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Epidermal Properties and Stomatal Abnormalities of two Amaryllidaceae Taxa (*Crinum album* and *Scadoxus multiflorus*) Growing in Yemen and their Taxonomic Significance

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Abstract:

This study investigated the epidermal properties and Stomatal abnormalities of two Amaryllidaceae taxa (*Crinum album* and *Scadoxus multiflorus*) and their taxonomic significance. Our analysis revealed twelve quantitative properties out of a total of 53 (17 qualitative and 36 quantitative) studied properties, which showed significant differences between *C. album* and *S. multiflorus*. These properties includes eight quantitative properties observed on both the adaxial and abaxial surfaces (Number of epidermal cells per $\times 250$ field of view, Width of epidermal cells, Size of the epidermal cells, Wall thickness of the epidermal cells, Number of stomata per $\times 250$ view, Stomata Index, Guard cells area and the Size of stomata), two quantitative properties specific to the adaxial surface (Size of epidermal cell, and Guard cell width), and two quantitative properties particular to the abaxial surface (Percentage of Tetracytic stomata and Guard cell length). Moreover, the 53 epidermal characters (17 qualitative and 36 quantitative) were subjected to numerical analysis using Two-way cluster analysis (TWCA), and the resulting dendrogram showed that the relative similarity level between *C. album* and *S. multiflorus* is about 87.92%.

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INTRODUCTION

Anatomically, plants are relatively simple organisms composed of a limited number of distinct cell types (Goldberg, 1988; Ibrahim *et al.*, 2018). The main tissue systems in plants—vascular, ground, and dermal tissues—arise from the three primary meristems: procambium, ground meristem, and protoderm, respectively. The protoderm, which is a single layer of cells, later differentiates to form the outermost layer known as the epidermis (Dickison, 2000; Bahadar *et al.*, 2018). Typically, the mature epidermis consists of a single layer of cells; however, in certain species, cell divisions can occur early in the epidermis during leaf development, resulting in a multilayered epidermis. Epidermal cells vary in shape among different species. In many monocotyledon species with strap-shaped or axially elongated leaves, the epidermal cells are elongated and organized into well-defined longitudinal files. These cells can have 4–6 or more sides, and their anticlinal walls may be straight, curved, or sinuous. The epidermis, along with a cuticle, functions to prevent water loss and allow gas exchange. Small pores in the epidermis of most leaves and young stems can open and close; these pores are known as stomata. Stomata regulate the movement of water in and out of the plant and consist of a pair of guard cells (often kidney-shaped) with a pore between them. The size of the pore is controlled by changes in the shape of the guard cells. Stomata may be found on both surfaces of the leaf (amphistomatic) or only on the upper (hyperstomatic) and lower (hypostomatic) surface (Cutler *et al.*, 2008; Ibrahim *et al.*, 2018). Measurements of epidermal cells are valuable for distinguishing between closely related species; with sufficient careful measurements and statistical analysis, significant differences can often be recognized (Ibrahim *et al.*, 2016; Ibrahim *et al.*, 2024).

Additionally, the arrangement of subsidiary cells is of great interest to taxonomists who aim to identify small leaf fragments (Ibrahim *et al.*,

2021; Aqlan *et al.*, 2025). Abnormal stomatal patterning, such as cytoplasmic connections between stomata, polarly contiguous stomata, and stomata that possess a single guard cell without a pore, has been reported in certain species belonging to the dicot and monocot families, including Begoniaceae, Crassulaceae, Sonneratiaceae, Moraceae, and Amaryllidaceae (Metcalfe and Chalk, 1939; Hashemloian and Azimi, 2014). These abnormal patterns differ significantly from standard stomatal patterns (Pandey *et al.*, 2007).

The Amaryllidaceae family, one of the monocotyledon families, comprises 61 genera and approximately 850 species, primarily found in tropical and subtropical regions (Takhtajan, 2009). All members of this family are bulbous, perennial herbs that display a basal rosette of leaves (Boulos, 2005). In Yemen, the Amaryllidaceae family is represented by five genera: *Crinum* L., *Hypoxis* L., *Pancratium* L., *Polyanthes* Hill, and *Scadoxus* Raf., and 10 species (Al Khulaidi, 2013). Most members of this family thrive in the highlands and mountainous regions of Yemen, including Al-Ahjour County, where two taxa (*C. album* and *S. multiflorus*) of Amaryllidaceae grow (Ibrahim *et al.*, 2014).

