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RESEARCH ARTICLE

Nutlets and foliar micromorphological investigation for some cultivated taxa of Lamiaceae Martinov

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Abstract

To study the micromorphology of leaf surfaces and nutlets, this investigation was conducted on a few *Lamiaceae* taxa from Upper Egypt. The taxa studied included *Mentha longifolia*, *M. spicata*, *Plectranthus scutellarioides*, *Ocimum basilicum*, and *O. basilicum* var. *purpurascens*, in addition to *P. amboinicus*, which was not included in the investigation of nutlets. The following microscopy techniques were used in this work: scanning electron microscopy (SEM), light microscopy (LM), and stereomicroscopy (SM). The results of foliar micromorphology proved that it has a high taxonomic impact, especially on leaf surface texture. The results also demonstrated two shapes of glandular hairs: linear, with two subforms: flattened and articulated, as well as slender. Glandular trichomes differed in shape depending on the number of head cells and the shapes of the stalk and base cells. Glandular hairs are characterized by two shapes: firstly, mushroom-shaped multicellular heads. Multicellular heads are distinct as large glands, "umbrella heads," or small glands. The second was simple, unicellular-head glandular hair. The nutlet micromorphology of the five taxa showed a significant taxonomic diagnosis in terms of shape, dimensions, coloration, areole position, and coat surface characteristics, especially the texture pattern and hairiness of the nutlet surface. Three different artificial keys based on foliar and nutlet micromorphology and trichome types were introduced to identify the taxa under study. Additionally, principal component analysis (PCA) was employed to determine the degree of genetic variation and ascertain the axes' and their corresponding characteristics' respective discriminative powers, as well as the categorization using a bi-plot diagram.

Keywords: Head; Multicellular; PCA; Stomatal Index; Trichomes; Umbrella.

1. Introduction

Various plant organs, such as nultlets and leaves, including their trichomes, stomata, epidermal cells, and surface texture represent the most useful taxonomic traits differentiating cultivated taxa of Lamiaceae Martinov. The plant species in the *Lamiaceae* family include flowering herbs and shrubs. These species are commonly cultivated for several purposes. The genus *Mentha* belongs to this family and includes 25-30 species which are widely distributed in temperate regions (Celenk et al*.*, 2008; Ahmad *et al.*, 2018). The mint plant is a perennial herb that is mainly grown to produce volatile oil as a secondary metabolic product. Its oil is characterized by a strong pleasant smell and is often used for medicinal and aromatic purposes (Pichersky *et al*, 2006; Broza *et al.*, 2009). However, for more than 100 years, the taxonomy of this genus has constantly changed (Tucker and Naczi, 2007; Salama *et al.*, 2018). Sweet basil (*Ocimum basilicum* L.) is another member of the *Lamiaceae* family and one of the most famous herbs in the world. The biological effects of basil plants are attributed to its essential oil. Basil oil is often used as an antioxidant, antilipidemic,

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anticarcinogenic, antimicrobial, and insecticidal agent (Bilal *et al.*, 2012; Abhay *et al.*, 2014). Moreover, basil leaves, and young branches can be used in wine and flavoring as well as in the production of tea (Vînătoru *et al.*, 2019; Govoreanu *et al.*, 2022). The common Coleus is a perennial herbaceous plant belonging to the Lamiaceae family that is used as a medicinal, food, and ornamental plant (Duke *et al.*, 2002; Desai and Thirumala, 2014). It is a close relative of basil, Salvia, oregano, peppermint, spearmint, and thyme. Even though this genus is important in horticultural and floricultural aspects, 85% of its documented uses are in medicine (Lukhoba, 2006). It occupies the previous name of the genus *Plectranthus* (WFO). There are morphological and anatomical differences in various plant parts, such as leaves, stems, fruits, and pollen, between plant species within the genera of *Lamiaceae*. The micro-morphological traits of different plant parts have been used as tools in the taxonomical appreciation of different plant species (Kamal, and Kumar, 2022). From a biosystematics perspective, the foliar epidermis is one of the most notable taxonomic characteristics, and taxonomic investigations of several families have been undertaken based on the leaf epidermis (Albert and Sharma, 2013). The leaf epidermis has many differentiated cell types, such as trichomes, basic epidermal cells, and cells of stoma complexes, which have many functions in plants. The density, structure, and distribution pattern of trichomes and stomata on the leaf surface greatly vary among plant species (Nikolić *et al.*, 2003). The indumentum described by light and scanning electron microscopy of *Mentha pulegium* L. was characterized by non-glandular and glandular trichomes, which corresponded to the common arrangement described for the Lamiaceae family (Rodriguesa *et al.*, 2012). The epidermal pattern was quite different among the four *Coleus* species, that is, *Coleus malabaricus*, morphotype of *C. malabaricus*, *C. zeylanicus*, and *C. amboinicus*, whereas diacytic stomata and the presence of both glandular and non-glandular trichomes are common (Kamal and Kumar, 2022). The morphological features of ten basil (*Ocimum basilicum* L.) cultivars under different growing conditions were evaluated and the tested cultivars differed according to their morphological parameters (Juškeviˇciene *et al.*, 2022). Therefore, the aim of the current study is to investigate and evaluate the different micro-morphological of vegetative leaves and nutlets features of three cultivated genera *i.e.*, *Mentha*, *Plectranthus*, and *Ocimum* and six taxa included (*Mentha longifolia* (L.) L., *M. spicata* L., *Plectranthus amboinicus* (Lour.) Spreng., *P. scutellarioides* R. Br., *Ocimum basilicum* L., and *Ocimum basilicum* var. *purpurascens* Benth.) distributed throughout temperate and tropical areas.

2. Materials and Methods

2.1. Plant Material

Six selected herbal cultivated taxa of family Lamiaceae Martinov belonging to three genera were collected from the Al-Marashda farm of Agriculture Research Station (MARS) at the co-ordinations: 26°05'32"N & 32°28’50"E, and from a private garden of flowering plants nursery (PFPN) in Nagaa Al-Mansouri, Qena at the co-ordinations: 26°08'43"N & 32°44’09"E (Fig. 1). Both sites are in Qena Governorate, Egypt, specimens were collected during their flowering and fruiting seasons 2022-2023 (Table 1).

Identification of different specimens was achieved using available floristic and taxonomic literature (Chaudhary, 2001; Boulos, 2002; Ibrahim, 2013). The scientific names and synonyms are checked according to (WFO). The nomenclature, citation, and the locality of the studied taxa are presented in Table (1).

Figure 1. Locality of collected sampling of the different sampling of Six herbal cultivated taxa of family Lamiaceae Martinov.

Table 1. The nomenclature, citation, and the collecting information the studied taxa.

No.	Taxon	Synonym(s)	Common names	Locality	
				Site	Year
1	*Mentha longifolia* (L.) L. Fl. Monsp. 19 (1756)	*M. longifilia* f. *angustifolia* Prodàn.	Horse mint, St. John's horsemint	MARS & PFPN	2022
2	*Mentha spicata* L. Sp. Pl.: 576 (1753)	*M. virdis* subsp. *angustifolia* (Lej. & Courtois) Briq.	Spearmint, garden mint, common mint	MARS & PFPN	2022
3	*Plectranthus amboinicus* (Lour.) Spreng. Syst. Veg. 2: 690 (1825)	*Coleus amboinicus* Lour.	Indian mint, Indian borage, soup mint	PFPN	2022
4	*Plectranthus scutellarioides* R. Br. Prodr. Fl. Nov. Holland.: 506 (1810)	*Coleus scutellarioides* Benth. *Ocimum scutellarioides* L.	Common coleus, painted nettle	PFPN	2022/ 2023
5	*Ocimum basilicum* L. Sp. Pl.: 597 (1753)	*O. basilicum* var. *basilicum*	Sweet basil, cinnamon basil, Saint Johseph's Wort	PFPN	2022
6	*Ocimum basilicum* var. *purpurascens* Benth. Pl. Asiat. Rar. 2: 13 (1830)		Purple basil, Rupin basil, purple ruffles	PFPN	2022/ 2023

MARS and PFPN: Al-Marashda cultivated plants farm and a private garden of flowering plants nursery, respectively.

2.2. Microscopic techniques and measurements

Healthy leaf pieces were selected from 3-5 plants of each taxon and placed in test tubes then boiled with lactic acid 88% in a water bath at 100 °C to remove the cuticle and soften the leaf tissue to investigate foliar micromorphology (Clarke, 1960). The samples were transferred to Petri dishes and with the use of fine forceps and dissecting needle the upper and lower epidermises were separated and stripped on dry clean glass slides (Ibrahim and Ayodele, 2013). This made it easy to prepare semi-permanent slides for epidermal layers from the leaf's upper and lower surfaces which were mounted with Canada Balsam. Foliar epidermal cells, stomata characters and trichome types were investigated subsequently. Photomicrographs were taken using Leica DM1000 light microscopy (LM) equipped by ToupView digital camera, two magnifications power were applied and expressed by (×10 & ×40).

For studying nutlets' macro- and micro-morphological characters, 15-30 nutlets for each taxon were taken from 3-5 healthy and fully mature plants. The specimens were studied by a conventional technique using a stereomicroscope (SM) and scanning electron microscope (SEM). Nutlets samples were placed on a millimetre paper to adjust the calibration of size when investigated by SM. Specimens were examined with an Olympus SZX7 dissecting stereomicroscope and photographed with the aid of Samsung ST72 HD digital camera. This investigation was applied for all the studied taxa except for the species *Plectranthus amboinicus*, since its nutlets were not available; this is mentioned in the data tables by NA- abbreviation. Both leaf upper and lower surfaces and mature nutlets were selected and prepared for SEM studies. They mounted directly on 12.5 nm diameter metallic stubs using portions of a double adhesive tape. The specimens were coated in a sputter coater with approximately 25 nm of gold-platinum for 10-20 seconds in a sputtering chamber of a JEOL JFC 1100 E ion sputtering equipment. They were observed and photographed by a JEOL JSM 5500 LV scanning electron microscope at the SEM-unit in the Central and Environmental Laboratory, South Valley University, Qena, Egypt. The quantitative measurements of foliar epidermal cells, trichomes, stomata, and nutlets characters from LM and SEM micrographs were obtained utilizing the program ImageJ v1.45 (Schneider *et al.,* 2012). The average of measurements was obtained from 30-35 readings per specimen. The quantitative values were introduced in a range on Minimum-Maximum then Figures were created by the average value.

2.3. Terminology

The terminology of foliar epidermal cells and nutlets traits based on (Dilcher, 1974; Stearn, 1983) with some modifications by the author if needed.

2.4. Statistics

Statistical software ASSISTAT Version 7.7 was used to carry out principal component analysis (PCA). To determine the degree of genetic variation, the PCA was employed. The PCA yielded eigenvalues, which were utilized to ascertain the axes' and their corresponding characters' respective discriminative powers (Pradhan *et al.,* 2011). The genotypes were compared using cluster analysis and categorized using a biplot diagram.

3. Results

3.1. Foliar micromorphology

The foliar epidermal cell shape showed a moderate significant variation among the studied taxa (Table 2). However, it is polygonal in *Mentha longifolia*, elongated in *Plectranthus scuttelarioides*, and irregularly in both plants. In the rest taxa they are 4-5-gonal except in *M. spicata* these are elongated to 4-5-gonal and irregularly (Fig. 2).

