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Original Research

Foliar LM and SEM Insights: Investigating Epidermal Features and Its Taxonomic Implications in Some Verbenaceae Taxa

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Abstract

The epidermal characteristics of the foliar of five species of the Verbenaceae family were examined using light and scanning electron microscopy. The use of the SEM method to investigate the foliar epidermal features of Verbenaceae family has not yet been reported in Pakistan. Therefore, the current study aims to investigate the anatomical characters for correct identification and taxonomic significance of the species within the family Verbenaceae. The result revealed that the plants exhibited variations in leaf epidermal features, including leaf cell size, stomata size, subsidiary cell size, and stomatal index. The studied species, with the exception of *Duranta repens,* are amphiostomatic i.e. they have stomata on both leaf surfaces. Normally, the stomata are anisocytic and animocytic and were observed on both leaf surfaces in most species. In *Vitex negundo*, the stomata were not visible as there were many tomentosa

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and glandular hairs. The shape of the epidermal cells was quite different on both leaf surfaces, with thick and straight epidermal walls in most species, except in *Phyla nodiflora* (L.) Greene, which has smooth epidermal walls. Glandular hairs were observed in *Lantana indica* Roxb*, Vitex negundo* L.*,* and *Verbena officinalis* L. The trichomes in *V. negundo* are segmented and branched. The largest length (30-33 µm) of stomata was found on the abaxial surface of *P. nodiflora,* while the smallest length (6-12.5 µm) of stomata was observed in *Lantana indica* Roxb. The size of epidermal cells varied in both length and width on both surfaces of the leaves. The mean length of epidermal cells was higher on the upper epidermal cell than on the lower surface. The length of the epidermis on the abaxial leaf surface was greatest in Verbenaceae members (42-47.5 µm) in *V. officinalis* and not in the other species. These anatomical features were given special consideration in the characterization and identification of Verbenaceae taxa.

Keywords: foliar assessment, morpho-anatomy, Pakistan, scanning electron microscopy, Verbenaceae

Introduction

The Verbenaceae family comprises a wide range of flowering plants with considerable significant morphological differences that challenge established taxonomic classifications. In recent years, researchers have focused on the morphological aspects of leaves, especially the characteristics of the leaf epidermis, to better understand the systematic relationships and ecological adaptations of the different Verbenaceae species [1]. Verbenaceae, commonly known as Verbena or Vervain family, is the largest group of angiosperms distributed in a wide range of habitats. It belongs to the order Lamiales and comprises mainly tropical flowering plants [2, 3]. It was established in 1805 by J. St. Hil. The family is mainly distributed in tropical and subtropical regions and comprises 34 genera and 1,200 species [4, 5]. About 17 genera and 35 species have been recorded in Pakistan [6]. However, the geographical distribution of species and phylogenetic studies show that the Verbenaceae comprises 32 genera and 800 species [7, 8]. Recently, a taxonomic and phylogenetic study of specific groups within the Verbenaceae proposed that the subfamily Verbenoideae comprises eight tribes [2, 9-11]. Molecular and phylogenetic studies on the Verbenaceae have resolved many problems concerning the major lineages and genetic relationships in most parts of this group [8]. The species of the Verbenaceae family are mostly found in tropical and subtropical regions, with a few species in temperate regions, commonly distributed in Africa, Europe, Southeastern Asia, South America, and Pakistan [12]. The species of the family are well represented in arid and semi-arid regions [13], but only a few species have been found in moist to dry tropical forests, cloud forests, and grasslands of the high Andean [14]. Members of the Verbenaceae are closely related to the family Lamiaceae (Labaiateae), which is characterized by the presence of a bilabiate corolla and a persistent calyx [15]. The Verbenaceae are also related to the Bignoniaceae and Martyniaceae due to the nature of the calyx, inflorescence, and fruit [16]. In addition to teak, which is very highly valuable because of its wood, Verbenaceae also includes many medicinal and ornamental plants [17].

The distribution of stomata is an important characteristic of the foliar epidermis [18, 19]. Differences in stomatal density and arrangement provide useful information for the identification of closely related species and the understanding of their physiological adaptations to different environmental situations [20]. Trichomes, hair-like structures on the leaf surface, are another important component of the foliar epidermis [21]. Trichome density, form, and the presence of glandular structures have been shown to be effective in distinguishing species and can be used as reliable taxonomic identifiers [22, 23].

The study of anatomical characteristics is a useful tool for the identification and systematic modification of taxa [24, 25]. It enables the correct identification of herbal medicines [26]. Morphological and anatomical characteristics of a leaf are used as markers to assist in the correct recognition of the plant species. The role of anatomical data of leaves has long been recognized in traditional taxonomy, as anatomical features have been used to reflect the variations within species, genera, or a family. Leaf anatomical features such as trichomes, types of stomata, and other features are useful anatomical tools [27, 28]. Although many studies have been conducted on the wood anatomy and gross morphology of plants, they have proven to be valuable for plant identification and differentiation because identification criteria would be incomplete without considering the epidermal morphological features of leaves [29, 30]. Scientists particularly focused on the anatomical features to identify small scraps of plant material. The epidermal cells usually show variations in their configuration. The epidermal features of leaves, including cuticular characters, trichomes, epidermis, subsidiary cells, and stomatal types, have been proven to be systematically valuable [31]. Most anatomical studies of the Verbenaceae genera such as *Lippia* [32-34], *Aloysia* [35], *Acantholippia* [36], *Glandularia* [37], and *Junellia* [38]. The anatomical revisions of Verbenaceae

were comprehensively performed by [39]. A study on the ontogeny of stomata and leaf epidermal was described by [40]. The most frequently found stomata patterns are paracytic, anomcytic, and diacytic. The trichomes were found to be uniseriate glandular capitate, multicellular glandular capitate, and non-glandular uniseriate filiform [41]. The most common stomata types are diacytic as found in *Gmelina arborea* Roxb. and occasionally anomocytic in *Duranta erecta* L. [42]. In Pakistan, the epidermal anatomy of the Verbenaceae family leaves has been least studied. The current study aims to investigate the distinguishing features of the leaf epidermis to prove its major role in establishing the taxonomic position of five species of the Verbenaceae family. Several research papers have focused on the morphology of epidermal cells, such as their shape, size, and arrangement. This study explores comprehensive insights from foliar characteristics using Light Microscopy (LM) and Scanning Electron Microscopy (SEM) for selected Verbenaceae taxa from Pakistan. The investigation explores the taxonomic significance of these features, contributing valuable knowledge to the understanding of Verbenaceae taxa.

Materials and Methods

Site Description and Climate

The district of Peshawar covers an area of 1257 square kilometers and lies at approximately 34°15 north latitude and 71°42 east longitude, with an elevation of about 1173 feet above sea level [43]. In the west, it borders Mohmand and Khyber Agency, while the northern border is shared with the Charsadda district [44]. In the east, it is bordered by the Nowshera district, and the southeastern parts are connected to tribal areas linking Kohat and Peshawar districts [44, 45]. The climate in the Peshawar district is semi-arid and is characterized by hot summers and mild winters. The average temperature fluctuates between 5ºC and 39ºC in the months of January to February and June to July respectively, accompanied by an annual rainfall of approximately 513 mm. July and August are the months with the highest rainfall [46].

Floristic Studies

The plants were collected from various territories of Peshawar University and surrounding areas. Information about the plants was collected, and specimens were dried in newspapers and identified with the help of (http://www.wfoplantlist.org/) and Flora of Pakistan [47, 48]. They were labeled with voucher numbers and deposited in the herbarium of the Department of Botany, University of Peshawar.