The genus *Crinum* L. consists of around 120 species (Chaudhary, 2001), of which two are found in Yemen: *C. album* (Forssk.) Herb., endemic to the southwest Arabian Peninsula (POWO, 2025; WFO, 2025), and *C. balfourii* Baker, which is endemic to Socotra Island (Al Khulaidi, 2013).

Few studies have focused on the epidermal features of members of the Amaryllidaceae family. Awasthi *et al.* (1984) investigated the epidermal properties, including epidermal cell characteristics and stomatal features, of several Amaryllidaceae species. They documented a variety of abnormal stomatal structures, such as laterally contiguous stomata in *Amaryllis belladonna*, *Cooperia pedunculata*, *Narcissus tazetta*, and *Scadoxus multiflorus*; polarly

contiguous stomata in *Hippeastrum multiflorum* and *A. belladonna*; cytoplasmic connections between adjacent stomata in *Amaryllis vittata* and *S. multiflorus*; and stomata featuring a single guard cell in *C. pedunculata*. They also noted stomata with a single guard cell lacking a pore in *Pancratium verecundum* and *S. multiflorus*.

Furthermore, Hashemloian and Azimi (2014) observed the presence of laterally contiguous stomata and stomata with a single guard cell in *Narcissus pseudonarcissus*. They identified polarly contiguous stomata in *Amaryllis reticulata* and *Ixiolirion tataricum*, as well as degenerated guard cells and cytoplasmic connections between two to six adjacent stomata in *A. reticulata* and *N. pseudonarcissus*.

In addition, Awasthi et al. (1984), Cutler et al. (2008), and Hashemloian and Azimi (2014) noted that the epidermal characteristics can be utilized to differentiate between species such as *A. belladonna*, *A. reticulata*, *A. vittata*, *C. pedunculata*, *I. tataricum*, *S. multiflorus*, *N. pseudonarcissus*, *N. tazetta*, *P. verecundum*, and other closely related species.

Based on previous studies no attempts seem to have been made to study the epidermal features (epidermal cell properties and stomatal properties including abnormal stomatal structures) of *C. album* and *S. multiflorus* leaf in details, therefore the present study aims to investigate in details the epidermal properties (epidermal cell properties and stomatal properties including abnormal stomatal structures) of *C. album* and *S. multiflorus* leaf and its taxonomical significance in distinguishing between the two species.

MATERIALS AND METHODS

Plant material

Fresh leaves and plant specimens of two Amaryllidaceae taxa, *C. album* and *S. multiflorus* (Figure 1), were collected during the period March-April 2025 from Al-Ahjour County, Al-Mahweet, governorate, which is 50 kilometers

northwest of Sana'a, Yemen's capital. According to Ibrahim et al. (2014), Al-Ahjour county is located in the country's highland and high mountain region between the longitudinal ranges of 43°51'25.07" E–43°53'57.02" E and the latitudinal ranges of 15°27'51.01"N–15°28'22.01"N, with an altitudinal range of 2302–2572 m asl.