Table 2. Foliar epidermal cell and stomata characterization in some studied cultivated taxa of Lamiaceae Martinov

Taxa	Epidermal cells (EPC)						
	Anticlinal walls		Periclinal walls		Leaf surface texture	EPC-Shape	Epicuticular waxes
	Pattern	Surface	Shape	Surface			
M. longifolia	Raised-channelled/ sinuate	Smooth	Flat to convex	Finely verrucate	Crested to ruminate	Polygonal irregularly	Few/ particles
M. spicata	Raised/ straight to undulate	Smooth	Flat	Smooth	Regulate to reticulate	Elongated to 4-5-gonal/ irregularly	Dense/ particles & granules
P. amboinicus	Raised/ straight to slight undulate	Smooth	Flat	Tuberculate	Rugose to reticulate	4-5-gonal/ irregularly	± Dense/ particles & granules
P. scutellarioides	Raised-channelled/ straight	Smooth to foveolate	Flat	Rugulate	Rugose to reticulate	Elongated irregularly	Dense/ particles & granules
O basilicum	Raised/ straight to slight undulate	Smooth	Flat to concave	Smooth	Ruminate	4-5-gonal	± Dense/ particles & granules
O. basilicum var. *purpurascens*	Raised/ straight to slight undulate	Smooth	Flat to convex	Tuberculate to regulate	Reticulate	4-5-gonal	Few/ particles & granules

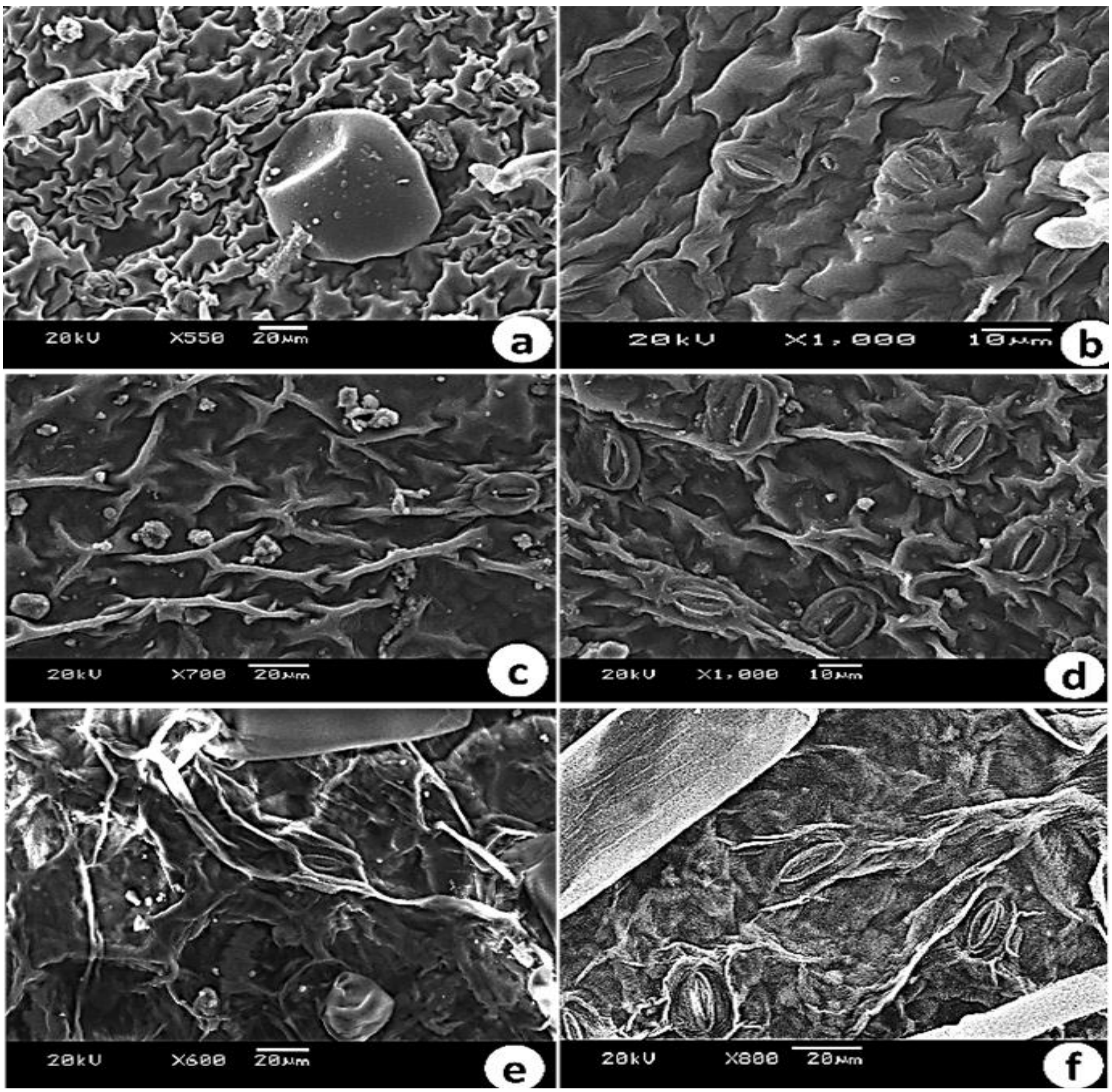
The surface of leaves is characterized by the presence of epicuticular waxes in shapes between particles and granules with variable density (Fig. 2-c; Fig. 2-a, b, d). Furthermore, there is a noticeable variation in the whole leaf surface texture (Fig. 2). Leaf surface has a high taxonomic impact. It varied from crested to ruminate in *Mentha longifolia*, rugulate to reticulate in *M. spicata*, ruminate in *Ocimum basilicum* and reticulate in *O. basilicum* var. *purpurascens.* As well as rugose to reticulate in both *Plectranthus amboinicus* and *P. scutellarioides* (Table 2).

The pattern of the anticlinal walls boundaries is of less systematic value among the studied taxa. Meanwhile, they raised in all taxa and sometimes channeled as in *M. longifolia* and *P. scutellarioides* (Table 2, Fig. 2). Nonetheless, there is a clear taxonomic heterogeneity in the orientation of these walls. *P. scutellarioides* had straight walls, but *M. longifolia* had sinuate walls (Fig. 3). In two taxa of *Ocimum basilicum* and *P. amboinicus*, there were straight to slightly undulate. In *M. spicata*, it was straight to undulate (Fig. 2).

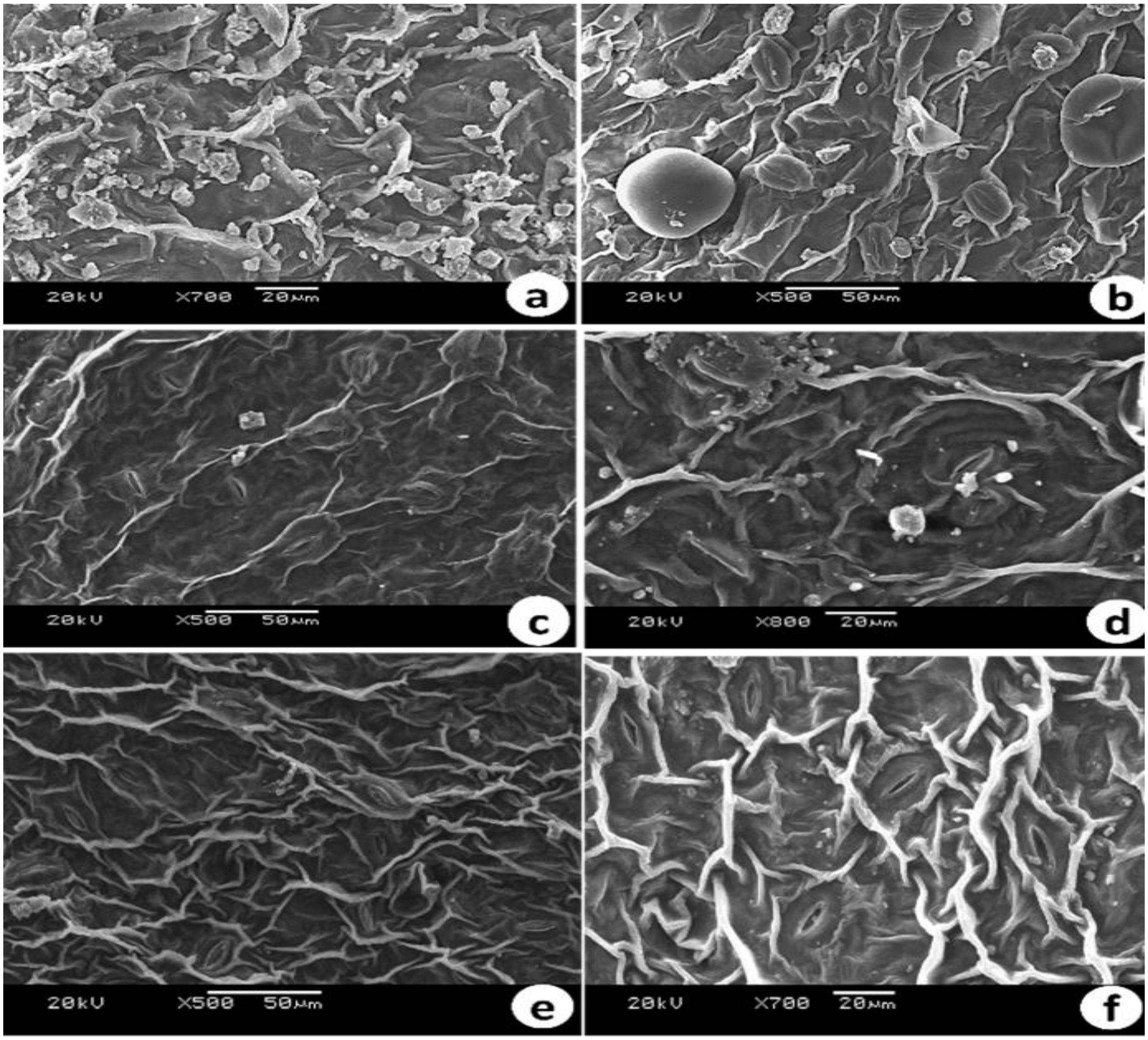
The anticlinal walls surface has a less systematic value, was smooth in all studied taxa except in *P. scutellarioides,* was smooth to foveolate. As shown in Table (2), three shapes of the periclinal walls are resulted in Flat (*M.spicata*, *P. amboinicus*, and *P. scutellarioides*), flat to convex (*M. longifolia* and *O.basilicum var. purpurascens*), and flat to concave (*O. basilicum*). Figure 1 illustrated that the surface of these walls is also of a diagnostic value, *i.e*., smooth (*M. spicata* and *O. basilicum*), Finely verrucate (*M. longifolia*), tuberculate (*P. amboinicus*) and rugulate (*P. scutellarioides*) as well as tuberculate to rugulate (*O. basilicum var. purpurascens*). The stoma types that were presented in the species under study did not differ all that much. All taxa have diacytic stomata, except for *P. scutellarioides*, which possesses anomocytic stomata (Fig. 2-f). *O. basilicum* var. *purpurascens* has anomocytic stomata in addition to diacytic stomata, while *O. basilicum* possesses anicocytic stomata as well. (Fig. 2-d., f).

The stomata complex size varied from the largest 29.69 x 19.48 µm in *P. scutellarioides* to the smallest 16.88 × 12.08 µm in *M. longifolia*. The stomatal index is of a high taxonomic relevance. It’s almost varying between the lower and upper surfaces of the same taxa, and conventionally it’s more among the lower surface in all taxa except in *O. basilicum* var. *purpurascens* (Fig.2). In this variety the upper surface stomatal index was 27.27 % while the lower index was 24.99 % (Fig.3). The highest stomatal index was among lower and upper surfaces of *O. basilicum*; 47.72 % and 35.77 % respectively. The lowest index is in *M. longifolia* lower and upper surfaces as 19.58 % and 8.65 % respectively.