Light Microscopy

The modified technique of [36, 49] was used for epidermal studies. A leaf sample was collected in a test tube and mixed with 2 g potassium chloride and 1 mL of distilled water. The test tube was poured with 4 mL of concentrated nitric acid and the mixture was then carefully boiled according to the method of [50]. After a few minutes, the epidermal peels were washed with water after separation and then placed on a slide, to which one to two drops of bleach were added for 30 seconds to remove chloroplasts. After bleaching, the strips were washed again with distilled water and then treated with one to two drops of lactic acid to cover the entire mixture with a coverslip. Peeling the epidermis by hand is the most conventional method for peeling a large number of specimens. Prepare the abaxial surface by placing the leaf blade on a cutting plate or tile so that the adaxial surface is facing upwards. Using a sharp razor, carefully scrape the adaxial surface until the abaxial surface remains, and then use a camel hair brush hairbrush to remove all the above materials following the procedure of [51]. The epidermal peels were treated with one to two drops of bleach to remove additional chloroplast material and washed again with water. The leaf epidermis materials treated with lactic acid drops were placed on a clean glass, covered with slip, and observed under a light microscope.

Scanning Electron Microscopy

The dried leaves of the samples were used for scanning electron microscopy (SEM). For the examination, a mature, undamaged leaf section was cut from the upper middle part. The epicuticular wax on the upper and lower surface of the epidermis was removed by soaking the material in xylene for 12 to 24 h [50, 51]. Two leaf pieces were removed and then mounted on stumps with double-coated adhesive tape. One leaf piece with the lower surface was mounted to expose the upper surface and the other piece was taped to stumps from the upper side to expose the lower surface in stumps [25, 45]. The gold-palladium samples were coated by sputtering and then observed under a scanning electron microscope at the Central Resource Library (CRL) of the University of Peshawar.

Measurement of Stomatal Index

The stomatal index was calculated using the following formula:

$$S.I = \frac{S}{S + E} x\ 100$$

Where,
S.I = stomatal index; S = No. of stomata per unit, E = No. of epidermal cells per unit area

Results

In the present study, the foliar anatomical features of five species of the Verbenaceae were investigated using light and scanning electron microscopy. The features recorded were based on the qualitative and quantitative characteristics shown in Tables 1 and 2. Fig. 1 and 2 show statistical graphs generated through foliar quantitative characters, whereas Fig. 3 and 4 depict foliar epidermal features through LM and SEM. These features were used as taxonomic tools for the identification of complex taxa.

Leaf Epidermal Morphology and Epidermal Cell Size

The leaf epidermis of five species of the Verbenaceae has various shapes and sizes. The shape of the epidermal cells is pentagonal, rectangular, square, and polygonal on both leaf surfaces of the examined representatives of the Verbenaceae. However, the hexagonal and oval shape of the epidermis was observed in *V. negundo*. Polygonal shapes with thick epidermal walls were observed in most species except *P. nodiflora*, which has smooth epidermal walls on both adaxial and abaxial surfaces (Table 1). In *P. nodiflora*, the unicellular trichomes are trailed by a layer of palisade cells with smooth edges.

On the abaxial surface of *V. negundo*, tomentose and glandular hairs were observed in enormous numbers as well as microtrichomes. Glandular hairs were observed in *L. indica, V. negundo and V. officinale*. The trichomes were divided and branched in *V. negundo.* In this study, the size of epidermal cells was found to vary in both length and width on both leaf surfaces. The mean length of epidermal cells was higher on the upper epidermis cell than on the lower surface. The highest epidermal length (32-40 µm) on the adaxial surface was observed in *V. officinalis*, followed by *P. nodiflora* (24-28 µm), *D. repens* (24-27 µm), and *L. indica* (22-24 µm). The maximum value (17.9-20 µm) of the leaf epidermal width on the upper surface of the leaf was found in *V. officinalis*. The length of the epidermis on the abaxial leaf surface was greatest (42-47.5 µm) in *V. officinalis* among the Verbenaceae members compared to the other species. However, the width of the epidermis ranged from 22.5-25 to 8-13 µm on the lower epidermis.

Stomata Type and Size

The leaves of *L. indica, V. officinalis, V. negundo* and *P. nodiflora,* were classified as amphistomatic, having stomata on both the upper and lower epidermis. In *D. repens*, however, the stomata were absent on the adaxial surface. Normally, the stomata are anisocytic and animocytic and were observed on both leaf surfaces in most species. In *V. negundo*, the stomata were not visible because the stomata were covered with an enormous number of glandular hairs. *V. officinalis* with diacytic stomata on the upper leaf surface. The stomata on the lower epidermis were smaller and anomocytic and paracytic. In *P. nodiflora*, the stomata are located more on the adaxial than on the abaxial surface and were anisocytic, anomocytic, and diacytic. The lower stomata are diacytic with some guard cells and two subsidiary cells (Table 1). In this study, we also measured the size of the stomata on both leaf surfaces. The largest length

Table 1. Qualitative leaf epidermal characters of family Verbenaceae based on LM.

Plant Species	Leaf Surface	Shape of epidermal cell	Epidermal wall pattern	Stomatal Type	Stomata present/Absent	Trichomes present/ Absent
Duranta repens L.	Adaxial	Pentagonal and Rectangular	Thick and straight	-	A	A
	Abaxial	Polygonal	Thick and straight	Anisocytic	P	A
Lantana indica Roxb.	Adaxial	Polygonal or pentagonal	Thick and straight	Anisocytic Anomocytic	P	P
	Abaxial	Square, rectangular	Thick and straight	Anisocytic and anomocytic	P	P
Phyla nodiflora (Linn.) Greene in Pittonia.	Adaxial	Sporadic polygonal	Smooth and straight edges	anisocytic, anomocytic and diacytic -	P	P
	Abaxial	Rectangular or pentagonal	Smooth and straight edges	Diacytic	P	P
Verbena officinalis L.	Adaxial	Rectangular, polygonal and square	Thick	Diacytic	P	P
	Abaxial	Sporadic and polygonal	Thick	anomocytic and paracytic	P	P
Vitex negundo L.	Adaxial	Rectangular and hexagonal	Thick	-	P	P
	Abaxial	Square or oval	Thick	-	P	P

Table 2. Quantitative leaf epidermal characters of family Verbenaceae under light microscopy.

