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Original article

Micromorphological Characters of the Leaf Epidermis for Identification of certain Solanoideae (Solanaceae)

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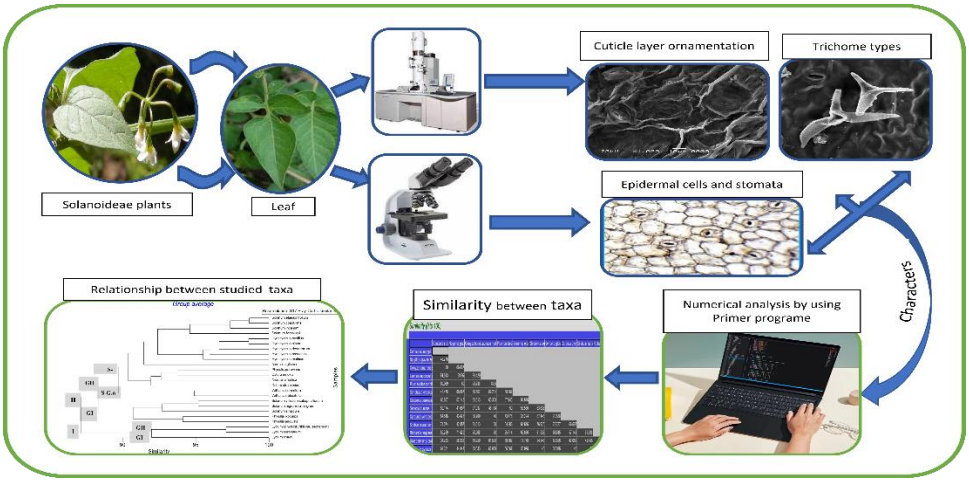
ABSTRACT

The foliar epidermis has great value in plant taxonomy studies. Light microscopy and scanning electron microscopy were conducted on the leaf surface micromorphological features, such as types of trichome (glandular and nonglandular) and epidermal cells and stomatal characteristics, of 21 species from Solanoideae belonging to the family Solanaceae. The studied species showed diversity in cuticle ornamentation, epidermal cell shape, and surface sculpture. Trichomes have four types: glandular, nonglandular, stellate, and dendritic. The studied *Hyoscyamus* species were characterized by the absence of nonglandular trichomes. The anticlinal walls are undulate in most species, but some species have sinuous and straight wall. The wax on leaves was detected in four patterns: smooth, finely warty, warty-crustose, and finely striate. The stomatal size, shape, and aperture also varied among species. Nearly all the investigated species had anisocytic and anomocytic types of stomata. *Hyoscyamus* species had anomocytic type of stomata. Numerical analysis of the studied species was conducted using the PRIMER and PCORD programs. Clustering analysis revealed the separation of these species into two major clusters. Cluster I consist of three species, whereas cluster II consists of 18 species. On the basis of numerical analysis, this study provided nearly sufficient information on the taxonomic importance of leaf micromorphology, validating its usefulness in the studied species and genera of Solanoideae discrimination. An identification key using micromorphological characters are provided to distinguish studied genera and species of Solanoideae. This study revealed that the types of trichome and epidermal cells, stomata, and cuticle ornamentation within the species show significant variation and can be helpful for taxonomic purposes.

Graphical abstract



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1. Introduction

Solanoideae is a subfamily belonging to the family Solanaceae (1). Solanoideae contains some of the most economically important genera and species, such as potato (*Solanum tuberosum*), eggplant or aubergine (*Solanum melongena*), tomato (*Solanum lycopersicum*), and jimson weed (*Datura stramonium*). This subfamily consists of several tribes: Solaneae, Capsiceae, Physaleae, Datureae, Hyoscyameae, Juanulloeae, and Lycieae (2). Solanaceae is widely distributed in various ecological habitats in tropical and temperate regions. It consists of 98 genera and 2300 species (3–5). In Egypt, Solanaceae consists of 30 to 33 wild species belonging to eight genera (6–9).

The subfamily and tribe group classification of Solanaceae has various revisions. Traditional taxonomy divides it into two subfamilies (Cestroideae and Solanoideae) and 19 tribes (10,11). Tetenyi (12) and Olmstead et al. (13) classified Solanaceae into four to seven subfamilies and 16 to 20 tribes. Recent molecular phylogenetic studies divided Solanaceae into four subfamilies and 18 tribes, but some subfamilies were not divided into tribes (2,14,15).

Trichomes in plants have many forms and shapes and a very complex structure for some plants. The micromorphological characteristics of foliar trichomes played an important role in comparative investigation in angiosperms and plant systematics, especially of groups at generic and specific levels (16,17). This field study has fascinated plant morphologists and diversity systematists of trichome features (18–20). There are various studies on trichomes and epidermal characteristics for different Solanaceae species growing elsewhere other than Egypt.

Ghahreman et al. (21) studied the foliar epidermis of 12 Hyoscyamus species. Aworinde et al. (22) studied the morphological and leaf epidermal features of some *Capsicum* species. Adedeji et al. (23), Wahua and Edwin-Wosu (24), Haruna et al. (25), and Sharma et al. (26) observed the trichomes and stomata of some species of the family Solanaceae. Illoh and Inyang (27) described the epidermal characteristics of six *Solanum* species in the subgenus Leptostermonum. Benitez De Rojas and Ferrarotto (28), Thongpukdee and Thepsithar (29), Anil Kumar and Murugan (30), and Bello et al. (31) observed the leaf epidermis of some *Solanum* species via light microscopy (LM) and scanning electron microscopy (SEM). Bhat et al. (32) and Ekeke et al. (33) studied the leaf anatomy and epidermis of some *Physalis* L. species, Mohamed and Al-Gohary (34) studied the leaf architecture of *Solanum* species.

For Solanaceae species in Egypt, there are some studies on trichomes and epidermal characteristics. El-Ghamery et al. (35) studied the seed protein of some Solanaceae species. Al-Nowaihi and Mourad (4) and Al-Gohary and Mohamed (36) studied the morphology and anatomy of some Solanaceae species. Al-Nowaihi and Khalifa (37), El-Gazzar et al. (38,39), Fawzi and Habeeb (40), and Khafagi et al. (41) conducted a taxonomic and numerical study of Solanaceae species.

This study aimed to clarify the aspects of diversity in foliar trichome and stomatal characteristics and leaf epidermal cells within the studied species as significant tools in the differentiation and identification of Solanaceae.