Figure 2. SEM-micrographs for magnifications showing the structures of the foliar epidermal cells, anticlinal and periclinal epidermal walls and stoma types and structures in the taxa:



Mentha longifolia (a, b), *M. spicata* (c, d), P. *amboinicus* (e-f). Upper leaf surfaces (a, c, e), lower surfaces (b, d, f).



P. scutellarioides (a-b), *Ocimum basilicum* (c-d) and *O. basilicum* var. *purpurascens* (e-f). Upper leaf surfaces (a, d, f), lower surfaces (b, c, e).

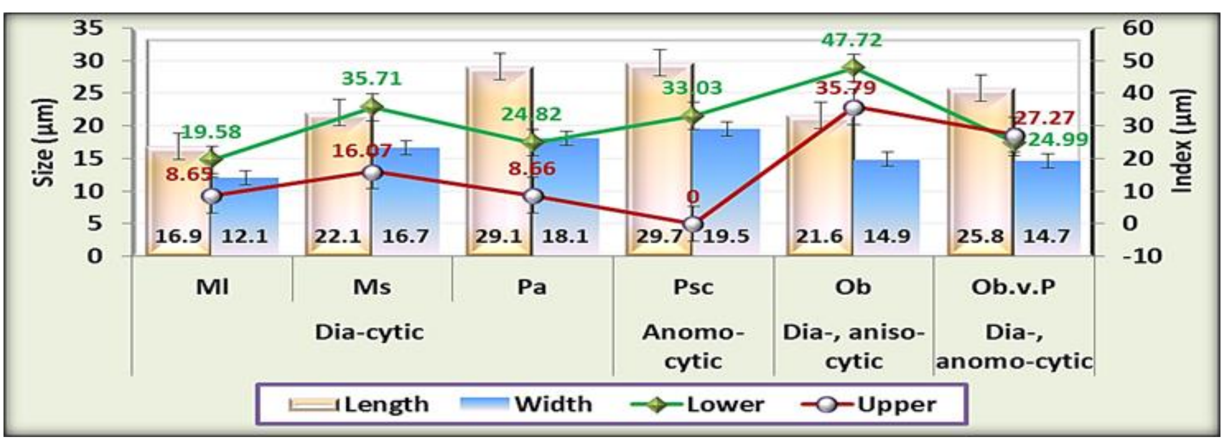


Figure 3. Type, size (length and width) and index % (Lower and Upper) of stomata. Ms, Ml, Pa, Ps, Ob and ob.v.p: *M. Spicata*, *M. longifolia*, *P. amboinicus*, *P. scutellarioides*, *O basilicum* and *O. basilicum* var. *purpurascens*, respectively.

Artificial key to taxa of Lamiaceae was created based on the foliar micromorphological characteristics as followed:

1a: Leaf texture reticulate, stomatal index on upper surface more than the lower surface included *O. basilicum* var. *purpurascens*

1b: Leaf texture otherwise than reticulate, stomatal index on lower > upper surface included *M. spicata*

2a: Leaf texture rugulate or rugose to reticulate, stomata of one type: diacytic or not included *P. scuttellarioides*

2b: Leaf texture ruminate or crested to ruminate, stomata of one or two types: diacytic or diacytic and another type included *P. amboinicus*

3a: Texture ruminate, stomata diacytic and anisocytic, anticlinal walls straight to slight undulate included *O. basilicum*

3b: Texture crested to ruminate, stomata diacytic, anticlinal walls sinuate included *M. longifolia*

4a: Texture rugose to reticulate, stomata anomocytic included *P. scuttellarioides*

4b: Texture rugulate or rugose to reticulate, stomata diacytic included *O. basilicum*

5a: Periclinal walls smooth, stomata length more than 25 µm, texture rugulate to reticulate included *P. amboinicus*

5b: Periclinal walls tuberculate, stomata length less than 25 µm, texture rugose to reticulate included *M. spicata*

3.2. Trichomes

Both eglandular and glandular trichomes were found in the six studied taxa with relatively variant distributions (Table 3). Different shapes of resulting trichomes have been introduced in the following synopsis:

3.2.1. Eglandular hairs

Two types of eglandular hairs were recognized, unicellular hairs and multicellular hairs. Unicellular hairs were of slender-shaped body and embedded non-globose basal cells. These were rarely present in the lower surface of *Mentha spicata* Fig (4- b). Secondly, the multicellular linear-shaped hairs range from 2 to 5 or 3-5-celled with a normal, embedded, and globose base cell. This type of linear hair was articulated in *Plectranthus amboinicus* Figs (4-c, 4-e) while flattened in all the rest taxa. Another form was relatively longer linear-shaped hairs (3-6-celled) with a rosette-shaped multicellular of 5-6 epidermal basal cells surrounding the normal globose embedded base.

3.2.2. Glandular hairs

Two distinct glandular hairs form and two subforms were defined in the taxa of Lamiaceae here. The hair of a simple head was unicellular subglobose gland with neck-shaped short stalk (Figs 5-d, e). This form was distinguished in both leaf surfaces of *P. amboinicus*. Mushroom-shaped glandular hairs were of unique form found in almost all the investigated taxa except *P. amboinicus*. This mushroom form had a short cylindrical-shaped stalk cell with an annulus Fig (5- a). Its head was multicellular gland in two distinct forms. The first common form was an umbrella-shaped multicellular head of mostly 7-8 or 8-9 celled Figs (4- a, f), (2- b) and (6), this form was identified by the term large glands (Table 3) as were observed in the species *Mentha longifolia* (7.5 cells), *M. spicata* (8.5 cells) and *Ocimum basilicum* (upper surface, 7.5 cells). The second form, the small glands, was of a smaller number of head cells, 2-4 celled with different-shapes head Fig (2- c) as in *P. scutellarioides* (globose to elliptic-shaped, 3 cells), *O. basilicum var. purpurascens* (umbrella-shaped, 3 cells) and *O. basilicum* (Elliptic- shaped in lower surface, 2.5 cells).

This form was found in the upper surface of *Ocimum basilicum* leaf and in fewer amounts in its lower surface. Observations pointed out that the direction of the eglandular hairs takes one of three directions, i.e., curved or upright, curved, and/or appressed.

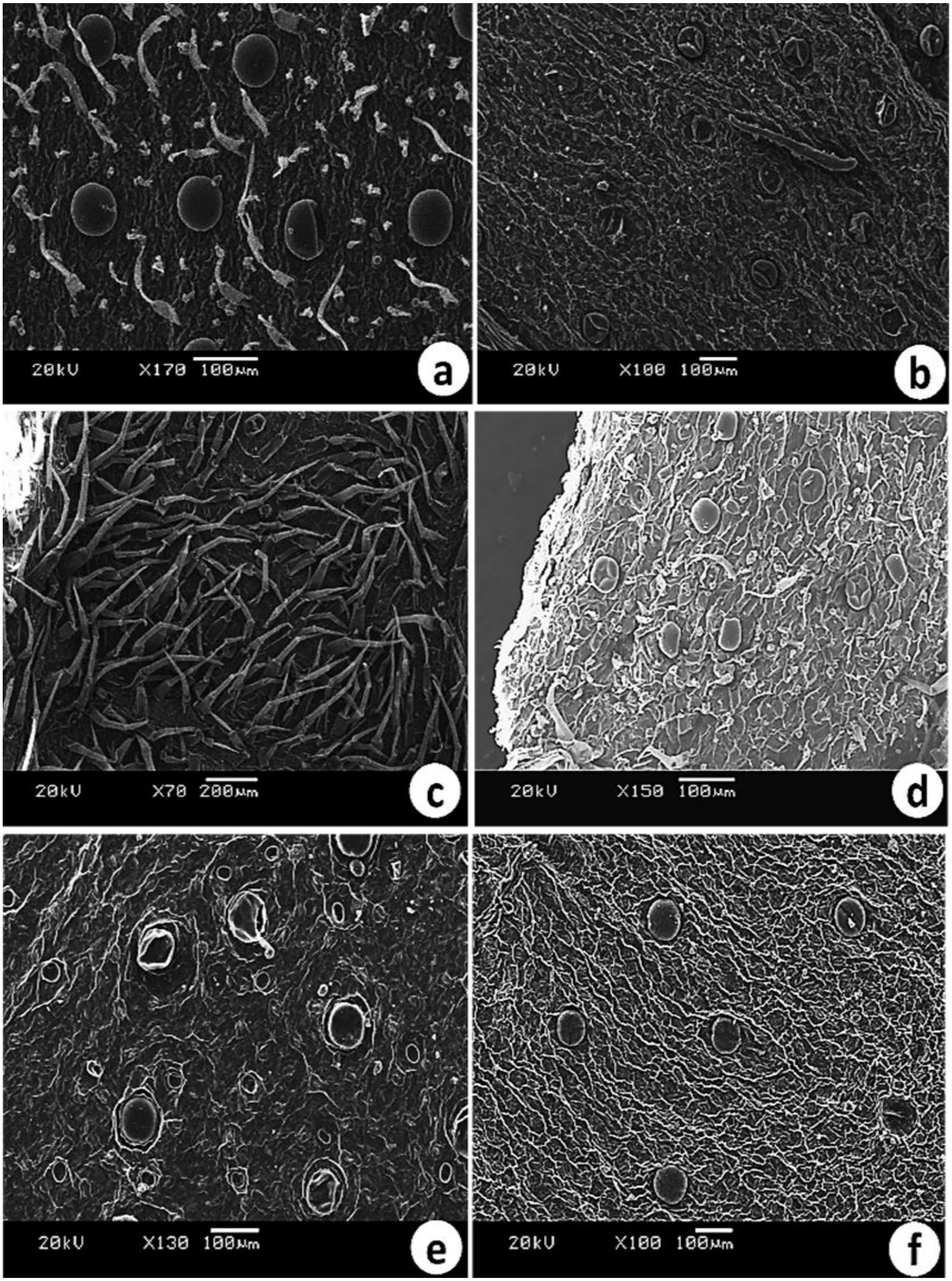


Figure 4. SEM-micrographs for the overview of leaf lower surfaces of G1 (a), G2 (b), G3 (c), G4 (d), G5 (e), and G6 (f). G1: *Mentha longifolia*, G2: *M. spicata*, G3: *Plectranthus amboinicus*, G4: *P. scutellarioides*, G5: *Ocimum basilicum* and G6: *O. basilicum* var. *purpurascens*.