Plant Taxa	LxW	Leaf epidermal in **µm**		Stomata in **µm**		Stomatal pore in **µm**		Subsidiary cell in µm		Stomatal index %	
		Min-Max = Mean±SE		Min-Max = Mean±SE		Min-Max = Mean±SE		Min-Max = Mean±SE			
		Ad	Ab	Ad	Ab	Ad	Ab	Ad	Ab	Ad	Ab
Duranta repens L.	L	24-27 =25.9±0.46	30-40.2 =35.17±2.84	A	15.9-18.1=16.875±0.46	A	21-27=23.8±1.247	A	43-47.5=44.67±1.00	33	19.7
	W	12.9-14=13.375±0.24	19.9-22=20.87±0.47	A	7.9-10.1=8.87±0.466	A	7.5-9.5=8.22±0.460	A	18.5-22.5=20.02±0.88	A	A
Lantana indica Roxb.	L	22-24=23±0.57	9.5-12=10.75±0.59	13-17=14.87±0.82	6-12.5=10.12±1.47	36-40.8=38.45±1.15	29-30.6=29.87±0.33	53-55=54.2±0.45	14.6-16=15.55±0.32	17	14
	W	10.1-14=12.4±0.87	9-12=10.57±0.653	4-6=5.12±0.42	3-8=5.75±1.10	10-11.9=11.15±0.4	3.9-5=4.42±0.22	22.9-24=23.65±0.25	15.9-17=16.42±0.22	A	A
Phyla nodiflora (Linn.) Greene in Pittonia.	L	24-28=25.77±0.84	10-14=12.15±0.88	28-31=29.5±0.64	30-33=31.625±0.62	24-26=25±0.41	28-31.5=29.75±0.721	18-21.1=19.65±0.64	50.9-52=51.5±0.26	19	16.6
	W	13-19=15.75±1.25	8-13=10.75±1.10	22.1-25=23.66±0.66	20-24=22.5±0.853	72.9-74.1=73.4±0.27	9-12.1=10.65±0.643	35-43.6=39.02±1.83	19-22.1=20.75±0.64	A	A
Verbena officinalis L.	L	32-40=36±1.68	42-47.5=44.87±1.19	8-13=10.87±1.161	22-27.7=24.9±1.64	39.9-44.5=41.6±1.092	26-28=27.5±0.43	55-61=58.25±1.25	20-25=22.1±1.03	11.2	9.8
	W	17.9-20=18.7±0.49	22.5-25=23.62±0.55	9-11.9=10.475±0.62	17-25=21±1.68	3-9=5.5±1.31	71-77=74.5±1.32	25-32=29.76±1.48	39.9-42=41.05±0.52	A	A
Vitex negundo L.	L	A	A	A	A	A	A	A	A	A	A
	W	A	A	A	A	A	A	A	A	A	A

*L: Length, W: Width, Min: least, Max: Maximum, SE: Standard mistake, Ad: Adaxial surface, Ab: Abaxial surface, A: Absent

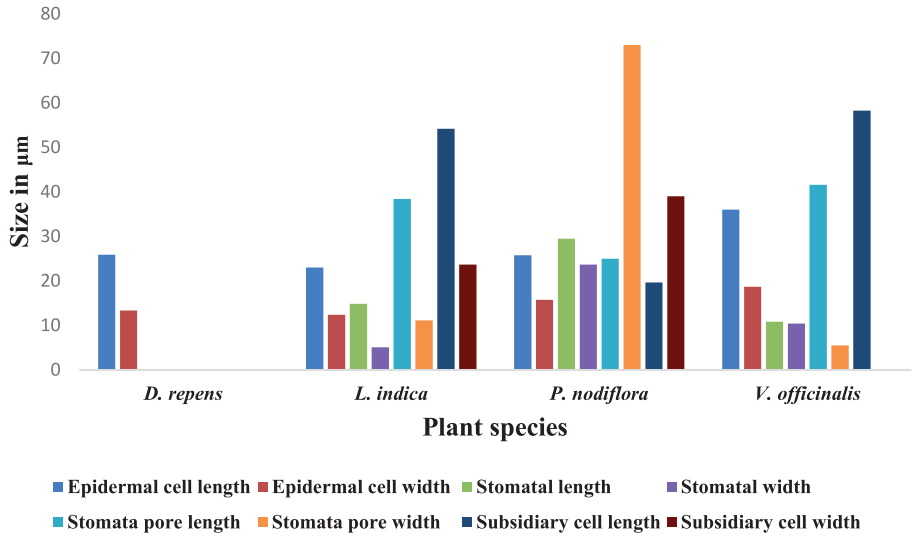

Fig. 1. Quantitative characters of the upper surface of selected species of the family Verbenaceae.

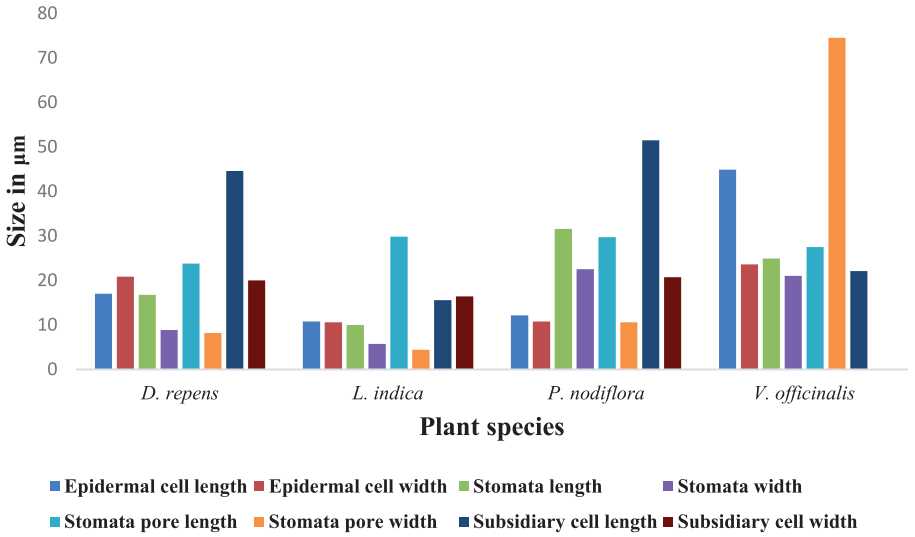

Fig. 2. Quantitative characters of the upper surface of selected species of the family Verbenaceae.

(28-31 µm) of stomata on the adaxial surface was found in *P. nodiflora*, followed by *L. indica* (13-17 µm) and *V. officinalis* (8-13 µm). The highest length (30-33 µm) of stomata was found on the abaxial surface of *P. nodiflora*, whereas the lowest length (6-12.5 µm) of stomata was recorded in *L. indica*.

The width of stomata on both leaf surfaces ranged from 3-8 to 22.1-25 µm. The length was quite variable from species to species. In this study, the length of the stomatal pore was larger on the adaxial surface than on the abaxial surface. Larger (39.9-44 µm) stomata were observed in *V. officinalis* and smaller (24-26 µm) stomata in *P. nodiflora*. The long width of stomata varied from a maximum (72.9-74 µm) in *P. nodiflora* to a minimum (3-9 µm) in *V. officinalis* on the upper surface and from a maximum of 71-77 µm (*V. officinalis*) to a minimum of 3.9-5 µm (*L. indica*) on the lower surface.

Subsidiary Cell Size and Stomatal Index

The size of the subsidiary cells was quite variant in various species. A long subsidiary cell was found in *V. officinalis* and a small subsidiary cell in *P. nodiflora* on the upper surface, while a large subsidiary cell in *P. nodiflora* and a small one in *L. indica* on the lower surface. It was investigated that the maximum length of the subsidiary cell is 55-61 µm and the minimum length of a subsidiary cell is 18-21.1 µm on the upper surface.

