Hex	M	St	Elv	Flat	Sm	+	+	Gr
Asphodelus fistulosus	El	Hex	Thn	St	Sn	SCov	Sm	+	++	Gr
A. ramosus	El	Hex	Thc	St	Sn	SCov	Pap	+	+	Gr
A. tenuifolius	El	Hex	M	St	Elv	Flat	Sm	+	+	Gr
Colchicum cornigerum	El	Rec	Thc	St	Elv	Flat	Sm	+	++	Gr
C. ritchii	El	Rec	M	Slu	Sn	Cov	Sm	+	+	Gr
Calochortus amabilis	El	Rec	Thn	St	Elv	Flat	Gr	+	+	Gr
C. monophyllus	Iso	Irg	Thn	Slu	Elv	Flat	Gr	+	++	Gr
C. pulchellus	El	Rec	Thn	St	Elv	Flat	Sm	+	++	Fl
C. uniflorus	El	Rec	M	St	Sn	Flat	Sm	--	--	--
Gagea dayana	El	Rec	M	St	Sn	Cov	Sm	+	++	Gr
G. fibrosa	Iso	Hex	Thc	Slu	Sn	Flat	Sm	+	++	Gr
Toxicoscordion fremontii	**El**	**Hex**	**M**	**St**	**Sn**	**Cov**	**Sm**	**+**	**++**	**Gr**
Smilax aspera	**Iso**	**Irg**	**Thc**	**Un**	**Sn**	**Cov**	**Sm**	**+**	**+**	**Gr**

Abbreviations: Cov=Convex, El= Elongate, Elv=Elevated, Fl=Flakes, Gr=Granulate, Hex=Hexagonal, Th.=Thickness, Thc=Thick, Thn=Thin, Iso=Isodiametric, Irg= Irregular, M=Moderate, Out.=Outline, Pap=Papillate, Rec=Rectangular, SCov=Slightly convex, Sculp.=Sculpture, Slw=Slightly wavy, Sm=Smooth, Sn=Sunken, St=Straight, Str=Striate, Slu=Slightly undulate, Un=Undulate, W=Wavy.

TABLE 4. Summary of the epidermal characters

	Prickle hairs		Soft Hairs			Stomata				
	Pr.	Den.	Den.	Type	Position	Type	Shape of guard cells	G.C Size µm	Index %SI	Density %SD µm²
Dipterostemon capitatus	+	+++	Gl	--	--	Par	Dum	18.0-22.2 20.8±0.9	24.9-27.6 26.9±2.8	6.9-9.4 8.6±1.2
Maianthemum racemosum	--	--	VD	MMP	All over	Par	Kid	17.8 -20.4 19.1±1.2	19.1-25.0 21.1±3.5	4.8-6.2 5.5±0.8
Dipcadi erythraeum	+	+	Gl	--	--	Par	Dum	10.3-12.5 10.8±0.6	15.2-17.4 15.8±1.4	5.6-7.5 6.6±0.8
Drimia purpurascens	--	--	Gl	--	--	Par	Kid	20.2-22.2 21.4±0.9	20.8-30.3 25.0±3.9	3.1-5.2 4.2±1.4
Muscari comosum	+	+++	M	Papillae	Margin	Par	Kid	20.0-25.4 23.2±1.4	19.8-25.3 22.0±3.6	3.8-5.6 4.8±0.9
Ornithogalum arabicum	--	--	M	Papillae	Margin	Anom	Kid	17.5-22.0 19.4±1.9	28.0-31.4 29.4±1.5	8.1-10.0 9.2±0.8
Scilla peruviana	--	--	SpH	UUP	Margin	Par	Kid	22.4-25.8 23.4±1.6	25.2-29.8 26.4±2.9	8.1-10.1 9.4±1.2
Asphodelus fistulosus	--	--	D	Papillae	All over	Par	Kid	17.8-20.4 19.2±2.1	25.0-33.3 29.9±4.3	3.8-7.5 5.8±1.9
A. ramosus	--	--	Gl	--	--	Par	Kid	17.5-20.8 18.2±1.8	13.3-15.0 14.2±0.8	6.2-8.1 7.2±0.8
A. tenuifolius	--	--	D	MMP	Veins	Par	Kid	10.5-12.5 11.2±0.9	17.5-23.8 20.9±2.7	4.4-6.2 5.5±0.8
Colchicum cornigerum	--	--	Gl	--	--	Par	Kid	22.2-24.5 23.2±0.9	26.4-31.2 29.5±2.1	9.8-13.1 11.1±1.8

TABLE 4. Cont.

	Prickle hairs		Soft Hairs			Stomata				
	Pr.	Den.	Den.	Type	Position	Type	Shape of guard cells	G.C Size µm	Index %SI	Density %SD µm^2
C. ritchii	--	--	Gl	--	--	Par	Kid	25.0-27.2 25.8±1.4	40.3-40.4 40.3±0.02	14.4-15.4 15.0±0.9
Calochortus amabilis	+	++	Gl	--	--	Par	Kid	25.4-27.2 26.2±1.2	15.3-25.0 19.7±4.0	1.2-2.5 1.9±0.4
C. monophyllus	+	+++	Gl	--	--	Anom	Kid	25.4-27.5 26.4±1.8	25.4-33.3 29.5±3.8	5.6-9.4 7.5±1.8
C. pulchellus	+	+++	Gl	--	--	Par	Kid	13.5-17.8 14.6±1.2	4.8-9.5 7.5±2.5	0.6-1.2 1.0±0.4
C. uniflorus	+	+++	Gl	--	--	Anom	Dum	24.6-28.2 26.2±1.8	40.0-43.7 41.4±2.0	10.8-13.1 11.7±1.3
Gagea dayana	+	+++	SpH	MMP	Margin	Par	kid	23.5-27.2 25.2±2.1	34.3-40.9 37.7±2.7	7.5-10.2 9.0±1.8
G. fibrosa	+	++	Gl	--	--	Par	Dum	24.2-27.8 26.2±1.4	16.7-20.1 18.2±1.5	6.2-9.4 7.8±1.5
Toxicoscordion fremontii	--	--	D	Papillae	Margin	Anom	Kid	22.4-27.2 23.8±1.8	8.0-13.8 9.1±3.9	0.6-2.5 1.5±0.7
Smilax aspera	--	--	Gl	--	--	Par	Kid	22.8-25.2 24.2 ±1.6	10.7-13.0 11.8±1.1	7.0-10.5 9.2±1.2