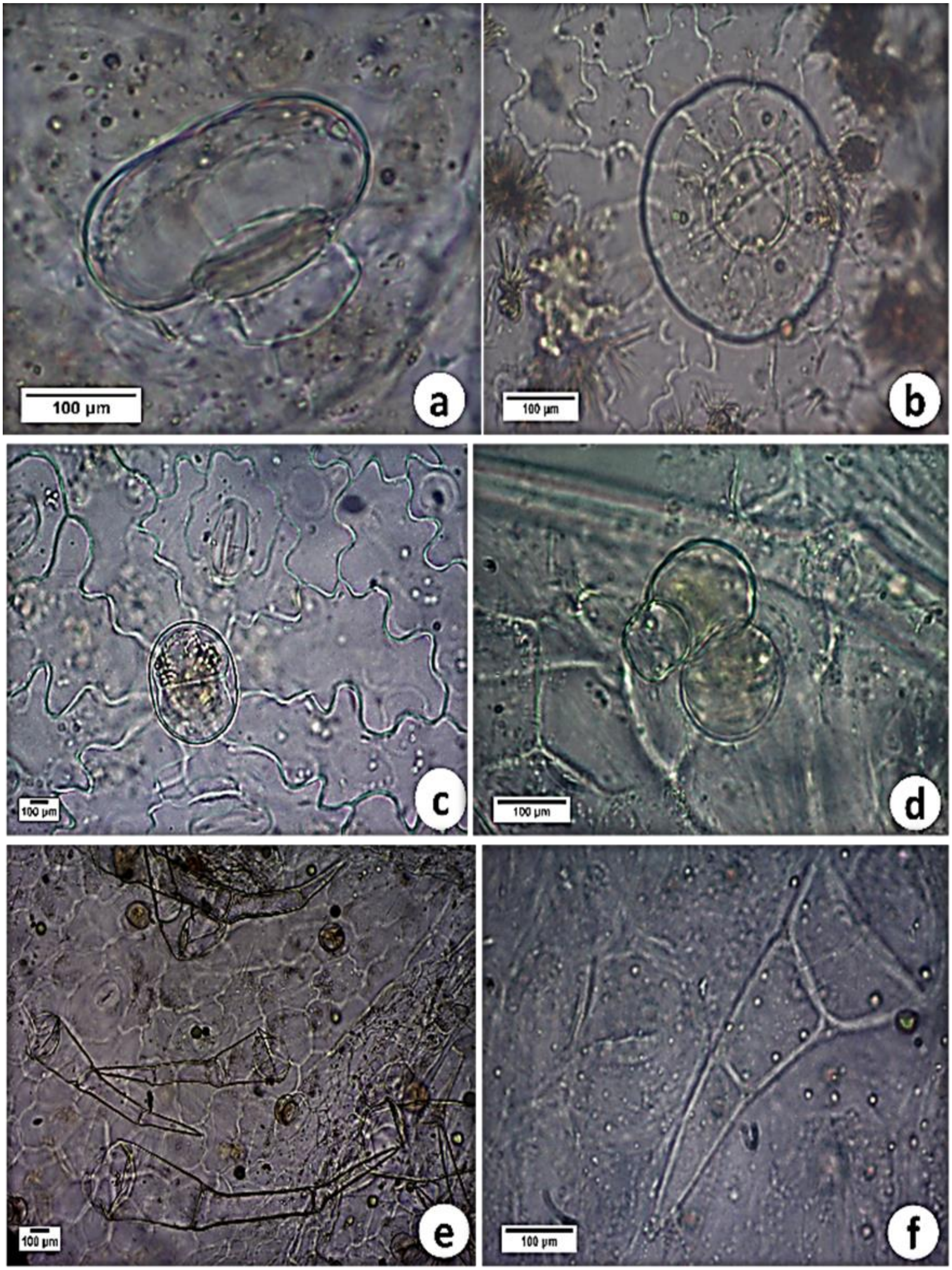


Figure 5. LM-photomicrographs illustrate the types of trichomes investigated within six cultivated taxa of Lamiacea. Glandular hairs with multicellular heads & multicellular basal cells; Rosette-shaped base (a, b, c). Upper leaf surface of G2 (a) and G1 (b), G3 (d, e) and G4 (f) as well as lower leaf surface of G5 (c**)**.

Table 3. Morphological characterization of trichomes in leaf surfaces of some studied cultivated taxa of Lamiaceae Martinov

Taxa	Eglandular hairs (EG)		Glandular hairs (G)		Distribution/ density		
	Direction	Base	Stalk	Base	Surface	EG-Hairs	G-Hairs
M. longifolia	Curved or upright	Normal**	SCWA*	normal	U L	dense denser	+ -
M. spicata	Appressed	Embedded/ not globose	SCWA	normal	U L	- rare	+ -
P. amboinicus	Curved and appressed	normal	Neck-shaped/ short	normal	U L	dense denser	+ +
P. scutellarioides	Curved and appressed	normal	SCWA	normal	U L	+ dense	+ +
O basilicum	Curved or upright	Rounded-globose with a rosette of 5-6-epidermal cells	SCWA	normal + 13-15-rosette epidermal base	U	+	+
			SCWA	normal + 7-8- rosette epidermal base	L	fewer	+
O. basilicum var. *purpurascens*	Curved or upright	normal	SCWA	normal	U L	few -	+ +

**SCWA: Short cylindrical with annulus, **normal: Embedded/ globose cell*

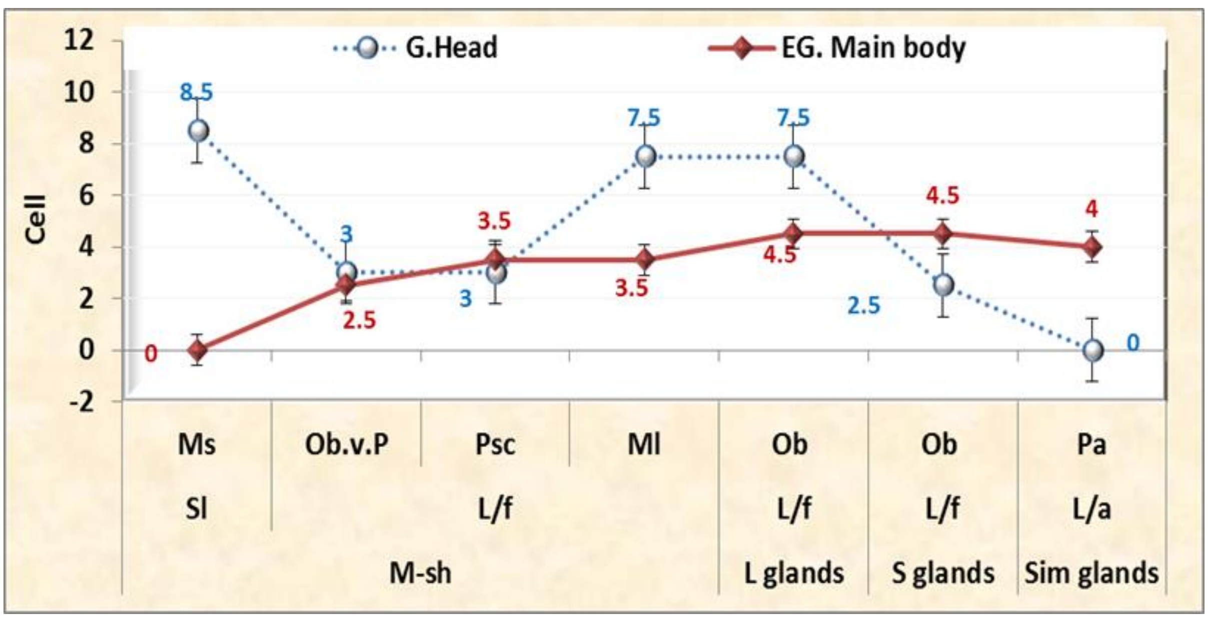


Figure 6. Shape and main body of Eglandular (EG) as well as shape and head of Glandular (G) hairs, zero refers to unicellular hair, L/f and L/a: linear flattened and articulated, respectively, L, S and Sim glands: large, small and simple glands, respectively, M-sh: Mushroom-shaped. Ms, Ml, Pa, Ps, Ob and ob.v.p: *M. Spicata, M. longifolia, P. amboinicus, P. scutellarioides, O basilicum* and *O. basilicum* var. *purpurascens*, respectively.

An artificial trichomes taxonomic key for separating the Lamiaceae taxa is presented as followed:

1a: Eglandular hairs multicellular: linear-shaped included *M. longifolia*, *P. amboinicus*, *P. scutellarioides*, *O basilicum* and *O basilicum var. purpurascens*

1b: Eglandular hairs unicellular: slender-shaped included *M. spicata*

2a: Glandular hairs of unicellular head of simple gland included *P. amboinicus*

2b: Glandular hairs of multicellular head of mushroom-shaped gland included *M. longifolia*, *M. spicata*, *P. scutellarioides* and *O basilicum var. purpurascens*

3a: The head of the gland large, 7-8-celled included *M. longifolia*, *M. spicata* (both surfaces) and *O basilicum* (Upper)

3b: The head of the gland small, 2-4-celled included *O. basilicum* (lower) and *P. scutellarioides*, *O basilicum var. purpurascens* (both surfaces)

4a: Hair base cell normal: embedded globose cell included *M. longifolia*, *P. amboinicus*, *P. scutellarioides* and *O basilicum var. purpurascens*

4b: Hair base cell normal: embedded not globose cell included *M. spicata*

4c: Hair base cell normal + rosette: multicellular base included *O. basilicum*

5a: The head is Subglobose included *P. amboinicus*

5b: The head is globose to elliptic-shaped included *P. scuttellarioides*

5c: The head is Umbrella-shaped included *M. longifolia*, *M. spicata*, *O basilicum var. purpurascens* and upper surface of *O basilicum*.

3.3. Nutlets

The evaluation of the tested *Lamiaceae* taxa under a light microscope and scanning electron microscopy revealed that their outline, size, areole, color and nutlet coat was different (Table 4 and Figs. 10 &11). The outline shape of nutlet varied depending on the species, as there is an oblong-elliptic in *M. longifolia*, ovoid in *M. spicata*, sub-globose/ constricted basally (pearl-shell shaped) in *P. scutellarioides*, oblong-ovoid in *O. basilicum*, or elliptic ovoid in *O. basilicum* var. *purpurascens* (Figs. 7, 8, 9). Nutlets general shape varied between dorsal and ventral sides. Dorsal sides ranged from convex, flat or flat & keeled only in *O. basilicum* var. *purpurascens* (Figs. 9-f, g). The ventral side ranged between flat & keeled only in *Mentha longifolia* (Table 4), concave, or flat & concave basally.

Areole position was not of high diagnostic impact. It commonly basal to sub-basal in most taxa, and caruncled specially in *P. scutellaroides* and *Ocimum basilicum* (Table 4), while not caranuncled in two species of genus *Mentha*. Areole was caruncled or not in *O. basilicum* var. *purpurascens* (Table 4). The pattern of colouring was distinct feature since the nutlets of genus *Mentha* only were dichromed of brown and beige shades (Fig. 9) and the other taxa had monochromed nutlets (Fig. 9). The variety purpurascens of *O. basilicum* was characterized by brown colour while the nutlets of species *O. basilicum* was mostly black (Fig. 9-d, f). *P. scutellaroides* is characterized by differently greyish-brown shade. The size of the investigated nutlets ranged from large in *Ocimum basilicum* var. *purpurascens* (2.15 × 1.40= 3.01 mm^2) and O. *basilicum* (2.23 × 1.33 = 2.966 mm^2) to Smallest one in *Mentha longifolia* (0.58 × 0.40= 0.232 mm^2). However, both *M. spicata* (0.378 mm^2) and *Plectranthus scutellaroides* (0.469 mm^2) exhibited slightly larger nutlets size than those of *M. longifolia* (Fig. 11). The nutlets/seeds epidermal cells were commonly 4-6 or 5-6-gonal in almost all taxa. However, some variation was observed, epidermal cells of *Mentha longifolia* nutlets were circular to 5-6-gonal (Tab. 4; Fig. 7-b). The epidermal cells of the nutlets coat were irregularly in *Plectranthus scutellarioides* (Fig. 7-f), *Ocimum basilicum*, and *O. basilicum* var. *purpurascens* (Fig. 7-e, f). Moreover, the number of boundaries of cells up to polygonal in both taxa of *Ocimum basilicum*.