On the other hand, the maximum length of the subsidiary cell in *P. nodiflora* is 50.9-52 µm, while the minimum length of the subsidiary cell in *L. indica* is 14.6-16 µm on the lower surface. The width of the subsidiary cell ranged from 35-43.6 µm to 22.9-24 µm on the upper surface and from 39.9-42 µm to 15.9-17 µm on the lower surface of a leaf. In this study, the stomatal

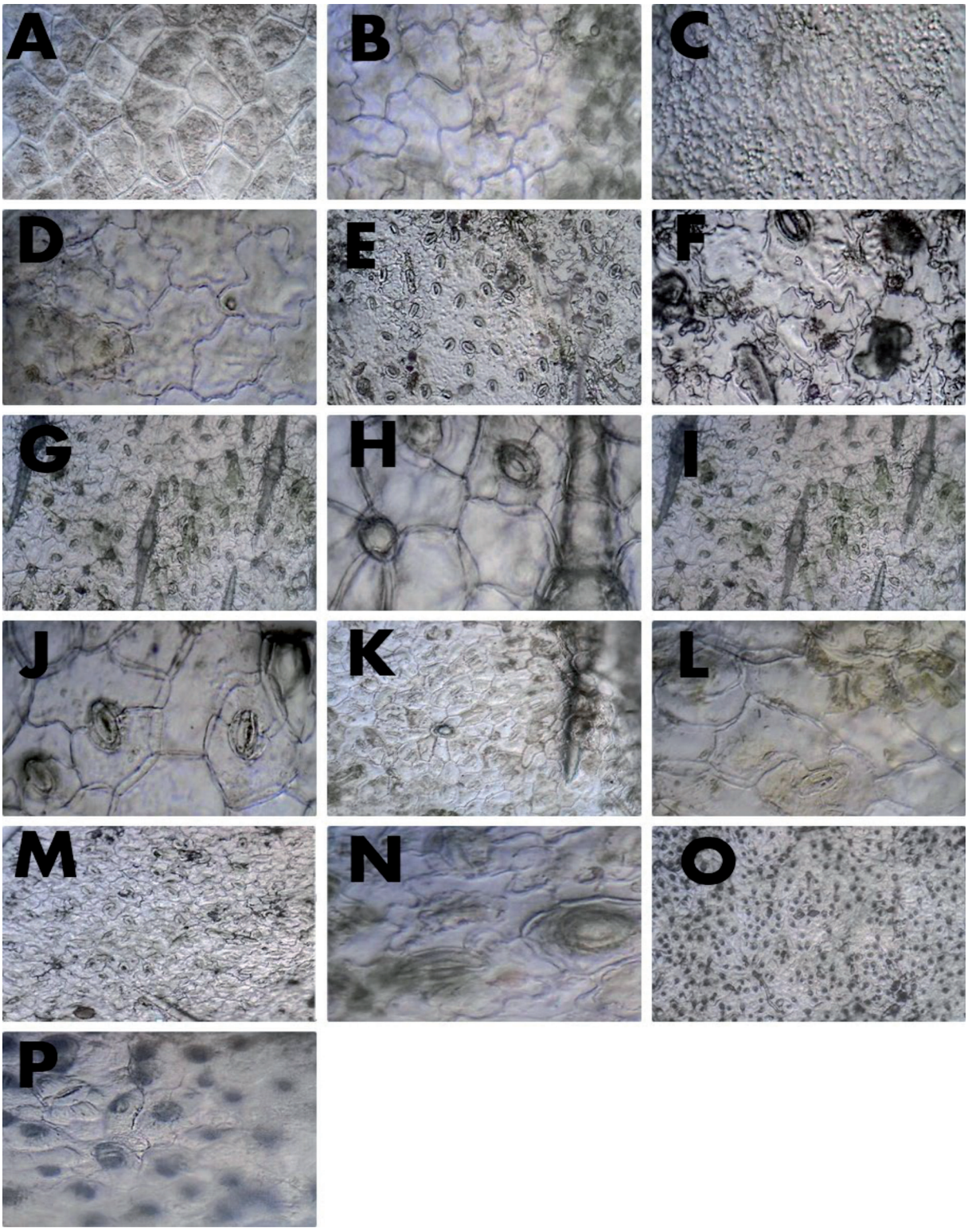

Fig. 3. Light micrograph of foliar epidermal characteristics of Verbanaceae taxa, A) *D. repens* (upper epidermis), B) *D. repens* (lower epidermis), C) *L. indica* (upper epidermis), D) *L. indica* (upper epidermis), E) *L. indica* (lower epidermis), F) *L. indica* (lower epidermis), G) *P. nodiflora* (upper epidermis), H) *P. nodiflora.* (upper epidermis), I) *P. nodiflora* (lower epidermis), J) *P. nodiflora* (lower epidermis), K) *V. officinalis* (lower epidermis), L) *V. officinalis* (lower epidermis), M) *V. officinalis* (upper epidermis), N) *V. officinalis* (lower epidermis), O) *V. negundo* (upper epidermis), P) *V. negundo* (upper epidermis).

index is an important tool to differentiate the complex taxa. The stomatal index shows different variations, with the largest stomatal index observed in *D. repens* and the smallest in *V. officinalis* on both surfaces of the epidermis.

Discussion

The leaf prepares the food for plant growth and development of the plant through the process of photosynthesis and respiration, and is therefore considered an important organ of the plant [52, 53]. Although it is the site where carbohydrates are formed, it is also the source of important features that have been used as the basis for the correct classification of important complicated plant taxa [53, 54]. Leaf

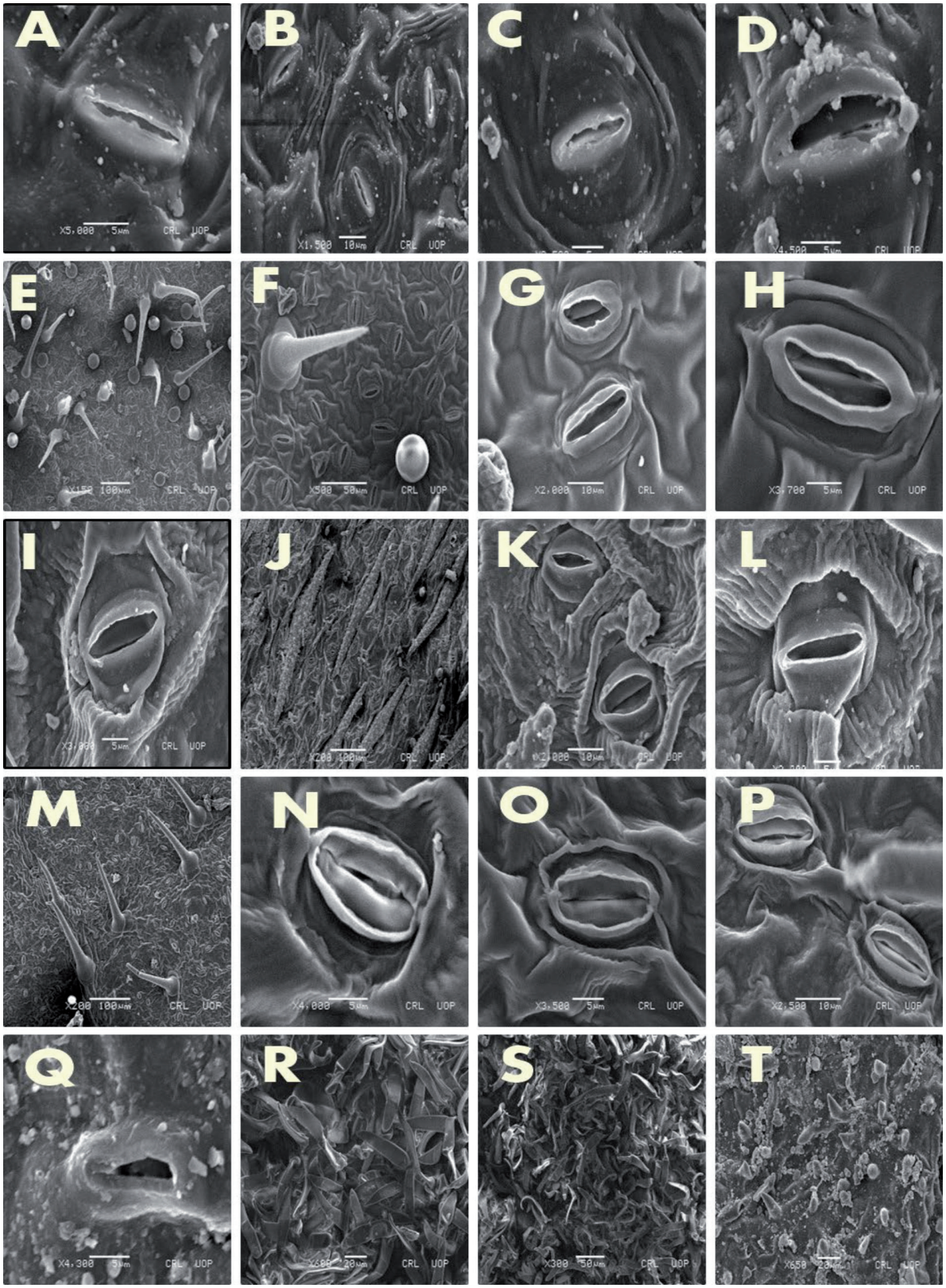

Fig. 4. Electron micrographs of Verbancaeae taxa, A-D) *D. repens*; E-H) *L. indica*; I-L) *P. nodiflora*; M-P) *V. officinalis*;Q-T) *V. negundo.*