2. Materials and Methods

The study was based on 21 species of Solanoideae. Fresh materials were collected from different localities in Egypt. Table 1 shows the collected materials.

The specimens were identified by comparing them with the specimens kept in the herbarium of the Agricultural Museum (CAIH) and Cairo University (CAI). Additionally, keys from Tăckholm (6) and Boulos (8) also were used. The stomatal and trichome types were determined according to Nassar and El-Sahhar (42). Leaf epidermis was examined via LM and SEM and photographed using JEOL JSM-100 SEM at an accelerating voltage of 18 kV (Plates 1 and 2). The terminology for the stomata and trichomes was adopted according to Metcalfe and Chalk (43) and Prabhakar (44).

All foliar epidermal characteristics of each species documented and used in a classic key construction. The relationships among the studied taxa of Solanoideae have been demonstrated as two dendrograms (Fig. 6A and B) using the statistical programs PRIMER version 6.0 and PCORD version 5.0.

Table 1: Collection data of specimens representing 21 species belonging to genera of Solanoideae in Egypt.

No.	Species	Collection data
1	*Datura innoxia* Mill.	Al Azhar University Cairo, 05 /11/2016; Osama Ragab (Al Azhar Univ.)
2	*Datura stramonium* L.	Al Azhar University Cairo, 05/23/2017; Osama Ragab (Al Azhar Univ.) Kafr El-Dwar, 06/29/2017; Osama Ragab (Al Azhar Univ.)
3	*Hyoscyamus albus* L.	Burg El Arab-El Hammam Coastal Road, 05/15/2017; O.N. Ghaly (Al Azhar Univ.)
4	*Hyoscyamus boveanus* (Dunal) Asch. & Schweinf.	Wadi El Messerdy, Saint Katherine, South Sinai, 05/02/2010; O.N. Ghaly (CAIH)
5	*Hyoscyamus desertorum* (Asch. & Boiss.) Täckh.	Wadi El-Arish, 09/14/1965; L. Boulos (CAI)
6	*Hyoscyamus muticus* L.	Cairo-Alexandria Road, 100 km, 12/13/2016; Osama Ragab (Al Azhar Univ.) Shalateen-Abu Ramad Road, 60 km, 04/07/2017; O.N. Ghaly (Al Azhar Univ.)
7	*Hyoscyamus pusillus* L.	Wadi Tiniya, Saint Katherine Protectorate, 04/20/2008; H. Shabana (CAIH)

Table 2 continued

No.	Species	Collection data
8	*Lycium europaeum* L.	Alexandria-Matruh Coastal Road, 237 km, 03/12/2017; Osama Ragab (Al Azhar Univ.)
9	*Lycium schweinfurthii* Dammer	Alkom Alakhdar Island, El Brullus, 03/20/2017; Osama Ragab (Al Azhar Univ.)
10	*Lycium shawii* Roem & Schult.	Wadi Hagul, 12/26/2017; Osama Ragab (Al Azhar Univ.)
11	*Physalis angulata* L.	Pajour, Minufiyah, 11/07/2017; Osama Ragab (Al Azhar Univ.)
12	*Physalis ixocarpa* Brot. ex Hornem.	Kafr El-Dwar, 10/27/2016; Osama Ragab (Al Azhar Univ.)
13	*Solanum coagulans* Forssk.	Wadi Darweena, Gabel Elba, 04/01/2000; Iman Al-Gohary (CAIH)
14	*Solanum elaeagnifolium* Cav.	Burg El Arab City, 05/15/2017; O.N. Ghaly (Al Azhar Univ.)
15	*Solanum forskalii* Dunal.	Wadi Maarafaii, Gebel Elba, 04/08/2017; O.N. Ghaly (Al Azhar Univ.)
16	*Solanum incanum* L.	Wadi Kanthesrob, Gebel Elba, 04/09/2017; O.N. Ghaly (Al Azhar Univ.)
17	*Solanum nigrum* L.	Kafr El-Dwar, 10/27/2016; Osama Ragab (Al Azhar Univ.)
18	*Solanum sinaicum* Boiss.	Sinai, Saint Katharine, Wadi El-Arba'een 4/2017; Osama Ragab (Al Azhar Univ.)
19	*Solanum villosum* Mill.	Minufiyah, 04/05/2017 and Burg El Arab City, 15/5/2017; Osama Ragab (Al Azhar Univ.)
20	*Withania obtusifolia* Täckh.	Wadi Akaw, Gebel Elba, 04/26/2013; O.N. Ghaly and M. Abutaha (CAIH)
21	*Withania somnifera* (L.) Dunal	Cairo University, 04/10/2017; Minufiyah, 4/2017 (Al Azhar Univ.) Nasr City-Cairo, 04/03/2018; Osama Ragab (Al Azhar Univ.)

3. Results

3.1. Detailed descriptions

Tables 2 and 3 summarize the foliar epidermal characteristics and qualitative and quantitative examination of the 21 species of the subfamily Solanoideae using LM and SEM. The chosen SEM and LM micrographs of the foliar epidermis are displayed in Plates 1 to 5.

3.1.1. Epidermal cells (Table 2; Fig. 1)

On the abaxial surface, a significant variation was found in epidermal cells. In most species, epidermal cells were irregular and regular in *H. muticus, L. europaeum* (Fig. 1b*), L. schweinfurthii, L. shawii (Fig. 1a), S. coagulans, S. elaeagnifolium, and S. incanum.* Anticlinal wall patterns also varied from species to species. For example, most species have an undulate (Fig. 1c*)* anticlinal wall pattern, but it was sinuous in *P. ixocarpa* (Fig. 1e), *S. forskalii, S. nigrum, S. sinaicum,* and *S. villosum* and straight in *L. europaeum* (Fig. 1b), *L. schweinfurthii, L. shawii, S. coagulans, S. elaeagnifolium,* and *S. incanum.*

3.1.2. Cuticular and wax ornamentation: (Table 2; Figs. 2 and 3)

According to Metcalfe and Chalk (43) and Mohamed *et al.* (34) stated that the difference in the striated of the leaf surface might be of taxonomic value. Four patters on the adaxial surface of the leaf are recorded: A. Wrinkled: Wrinkled covered with warty-crustose wax particles: *L. schwii*; (Fig. 3B), Wrinkled with tuberculate wax: *L. schweinfurthii* (Fig. 2F) and wrinkled covered with finely warty wax: *P. angulata* (Fig. 3C). B. Striated covered with finely warty wax: *H. boveanus* (Fig. 2D), C. Reticulate: Irregularly reticulate covered with finely warty wax: *L. schwii* and *Physalis ixocarpa* (Fig. 3B and D) and reticulate-foveate covered with smooth wax: *D. innoxia* and *H. muticus* (Fig. 2A and E). D. Favulariate: Favulariate covered with finely warty wax, e.g., *H. albus* (Fig. 2C), *H. desertorum*, and *S. sinaicum* and favulariate covered with finely striate wax, e.g., *L. europaeum* (Fig. 3A and F).