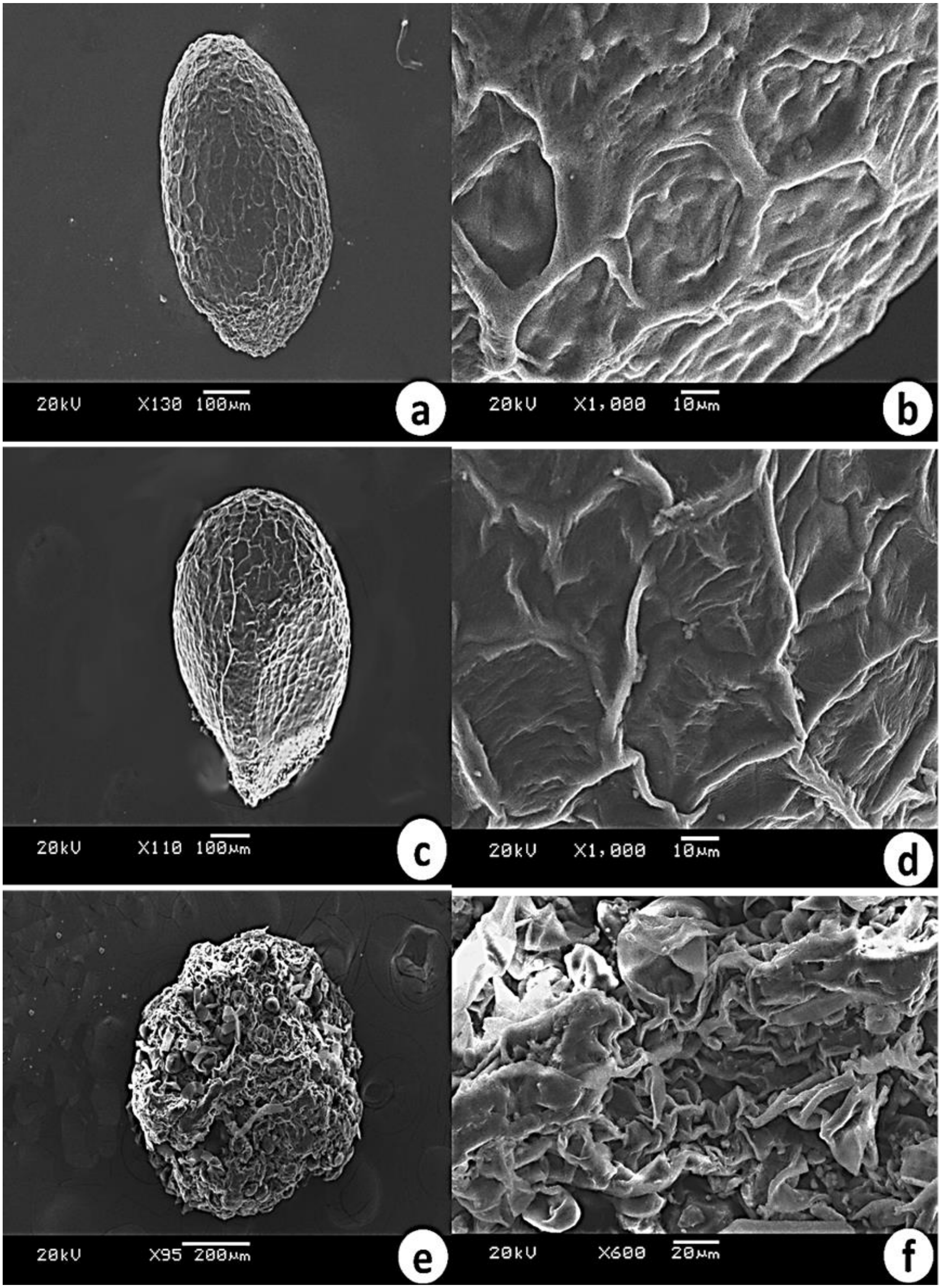


Figure 7. SEM micrographs of Lamiaceae taxa nutlets. Side-dorsal view of whole nutlet (a, c, e), nutlet coat texture (b, d, f). *Mentha longifolia* (a–b), *M. spicata* (c–d), *Plectranthus scutellarioides* (e–f).

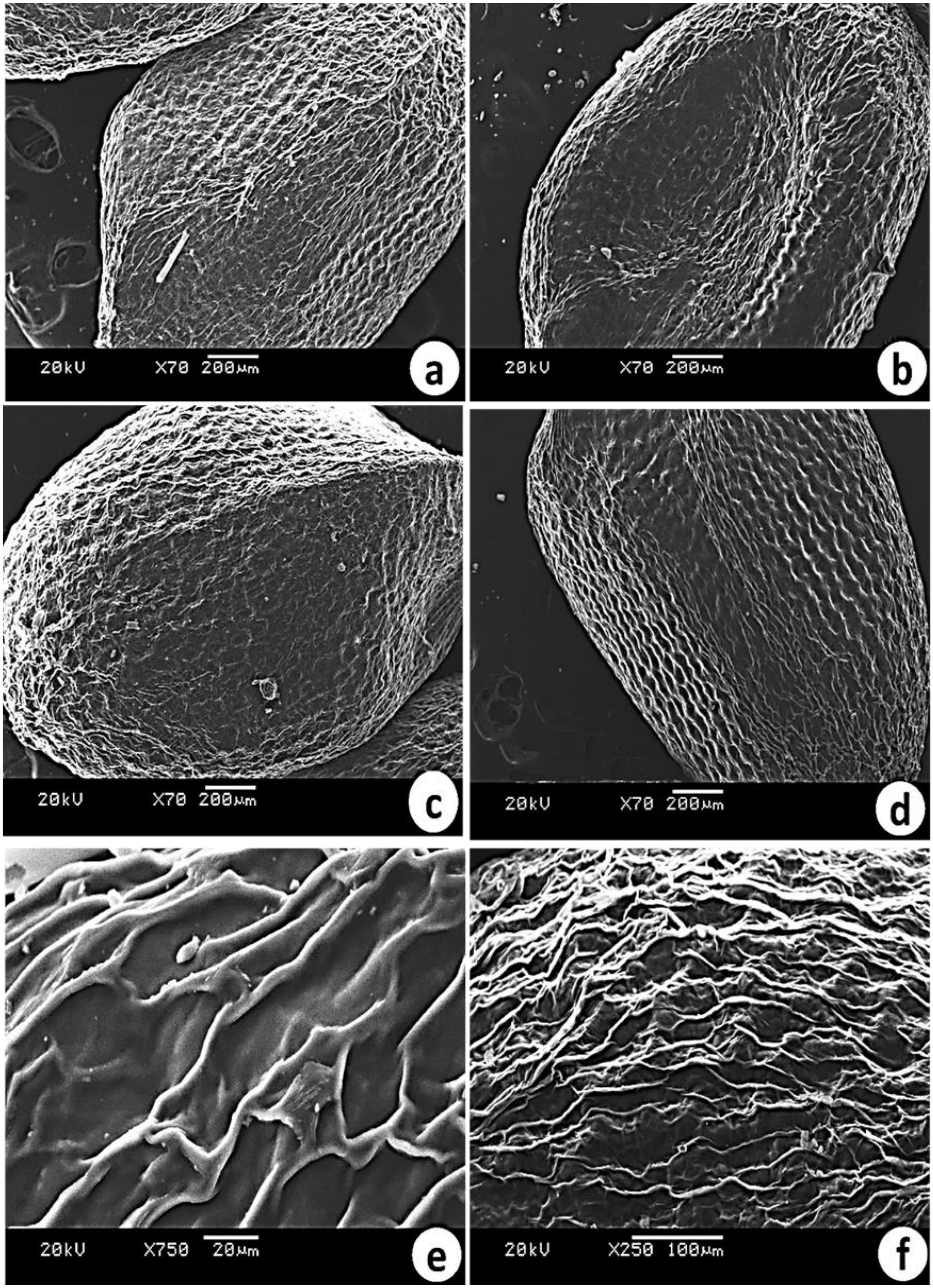


Figure 8. SEM micrographs of Lamiaceae taxa nutlets. Dorsal view of whole nutlet (a, c), ventral view of whole nutlet (b, d), nutlet coat texture (e, f). *Ocimum basilicum* (a, c, e), *O. basilicum var. purpurescens* (b, d, f).

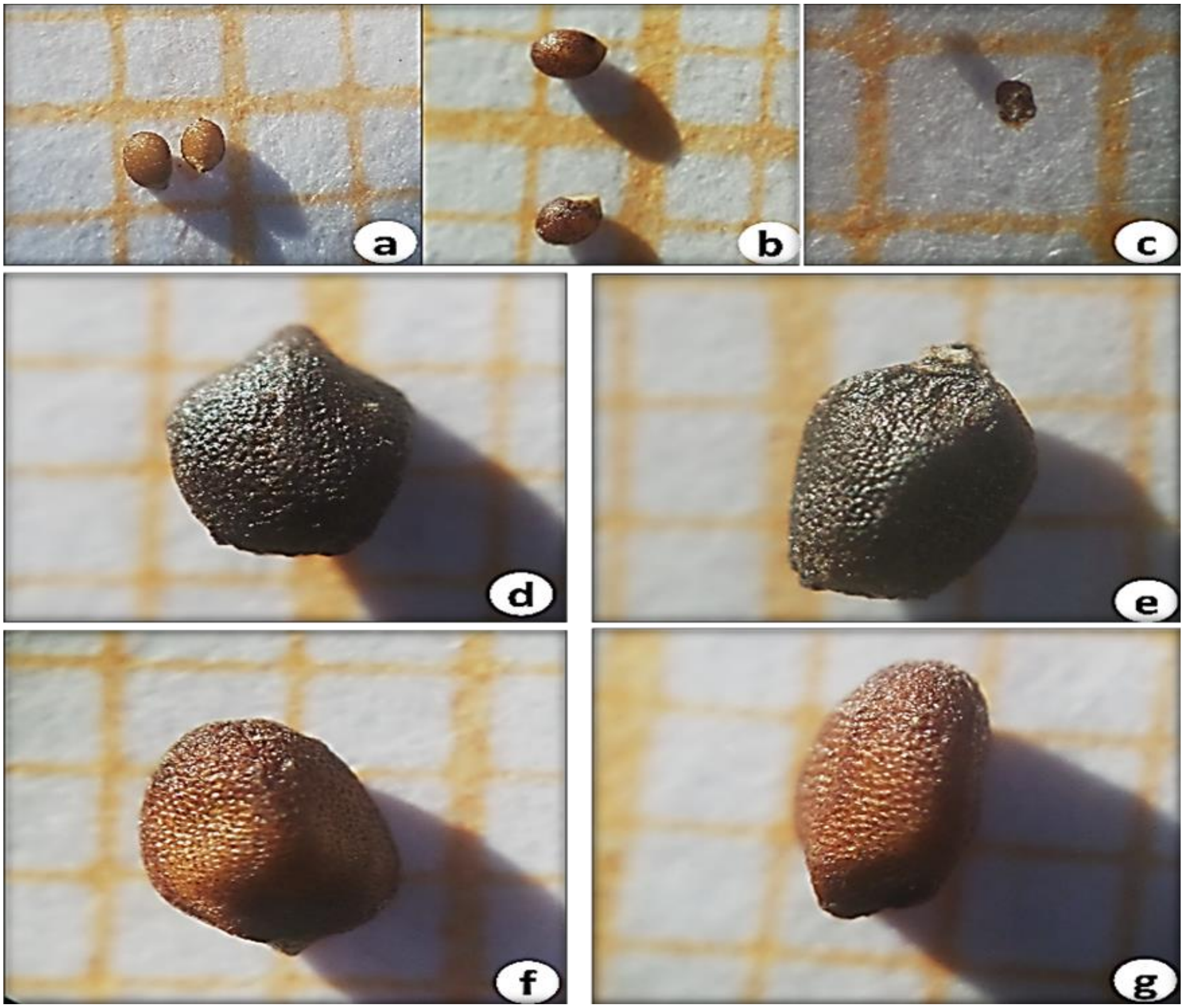


Figure 9. SM photomicrographs of whole nutlets of taxa of Lamiaceae. Dorsal and ventral views (a,b), dorsal view (c, d), ventral view (e, f), side-dorsal view (g). *Mentha longifolia* (a), *M. spicata* (b), *Plectranthus scutellarioides* (c), *Ocimum basilicum* (d, e), and *O. basilicum* var. *purpurascens* (f, g).