anatomical studies are now considered an important aspect of plant systematics, and the epidermal features of leaves have been studied in many angiosperm families [30, 54-56]. Leaf anatomical features are important for the delimitation of various plant species [28]. The use of foliar epidermal characters is helpful in the identification and delimitation of taxa identified by previous studies [57, 58]. Leaf anatomical variations are important tools in the classification and differentiation of complex taxa [59]. Foliar anatomical characters provide a basis for the delimitation of complex taxa of the family Verbenacaeae, which have been described by many authors [42, 60-63]. The use of light and scanning electron microscopy can play an important role in the study of anatomical features of leaves, and these epidermal features of leaves are important for the characterization of groups abroad within subgroups and tribes [45, 51].

The present study shows that the five species of the Verbenaceae family exhibit marked differences in the size of the leaf epidermal cells, the size and shape of the stomata, the subsidiary cells, and the stomatal index. Two types of trichomes, glandular and non-glandular trichomes, were observed in the members of Verbenaceae as documented by [60, 61, 64]. Many studies have reported that the presence of non-glandular trichomes in Verbenaceae species shows considerable differences in their chemical composition and morphological features [65, 66]. [32] reported at least five species of glandular trichomes and one species of non-glandular trichomes in the leaves of *Lippia citriodora L*. Uniseriate and tomentosa, non-glandular trichomes were observed on the leaves of *Lippia javanica* (Burm.f.) Spreng. [67]. However, the number and shape of trichomes vary from species to species [33] in contrast to the study of [33], in which six types of glandular trichomes ranging from unicellular to tetracellular trichomes and three types of tectorial trichomes were observed in the leaves of *Lippia* species, [68] observed only two types of glandular trichomes in *L. scaberrima* Sond. The presence of trichomes in Verbenaceae species is a characteristic feature of this family [60, 68, 69]. Identification on the basis of trichome characteristics has a high taxonomic value and their classification can even be made to the specific level [70, 71]. Features of the epidermal wall and ontogeny of stomata are important taxonomic characters to distinguish between advanced and primitive taxa and are used for morphological and ontogenetic classification [61, 72, 73]. In this study, the leaf epidermal cells of five different species of Verbenaceae show variation in their morphology. Polygonal with thick epidermal walls and straight edges were the common features of most species. The results are in agreement with those of [61, 74-76]. However, rectangular, sporadic, square, pentagonal, and hexagonal epidermal shapes with smooth edges were also observed in the studied species. Stomata are small pores on the adaxial and abaxial surfaces of leaves and plants that play an important role in plant identification and classification and also have a significant role in plant physiological processes [77]. The anisocytic, anomocytic, and diacytic stomata were commonly observed on both leaf surfaces in the Verbenaceae family [67]. Our results were in agreement with the findings of [75], who also reported the same types of stomata in the Verbenaceae in Egypt. However, [74] reported tetracytic types of stomata in Clearodendrum species. In our result, the leaves of *L.indica*, *V. officinalis*, *V. negundo*, and *P. nodiflora*, were classified as amphistomatic, with stomata on both the upper and lower epidermis- a result similar to that of [67, 78]. In our finding, the stomata were not seen on both surfaces of the epidermal cells of *V. negundo* due to the presence of an enormous number of tomentose and glandular hairs. This result was in agreement with [79]. The leaf anatomical features of the leaves provided information on the fossil phylogeny and revealed taxonomic relationships [80]. The larger epidermal cell was observed on the adaxial surface in *V. officinalis* with 32-40 µm and smaller epidermal cells on the adaxial surface were found in *L. indica* with 22-24 µm. On the abaxial surface, the large stomata were observed in *Phyla nodiflora* with a size of 30-33µm, while smaller stomata were observed in *L. indica* with a size of 6-12.5 µm. In *P. nodiflora*, the stomata are located more on the adaxial than on the abaxial surface and were anisocytic, anomocytic, and diacytic. This finding differs from the results of [73, 81], who reported that the stomata are larger on the abaxial surface than on the adaxial surface.

The subsidiary cells have a disjunct role on the upper surface. It is investigated that the maximum length of a subsidiary cell is 55-61 µm and the minimum length of a subsidiary cell is 18-21.1 µm on the upper surface. A long subsidiary cell was found in *V. officinalis* and a small subsidiary cell in *P. nodiflora* on the upper surface. On the other hand, the large subsidiary cell in *P. nodiflora* and the small one in *L. indica* are on the lower surface of a leaf. The stomatal index shows different variations, with the largest stomatal index observed in *D. repens* and the smallest observed in *V. officinalis* on both surfaces of the epidermis. The stomatal index can affect plant-environment interactions such as assimilation and transpiration [50, 82]. Overall, this study is important for the delimitation of Verbenaceous species based on leaf anatomical features and provides us with important information on the relevance and importance of foliar epidermal features in solving many problems related to the classification of complex taxa [30, 83, 84].

Conclusion

The above study suggests that examination of the leaf epidermis can be helpful in identifying and differentiating different Verbenaceous species. Various characteristics of the leaf epidermis such as the absence or presence of stomata, size, and shape of epidermal cells, the absence or presence of trichomes, and the

types of trichomes are preliminary steps to delimit different taxa at generic and specific levels. Finally, the current work deals with the complex architecture and morphology of the leaf epidermis in Verbenaceae species. Through a thorough examination of the leaf epidermis of different species of this plant family, we gained important insights into the unique adaptations and features that these structures exhibit. The different epidermal characters discovered in this study may be useful diagnostic features for taxonomists and botanists attempting to classify the Verbenaceae species. It is crucial to highlight that, while this work provides insight into the epidermal anatomy and morphology of the leaves of the Verbenaceae taxa, further research is needed to investigate the functional significance of these morphological differences.

Future Perspectives

As we continue to explore the complex field of Verbenaceae foliar epidermis, future research should attempt to fill information gaps and uncover the larger ecological consequences of the observed diversity. Understanding the adaptive importance of different epidermal characteristics could shed light on the dynamic interactions between Verbenaceae taxa and their habitats. Investigating the relationship between certain epidermal features and environmental conditions, such as climate, soil composition, or altitude, could shed light on the adaptive methods of Verbenaceae species. Deciphering these relationships could help us to understand plant ecology and improve conservation measures. Exploring potential applications of the foliar diversity of Verbenaceae in industries such as medicine, agriculture, and industry could be a promising direction. Certain features of the epidermis could have medicinal properties, and the research could inspire improvements in biomimicry for agricultural practices or materials science. In summary, research into the leaf epidermis of Verbenaceae is not a goal in itself, but rather a step towards a more comprehensive understanding of plant biology and its practical applications. The combination of ecological, genetic, and evolutionary perspectives promises a fertile ground for future research, providing both theoretical insights and potential applications in practice.