3.1.3. Stomata (Table 2; Figs. 2 and 3)

Solanoideae species showed remarkable variation in the number, size, and type of stomata. Most species had amphistomatic leaves. The most common stomatal type was anomocytic and ansiocytic (Table 2).

The maximum length of stomata "41" to "44" mm. was observed in *L. europaeum*, and the maximum width of stomata 30–35 mm was observed in *L. europaeum*, *L. schweinfurthii*, and *W. obtusifolia* at the abaxial surface (Table 2).

The stomata level was superficial, at a level, semidepressed, or depressed. It was superficial in *D. innoxia*, *H. boveanus*, *P. angulata*, and *S. sinaicum*; semidepressed in *H. albus, H. desertorum*, *H. pusillus, P. ixocarpa, W. obtusifolia, and W. somnifera*; depressed in *D. stramonium* and *H. muticus*; and at a level in the rest.

The stomatal shape was suborbiculate, elliptic, or elongate. It was elliptic in most studied species; suborbiculate in *D. innoxia, D. stramonium, H. pusillus, L. shawii, S. coagulans, S. elaeagnifolium, W. obtusifolia*, and *W. somnifera*; and elongate in *Physalis*

angulate (Fig. 3C), *S. nigrum, S. sinaicum*, and *S. villosum*.

The aperture shape was elliptic, ovate, or linear. It was linear in *L. europaeum* and *L. schweinfurthii* (Fig. 2F), ovate in *H. albus, H. boveanus, H. desertorum, H. muticus*, and *H. pusillus* and elliptic in the rest.

The stomatal rim was raised in 10 species, namely, *D. innoxia, D. stramonium, H. albus, H. pusillus, L. europaeum, L. schweinfurthii* (Fig. 2F), *P. angulate* (Fig. 3C), *P. ixocarpa, W. obtusifolia*, and *W. somnifera*, and flat in the rest.

3.1.4. Trichomes (Table 3; Figs. 4 and 5)

Twenty-six trichome types were observed. Some occurred in only one species, whereas others occurred in more than one species in varied combinations (Table 3). All types were not found in any one species. Aside from the qualitative and quantitative differences trichomes exhibit, their dispersion was variable from one species to another (Table 3). The general description of the trichome types is as follows:

A. Nonglandular trichomes

Unicellular papillose, e.g., *Ph. ixocarpa* (Fig. 4C), unicellular basal cell with long narrow apical cell, e.g., *D. stramonium* (Fig. 4A), bicellular uniseriate with hooked apical cell, e.g., *S. villosum* (Fig. 4J), unicellular basal cell with long straight acute apical cell, e.g. *S. villosum* (Fig. 4E), bicellular uniseriate, with obtuse apical cell e.g. *Ph. ixocarpa* (Fig. 4G), multicellular uniseriate with acute apical cell, e.g., *L. europaeum* (Fig. 4D), multicellular uniseriate with oblique apical cell (Fig. 4B), multicellular basal cell with unicellular obtuse apical cell, e.g. *S. forskalii* (Fig. 4H), multicellular uniseriate with obtuse apical cell, e.g., *L. shawii* (Fig. 4I), unicellular stalk and biarmed unequal apical cell, multicellular (2–4) stalk and equal biarmed apical cell, e.g. *W. sominfera* (Fig. 4F) and multicellular (2–4) stalk and unequal biarmed apical cell e.g., *L. shawii*.

B. Stellate trichomes

short stalk, apical with triradiate, e.g. *W. obtusifolia* (Fig. 5B), short stalk, apical with tetraradiate, e.g., *W. obtusifolia* (Fig. 5C), multicellular stalk radiating (5–8) with long central ray, e.g. *S. incanum* (Fig. 5D), and multicellular stalk radiating (12–16) with short central ray, e.g. *S. elaeagnifolium* (Fig. 5E).

C. Dendritic trichomes:

Dendritic trichome with unicellular stalk, e.g. *W. obtusifolia* (Fig. 5F) and dendritic trichome with multicellular stalk,

D. Glandular trichomes

Unicellular stalk and bicellular head, e.g. *Ph. angulate* (Fig. 5G), unicellular stalk and multicellular head *D. stramonium* (Fig. 5H), bicellular uniseriate stalk and multicellular head, bicellular uniseriate stalk and unicellular head, multicellular uniseriate stalk and unicellular head, e.g. *W. obtusifolia* (Fig. 5F), multicellular biseriate stalk and multicellular head, e.g. *L. schweinfurthii* (Fig. 5L) and unicellular stalk, biarmed with unicellular head, e.g. *S. villosum* (Fig. 5I) and dendritic trichome with unicellular head, e.g., *H. muticus* (Fig. 5k).

3.2. Constructed key based on the leaf micromorphological characteristics

Data from Tables 2 and 3 were used to produce the bracketed key for the 21 Solanoideae species, which can help validate their identity.

1- Stomata mixed (anomocytic and anisocytic)…..…… 2
1- Stomata anomocytic or anisocytic……..…….…… 15
2- Dendritic trichomes present…….…….. 3
2- Dendritic trichomes absent…….…… 4
3-Triradiate and tetraradiate stellate present, ……..…… *W. obtusifolia*
3-Triradiate and tetraradiate stellate absent, ……..……..…….. *W. somnifera*
4-stellate trichomes present…… 5
4- stellate trichome absent…….. 8
5- Multicellular stalk radiating (12–16) with short central ray present…… *S. elaeagnifolium*
5-Multicellular stalk radiating (5–8) with long central ray present ……..…….. 6
6- Trichome with tetraradiate apical cell present ……..……... 7
6- Trichome with tetraradiate apical cell absent ……..…….. *S. incanum*
7- Epidermal cell irregular; Stomata elliptic…….……..…… *S. forskalii*
7- Epidermal cell regular; Stomata suborbiculate …….. *S. coagulans*
8- Glandular trichomes present…….. 9
8- Glandular trichomes absent…….. *S. nigrum*
9- Epidermal cell regular, anticlinal cell wall straight ……. *L. shawii*
9-Epidermal cell irregular, anticlinal cell wall undulate or sinuous……. 10
10- Stomata elongated or elliptic ……. 11
10- Stomata suborbiculate ……... 14
11- Stomatal rim flat; uniseriate stalk and unicellular head trichome present……. 12
11- Stomatal rim raised; uniseriate stalk and unicellular head trichome absent.…… 13
12- Favulariate sculpture; uniseriate trichome with oblique apical cell present…….. *S. sinaicum*
12-Irregularly reticulate sculpture; uniseriate with oblique apical cell absent.…… *S. villosum*
13- Stomata outline elongate, superficial; anticlinal cell wall undulate…….…… *P. angulata*
13- Stomata outline elliptic, semi depressed; anticlinal cell wall sinuous…..…..…… *P. ixocarpa*