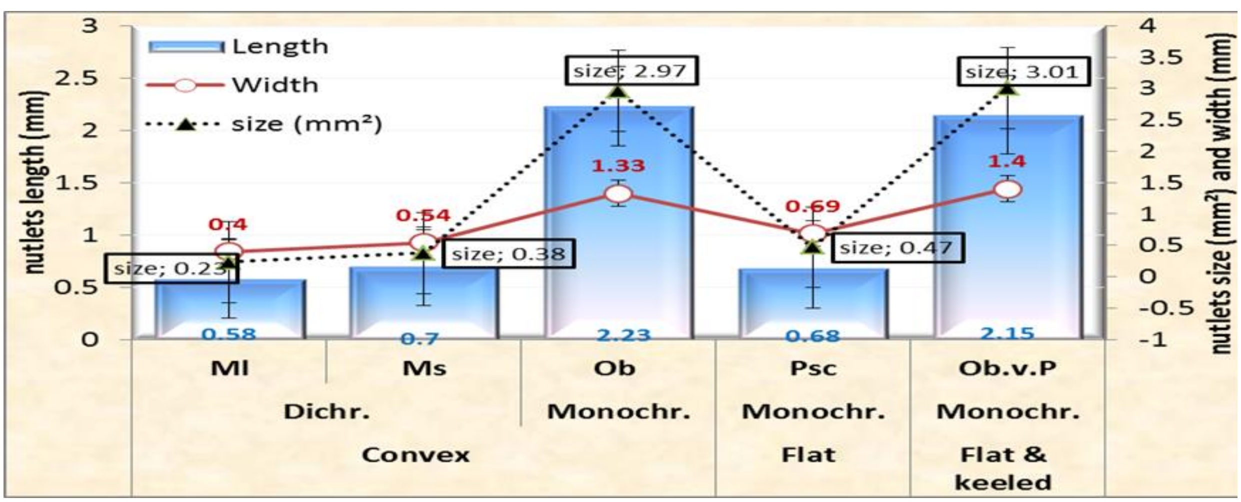


Figure 10. Nutlets length (mm), width (mm), Size (mm^2), Outline (Dorsal) and Coloration (Pattern). Di-and Mono-chr.: Dichchromed and Monochromed. Ms, Ml, Pa, Ps, Ob and ob.v.p: *M. Spicata*, *M. longifolia*, *P. amboinicus*, *P. scutellarioides*, *O basilicum* and *O. basilicum* var. *purpurascens*, respectively.

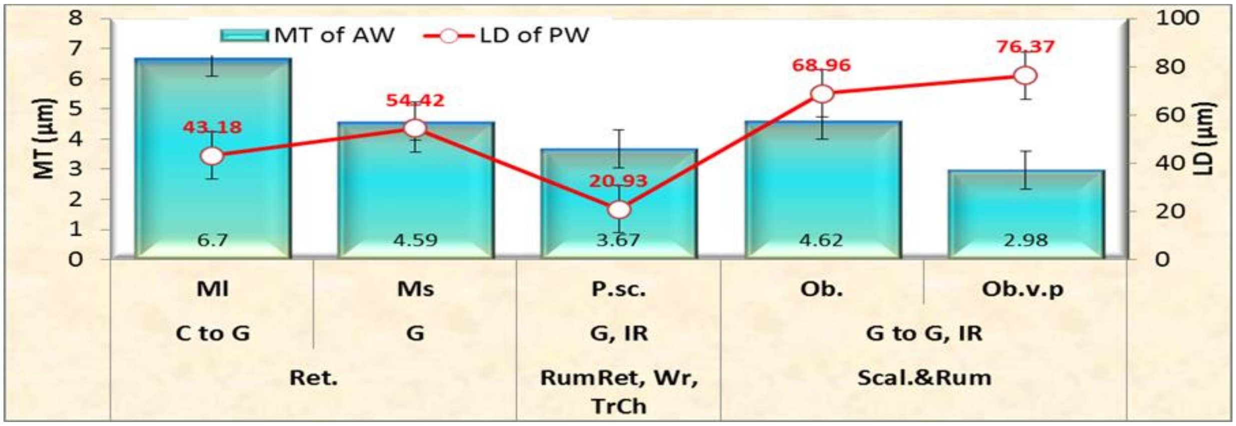


Figure 11. Testa texture pattern and EPC-Shape as well as Muri thickness (MT) and lumen diameter (LD) of Anti-(AW) and peri-(PW) clinal walls, respectively of nutlet coat; *C: Circular, G: gonal, IR: irregularly, Rum: ruminate, Ret: reticulate, Wr: wrinkled, TrCh: trichomes, Scal: scalariform*. Ml: *M. longifolia*, Ms: *M. spicata*, P.sc.: *Plectranthus scutellarioides*, Ob. *O. basilicum* and Ob.v.p: *O. basilicum* var. *purpurascens.*

Table 4. Nutlets macro- & micromorphological characteristics in some studied cultivated taxa of Lamiaceae Martinov; G1: *M. longifolia*, G2: *M. spicata*, G3: *P. amboinicus*, G4: *P. scutellarioides*, G5: *O basilicum* and G6: *O. basilicum* var. *purpurascens*

Taxa	Outline		Areole position	Color Shade	EPC-Shape	Nutlet coat			
						Anticlinal walls (AW)		Periclinal walls (PW)	
	Shape	Ventral				Pattern	Surface	Form	Surface
G1	Oblong-elliptic	Flat to keeled	Sub-basal to basal/ not caruncled	Brown & beige basally	Circular to 5-6-gonal	± Raised	Smooth	Flat	Tuberculate-rugulate
G2	Ovoid	Concave	Sub-basal / not caruncled	Brown & beige basally	5-6-gonal	Slightly raised	Smooth	Flat to concave	Tuberculate-rugulate; tubercles finely striated
G3	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
G4	Sub-globose/ constricted basally (pearl-shell shaped)	Concave	Basal/ caruncled	Greyish-brown	4-6-gonal, irregularly	± Undulat, slightly raised	Smooth to finely granulate	Flat to concave	Rugulate-ruminate, finely tuberculate
G5	Oblong-ovoid	Concave	Basal/ caruncled	Dark brown to black	4-6- to polygonal, irregularly	Slightly raised	Smooth	Flat to concave	Smooth
G6	Elliptic ovoid	Flat & concave basally	Basal/ caruncled or not	Brown, notched	4-6- to polygonal, irregularly	Undulate	Smooth	Concave	Tuberculate

Three different patterns of nutlets coat texture have been recognized: reticulate, ruminate-reticulate, and scalariform and ruminate (Fig.11). However, it was reticulate in the two species of genus *Mentha* (Fig. 7-b, d). *M. spicata* had larger diameter of reticulum lumen (54.42 µm) than that of *M. longifolia* (43.18 µm) as shown in Fig.11. The largest lumen diameter has been recorded in the scalariform-ruminate in *Ocimum basilicum* (68.96 µm), and *O. basilicum* var. *purpurascens* (76.37 µm). The unique texture of nutlet coat of *Plectranthus scutellarioides* was shown in (Fig. 7-f). This species was characterized by ruminate-reticulate wrinkled texture pattern, while the surface of the nutlets has some attached trichomes (Fig.11). All nutlets of the taxa studied are glabrous except the surface of *P. scutellarioides*. Trichomes have been observed on its surface. The anticlinal walls (AW) boundaries of the nutlet coat were mostly smooth in all the studied taxa, tended to finely granulate only in *Plectranthus scutellarioides* (Fig. 7- f). These walls were slightly/ more or less raised; this was not a valuable difference (Table 4). However, the boundaries muri thickness (MT) varied from 2.98 µm in *O. basilicum* var. *purpurascens* to 6.70 µm in *Mentha longifolia* (Fig.11). While this thickness differed in *O. basilicum* to be 4.62 µm and obviously differed from variety purpurascens.

The periclinal walls (PW) form wasn't highly variant in the studied taxa. They were flat to concave in all of them except in *M. longifolia* (flat) and in *O. basilicum* var. *purpurascens* (concave) (Table 4). These walls have been precepted that they were highly variant in their surface (Table 4). That is of high taxonomic value. Periclinal walls were smooth in *O. basilicum*, tuberculate in *O. basilicum* var. *purpurascens* (Fig. 8-e, f); distinctly rugulate-ruminate and finely tuberculate in *P. scutellarioides*. Otherwise, they were tuberculate-rugulate in *Mentha* species with some difference in *M. spicata* (Table 4; Fig. 7-b, d, f and Fig.11).

An artificial nutlets taxonomic key for separating the *Lamiaceae* taxa is presented as follows:

1a: Nutlets coloring monochrome, glabrous, or hairy included *P. scutellarioides, O basilicum* and *O. basilicum var. purpurascens*

1b: Nutlets coloring dichromed, glabrous included *M. longifolia* and *M. spicata*

2a: Outline ventrally concave, nutlet long < 0.75 mm included *M. spicata* and *P. scutellarioides*

2b: Outline ventrally concave, nutlet long >2 mm included *O basilicum*

2c: Outline ventrally flat to keeled, nutlet shorter up to 0.60 mm, oblong-elliptic included *M. longifolia*

3a: Nutlets dorsally and ventrally similar, size >1 mm2 included *O. basilicum var. purpurascens*

3b: Nutlets dorsally and ventrally different, size< 1 mm2 included *M. longifolia* and *M. spicata, P. scutellarioides*

4a: Nutlets oblong-ovoid, big size (about 3 mm2), dark brown tends to black included *O. basilicum*

4b: Nutlets elliptic-ovoid, big size (about 3 mm2), brown, notched included *O. basilicum var. purpurascens*.

3.4. Principal component analysis

The principal component analysis is important and could define most of the analyzed parameters, especially macro- & micromorphological parameters. PCA diagnosis is a multivariate approach providing a theoretical basis for further improvements in taxa classification diagnosis. Cluster analysis (based on the ten quantitative characteristics of stomata, trichomes in leaf surfaces and nutlet) grouped five cultivated Lamiaceae taxa into four clusters as shown in Table 5 and the mean value of all variables in each cluster is presented in Table (6).

Table 5. Clustering patterns of the five cultivated taxa of Lamiaceae Martinov based on some quantitative characteristics.