Experimental investigation

The plant materials were classified by using the available taxonomic references, particularly Wood (1997) and Al Khulaidi (2013). The identified specimens were designated herbarium numbers, with BHSS 1178 assigned to *C. album* and BHSS 1201 to *S. multiflorus*, and preserved at the Faculty of Science Herbarium for future reference. For each species, *C. album* and *S. multiflorus*, about ten fully grown and mature leaves were cut into 2-cm-long pieces (Ibrahim et al., 2021; Aqlan et al., 2025). The leaf pieces were then submerged in concentrated nitric acid until air bubbles formed on the surface, indicating that the adaxial and abaxial epidermal layers were ready to be separated. The leaf fragments were then put in a water-filled Petri dish, and using a dissecting needle and delicate forceps, the epidermal layers were carefully separated. Finally, a camel hair brush was used to gently clean each layer of the epidermis while it was submerged in water (Ibrahim et al., 2016; Aqlan et al., 2025). The layers were then mounted in glycerol on clean slides after being stained with Safranin and rinsed with clean water to remove any leftover stain. Then each slide was covered by a cover slide. About 30 duplicates of the upper (adaxial) and lower (abaxial) epidermal layers for each species under investigation were made. The slides were examined at $\times 250$ magnification using a light microscope (Leica ATC 2000). To evaluate the qualitative and quantitative properties of the epidermal layers, Photographs of the epidermal views were captured with a Canon (IXUS255 HS) digital camera. Moreover, an ocular micrometer calibrated with a stage micrometer was used with Image J software to measure quantitative parameters at a magnification of $\times 400$, where one ocular minor division is equivalent to 2.5 μm (modified after Ibrahim et

al., 2024; Aqlan *et al.*, 2025). About 53 epidermal characteristics (17 qualitative and 36 quantitative) were investigated (Tables 1 and 2), based on the nomenclature of epidermal layers as defined by Dilcher (1974) and the Leaf

Architecture Working Group (1999), while the terminology of stomatal abnormalities was taken from Awasthi *et al.* (1984) and Hashemloian and Azimi (2014).



Fig. 1. General view of the two Amaryllidaceae taxa: A. *Crinum album*, B-C. *Scadoxus multiflorus*; B. Inflorescence of *Scadoxus multiflorus*, C. fruits and leaves of *Scadoxus multiflorus*.

Table 1. Examined leaf epidermal qualitative characters of the two Amaryllidaceae taxa (*C. album* and *S. multiflorus*) under investigation.

1	Shape of Epidermal Cells	Linear, elongate [1]	Rectangular [2]
2	Epidermal Cell Wall Patterns	Straight with an end wrap around the cell wall enclosing adjacent cells [1]	Sinuously [2]
3	Type of leaf based on the stomata ratio	Hypoamphistomatic [1]	Not so [2]
4	Type of stomata.	3 types of stomata (Amniocytic, Tetracytic, Polycytic (5 subsidiary cells)) in the adaxial and abaxial layer [1]	2 types of stomata (Tetracytic and Polycytic (5 subsidiary cells)) at the adaxial layer and 3 types of stomata (Tetracytic, Polycytic (5 subsidiary cells), and Polycytic (> 5 subsidiary cells)) at the abaxial layer. [2]

Stomatal Abnormalities	5	Poles of guard cells marked with a small area of Cutin	Absent [0]	Present [1]
	6	Cytoplasmic connection between one guard cell of the stomata and an epidermal cell	Absent [0]	Present [1]
	7	Cytoplasmic connection between one guard cell of the stomata and an epidermal cell, and the cytoplasmic connection between 2 adjacent stomata	Absent [0]	Present [1]
	8	Cytoplasmic connection between one guard cell of the stomata and an epidermal cell, and the cytoplasmic connection between 3 adjacent stomata	Absent [0]	Present [1]
	9	Cytoplasmic connection between two guard cells of the stomata and epidermal cells	Absent [0]	Present [1]
	10	Cytoplasmic connection between two adjacent stomata	Absent [0]	Present [1]
	11	Cytoplasmic connection between three adjacent stomata	Absent [0]	Present [1]
	12	Cytoplasmic connection between four adjacent stomata	Absent [0]	Present [1]
	13	Degenerated stomata	Absent [0]	Present [1]
	14	Obliquely oriented Stomata	Absent [0]	Present [1]
	15	Polarly contiguous stomata	Absent [0]	Present [1]
	16	Stomata with unequal guard cells	Absent [0]	Present [1]
	17	Stomata with one guard cell	Absent [0]	Present [1]

Table 2. Examined leaf epidermal quantitative characters of the two Amaryllidaceae taxa (*C. album* and *S. multiflorus*) under Investigation.