Abbreviations: Anom=Anomocytic, D=Dense, Den=Density, Dum=Dumbbell-shaped, El=Elongate, G.C= Guard cell, Kid=Kidney-shaped, M=Median, MMP=Multicellular Multiseriate Pointed, Par=Paracytic, Rec=Rectangular, SD=Stomatal density, SI=Stomatal index, SpH=Sparsely hairy, Th.=Thickness, Thc=Thick, Thn=Thin, UUP=Unicellular Uniseriate Pointed, VD=Very dense. Yellow color= small G.C., Blue color= low %SI & %SD, Pink color= Medium %SI & %SD

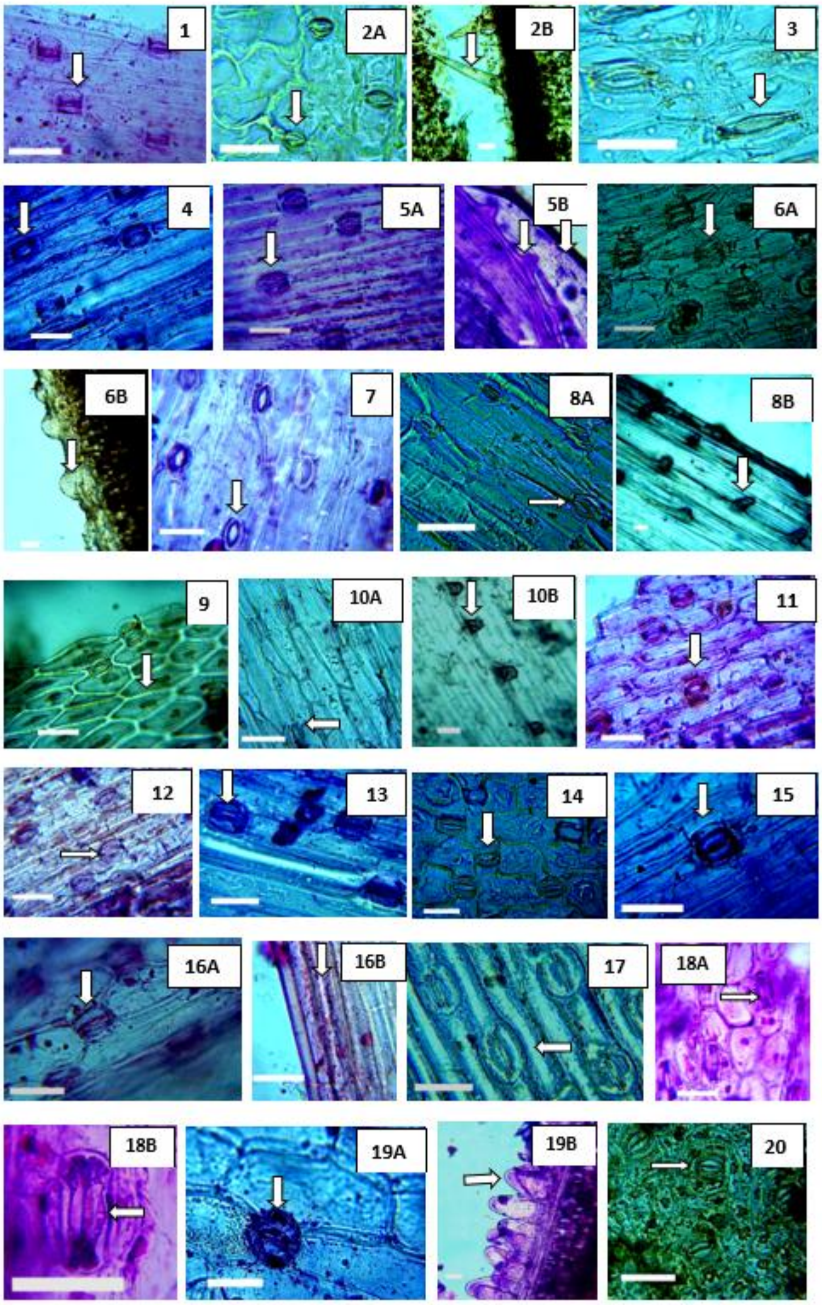

Plate 1. Leaf surfaces of some monocot taxa under light microscope (Scale bar = 50 µm). 1- *Dipterostemon capitatus*, stomata and elongated epidermal cells; 2-*Maianthemum racemosum*, 2A. Stomata and wavy anticlinal wall & 2B. Soft multiseriate multicellular pointed hairs; 3- *Dipcadi erythraeum*, stomata and isodiametric epidermal cells; 4- *Drimia purpurascens*, stomata and elongated epidermal cells; 5- *Muscari comosum*, 5A. Stomata and elongated epidermal cells & 5B. Marginal papillae & elongated pavement cells; 6- *Ornithogalum arabicum,* 6A. Stomata and elongated epidermal cells & 6B. Marginal papillae; 7- *Scilla peruviana*, stomata and elongated epidermal cells; 8- *Asphodelus fistulosus*, 8A. Stomata and elongated epidermal cells & 8B. Surface papillae; 9- *A. ramosus*, hexagonal straight glabrous walls; 10- *A. tenuifolius*, 10A. Stomata and elongated epidermal cells & 10B. Veins papillae; 11- *Colchicum cornigerum*, stomata and elongated epidermal cells; 12- *C. ritchii*, stomata and elongated epidermal cells; 13- *Calochortus amabilis*, stomata and elongated epidermal cells; 14- *C. monophyllus*, isodiametric irregular epidermal cells; 15- *C. pulchellus,* stomata and elongated epidermal cells; 16- *C. uniflorus*, 16A. Stomata and elongated epidermal cells & 16B. Dense prickles; 17- *Gagea dayana*, stomata and elongated epidermal cells; 18- *G. fibrosa,* 18A. Stomata and isodiametric epidermal cells & 18B. Dumbbell-shaped guard cells; 19- *Toxicoscordion fremontii*, 19A. Stomata and elongated epidermal cells & 19B. Marginal papillae; 20- *Smilax aspera*, stomata and isodiametric epidermal cells