Clusters		Genotypes		
		No.	Percentage	included
I	(Y1 >= 0, Y2 >= 0)	1	20%	*O. basilicum var. purpurascens*
II	(Y1 >= 0, Y2 < 0)	1	20%	*O. basilicum*
III	(Y1 < 0, Y2 < 0)	2	40%	*M. longifolia* and *M. spicata*
IV	(Y1 < 0, Y2 >= 0)	1	20%	*P. scutellarioides*

The third cluster (III) has two genotypes accounting for 40% of the total 5 genotypes followed by the other three clusters (20% each) as shown in Table (5). As shown in Table 6, the *O. basilicum* genotype of cluster II was prevalent in 70% compared to the other clusters.

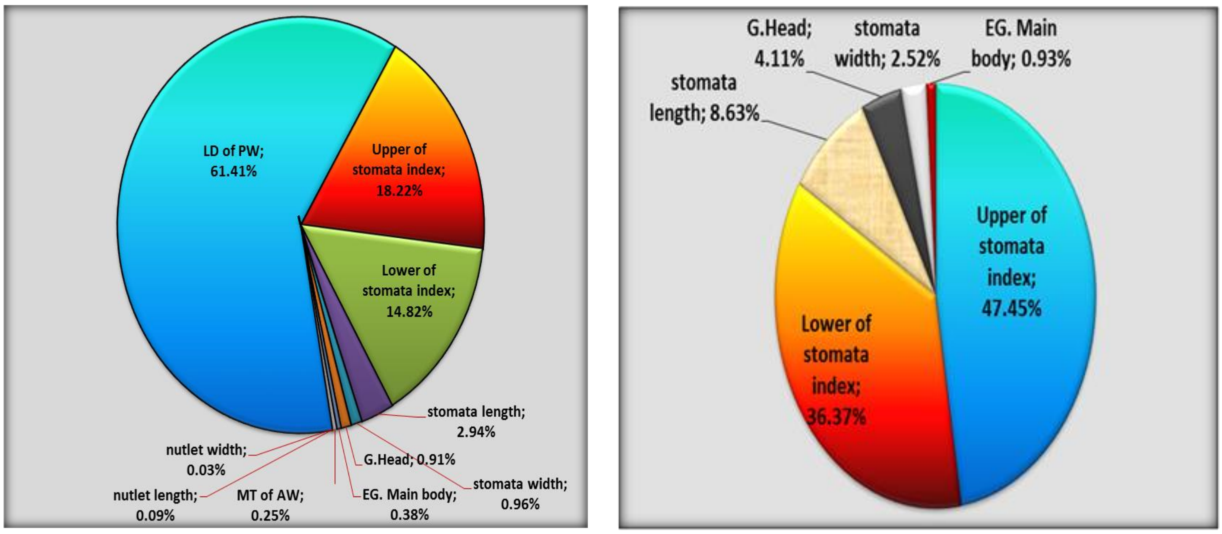
Table 6. The mean value of all studied variables for each cluster along with the difference between each cluster mean and total mean.

Characters*	PC1		PC2		PC3		PC4	
	Value	difference	Value	difference	Value	difference	Value	difference
SL	25.77	2.58	21.56	-1.63	19.48	-3.71	29.66	6.47
SW	14.65	-0.892	14.85	-0.692	14.365	-1.177	19.48	3.938
LSI	24.99	-7.216	47.72	15.514	27.645	-4.561	33.03	0.824
USI	27.27	7.976	35.79	16.496	12.36	-6.934	8.69	-10.604
GH, head	3	-2.9	7.5	1.6	8	2.1	3	-2.9
EG. Main body	2.5	-0.3	4.5	1.7	1.75	-1.05	3.5	0.7
NL	2.15	0.882	2.23	0.962	0.64	-0.628	0.68	-0.588
NW	1.4	0.528	1.33	0.458	0.47	-0.402	0.69	-0.182
MT of AW	2.98	-1.532	4.62	0.108	5.645	1.133	3.67	-0.842
LD of PW	76.37	23.598	68.96	16.188	48.8	-3.972	20.93	-31.842
High	50%		70%		20%		30%	
Medium	0%		10%		0%		10%	
Low	50%		20%		80%		60%	

***SL: stomata length, SW: stomata width, LSI: Stomata index in Lower, USI: Stomata index in upper, GH: Glandular hairs, EG, Eglandular hairs, NL: nutlet length, NW: nutlet width, MT: Muri thickness, AW: Anticlinal walls, LD: Lumen diameter, PW: Periclinal walls.**

However, it had the highest in cluster mean values for stomata index% in lower and upper surfaces, the main body of glandular hairs, head of the glandular hair, nutlet length and width as well as Lumen diameter of Periclinal walls indicating that this genotype can be extensively used for further planting programs to obtain good characteristics under upper Egypt conditions followed by PC-1 (*O. basilicum* var. m) which had the highest values for nutlet length and width whereas cluster IV (*P. scutellarioides*) was the longest and widest of stomata, however, the third cluster (PC-III) which included both *M. Longifolia* and *M. spicata* has an umbrella-shaped multicellular head of mostly 7-8 or 8-9 celled and the highest Muri thickness in anticlinal walls.

On the other hand, the current study found that out of the ten characteristics and their contributing traits, the proportionate contribution of the Lumen diameter of Periclinal walls towards the variance was found at 61.41% (Fig.12a) followed by stomata index in upper (18.22%) and lower (14.82%) surfaces as well as stomata length (2.94%) in descending order. However, other characteristics' proportionate contributions do not skip 0-<1%. Therefore, the trait of Lumen diameter of Periclinal walls would be an important parameter for selecting divergent genotypes based on stomata, trichomes in leaf surfaces, and nutlet macro- & micromorphological characteristics. As for the cluster analysis based on the only six variables (Fig.12b) of stomata and trichomes in leaf surfaces (stomata length, stomata width, stomata index in Lower and Upper surfaces, Head of glandular hairs, and Main body of glandular hairs with absent nutlets traits) which are taken included *P. amboinicus*, the study found that out of the 6 characteristics and their contributing traits, the proportionate contribution of the stomata index of upper (47.45%) and lower (36.37%) surfaces towards variance was found the highest (Fig. 12b) followed by stomata length (8.63%), Head of glandular hairs (4.11%), stomata width (2.52%) and EG. Main body (0.93%) in descending order. Therefore, the stomata index of upper or lower surfaces would be an important parameter for selecting divergent genotypes based on stomata and trichomes in leaf surfaces with or without including the nutlet macro- & micromorphological characteristics. Principal components analysis (PCA) was conducted to determine which traits were the major sources of variation within the treatment groups. The results of the PCA of the five genotypes based on 10 characteristics are presented in Figs.13, 14, 15. The results of Fig. 13 showed that nutlet width (NW), nutlet length (NL) and stomata index in upper (USI) leaf surface had the highest loadings in PC1, indicating their significant importance for *O. basilicum var. purpurascens*. The second component was negatively correlated with length (SL) and width (SW) of stomata (Fig. 13). Thus, the effect of the variation factor of these variables led to a reduction of its values while the values of other variables increased. G. Head had the highest positive loadings in both PCII (*O. basilicum*) and PCIII (*M. longifolia* and *M. spicata*) components. The third PC has a positive association with both Lower of stomata index along with G. Head and negative association with EG. Main body. LD of PW had the highest loadings in PCIV (*P. scutellarioides*) which has negative association with both stomata index in Lower surface and EG. main body.



a. stomata, trichomes and nutlet

b. stomata, trichomes without nutlet

Figure 12. Graphical representation of the proportionate contribution of studied stomata, trichomes and nutlet morphological characteristics toward different taxa of Lamiaceae Martinov

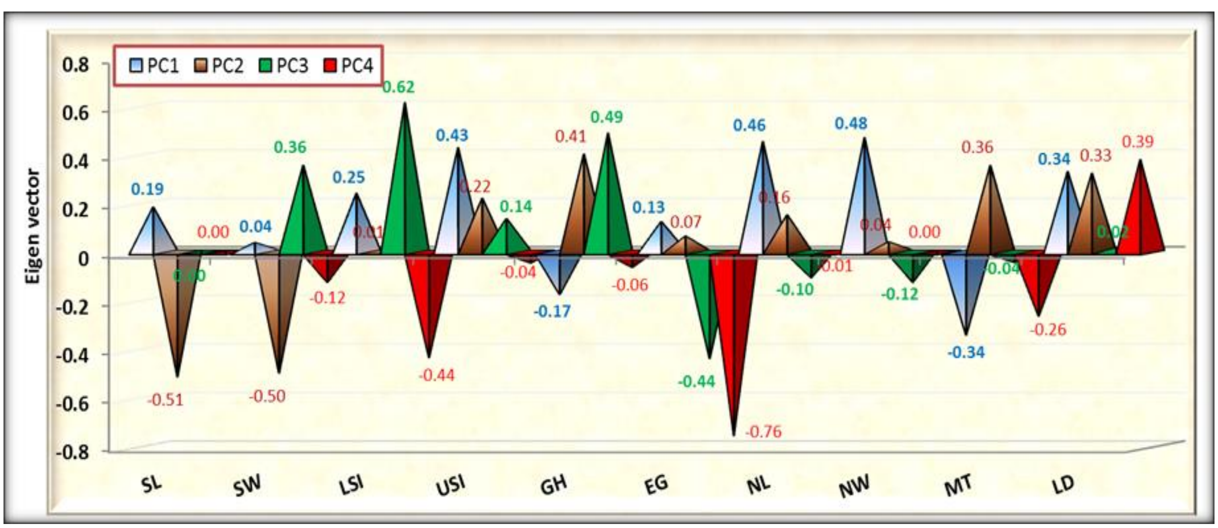


Fig. 13: Scree plot of Eigen vector for 10 stomata, trichomes and nutlet morphological characteristics. SL: stomata length, SW: stomata width, LSI: Stomata index in Lower, USI: Stomata index in upper, GH: Glandular hairs, EG, Eglandular hairs, NL: nutlet length, NW: nutlet width, MT: Muri thickness, LD: Lumen diameter.

The principal component analysis considered the first two factors, which had an accumulated eigenvalue of 74.95 % (Fig.14). The first and second principal components are a result of the linear combination of the 10 studied variables, and both explained 42.97 % and 31.98 % of the variance, respectively. The results obtained from biplot-PCA (Fig. 15) indicated the presence of high variations among the studied genotypes based on the 10 studied characteristics resulting in amplitude that may appear their affect the growth performance in Upper Egypt conditions. The biplot enabled the evaluation of the correlation level among the variables, with genotypes presenting the same site being more closely correlated.

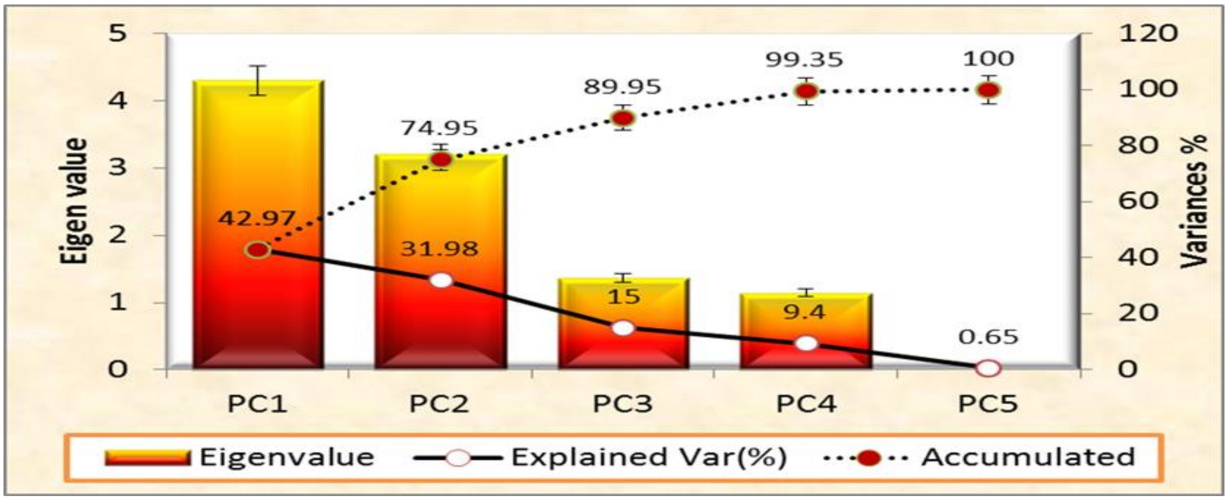


Figure 14. Scree plot of Eigen values explained (%) and accumulative variability (%) for 10 morphological related parameters of 5 Lamiaceae Martinov taxa.