Author Contributions

Conceptualization: F.H., S.D, B.U & M.N.K; Methodology: K.H, S.N.S & M.I; Data Curation: S.W, M.N.K & F.R.B; Writing-original draft preparation: S.D; Writing-Review and Editing: A.K., S.A.R., & S.E; Supervision: F.H & A.R.; Funding Acquisition: M.A.A & J.A.

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Conflict of Interest

The authors declare no conflict of interest.

Reference

1. ATKINS S. Verbenaceae. Springer, **2004**.
2. MARX H.E., O'LEARY N., YUAN Y.W., LU-IRVING P., TANK D.C., MÚLGURA M. E., OLMSTEAD R.G. A molecular phylogeny and classification of Verbenaceae. American Journal of Botany, **97** (10), 1647, **2010**.
3. REFULIO-RODRIGUEZ N.F., OLMSTEAD R.G. Phylogeny of lamiidae. American Journal of Botany, **101** (2), 287, **2014**.
4. O'LEARY N., CALVIÑO C.I., MARTÍNEZ S., LU-IRVING P., OLMSTEAD R.G., MÚLGURA M.E. Evolution of morphological traits in Verbenaceae. American Journal of Botany, **99** (11), 1778, **2012**.
5. THODE V.A., O'LEARY N., OLMSTEAD R.G., FREITAS L.B. Phylogenetic position of the monotypic genus Verbenoxylum (Verbenaceae) and new combination under Recordia. Systematic Botany, **38** (3), 805, **2013**.
6. MABBERLEY D.J. Mabberley's plant-book: a portable dictionary of plants, their classification and uses. Cambridge university press, **2017**.
7. CARDOSO P.H., O'LEARY N., OLMSTEAD R.G., MORONI P.A. An update of the Verbenaceae genera and species numbers. Plant Ecology and Evolution, **154** (1), 80, **2021**.
8. MALIK A., ARIF S., AKHTAR W., MAHMOOD T. Molecular phylogeny of different species of family verbenaceae using chloroplast rps14 gene. Pakistan Journal of Botany, **54** (1), 215, **2022**.
9. MORONI P., O'LEARY N. Insights into the Systematics of Tribe Duranteae (Verbenaceae): A Taxonomic Revision of the New World Genus Bouchea1. Annals of the Missouri Botanical Garden, **104** (3), 355, **2019**.
10. MORONI P., O'LEARY N. Insights into the Systematics of Tribe Duranteae (Verbenaceae) II: A Taxonomic Revision of the New World Genus Duranta1. Annals of the Missouri Botanical Garden, **105** (4), 502, **2020**.
11. YUAN Y.-W., LIU C., MARX H.E., OLMSTEAD R.G. An empirical demonstration of using pentatricopeptide repeat (PPR) genes as plant phylogenetic tools: Phylogeny of Verbenaceae and the Verbena complex. Molecular Phylogenetics and Evolution, **54** (1), 23, **2010**.
12. 12. KANWAL K., ZAFAR M., KHAN A.M., MAHMOOD T., ABBAS Q., OZDEMIR F. A., AHMAD

M., SULTANA S., ROZINA FATIMA A. Implication of scanning electron microscopy and light microscopy for oil content determination and seed morphology of Verbenaceae. Microscopy Research and Technique, **85** (2), 789, **2022**.
13. FROST L.A., TYSON S.M., LU-IRVING P., O'LEARY N., OLMSTEAD R.G. Origins of North American arid-land Verbenaceae: More than one way to skin a cat. American Journal of Botany, **104** (11), 1708, **2017**.
14. OLMSTEAD R.G. Phylogeny and biogeography in Solanaceae, Verbenaceae and Bignoniaceae: a comparison of continental and intercontinental diversification patterns. Botanical Journal of the Linnean Society, **171** (1), 80, **2013**.
15. XU Z., CHANG L., XU Z., CHANG L. Verbenaceae. Identification and Control of Common Weeds: Volume 3, pp. 163, **2017**.
16. OXELMAN B., KORNHALL P., OLMSTEAD R.G., BREMER B. Further disintegration of Scrophulariaceae. Taxon, **54** (2), 411, **2005**.
17. BRIDI R., VON POSER G.L., GÓMEZ M., ANDIA M.E., OYARZÚN J.E., NÚÑEZ P., ARIAS A.J.V., ESPINOSA-BUSTOS C. Hepatoprotective species from the Chilean medicinal flora: Junellia spathulata (Verbenaceae). Journal of Ethnopharmacology, **267**, 113543, **2021**.
18. PADMINI S., RAO S.R.S. Structure, distribution and taxonomic importance of foliar stomata in some Indian Amaranthaceae. Botanical Journal of the Linnean Society, **118** (2), 149, **1995**.
19. ZENG X., WANG M., CAI M., LUO P., PACE M.C., LI P. Characteristics and evolution of leaf epidermis in the genus Amana Honda (Liliaceae). Taxonomy, **3** (3), 435, **2023**.
20. HETHERINGTON A.M., WOODWARD F.I. The role of stomata in sensing and driving environmental change. Nature, **424** (6951), 901, **2003**.
21. WANG X., SHEN C., MENG P., TAN G., LV L. Analysis and review of trichomes in plants. BMC Plant Biology, **21** (1), 1, **2021**.
22. PATEL I., GORIM L.Y., TANINO K., VANDENBERG A. Diversity in surface microstructures of trichomes, epidermal cells, and stomata in lentil germplasm. Frontiers in Plant Science, **12**, 697692, **2021**.
23. WATTS S., KARIYAT R. Morphological characterization of trichomes shows enormous variation in shape, density and dimensions across the leaves of 14 Solanum species. AoB Plants, **13** (6), plab071, **2021**.
24. JIA W., WANG Y., QI Q., HE S., MI Z., ZHU X. Leaf epidermal morphology of ten wild tree Peonies in China and its taxonomic significance. Horticulturae, **8** (6), 502, **2022**.
25. SHAH S.N., AHMAD M., ZAFAR M., HADI F., KHAN M.N., NOOR A., MALIK K., RASHID N., KAMAL A., IQBAL M. Application of spore morphology to solve identification problems in certain species of family Dryopteridaceae from Malakand Division, Pakistan. Microscopy Research and Technique, **84** (8), 1897, **2021**.
26. BARBOZA G.E., CANTERO J.J., NÚÑEZ C., PACCIARONI A., ARIZA ESPINAR L. Medicinal plants: A general review and a phytochemical and ethnopharmacological screening of the native Argentine Flora. Kurtziana, **34** (1-2), 7, **2009**.
27. ULLAH F., AYAZ A., SAQIB S., PARMAR G., BAHADUR S., ZAMAN W. Taxonomic implication of leaf epidermal anatomy of selected taxa of Scrophulariaceae from Pakistan. Microscopy Research and Technique, **84** (3), 521, **2021**.
28. GUL S., AHMAD M., ZAFAR M., BAHADUR S., SULTANA S., ASHFAQ S., ULLAH F., KILIC O., HASSAN F.U., SIDDIQ Z. Foliar epidermal anatomy of Lamiaceae with special emphasis on their trichomes diversity using scanning electron microscopy. Microscopy Research and Technique, **82** (3), 206, **2019**.
29. ALOUSH R. Morphological and anatomical characterization of the species trifolium incarnatum l. Cultivated in Iraq. Sabrao Journal of Breeding & Genetics, **55** (4), **2023**.
30. ASHFAQ S., AHMAD M., ZAFAR M., SULTANA S., BAHADUR S., ULLAH F., ZAMAN W., AHMED S.N., NAZISH M. Foliar micromorphology of Convolvulaceous species with special emphasis on trichome diversity from the arid zone of Pakistan. Flora, **255**, 110, **2019**.
31. JAMIL RAZA M.A., SHINWARI Z.K., ZAFAR M., MAJEED S., JORAEV A., BOBOJANOV K. Leaf micromorphological traits of legumes from takkar wildlife sanctuary. Pakistan Journal of Botany, **56**, 1, **2024.**
32. ARGYROPOULOU C., AKOUMIANAKI-IOANNIDOU A., CHRISTODOULAKIS N. S., FASSEAS C. Leaf anatomy and histochemistry of Lippia citriodora (Verbenaceae). Australian Journal of Botany, **58** (5), 398, **2010**.
33. DE OLIVEIRA A.R.M.F., DE OLIVEIRA L.M., CARNEIRO J.S., DOS SANTOS SILVA T.R., DO BONFIM COSTA L.C. Leaf anatomy and essential oil production of lippia native species. Brazilian Journal of Agriculture, **93** (3), 324, **2018**.
34. MOOD K., JOGAM P., SIRIKONDA A., SHEKHAWAT M.S., ROHELA G.K., MANOKARI M., ALLINI V.R. Micropropagation, morpho-anatomical characterization, and genetic stability studies in Lippia javanica (Burm. f.) Spreng: a multipurpose medicinal plant. Plant Cell, Tissue and Organ Culture (PCTOC), **150** (2), 427, **2022**.
35. WERDIN GONZALEZ J.O., GUTIÉRREZ M.M., MURRAY A.P., FERRERO A.A. Biological activity of essential oils from Aloysia polystachya and Aloysia citriodora (Verbenaceae) against the soybean pest Nezara viridula (Hemiptera: Pentatomidae). Natural Product Communications, **5** (2), 1934578X1000500227, **2010**.
36. CARMONA C., ANCIBOR E. Anatomía ecológica de las especies de Acantholippia (Verbenaceae). Boletín de la Sociedad Argentina de Botánica, **31** (1-2), 3, **1995**.
37. BOTTA S. Notas en el género Glandularia (Verbenaceae-Verbenoideae) III. Estudio taxonómico de las especies patagónicas. Parodiana, **8** (1), 9, **1993**.
38. BOTTA S.M. Estudios en el género sudamericano Junellia (Verbenaceae, Verbenoideae) I. Delimitación y tratamiento infragenérico. Darwiniana, 371, **1989**.
39. METCALFE C., CHALK L. Anatomy of the dicotyledons. Vol I. Systematic anatomy of leaf and stem, with a brief history of the subject. Anatomy of the dicotyledons. Vol I. Systematic anatomy of leaf and stem, with a brief history of the subject, 2nd ed.; **1979**.
40. INAMDAR J. Epidermal Structure and Ontoģeny of Stomata in some Verbenaceae. Annals of Botany, **33** (1), 55, **1969**.
41. INGOLE S. Study of floral epidermal features in Gmelina arborea Roxb. International Journal of Scientific and Engineering Research, **3**, 85, **2013**.
42. AJUZIOGU G., EJEAGBA P., NWAFOR F., AYOGU V., NWEZE A., ASUZU C., EGONU S. Comparative anatomical studies of the stomatal patterns of some tree species of Sterculiaceae and Verbenaceae in Nigeria. Pakstan Journal of Botany, **50** (2), 679, **2018**.