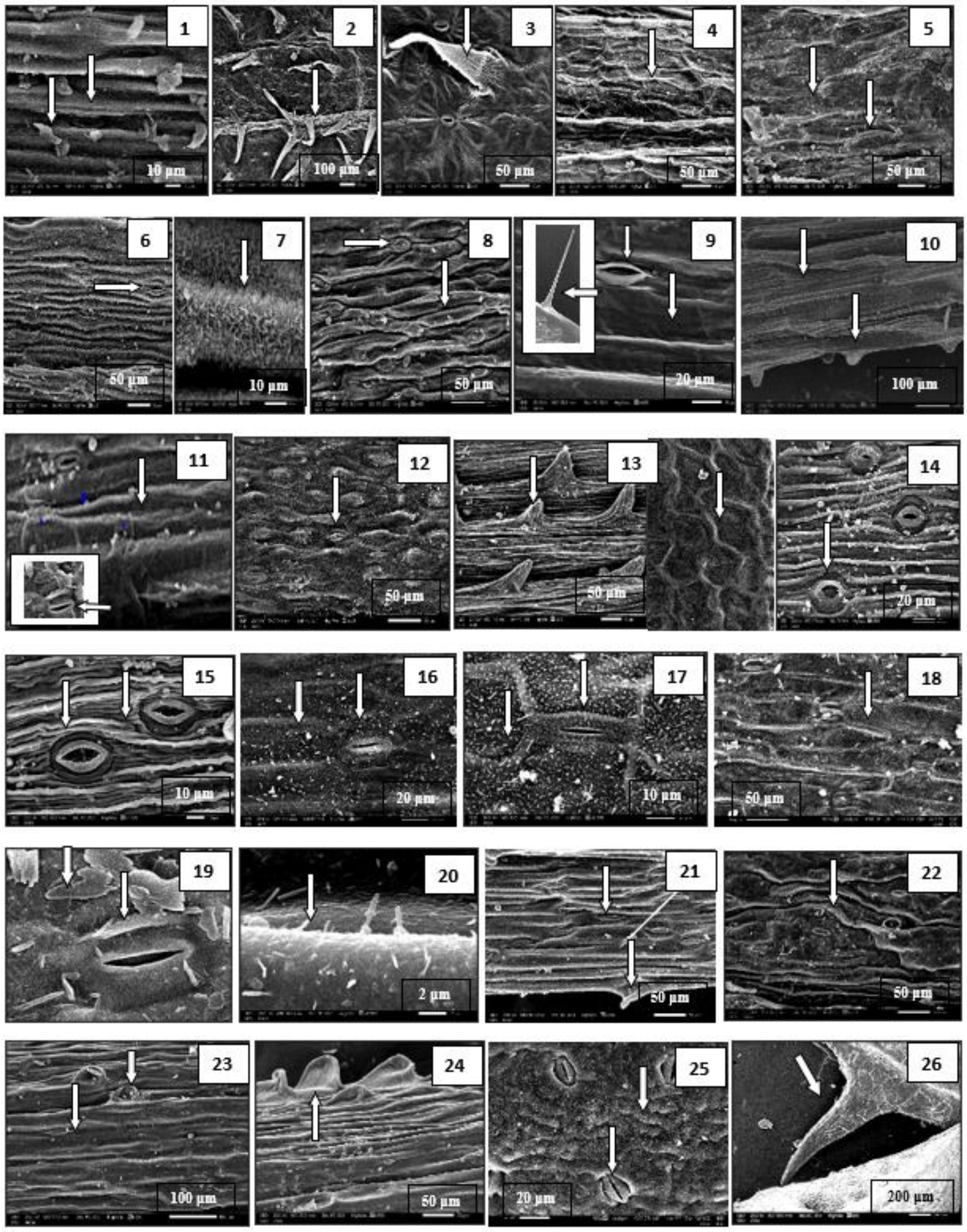

Plate 2. Leaf surfaces of some monocot taxa under SEM 1- *Dipterostemon capitatus*, sunken anticlinal wall and convex periclinal walls, broken prickles; 2, 3- *Maianthemum racemosum*, soft multicellular hairs on the leaf surface; 4- *Dipcadi erythraeum,* elevated anticlinal wall and straight periclinal wall; 5- *Drimia purpurascens*, elevated anticlinal wall, straight periclinal wall and epicuticular flakes secretions; 6, 7- *Muscari comosum*, sunken anticlinal wall, convex periclinal wall and very dense prickles; 8- *Ornithogalum arabicum*, sunken anticlinal wall, convex periclinal wall and granulated epicuticular secretions; 9- *Scilla peruviana*, elevated anticlinal wall, straight periclinal wall and unicellular pointed hairs; 10, 11- *Asphodelus fistulosus*, 10- Papillae, 11- Sunken anticlinal wall and slightly convex periclinal wall, prickles and granulated epicuticular secretions; 12- *A. ramosus*, sunken anticlinal wall, slightly convex periclinal wall and granulated epicuticular secretions; 13- *A. tenuifolius*, multicellular hairs, elevated anticlinal wall, flat periclinal wall and granulated epicuticular secretions; 14- *Colchicum cornigerum*, elevated anticlinal wall, flat periclinal wall and granulated epicuticular secretions; 15- *C. ritchii*, sunken anticlinal wall, convex periclinal wall and granulated epicuticular secretions; 16- *Calochortus amabilis*, elevated anticlinal wall, flat periclinal wall, prickles and granular epicuticular secretions; 17- *C. monophyllus*, elevated anticlinal wall, flat periclinal wall, prickles and granular epicuticular secrertions; 18, 19- *C. pulchellus,* 18- Elevated anticlinal wall, flat periclinal wall, 19- Flakes epicuticular secretions; 20- *C. uniflorus*, anticlinal wall, convex periclinal wall and granulated epicuticular secretions; 21- *Gagea dayana,* anticlinal wall, convex periclinal wall, marginal multicellular hairs and granulated epicuticular secretions; 22- *G. fibrosa*, anticlinal wall, flat periclinal wall and granulated epicuticular secretions; 23, 24- *Toxicoscordion fremontii*, 23- Sunken anticlinal wall, convex periclinal wall and granulated epicuticular secretions, 24- Marginal papillae; 25, 26- *Smilax aspera*, 25- Sunken anticlinal wall, convex periclinal wall and granulated epicuticular secretions, 26- Multicellular pointed hairs

Prickles, soft hairs, and papillae (Table 4)

Prickle hairs

Prickles are coarse hairs that extend from the cortex and epidermis, they were recorded in some of the studied taxa with different densities. They are sparse or dense, which are distributed throughout the costal and intercostal regions on the leaf blades. They are short or moderate, stiff hairs with sharply pointed apices, as in the studied species of family Liliaceae. These prickles are recorded in *Dipterostemon capitatus, Dipcadi erythraeum* and *Muscari comosum* from family Asparagaceae, also all the studied taxa belonging to family Liliaceae (Plate 2, Photos.1, 4, 7, 16, 17, 19, 20). Their lengths and density varied within the recorded species. These prickles are absent in the rest of the studied species from the rest of the studied families.