14- Irregularly reticulate sculpture; smooth cuticle layer ……..……. *D. innoxia*
14- Reticulate-foveate sculpture; finely warty cuticle layer……. *D. stramonium*
15- Stomata anomocytic and linear shape…… 16
15- Stomata anisocytic and ovate shape……..……. 17
16- Favulariate sculpture; trichome with biarmed apical cell present……..…….. *L. europaeum*
16- Wrinkled sculpture; trichome biarmed apical cell absent……...……..……... *L. schweinfurthii*
17-Dendritic trichome with unicellular head; epidermal cell regular……. *H. muticus*
17- Dendritic trichome with multicellular head; epidermal cell irregular…..…… 18
18-Branched glandular trichome present, stomata superficial……. *H. boveanus*
18-Branched glandular trichome absent, stomata semi depressed……..…..…… 19
19- Trichomes with multicellular head present, stomata rim flat…….. *H. desertorum*
19- Trichomes with multicellular head absent, stomata rim raised…….. 20
20- Stomata elliptic; unicellular stalk and head present.…….…… *H. albus*
20- Stomata suborbiculate; unicellular stalk and head absent……. *H. pusillus*

3.3. Numerical analysis

The epidermal characteristics obtained from the 21 studied species of Solanoideae were used for numerical analysis using the statistical programs PRIMER version 6 and PCORD version 5 as tools in their identification and differentiation.

To analyze the relationships among the 21 studied species using hierarchical cluster analysis, Tables 2 and 3 include the 38 characteristics used in the cluster analysis. PRIMER used the Bray Curtis similarity measure (Fig. 6A). PCORD used the Euclidean distance similarity measure and Ward's method of clustering (Fig. 6B).

The species were grouped into two major clusters in the dendrogram produced using PRIMER. Cluster I consisted of three species: *L. shawii*, *L. europaeum*, and *L. schweinfurthii*. Cluster II was divided into two groups. The first group (II, A) comprised *W. obtusifolia* and *W. somnifera*, whereas the second group (II, B) was divided into two subgroups. Subgroup "II, B, 1" contained the studied taxa of *Solanum* and *Hyoscyamus. S. sinaicum* separated into a distinct subgroup. Subgroup "II, B, 2" consisted of the studied taxa of *Physalis* and *Datura*.

The dendrograms produced using PRIMER and PCORD gave the same results.

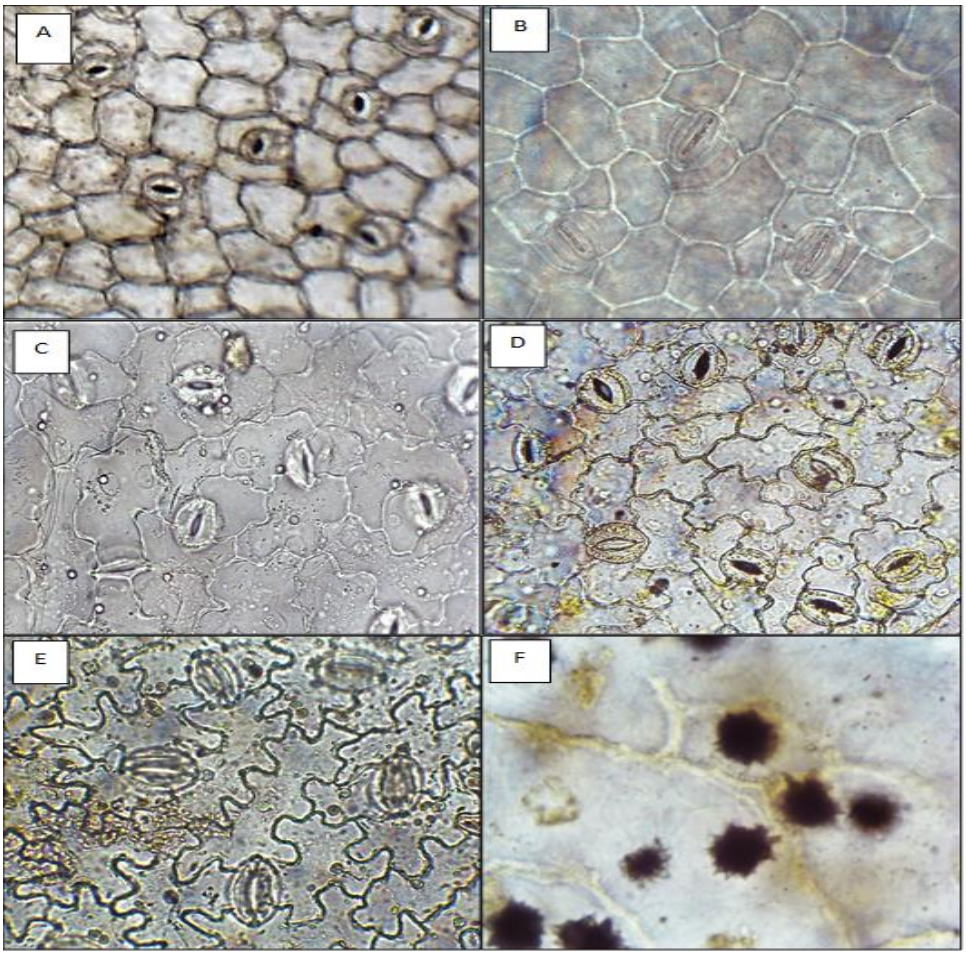

Figure 1. LM micrographs of the leaf epidermal cells in the studied taxa. A, Lycium shawii; B, Lycium europaeum; C, Datura innoxia; D, Withania somnifera; E, Physalis ixocarpa and F, Hyoscyamus desertorum.