No.	Characters		Epidermal layer
1	Epidermal cells	No. of cells in $\times 250$ view	Adaxial
2			Abaxial
3		Length (μm)	Adaxial
4			Abaxial
5		Width (μm)	Adaxial
6			Abaxial
7		Size (μm^2)	Adaxial
8			Abaxial
9		Wall thickness (μm)	Adaxial
10			Abaxial
11	Stomata	No. of Stomata in $\times 250$ view	Adaxial
12			Abaxial
13		Stomata Index (%)	Adaxial

14	Percentage of Stomata Type	% Polycytic (> 5 subsidiary cells)	Abaxial
15			Adaxial
16			Abaxial
17		% Polycytic (5 subsidiary cells)	Adaxial
18			Abaxial
19		% Tetracytic	Adaxial
20			Abaxial
21		% Anisocytic	Adaxial
22			Abaxial
23		Guard cell length (μm)	Adaxial
24			Abaxial
25		Guard cell width (μm)	Adaxial
26			Abaxial
27		Guard cells Size (μm ²)	Adaxial
28			Abaxial
29		Guard cell area (μm ²)	Adaxial
30			Abaxial
31		Stomata length (μm)	Adaxial
32			Abaxial
33		Stomata width (μm)	Adaxial
34			Abaxial
35		Size of stomata (μm ²)	Adaxial
36			Abaxial

RESULTS

A total of 53 leaf epidermal characters (17 qualitative and 36 quantitative properties) of *C. album* and *S. multiflorus* growing in Al-Ahjour County were examined to investigate in detail the epidermal cell properties and stomatal properties and to show their taxonomical significance in distinguishing between the two species.

The qualitative properties of the leaf epidermis, as shown in Table 3 and Figures 2, 3 and 4, comprise four typical structures and 13 stomatal abnormalities. The normal qualitative structures (Table 3, Figures 2 and 3) indicate that the epidermal cells of *C. album* are linear and elongate, while the epidermal cells of *S. multiflorus* are rectangular in shape. Moreover, the epidermal cell wall patterns of *C. album* are straight with an end wrap around the cell wall, enclosing adjacent cells, while *S. multiflorus* has sinuous epidermal cell wall patterns. Furthermore, the Type of leaf in the two studied taxa, based on the stomata ratio, is Hypoamphistomatic. In addition, *C. album* shows three types of stomata in the adaxial and abaxial surface based on the subsidiary cells

(Amniocytic, Tetracytic, Polycytic (5 subsidiary cells)). In comparison, *S. multiflorus* displays two types of stomata (Tetracytic and Polycytic (5 subsidiary cells)) at the adaxial layer and three types of stomata (Tetracytic, Polycytic (5 subsidiary cells), and Polycytic (> five subsidiary cells)) at the abaxial layer. On the other hand, Table 3 and Figure 4 illustrates that *C. album* displays 12 stomatal abnormalities; Cytoplasmic connection between one guard cell of the stomata and an epidermal cell, Cytoplasmic connection between the two guard cells of the stomata and two epidermal cell, Cytoplasmic connection between two adjacent stomata, Cytoplasmic connection between one guard cell of the stomata and an epidermal cell and cytoplasmic connection between 2 adjacent stomata, Cytoplasmic connection between three adjacent stomata, Cytoplasmic connection between one guard cell of the stomata and an epidermal cell and cytoplasmic connection between 3 adjacent stomata, Cytoplasmic connection four adjacent stomata, Degenerated stomata, Obliquely oriented Stomata, Polarly contiguous stomata, Stomata with unequal guard cells and Stomata with one guard cell. However, *S. multiflorus* exhibits only two stomatal abnormalities: Poles of guard cells

marked with a small area of Cutin and Cytoplasmic connections between two adjacent

stomata (Table 3 and Figure 4).

Table 3. Qualitative leaf epidermal characters of the two Amaryllidaceae taxa (*C. album* and *S. multiflorus*) under investigation.