Soft hairs

The soft hairs are recorded in different species by different densities and positions. In both *Maianthemum racemosum* and *Asphodelus tenuifolius* multicellular multiseriate pointed hairs covered the lamina very densely in the former and dense at the sides of the veins in the latter (Plate 1, Photo 2B, Plate 2, Photos 2, 3, 13). Sparse hairs are recorded at the margins of the leaves; it was unicellular in *Scilla peruviana* and multicellular multiseriate in *Gagea dayana* (Plate 2, Photos 9, 21). The rest of the studied taxa are glabrous or enriched by papillae appendages.

Papillae

Dense papillae were found at the margins of the leaf laminas in both *Muscari comosum* and *Ornithogalum arabicum* (Plate 1, photos 5B, 6B), while they were distributed all over the lamina in *Asphodelus fustulosus* (Plate 1, photo 8B).

Stomatal complex

The stomatal complex of the twenty studied species has been examined carefully with a light microscope. All the studied species were found to be hypostamic (i.e., stomata occurring on the abaxial surface only). The stomata were of the paracytic or tetra-anomocytic types. The tetra-anomocytic stomata possess four subsidiary cells as in *Ornithogalum arabicum, Calochortus monophyllus*, *C. uniflorus, Toxicoscordion fremontii* (Plate 1, Photos 6A, 14, 16A, 19A). The guard cells are either kidney-shaped in most of the studied taxa or dumbbell-shaped in *Dipterostemon capitatus, Dipcadi erythraeum* (Asparagaceae)*, Calochortus uniflorus* and *Gagea fibrosa* (Liliaceae) (Plate 1, Photos 1, 3, 16A, 18A). The sizes of guard cells are over 15 µm in most of the studied taxa, except in *Dipcadi erythraeum*, *Asphodelus tenuifolius* and *Calochortus pulchellus* they are less than 15µm (Plate 1, Photos. 3A, 10A, 15). The opening of the stomata is usually moderate lenticular in all the studied taxa, except in *Calochortus monophyllus*, *C. pulchellus*, *Gagea dayana, Gagea fibrosa, Toxicoscordion fremontii* and *Smilax aspera* the stomatal openings are small slits (Plate 2, Photos. 17, 19, 21, 22, 23, 24, 25). The percentage of the stomatal index lies under three categories, the first group with few stomata with index less than 15% in four taxa only (*Asphodelus ramosus, Calochortus pulchellus*, *Toxicoscordion fremontii* and *Smilax aspera*). The second group with moderate percentage of stomata between 15 to 25% in six taxa (*Maianthemum racemosum*, *Dipcadi erythraeum*, *Muscari comosum Asphodelus tenuifolius, Calochortus amabilis* and *Gagea fibrosa*). The third group has the rest of taxa with dense stomatal index exceed 25%. These groups did not coincide with the three groups of the stomatal density which was very few less than 2.5 % in *Calochortus amabilis*, *Calochortus pulchellus* and *Toxicoscordion fremontii*, from 2.5 to nearly 10% in most of the studied taxa and over than 10% in *Colchicum cornigerum, C. richii* and *C. uniflorus*.