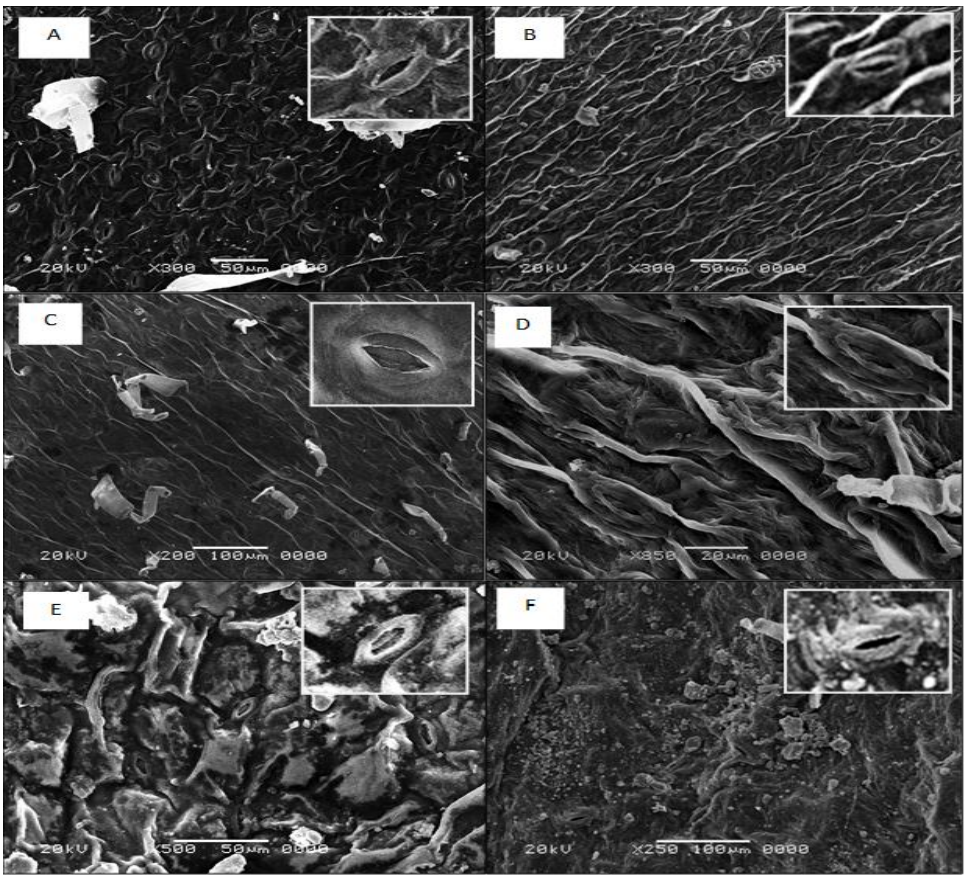

Figure 2. Cuticular ornamentation and stomatal features. A, *Datura innoxia*; B, *Datura stramonium*; C, *Hyoscyamus albus*; D, *Hyoscyamus boveanus*; E, *Hyoscyamus muticus;* and F, *Lycium schweinfurthii*.

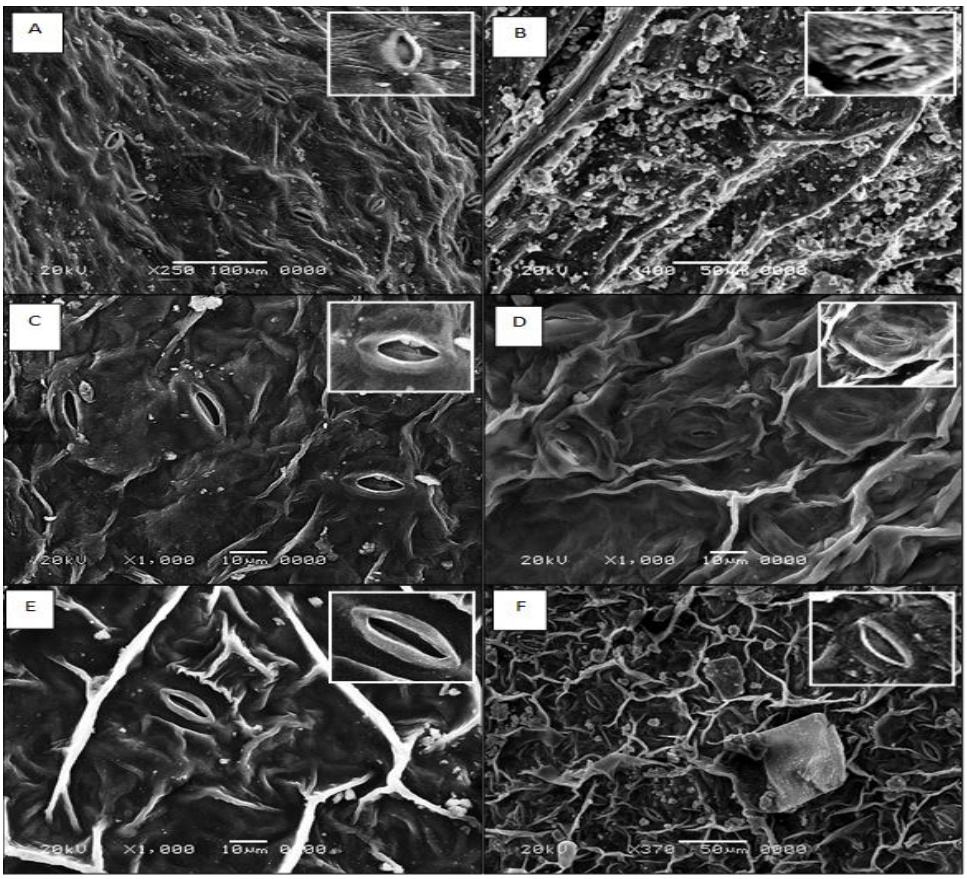

Figure 3. Cuticular ornamentation and stomatal features. A, *Lycium europaeum*; B, *Lyciumm schwii*; C, *Physalis angulata*; D, *Physalis ixocarpa*; E, *Solanum nigrum;* and F, *Solanum villosum*