43. NAZ R., KHAN M., HAFEEZ A., FAZIL M., KHAN M., ALI B., JAVED M., IMRAN M., SHATI A., ALFAIFI M. Assessment of phytoremediation potential of native plant species naturally growing in a heavy metal-polluted industrial soils. Brazilian Journal of Biology, **84**, **2022**.
44. KHAN R., KHAN M.N., ULLAH H., BASIT A., RAZZAQ A., AHMAD M., OZDEMIR F. A comparative assessment of proximate and elemental composition six weedy grasses for their potential use as fodder. Progress in Nutrition, **20** (1-S), 182, **2018**.
45. SHAH S.N., AHMAD M., ZAFAR M., HADI F., KHAN M.N., NOOR A., MALIK K., RASHID N., KAMAL A., IQBAL M. Spore morphology and leaf epidermal anatomy as a taxonomic source in the identification of Asplenium species from Malakand division Pakistan. Microscopy Research and Technique, **83** (11), 1354, **2020**.
46. ALI S., ULLAH S., KHAN M.N., KHAN W.M., RAZAK S.A., WAHAB S., HAFEEZ A., KHAN BANGASH S.A., POCZAI P. The effects of osmosis and thermo-priming on salinity stress tolerance in Vigna radiata L. Sustainability, **14** (19), 12924, **2022**.
47. NAZIMUDDIN S., QAISER M. In E. Nasir & SI Ali. Flora of West Pakistan, **148**, 10, **1983**.
48. PERVEEN A., QAISER M. Pollen flora of Pakistan-LIII. Verbenaceae. Pakistan Journal of Botany, **39** (3), 663, **2007**.
49. MUNIR M., KHAN M.A., AHMED M., BANO A., AHMED S.N., TARIQ K., TABASSUM S., MUKHTAR T., AMBREEN M., BASHIR S. Foliar epidermal anatomy of some ethnobotanically important species of wild edible fruits of northern Pakistan. Journal of Medicinal Plants Research, **5** (24), 5873, **2011**.
50. SHAH S., SHAH S.M., ULLAH A., ALI U., KHAN M.N., SULTANA S., HAYAT K., AZIZ R., ÇETIN M. Light and Scanning Electron Microscopic Investigation of Selected Taxa of Fabaceae and its Utilization for Taxonomic Classification. Journal of Xi'an Petroleum Institute (Natural Science Edition), 19, **2023.**
51. HAYAT K., KHAN W.M., KHAN M.N., SHAH S.N. Pollen morphological investigation of selected species of family Asteraceae from Pakistan by using light and scanning electron microscopy. Microscopy Research and Technique, **86**, 1258, **2023**.
52. BASHIR K., SOHAIL A., ALI U., ULLAH A., UL HAQ Z., GUL B., ULLAH I., SUNERA, ASGHAR M. Foliar micromorphology and its role in identification of the Apocynaceae taxa. Microscopy Research and Technique, **83** (7), 755, **2020**.
53. ABDALLA M.M., ELTAHIR A.S., EL-KAMALI H.H. Comparative morph-anatomical leaf characters of Nerium oleander and Catharanthus roseus family (Apocynaceae). European Journal of Basic and Applied Sciences, **3** (3), 68, **2016**.
54. ATTAR F., ESFANDANI-BOZCHALOYI S., MIRTADZADINI M., ULLAH F., ZAMAN W. Foliar and stem epidermal anatomy of the tribe Cynoglosseae (Boraginaceae) and their taxonomic significance. Microscopy Research and Technique, **82** (6), 786, **2019**.
55. GUL S., AHMAD M., ZAFAR M., BAHADUR S., CELEP F., SULTANA S., BEGUM N., HANIF U., ZAMAN W., SHUAIB M. Taxonomic significance of foliar epidermal morphology in Lamiaceae from Pakistan. Microscopy Research and Technique, **82** (9), 1507, **2019**.
56. ULLAH F., ZAMAN W., PAPINI A., ZAFAR M., SHAH S.N., AHMAD M., SAQIB S., GUL S., SOHAIL A. Using multiple microscopic techniques for the comparative systematic of Spergula fallax and Spergula arvensis (Caryophyllaceae). Microscopy Research and Technique, **82** (4), 352, **2019**.
57. AMEEN F. Taxonomic studies based on leaf epidermal microanatomy using high-resolution microscopy in Lamiaceous species and their antimicrobial effects. Microscopy Research and Technique, **86** (11), 1484, **2023**.
58. CHOI B., HWANG Y., MCADAM S.A., JANG T.S. Comparative microscopic investigations of leaf epidermis in four Ajuga species from Korea. Microscopy Research and Technique, **87** (3), **2024**.
59. JEHANZEB S., ZAFAR M., AHMAD M., SULTANA S., ZAMAN W., ULLAH F. Comparative petioler anatomy of tribe Mentheae subfamily Nepetoideae, Lamiaceae from Pakistan. Feddes Repertorium, **131** (3), 163, **2020**.
60. RODRIGUEZ MORCELLE M.I., GATTUSO M.A., APÓSTOLO N.M. Anatomical and micromorphological features of inflorescences of six Verbena L. species (Verbenaceae). Biologia, 1, **2023**.
61. ADEDEJI O. Systematic significance of trichomes and foliar epidermal morphology in the species of Stachytarpheta Vahl.(Verbenaceae) from Nigeria. Thaiszia-Journal of Botany, **22** (1), 1, **2012**.
62. ESMAEEL Z.A., AL-MUSAWI A.H. Comparative anatomical features of Lippia nodiflora (L.) Rich. and Verbena officinalis L., Verbenaceae. Journal of Education and Scientific Studies, (4), **2014**.
63. GRYTSYK A., POSATSKA N., SVIRSKA S. Macroscopic and microscopic morphological features of verbena l. Species. Sciences of Europe, (60-1), 35, **2020**.
64. KARAISMAILOĞLU M.C., GÜNER Ö. Trichome micromorphology of the genus Stachys sect. Fragilicaulis subsect. Fragilis and its taxonomic implications. Plant Biosystems-An International Journal Dealing with all Aspects of Plant Biology, **155** (4), 833, **2021**.
65. SANTOS TOZIN L.R.D., DE MELO SILVA S.C., RODRIGUES T.M. Non-glandular trichomes in Lamiaceae and Verbenaceae species: morphological and histochemical features indicate more than physical protection. New Zealand Journal of Botany, **54** (4), 446, **2016**.
66. TOZIN L.R.D.S., CARVALHO S.F., MACHADO S.R., RODRIGUES T.M. Glandular trichome diversity on leaves of Lippia origanoides and Lippia stachyoides (Verbenaceae): morphology, histochemistry, and ultrastructure. Botany, **93** (5), 297, **2015**.
67. ASOWATA-AYODELE A.M., AFOLAYAN A.J., OTUNOLA G.A. Foliar micromorphology of Lippia javanica (Burm. F) spreng. African Journal of Traditional, Complementary and Alternative Medicines, **13** (2), 237, **2016**.
68. COMBRINCK S., DU PLOOY G., MCCRINDLE R., BOTHA B. Morphology and histochemistry of the glandular trichomes of Lippia scaberrima (Verbenaceae). Annals of Botany, **99** (6), 1111, **2007**.
69. MARTÍNEZ-NATARÉN D.A., PARRA-TABLA V., DZIB G., CALVO-IRABIÉN L. M. Morphology and density of glandular trichomes in populations of Mexican oregano (Lippia graveolens HBK, Verbenaceae), and the relationship between trichome density and climate1. The Journal of the Torrey Botanical Society, **138** (2), 134, **2011**.
70. MURAVNIK L. The structural peculiarities of the leaf glandular trichomes: A review. Plant Cell and Tissue Differentiation and Secondary Metabolites: Fundamentals and Applications, 63, **2021**.
71. RASHID Z.S.A., AMRI C.N.A.C., SHAHARI R. The taxonomic study of trichome morphology in selected