Data analyses

The data analyses were according to the observable results as they give obvious variation between the studied taxa. The twenty characters listed in Tables 5 & 6 are subjected to clustering, principal component and coordinate component analyses as illustrated in Figs. 1, 2 and 3. The clustering dendrogram divided the studied taxa into two main categories at similarity distance of 6.5. The first group (I) includes 12 taxa, *Asphodelus ramosus* (Asphodelaceae) separates in a phenetic line at similarity distance 5.75, while the rest of the taxa are divided into two subgroups (A & B) at similarity distance 5.5. Subgroup (IA) has seven taxa divided into three categories: the first one at similarity distance 5.0 includes both *Calochortus uniflorus* and *Dipterostemon capitatus.* However, the two genera *Drimia purpurascens* and *Colchicum cornigerum* separated from the three genera *Dipcadi erythraeum, Calochortus amabilis* and *C. pulchellus* at similarity distance 4.25 (Fig.1). Subgroup (IB) has four taxa in two groups. *Colchicum ritchii* and *Smilax aspera* are separated from *Calochortus monophyllus* and *Gagea fibrosa* at similarity distance 5.25.

TABLE 5 . Characters, types and states employed in numerical analyses

No	Character	Type	States
1	Ep. C. Shape	Binary	1-Elongate 2-Isodiametric
2	Ep. C. Wall Outline	M.Ql.Unord.	1-Rectanguler 2-Irregular 3-Elongated 4-Hexagonal
3	Ep. C. Wall Thickness	M.Ql.Ord.	1-Thin 2-Moderate 3-Thick
4	Ant. Wall Shape	M.Ql.Ord.	1-Straight 2-Slightly wavy 3-Wavy 4-Slightly undulate 5-Undulate
5	Ant. Wall Relief	Binary	1-Sunken 2-Elevated
6	Per. Wall level	M.Ql.Ord.	1-Flat 2-Slightly convex 3-Convex
7	Per. Wall Sculpture	M.Ql.Unord.	1-Smooth 2-Striate 3-Granulate 4-Papillate
8	Epi. Sec. Presence	Binary	1-Abscent 2-Present
9	Epi. Sec. Density	M.Ql.Ord.	1-Abscent 2-Sparse 3-Dense 4-Very dense
10	Epi. Sec. Shape	M.Ql.Unord.	1-Abscent 2-Granulate 3-Flakes
11	Pri. Presence	Binary	1-Abscent 2-Present
12	Pri. Density	M.Ql.Ord.	1-Abscent 2-Sparse 3-Dense 4-Very dense
13	Soft H. Density	M.Ql.Ord.	1-Abscent 2-Sparse 3-Moderate 4-Dense 5-Very dense
14	Soft H. Type	M.Ql.Unord.	1-Absent 2-Papillate 3-Unicellular pointed 4-Multicellular multiseriate pointed
15	Soft H. Position	M.Ql.Unord.	1-Abscent 2-All-over 3-Margin 4-Veins
16	Stomata Type	Binary	1-Paracytic 2-Anomocytic
17	G. C. Shape	Binary	1-Kidney shaped 2-Dumbbell shaped
18	G. C. size	Continuous	1-(10-12.5 µm) 2-(13-17.5 µm) 3-(17.5-22.2µm) 4-Over 22.2 µm
19	Stomatal Index	Continuous	1-> 15 2-(15-20) 3-(20-35) 4-≤ 35
20	Stomatal Density	Continuous	1-(1-5) 2-(5-10) 3-(10-15)

Abbreviations: Ant. =Anticlinal, Ep. C.=Epidermal cell, Epi. Sec.=Epicuticular secretions, G. C.= Guard cell, H.= Hair, M.Ql.Ord.=Multistate qualitative ordered, M.Ql.UnOrd.=Multistate qualitative unordered, Peri =Periclinal, Pri.=Prickle.