No.	Characters	<i>C. album</i>	<i>S. multiflorus</i>
1	Shape of Epidermal Cells	1	2
2	Epidermal Cell Wall Patterns	1	2
3	Type of Leaf based on stomata ratio	1	1
4	Type of Stomata	1	2
5	Poles of guard cells marked with a small area of Cutin	0	1
6	Cytoplasmic connection between one guard cell of the stomata and an epidermal cell	1	0
7	Cytoplasmic connection between the two guard cells of the stomata and two epidermal cells	1	0
8	Cytoplasmic connection between two adjacent stomata	1	1
9	Cytoplasmic connection between one guard cell of the stomata and an epidermal cell, and the cytoplasmic connection between two adjacent stomata	1	0
10	Cytoplasmic connection between three adjacent stomata	1	0
11	Cytoplasmic connection between one guard cell of the stomata and an epidermal cell, and the cytoplasmic connection between three adjacent stomata	1	0
12	Cytoplasmic connection between four adjacent stomata	1	0
13	Degenerated stomata	1	0
14	Obliquely oriented Stomata	1	0
15	Polarly contiguous stomata	1	0
16	Stomata with unequal guard cells	1	0
17	Stomata with one guard cell	1	0

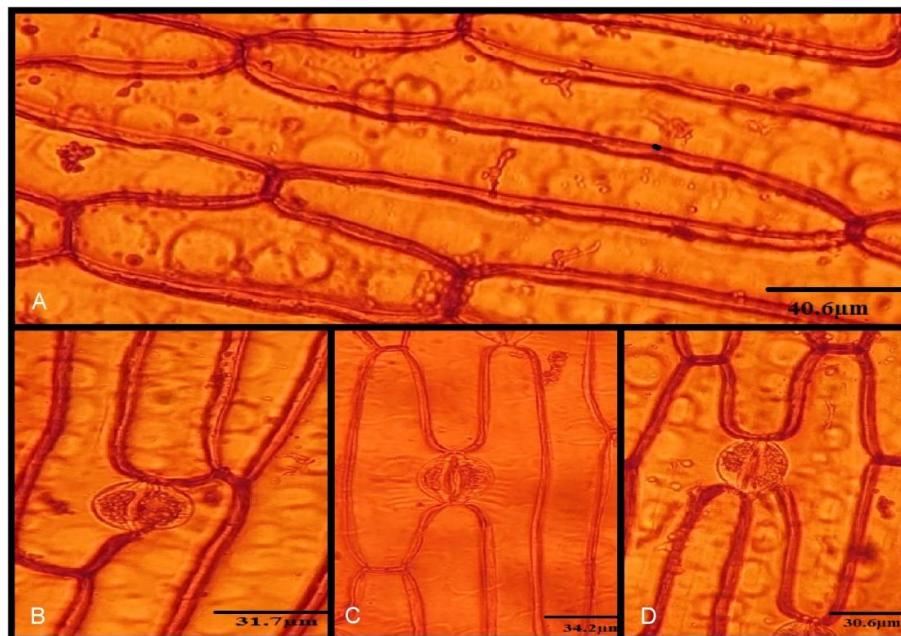


Fig. 2. Epidermal cells and Stomata type in *C. album*: **A.** Epidermal cells at the Adaxial and Abaxial surface, **B.** Anisocytic stomata, **C.** Tetracytic stomata, **D.** Polycytic stomata (5 subsidiary cells).