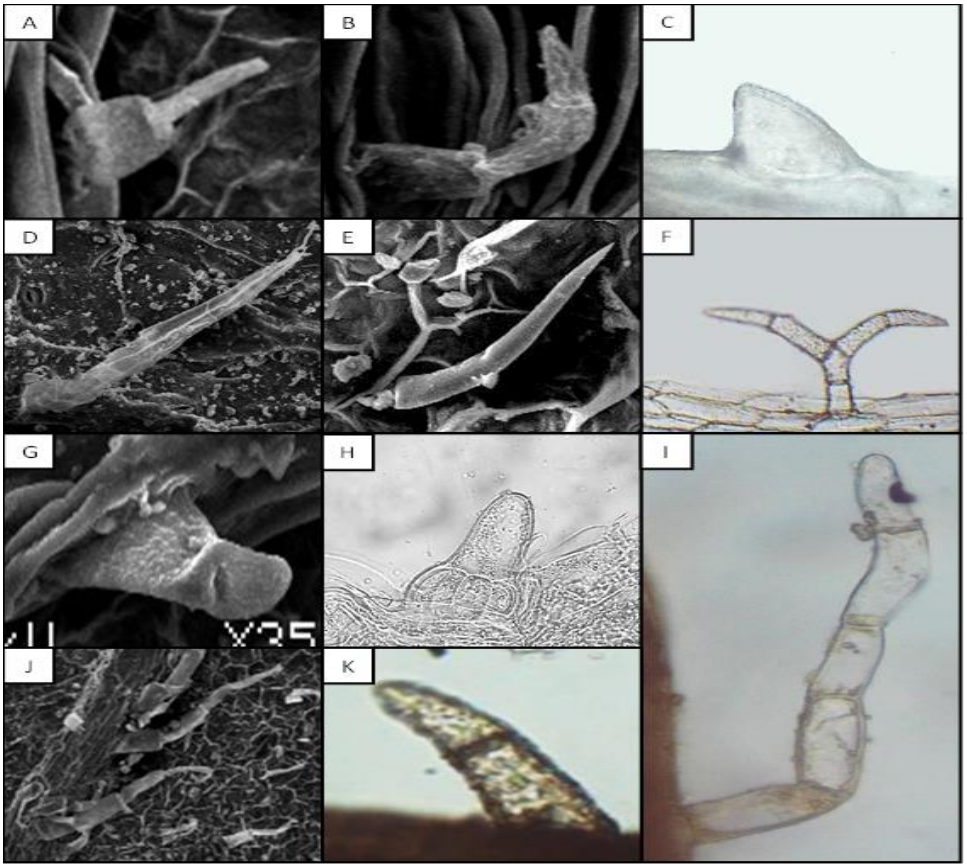

Figure 4. LM and SEM micrographs of trichomes on the leaf surface in the studied taxa. A, B, *Datura stramonium*; D, *Lycium europaeum*; C, G, *Physalis ixocarpa*; E**,** J, *Solanum villosum*; F, *Withania sominfera;* H, *Solanum forskalii;* I, *Lycium shawii***;** and K, *Solanum nigrum*..

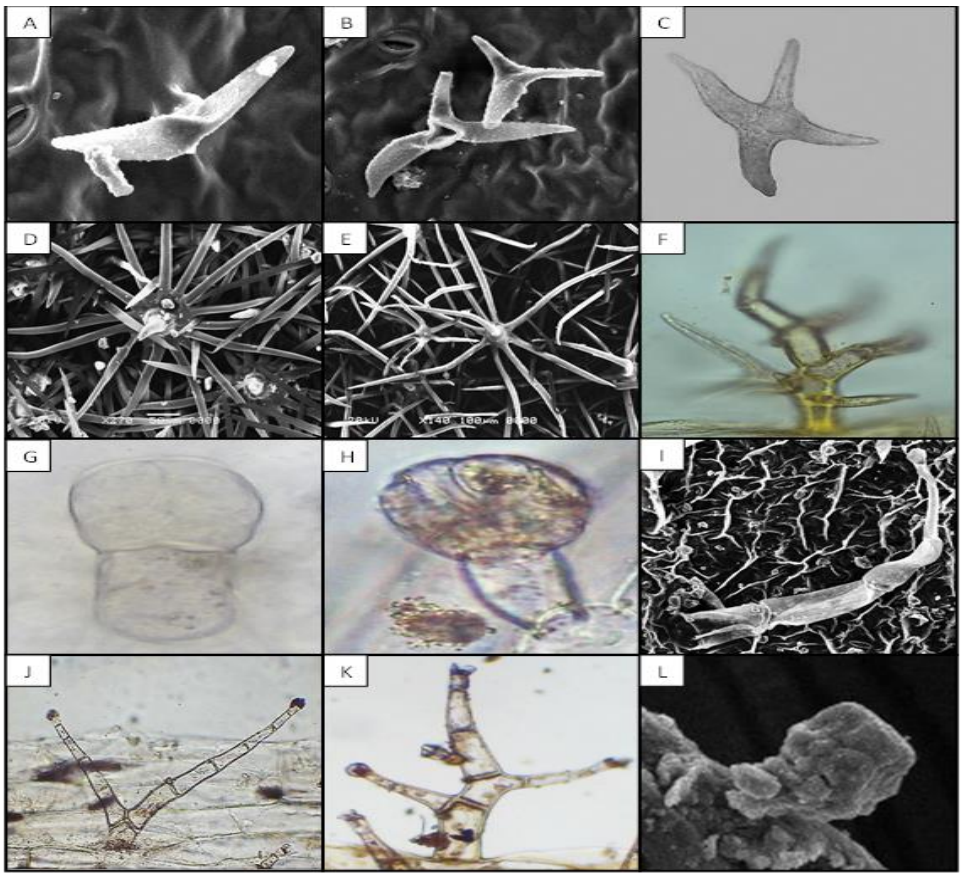

Figure 5. Cuticular ornamentation and stomatal features. A, *Lycium europaeum*; B, *Lyciumm schwii*; C, *Physalis angulata*; D, *Physalis ixocarpa*; E, *Solanum nigrum;* and F, *Solanum villosum*