TABLE 6 . Character states for numerical analysis

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
Dipterostemon capitatus	1	1	1	1	1	3	3	1	1	1	2	4	1	1	1	1	2	3	3	2
Maianthemum racemosum	2	1	2	3	2	1	2	1	1	1	1	1	5	4	2	1	1	3	3	2
Dipcadi erythraeum	2	2	1	1	2	1	2	2	2	2	2	2	1	1	1	1	2	1	2	2
Drimia purpurascens	1	1	2	2	2	1	1	2	4	3	1	1	1	1	1	1	1	3	3	1
Muscari comosum	1	3	3	1	1	3	1	2	4	2	2	4	3	2	3	1	1	3	3	1
Ornithogalum arabicum	1	1	1	1	1	3	2	2	2	2	1	1	3	2	3	2	1	3	3	2
Scilla peruviana	1	4	2	1	2	1	1	2	2	2	1	1	2	3	3	1	1	3	3	2
Asphodelus fistulosus	1	4	1	1	1	2	1	2	3	2	1	1	4	2	2	1	1	3	3	1
A. ramosus	1	4	3	1	1	2	4	2	2	2	1	1	1	1	1	1	1	3	1	2
A. tenuifolius	1	4	2	1	2	1	1	2	2	2	1	1	4	4	4	1	1	1	2	2
Colchicum cornigerum	1	1	1	1	2	1	1	2	3	2	1	1	1	1	1	1	1	4	3	3
C. ritchii	1	1	2	4	1	3	1	2	2	2	1	1	1	1	1	1	1	4	4	2
Calochortus amabilis	1	1	1	1	2	1	3	2	2	2	2	3	1	1	1	1	1	4	2	1
C. monophyllus	2	2	1	4	2	1	3	2	3	2	2	4	1	1	1	2	1	4	3	2
C. pulchellus	1	1	1	1	2	1	1	2	3	3	2	4	1	1	1	1	1	2	1	1
C. uniflorus	1	1	2	1	1	1	1	1	1	1	2	4	1	1	1	1	2	4	4	3
Gagea dayana	1	1	2	1	1	3	1	2	3	2	2	4	2	4	3	2	1	4	4	2
G. fibrosa	2	4	3	4	2	1	1	2	3	2	2	4	1	1	1	1	2	4	2	2
Toxicoscordion fremontii	1	4	2	1	1	3	1	2	3	2	1	1	4	2	3	2	1	4	1	1
Smilax aspera	2	2	3	5	1	3	1	2	2	2	1	1	1	1	1	1	1	4	1	3

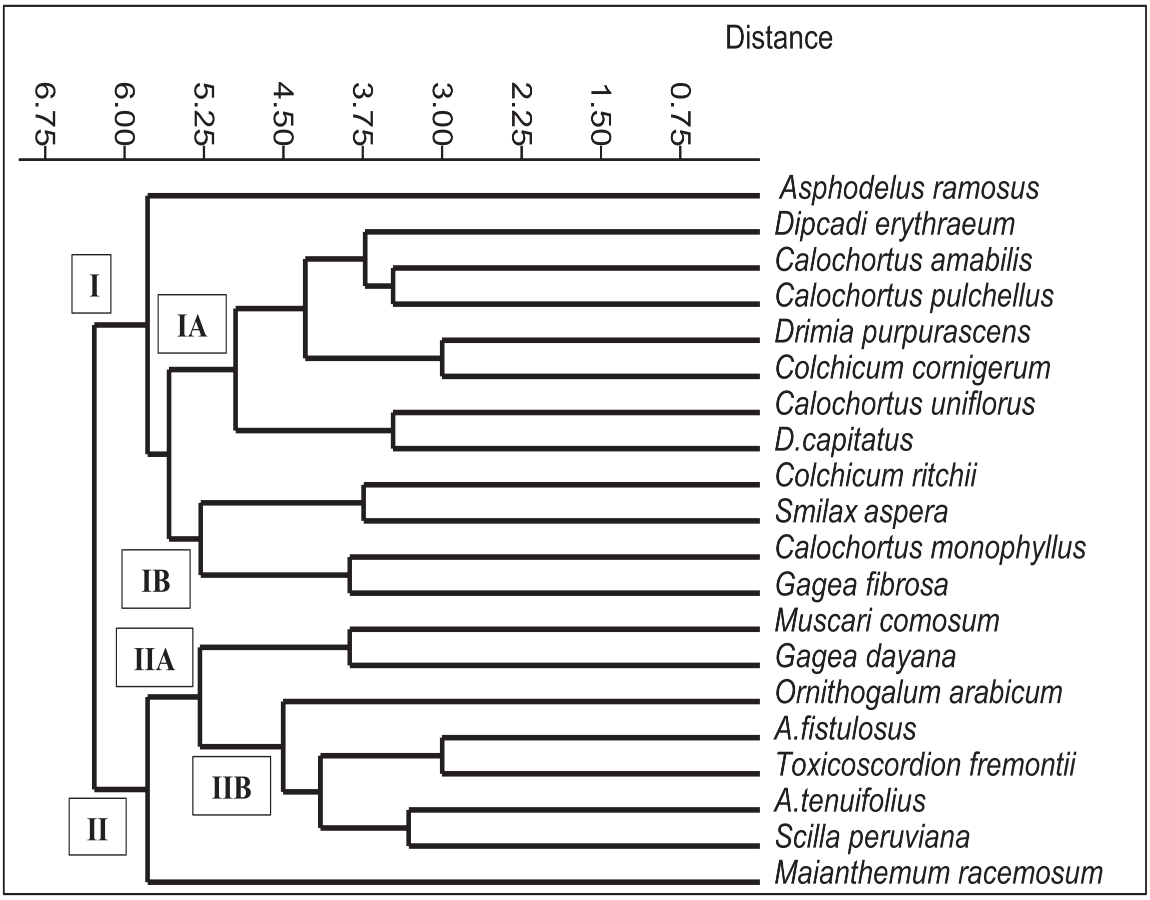

Fig. 1. Clustering analysis of the 20 studied taxa according to their similarity distance