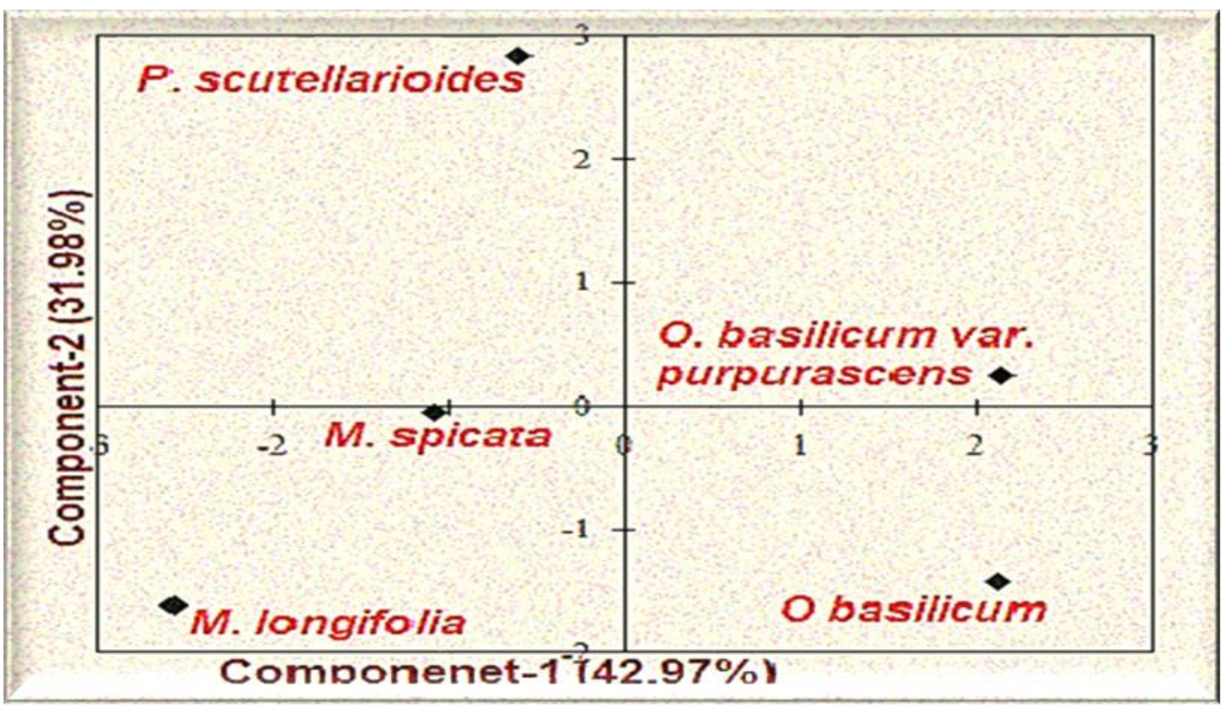


Figure 15. Principal component biplot of 5-5 Lamiaceae Martinov taxa based on 10 morphological related parameters.

4. Discussion

Six selected herbal cultivated taxa of family Lamiaceae Martinov belongs to three genera were collected from the Al-Marashda Agriculture Research Station (MARS), and a private garden of flowering plants nursery (PFPN) in Nagaa Al-Mansouri, Qena; both sites are in Qena Governorate, Egypt during their flowering and fruiting seasons 2022 and 2023.

The micro-morphological characteristics of foliar leaves' surfaces, trichomes diversity, stoma types, and nutlets micromorphology are investigated among 6 cultivated taxa of Lamiaceae: *Mentha longifolia*, *M. spicata*, *Plectranthus amboinicus*, *P. scuttellarioides*, *Ocimum basilicum* and *O. basilicum* var. *purpurascens*. The nutlets of *P. amboinicus* have not been investigated because they were not available in the plant specimens. Three artificial keys were introduced based on the results of different foliar and nutlet micromorphological traits. Among the different foliar micromorphological characteristics, the features of the leaf epidermis are one of the most significant for the taxonomy of various species in Lamiaceae (Sass, 1940; Kamal and Kumar, 2022). The result of foliar micromorphology revealed that it has a high taxonomic impact, especially in the variation in leaf surface texture or sculpture. It ranges from crested to ruminate and rugulate to reticulate in Mentha species, rugose to reticulate in *Plectranthus* species, ruminate in *Ocimum basilicum*, and reticulate in *O. basilicum* var. *purpurascens*. Clearly, leaf surface ornamentation differentiates between the two taxa at the species and varietal level of sweet basil. In this respect, (Moon, 2009) pointed out that the leaf micromorphology of *Mentheae* showed a high diversity in the stomatal complex and structure, epidermal cell outline, and trichome density, and the pattern of the epidermal anticlinal cell walls may be related to the effect of ecological conditions. They added that more than one type of anticlinal wall is found within the same leaf. On the other hand, (El-Gazzer, 1968; Metcalfe and Chalk, 1960) indicated that diacytics are common in Lamiaceae leaf surfaces and sometimes mixed with other types, such as anomocytic and this Confirms the current study since the diacytic stoma type is dominant. It is present singly in three taxa, i.e., *M. longifolia*, *M. spicata*, and *P. amboinicus* (Fig. 3) as well as mixed with anisocytic and anomocytic in *O. basilicum* and *O. basilicum var. purpurascens*, respectively whereas the anomocytic is clearly characterized in *P. scuttellarioides*. However, it has been reported that there are five different types of stomata in *Mentheae* (Cantino, 1990), indicating the taxonomic significance of the variation in stomata structures and types in *Lamiaceae.*

As shown in the current study, there was diversity in trichome shapes and types in the studied taxa where the observations exhibited the two main types of glandular and eglandular hairs. It was previously reported that the non-glandular/ eglandular trichomes are more common than glandular trichomes in Lamiaceae (Cantino, 1990; Osman, 2012; Akçin and Camili, 2018). The current study exhibited the shapes of eglandular hairs are linear (with two sub forms of flattened, L/f and articulated, L/a), as well as the slender (Sl) as shown in Fig. (6). Eglandular hairs ranged from unicellular (slender-shaped hairs) and multicellular (linear-shaped hairs). Additionally, the length of linear hair varies over a wide range of cells, as 2-(3)-(5)-6 cells with averages from 2.5 to 4.5 cells are held to construct the hair body (Fig. 6). Through their study of the genus *Teucrium* (Lamiaceae), (Eshratifar, 2011) reported that the shape, length, and surface of trichomes varied among different species. Moreover, the two main forms of glandular trichomes differ in the shape of head and stalk as well as the number of the head cells, base cells and the variability in the basal cells and surface of trichomes (Salmakia *et al.*, 2009; Xiang *et al.*, 2010; Osman, 2012, Ya'ni *et al.*, 2018). The first type is the distinct mushroom-shaped glandular hairs of multicellular umbrella-head, while the second type is a simple glandular hair of unicellular head. Glandular trichomes of peltate and capitate shapes are considered as the common glandular trichome arrangement in the Lamiaceae family (Werker *et al.*, 1993) and were as well described in *Mentha* genus (Rodrigues *et al.,* 2008) and other Lamiaceae species (Rodrigues *et al.*, 2006; Rodriguesa *et al*., 2012). Although the slender unicellular glandular hair is only present *in Mentha spicata*, the simple glandular hairs are in *Plectranthus amboinicus*. The normal globose embedded base cell is present in all the studied taxa except *M. spicata* and *Ocimum basilicum*. However, *M. spicata* is distinguished by the not globose embedded base cell whereas the multicellular rosette-shaped base cell is present in *Ocimum basilicum* only. This finding has been confirmed by different researchers (Abu-Asab and Cantino, 1987; Demissew *et al.*, 1992; Ayodele and Olowokudejo, 2006). Hence, variations in trichome type/structure and form are taxonomically significant at different taxonomic levels. However, trichomes are one of the most important features contributing to plant resistance to pests, pathogens, and drought (Levin, 1973), and they play an important role in environmental adaptation to high irradiance levels (Stenglein and Arambarri, 2005). However, trichome types are usually constant in species groups (Okpon, 1969). The identification impact of trichome variation on Lamiaceae members was evaluated by (Xiang *et al.*, 2010; Osman, 2012).

The nutlet micromorphology of the taxa revealed a significant taxonomic diagnosis. They varied among the features of shape, dimensions, color mode, areole position, coat surface characteristics, especially the texture pattern of the nutlet surface and the hairiness of nutlets. In the current study, the outline shape ranged from oblong-elliptic, ovoid to elliptic- or oblong-ovoid outlines with polygonal testa epidermal cells. Ovoid and its derivative shapes characterized *Mentha spicata* and two taxa of *Ocimum basilicum*, while *M. longifolia* was distinguished by different outline (oblong-elliptic). Hence, the results presented the variability of the nutlet characteristics at major taxonomic levels, generic and specific, and even at the varietal level. This diagnostic finding was emphasized, however, by differentiating the outline shapes of *Plectranthus scuttellarioides*, *i.e*., size, color and testa surface, and the presence of sparse hairs on the surface, while the other studied nutlets are glabrous. This agrees with the results of (Eshratifar *et al.*, 2011) in their investigation of the nutlets of *Teucrium* L. (Lamiaceae). They recorded that Lamiaceae taxa have glabrous or sparsely to densely hairy nutlets. The nutlet shape, morphology, and abscission scar (areole) have also been recorded as valuable systematic aspects of *Mentheae* (Lamiaceae) by (Moon *et al.*, 2009).

The nutlet/mericarps micro-morphological features are representative of taxonomic and diagnostic characteristics, and this agrees with several authors, such as (Kahraman *et al.*, 2005; Krawczyk and Głowacka, 2015). As for the investigated mericarps, *Mentha*, *Plectranthus*, and *Ocimum* gained different shades of coloring, ranging from brown to dark brown or black. The dichromed coloring pattern was distinctive for the two *Mentha* studied species (*longifolia* and *spicata*). The areole (abscission scar) was recorded to be basal or sub-basal in all studied taxa. Related and similar data about the shape of nutlets, color, and areole position resulted in the identification of different species of the genus *Nepeta* by (Amirmohammadi *et al.*, 2019). The overall outcome of the findings in this study agrees with those of other micromorphological investigations of different Lamiaceae members, such as (Akçin and Camili, 2018; Kamal and Kumar, 2022).

5. Conclusion

A detailed report described some micro-morphological features of the foliar leaves and nutlets of some cultivated taxa of Lamiaceae. The findings reported herein may contribute to future taxonomic research on other taxa at different taxonomic levels within this large and important family.

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is not applicable.
Declarations
Conflict of interest: none.
Ethical approval
The Institute Ethics Committee approved the study protocol.

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