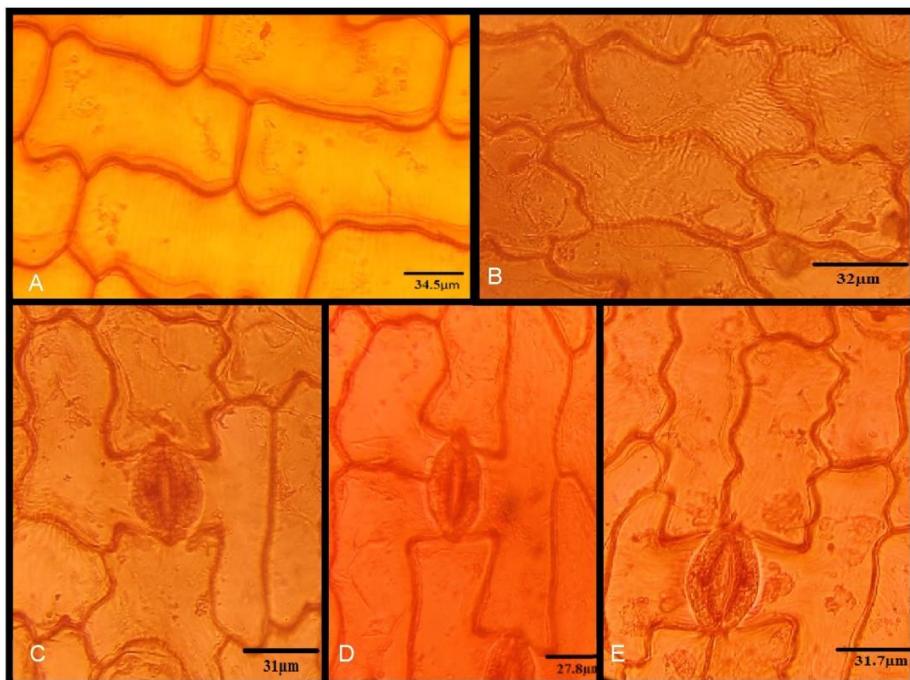


Fig. 3. Epidermal cells and Stomata type in *S. multiflorus*: **A.** Epidermal cells at the Adaxial surface, **B.** Epidermal cells at the Abaxial surface, **C.** Tetracytic stomata, **D.** Polycytic stomata (5 subsidiary cells), **E.** Polycytic stomata (> 5 subsidiary cells).