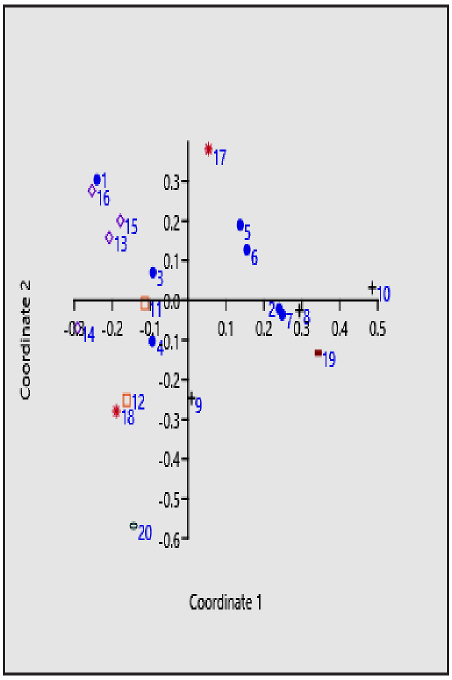

Fig. 2. Principal coordinates analysis

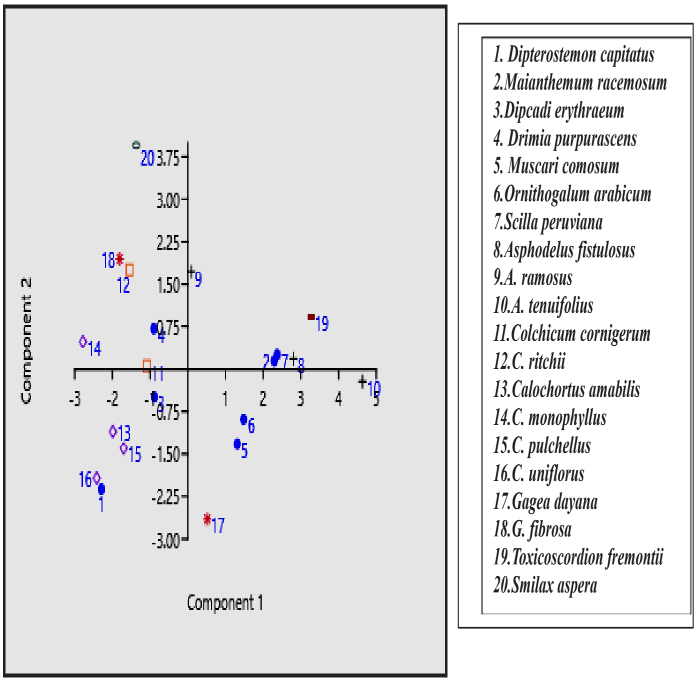

Fig. 3. Principal components analysis

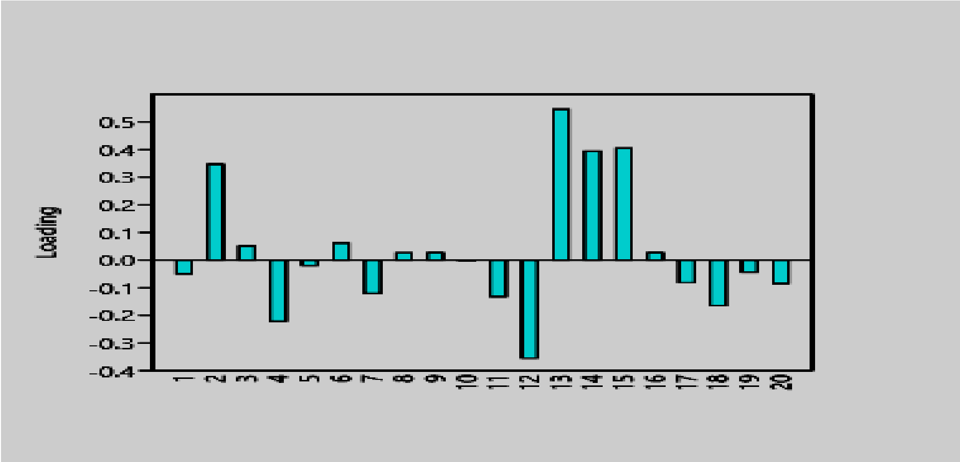

Fig. 4. The weight of the studied characters (1-20, Table 5) on the distribution of the taxa

Group (II) includes eight taxa, *Maianthemum racemosum* separates in a phenetic line at similarity distance 5.75, while the rest taxa are divided into two subgroups (A & B). Subgroup (IIA) has two taxa only: *Muscari comosum* and *Gagea dayana* which separate at similarity distance 5.25. Subgroup (IIB) includes five taxa, *Ornithogalum arabicum* separates in a phenetic line at similarity distance 4.5, while the rest four taxa divided into two categories, *Asphodelus fistulosus* and *Toxicoscordion fremontii* in one group at similarity distance 3.0 while *Asphodelus tenuifolius* and *Scilla peruviana* at similarity distance 3.25 (Fig. 1).

The principal coordinate and component analyses (Figs. 2, 3) did not give clear separations of the taxa according to families and even orders. All the taxa distributed randomly at all the four axes. Fig. 4 clarifies the weight of the studied characters on the distribution of the taxa. The most affected characters in the grouping of the studied taxa (Table 5) are the outline of the epidermal cell wall (2) and characters relating to the soft hairs (13, 14, 15). Meanwhile the negatively related characters are shape of the anticlinal wall (4), presence of the epicuticular secretions (7), presence and density of prickles (11, 12) and characters of guard cells and stomata (17, 18, 19, 20).