asteraceae species of pahang, Malaysia. Malaysian Applied Biology, **51** (4), 1, **2022**.
72. EKEKE C., AGBAGWA I.O. Epidermal structures and stomatal ontogeny in Terminalia catappa L.(Combretaceae). International Journal of Botany, **11** (1), 1, **2015**.
73. JIBRIL S., JAKADA B. Leaf epidermal structures and stomata ontogeny in some members of the family Cucurbitaceae. International Journal of Plant & Soil Science, **9** (2), 1, **2016**.
74. BANGAR M., PADAMWAR S., PATIL S., DHEMBRE A. Foliar epidermal studies in some clearodendrum species (Verbenaceae). International Journal of Plant Sciences (Muzaffarnagar), **6** (2), 243, **2011**.
75. BAKR A., EL-GENDY A., EL-TAHER A., EL-KAFAFI E. Comparative morphological and chemical studies on some verbenaceous plants in Egypt. Al-Azhar Journal of Agricultural Research, **47** (2), 1, **2022**.
76. PURI V.A., ANSARI N.Y., PURI A.P. Pharmacognostic, physicochemical and phytochemical investigation of leaves of Duranta repens Linn. Research Journal of Pharmacognosy and Phytochemistry, **9** (3), 141, **2017**.
77. SHAHEEN S., FATEH R., YOUNIS S., HARUN N., JAFFER M., HUSSAIN K., ASHFAQ M., SIDDIQUE R., MUKHTAR H., KHAN F. Light and scanning electron microscopic characterization of thirty endemic Fabaceae species of district Lahore, Pakistan. Microscopy Research and Technique, **83** (12), 1507, **2020**.
78. HARUNA H., ASHIR H. Leaf epidermal members of the epidermal structures and stomata. Journal of Pure and Applied Sciences, **10** (1), 670, **2017**.
79. ZAMAN W., ULLAH F., PARMAR G., SAQIB S., AYAZ A., PARK S. Foliar micromorphology of selected medicinal Lamiaceae taxa and their taxonomic implication using scanning electron microscopy. Microscopy Research and Technique, **85** (9), 3217, **2022**.
80. VENTO B., AGRAÍN F., PUEBLA G.G., PINZÓN D. Phylogenetic relationships in Nothofagus: The role of Antarctic fossil leaves. Acta Palaeontologica Polonica, **68** (1), 175, **2023**.
81. AJAYI B.A., KADIRI A.B., OLOWOKUDEJO J.D. Comparative Foliar Micro-Morphological Study of Six Cucurbitaceous Species. Asian Journal of Research in Botany, **7** (3), 26, **2022**.
82. YOUSAF Z., ZAFAR M., AHMAD M., SULTANA S., ROZINA, OZDEMIR F.A., ABIDIN S.Z.U. Palyno-anatomical microscopic characterization of selected species of Boraginaceae and Fabaceae. Microscopy Research and Technique, **85** (4), 1332, **2022**.
83. LLUGA-RIZANI K., ŠOLJAN D., RIZANI S., KURTESHI K. Morphological and anatomical studies on hill clover (Trifolium alpestre)(Fabaceae) from Sharri Mountain, Kosovo. Advances in Agriculture & Botanics, **11** (3), 121, **2019**.
84. LUCHIAN V., SĂVULESCU E., PĂDURARU J., POPA V., LAGUNOVSCHI-LUCHIAN V. Morphological and anatomical investigation of Aloysia citrodora Palau-new medicinal plant introduced in Romania. Scientific papers. Series B. Horticulture, **62**, **2018**.