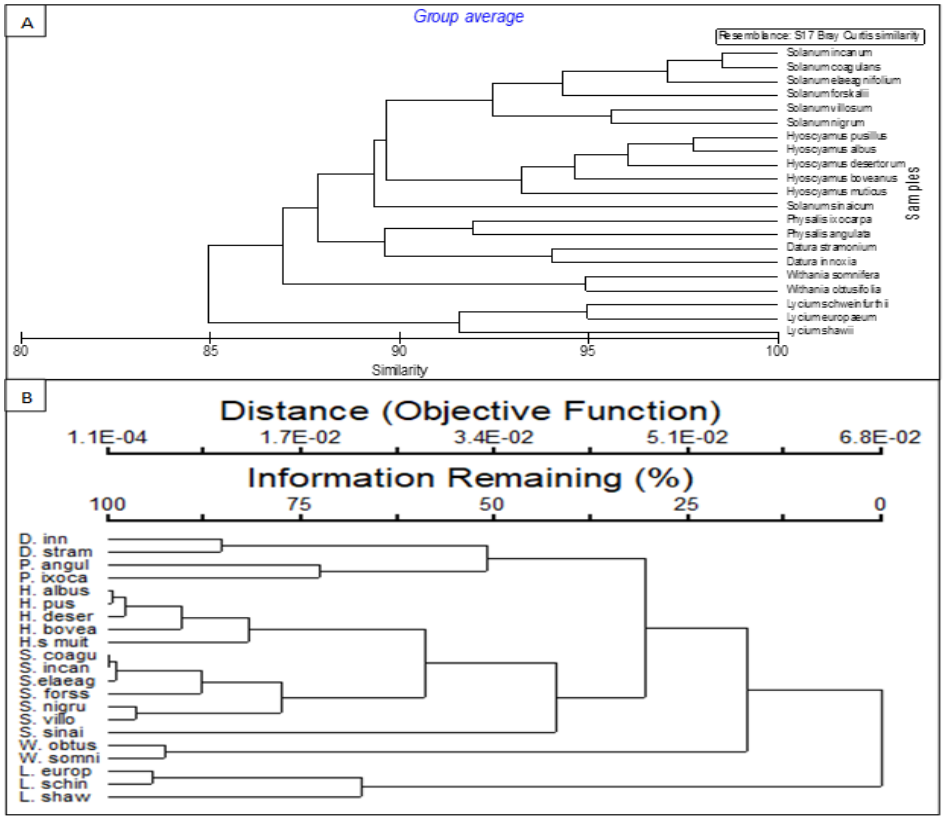

Figure 6. Dendrograms showing the interrelationships between 21 taxa of Solanoideae based on 38 characters of leaf epidermal characters by using (A): PRIMER program and (B): PCORD program

Table 2. Epidermal cell and stomatal characteristics in the studied species of Solanoideae.

Character / Taxa	Epidermal characteristics				Stomatal apparatus									
	Cell shape	Anticlinal cell wall	Surface sculpturing	Cuticle ornamentation	Type		Size		Level	Outline	Aperture			Stomatal rim
					Anomocytic	Ansiocytic	Length	Width			Shape	Length	Width	
D. innoxia	Ir	un	Rf	sm	1	1	22.4–24.7	19.2–21.1	sup	sub	ell	13.8–15.5	4.2–5.8	ra
D. stramonium	Ir	un	Rir	wt	1	1	24.5–28.8	18.4–22.7	dep	sub	ell	11.0–14.8	3.9–4.5	ra
H. albus	Ir	un	Fa	wt	2	1	29.6–34.4	23.2–26.2	sem	ell	ov	15.4–18.6	5.7–8.2	ra
H. boveanus	Ir	un	St	wt	2	1	34.2–37.6	20.8–23.8	sup	ell	ov	17.2–21.1	9.4–11.6	fl
H. desertorum	Ir	un	Fa	wt	2	1	36.2–37.7	23.2–28.9	sem	ell	ov	17.7–25.7	7.5–7.7	fl
H. muticus	R	un	Rf	sm	2	1	31.6–33.3	23.7–24.8	dep	ell	ov	16.5–19.2	4.7–6.4	fl
H. pusillus	Ir	un	Rir	wt	2	1	26.0–32.7	24.5–27.5	sem	sub	ov	15.2–20.1	6.8–8.3	ra
L. europaeum	R	st	Fa	stw	1	2	41.8–44.1	30.1–34.2	ata	ell	lin	24.1–31.2	5.0–6.0	ra
L. schweinfurthii	R	st	W	tu	1	2	38.6–39.5	30.5–31.1	ata	ell	lin	19.3–25.5	3.5–5.6	ra
L. shawii	R	st	W	wcr	1	1	27.8–32.2	22.6–25.6	ata	sub	ell	13.6–16.3	4.2–5.7	fl
P. angulata	Ir	un	W	wt	1	1	29.8–32.3	16.3–18.8	sup	elo	ell	19.5–22.5	3.7–6.3	ra
P. ixocarpa	Ir	si	Rir	wt	1	1	26.3–31.2	20.0–20.7	sem	ell	ell	15.5–19.5	5.3–6.3	ra
S. coagulans	R	st	Rir	wt	1	1	17.2–19.2	13.9–16.2	ata	sub	ell	8.5–11.2	2.9–3.9	fl
S. elaeagnifolium	R	st	Rir	wt	1	1	20.3–21.6	18.6–19.7	ata	sub	ell	8.2–12.2	4.0–4.5	fl
S. forskalii	Ir	si	Rir	wt	1	1	23.5–25.9	16.7–22.0	ata	ell	ell	12.7–15.9	5.2–5.8	fl
S. incanum	R	st	Rir	wt	1	1	20.5–21.3	15.4–18.4	ata	ell	ell	11.3–14.1	3.9–5.4	fl
S. nigrum	Ir	si	Rir	wt	1	1	33.6–36.5	23.2–26.3	ata	elo	ell	18.4–26.0	5.2–6.9	fl
S. sinaicum	Ir	si	Fa	wt	1	1	23.8–25.1	17.4–20.6	sup	elo	ell	13.0–16.7	3.9–5.3	fl
S. villosum	Ir	si	Rir	wt	1	1	26.9–31.3	20.1–21.7	ata	elo	ell	15.5–19.1	5.7–7.5	fl
W. obtusifolia	Ir	un	Rf	sm	1	1	38.9–45.0	30.7–33.3	sem	sub	ell	21.5–26.6	7.7–8.4	ra
W. somnifera	Ir	un	Rf	sm	1	1	30.4–33.1	26–28.3	sem	sub	ell	17.5–22.3	5.9–8.6	ra

1, present; 2, absent; Ir, irregular; R, regular. Un, undulate; st, straight; si, sinuous; W, wrinkled; St, striated; Rir, irregularly reticulate; Rf, reticulate-foveate; Fa, favulariate; wt, finely warty wax; wcr, warty-crustose wax; tu, tuberculate wax; sm, smooth wax; stw, finely striate wax; sup, superficial; sem, semidepresed; ata, at a level; dep, depressed; sub, suborbiculate; elo, elongate; ell, elliptic; lin, linear; ov, ovate; fl, flat; ra, raised.