Discussion

Many of taxonomical systems for classifying the angiosperms is available with many opinions in grouping plants into orders and families. Early systems relied mostly on morphological evidence to show relationships of the angiosperms, including the monocots. Two widely used works are those done by Cronquist's (1981), which divided the monocots (Class Liliopsida) into 19 orders and 65 families, and Takhtajan's (1997), which recognized 58 orders and 133 families within the monocots. Most of these taxonomic works used the leaf characters beside other morphological characters in grouping the taxa. Spaulding et al. (2019) found that many monocot taxa have common characters with the dicots, this opinion made more arguments about the relation between both monocots and dicots. In fact, the taxonomy of the monocots had undergone different opinions concerning the grouping of the taxa. Bentham & Hooker (1883) as well as Engler (1888) gathered all the trimerous, hypogenous flowers under family Liliaceae with many tribes and subtribes. Cronquist (1981) upgrades the ranking of the family into order Liliales with fifteen families while Dhalgren et al. (1985) reclassified the taxa to be under twenty-seven families representing five orders namely Discoreales, Asparagales, Melanthiales, Burmaniales and Liliales. The two orders, Asparagales and Liliales are intermingled and despite the sixteen micro-morphological characters listed by Dahlgren et al. (1985) to distinguish between the taxa belonging to each order.

The results obtained revealed that there are substantial variations in the type and shape of pavement cells. The cell shape under the light microscope examination appeared as elongated or isodiametric cells, with different thickness and outlines from irregular, rectangular to even the hexagonal. Substantial variations within the anticlinal walls observed under the SEM investigations within the studied taxa. The anticlinal wall shape appears straight, slightly wavy or undulates to even strongly wavy or undulate with either sunken or elevated positions. According to the level of the periclinal walls which are either flat or convex with smooth or striate surfaces or ornamented by granules or even papillae in *Asphodelus ramosus* only, with wax depositions in different densities. These variations within the species are not correlated within the studied families but can be of use in delimiting the related species. Among the most important characters in this study are the prickle density, soft hairs and papillae features which can be used in taxa discrimination. The prickles are recorded in all the studied species in family Liliaceae and three species from family Asparagaceae; *Dipterostemon capitatus, Muscari comosum* and *Dipcadi erythraeum*. It is worth noticing that species whose leaves covered with dense or moderate prickles are glabrous except *Muscari comosum* which has marginal papillae. *Gagea dayana* leaves, from the Liliaceae, ornamented by few multicellular multiseriate pointed hairs on the leaf margins. This result indicates that both the Asparagaceae and Liliaceae share common leaf morphological characters as mentioned by Dahlgren et al. (1985). Prychid et al. (2004) found that there are different forms and densities of silica in some monocots such as the Orchids and Commelinids, not in other Lilloids or basal monocots, which can be used in the phylogeny of the families. This opinion is in accordance with this study as the presence of prickles and their densities can be of use in the discrimination of many of the studied species.

The stomata are found in the lower surface only (hypostyomatic) as recorded previously by Zomlefer (1999), and Pradham & Bajrachyara (2016), stomata mainly paracytic with few tetra-anomocytic species in *Ornithogalum arabicum* (Asparagaceae), *Calochortus monophyllus, C. uniflorus* (Liliaceae) and *Toxicoscordion fremontii* (Melanthiaceae), with kidney-shaped guard cells in most of the species. The clustering analyses of the data obtained did not give clear separation between the families of the Asparagales and Liliales and considered both orders as close groups. This observation has been given by Chang-Gee & Pfosser (2002), as they found that members of the Liliales are closely related to the Asparagales according to *rbc* L analyses. The results obtained from this study do not give clear separations between the two orders or even the families and considered the studied families having common leaf characters. Meanwhile characters of epidermal cells, hairs, papillae, prickles, and stomata can give clear features in the identification of some species. Hence, we can conclude that the separation of the orders and families of the monocot families and their taxonomic ranking needs more investigation to clarify the perfect relation between them. So, we recommend in the future, more studies on the monocot plants on a large scale involving the use of Transmission Electron Microscope to observe more details that may show some differences. Recent studies should also involve other vegetative and floral parts of the taxa.

Competing interests: The authors report no conflicts of interest regarding this work.

Authors' contributions: Prof. Wafaa K. Taia supervised the practical work and wrote the research. Prof. Azza A. Shehata gathered the specimens and reviewed the writing. Dr. Eman M. Bassiouni did the practical work, did the analyses, and participated in writing.

Ethics approval: Not applicable.

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التحقيق المجهري الشكل في البشرة الورقية لبعض الأصناف أحادية الفلقة

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انه في ذلك العمل تم فحص عشرون نوع ينتمي إلي ثلاثة عشر جنس ضمن ست عائلات؛ اثنين منهم ينتموا إلي Asparagales، وأربعه ينتموا إلي Liliales. لقد تم دراسة الصفات المورفولوجية الدقيقة للورقة باستخدام كلا من الميكروسكوب الضوئي والالكتروني الماسح. لقد تم دراسة كلا من خلايا الرصف pavement cells، الشعيرات، الاشواك وصفات الثغور بجانب حساب معامل وكثافة الثغور. البيانات التم الحصول عليها قد تعرضت الي تحليلات عنقودية باستخدام برنامج PAST. ان النتائج التي تم التوصل لها من هذه الدراسة لم تعط فصل واضح ما بين الرتبتين وحتى العائلات، وقد اعتبرت العائلات اللاتي تم دراستهم لديهم صفات مشتركه للورقة. في غضون ذلك، صفات خلايا البشرة، الشعيرات، الاشواك والثغور ممكن تساعد في تعريف بعض الأنواع. أيضا يمكن ان نستنتج ان فصل رتب وعائلات النباتات ذوات الفلقة الواحدة ووضعها التصنيفي يحتاج إلى دراسات أكثر.