Table 3. Trichome types of the studied species

Characteristics \ Species		*D. innoxia*	*D. stramonium*	*H. albus*	*H. boveanus*	*H. desertorum*	*H. muticus*	*H. pusillus*	*L. europaeum*	*L. schweinfurthii*	*L. shawii*	*P. angulata*	*P. ixocarpa*	*S. coagulans*	*S. elaeagnifolium*	*S. forskalii*	*S. incanum*	*S. nigrum*	*S. sinaicum*	*S. villosum*	*W. obtusifolia*	*W. somnifera*
Nonglandular	1	-	-	-	-	-	-	-	+	+	+	+	+	-	-	-	-	+	+	-	-	-
	2	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	+	+	-	-	-
	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	-
	4	+	-	-	-	-	-	-	+	+	+	+	-	-	-	-	-	-	+	-	-	-
	5	+	+	-	-	-	-	-	+	+	+	+	+	-	-	-	-	+	+	+	-	-
	6	+	+	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	+	-	-	+
	7	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	+	-	-	-
	8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
	9	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
	10	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	+	+
	11	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+
	12	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	+	+
	13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
	14	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	+	-
	15	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	+	-	-	-	-	-
	16	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
	17	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+
	18	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+
Glandular	19	-	+	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	20	+	+	-	-	-	-	-	+	+	+	+	+	+	-	-	+	-	-	-	+	-
	21	+	+	-	-	-	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-
	22	+	-	+	-	-	+	+	-	-	-	-	-	-	-	+	-	-	+	+	-	-
	23	+	-	+	+	-	+	+	-	-	-	-	-	-	-	+	-	-	+	+	-	-
	24	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	25	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	26	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Density 1=sparsely hairy 2=tomentose 3=pubescent		3	3	3	3	3	3	3	1	1	1	1	1	2	2	2	2	1	3	3	3	3

+ = Present - = Absent

4. Discussion

The use of trichomes as anatomical features for systematic comparison and delimitation has been underlined by many research workers. Some families can be easily identified by the presence of a particular trichome (23,45). Trichomes have four types: glandular, nonglandular, stellate, and dendritic. Some trichomes are specific for some species.

In Table 3, some trichome types were restricted to a particular species, viz., multicellular uniseriate with obtuse apical cell hair (*L. shawii*), multicellular basal cells with unicellular obtuse apical cell hair (*S. forskalii*), short stalk, apical with triradiate hair (*W. obtusifolia*), multicellular head hair (*H. desertorum*), and unicellular stalk and biarmed with unicellular head and dendritic trichome with unicellular head hair (*H. muticus*). Hence, these species can be directly identified from the others. The studied *Hyoscyamus* species were characterized by the absence of nonglandular trichomes. While *S. elaeagnifolium*, *S. nigrum*, and *W. somnifera* were characterized by the absence of glandular trichomes. The presence of dendritic trichomes distinguished the studied species of *Withania.* Dendritic glandular trichome was specific to *H. muticus*. Stellate trichomes were recorded in *S. forskalii*, *S. incanum*, *S. coagulans*, and *S. elaeagnifolium*.

The appearance of the cuticle layer on leaves varied from thin films to thick crusts, producing considerable micromorphological diversity that may be taxonomically interesting (46,47). The cuticle layer was detected in four patterns: smooth, finely warty, warty-crustose, and finely striate wax.

The anticlinal cell wall was recorded in three patterns (undulate, sinuous, and straight). The cell wall pattern is one of those epidermal features of taxonomic importance at specific levels (31,46). Four surface sculpturing patterns were recorded in the studied species: wrinkled, striated, reticulate, and favulariate.

The types of trichome and cuticle ornamentation can differentiate between the two species of *Datura. D.*

innoxia characterized by reticulate-foveate leaf surface ornamentation, while *D. stramonium was* characterized by irregularly reticulate leaf surface ornamentation. Three trichome types (Bicellular uniseriate with a straight acute apical cell, bicellular uniseriate stalk, unicellular head, and multicellular uniseriate stalk and unicellular head) were recorded in *Datura innoxia,* while absent in *D. stramonium.*

The three studied species of *Lycium* were characterized by regular epidermal cells and straight anticlinal cell walls. *L. schweinfurthii* was more related to *L. europaeum*. They were characterized by anomocytic, elliptic stomata, stomatal aperture linear and stomatal rim raised, the two trichome types (multicellular uniseriate with acute apical cell, and multicellular stalk and biarmed unequal apical cell) were absent.

This study recommended that some taxa belong to separate tribes. Tribes Hyoscyameae (*H. boveanus, H. muticus, H. desertorum, H. albus,* and *H. pusillus*), Solaneae (*S. coagulans, S. elaeagnifolium, S. incanum, S. nigrum, S. sinaicum, S. villosum, S. forskalii, W. obtusifolia, W. somnifera, P. angulata,* and *P. ixocarpa*), and Datureae (*D. innoxia* and *D. stramonium*) were grouped in one cluster. *Hyoscyamus* taxa (tribe Hyoscyameae) was more related to *Solanum* taxa (tribe Solaneae). This result agreed with Wettstein (48). Additionally, *Physalis* species (tribe Solaneae) were more related to *Datura* species (tribe Datureae) and separated into a distinct subgroup. *W. obtusifolia* and *W. somnifera* were separated into a distinct subgroup.

Cluster I contained *L. shawii, L. europaeum*, and *L. schweinfurthii*, which were more related and separated into a distinct group. This group was supposed to represent the tribe Lycieae. This agreed with Hunziker (1) who classified these members into a distinct tribe. The tribal classification of the genus *Lycium* is controversial. Earlier works treated it as a member of the large tribe Solaneae based on morphological characteristics (48). However, *Lycium* has long been part of the tribe Lycieae (1,2). In this study, *Lycium* was distinct from the other groups. This result agreed with Hunziker (1).

On the basis of numerical analysis, this study provided nearly sufficient information on the taxonomic importance of leaf micromorphology, validating its usefulness in the studied species and genera of Solanoideae discrimination. The results indicated that the characteristics of surface sculpturing patterns, types of trichome, and outer stomatal rims would be helpful for the identification and differentiation among the studied species.

5. Conclusion

This study determined that the morphological characteristics and identification keys as classical taxonomy are still important and that numerical analysis is required to clarify taxonomic relationships among the studied species. Additional SEM studies are important to reveal the details of plant surfaces, especially epidermal features, such as trichomes and cell types, aside from gross morphological characteristics that are very useful in systematic plant anatomy.

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