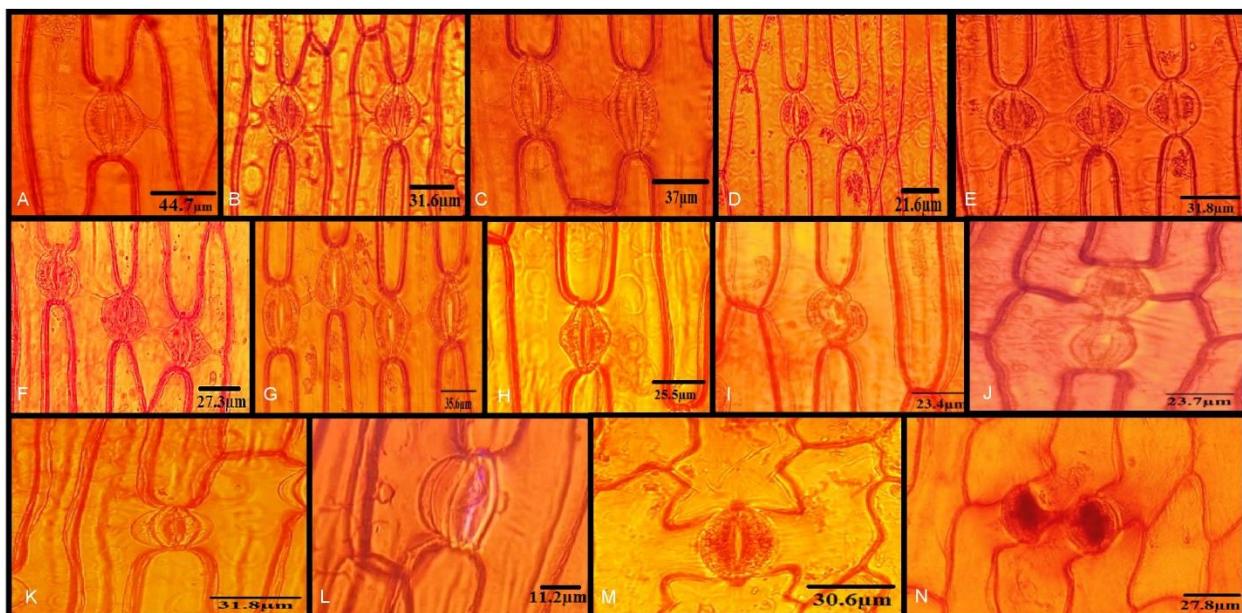


Fig. 4. Stomatal Abnormalities: **A-L** Stomatal Abnormalities in *C. album*, **M-N** Stomatal Abnormalities in *S. multiflorus*: **A.** Cytoplasmic connection between one guard cell of the stomata and an epidermal cell, **B.** Cytoplasmic connection between the two guard cells of the stomata and two epidermal cells, **C.** Cytoplasmic connection between two adjacent stomata, **D.** Cytoplasmic connection between one guard cell of the stomata and an epidermal cell, and the cytoplasmic connection between 2 adjacent stomata, **E.** Cytoplasmic connection between three adjacent stomata, **F.** Cytoplasmic connection between one guard cell of the stomata and an epidermal cell, and the cytoplasmic connection between 3 adjacent stomata, **G.** Cytoplasmic connection between four adjacent stomata, **H.** Degenerated stomata, **I.** Obliquely oriented stomata, **J.** Polarly contiguous stomata, **K.** Stomata with unequal guard cells, **L.** Stomata with one guard cell, **M.** Poles of guard cells marked with a small area of Cutin, **N.** Cytoplasmic connections between two adjacent stomata.

According to the quantitative characteristics illustrated in Table 4, the abaxial surface of *C. album* show the highest mean number of epidermal cells (111 epidermal cell) in a view field of $\times 250$ followed by the adaxial surface of *C. album* and the abaxial surface *S. multiflorus*

with a mean number of epidermal cells 99 and 78 in a view field of $\times 250$ respectively, while the adaxial surface *S. multiflorus* exhibited the lowest mean number of epidermal cell 43 epidermal cell) in a view field of $\times 250$.

Table 4. Quantitative leaf epidermal characters of the two Amaryllidaceae taxa (*C. album* and *S. multiflorus*) under investigation.

No.	Characters	Epidermal layer	<i>C. album</i>	<i>S. multiflorus</i>	P. value	
1	No. of cells in $\times 250$ view	Epidermal cells	Ad	89 (99 \pm 6.5) 111	36 (43 \pm 4) 50	< 0.0001 ^(****)
2	Min (Mean \pm SD) Max		Ab	100 (111 \pm 8.1) 124	70 (78 \pm 5.2) 86	< 0.0001 ^(****)
3	Length (μm)		Ad	72 (156.1 \pm 34.8) 218.4	72.9 (145.4 \pm 45.2) 234.8	0.3109
4	Min (Mean \pm SD) Max		Ab	55.2 (130.2 \pm 39.8) 224.3	50.1 (106.2 \pm 40.7) 183.7	0.0246 ^(*)
5	Width (μm)		Ad	24.747(45.2 \pm 17.8) 109.1	58.4 (87.7 \pm 22.5) 158.2	< 0.0001 ^(****)
6	Min (Mean \pm SD) Max		Ab	20.1 (36.5 \pm 11.6) 67.7	33.4 (59.5 \pm 12.6) 90.6	< 0.0001 ^(****)
7	Size (μm^2)		Ad	2812.2 (7200.1 \pm 3704.2) 20677.1	6032.1 (12643.6 \pm 4913.8) 26204.1	< 0.0001 ^(****)
8	Min (Mean \pm SD) Max		Ab	1108.3 (4829.7 \pm 2204.6) 9609	2285.2(6251.7 \pm 2593.1) 13214	0.0258 ^(*)
9	Wall thickness (μm)	Ad	3.2 (5.3 \pm 1.7) 9.3	2.4 (3.5 \pm 0.7) 4.9	< 0.0001 ^(****)	
10	Min (Mean \pm SD) Max	Ab	2.9 (4.7 \pm 1.2) 6.8	2 (2.9 \pm 0.6) 3.9	< 0.0001 ^(****)	
11	No. of Stomata in $\times 250$ view	Ad	20 (23 \pm 2.2) 27	0 (3 \pm 2.4) 7	< 0.0001 ^(****)	
12	Min (Mean \pm SD) Max	Ab	22 (27 \pm 4) 36	13 (14 \pm 1.3) 17	< 0.0001 ^(****)	
13	Stomata Index (%)	Ad	15.8 (18.8 \pm 1.7) 21.9	0 (6.6 \pm 5.3) 15.6	< 0.0001 ^(****)	
14	Min (Mean \pm SD) Max	Ab	17.3 (19.7 \pm 1.8) 23.2	14 (15.4 \pm 1) 17.2	< 0.0001 ^(****)	
15	% Polycytic (> 5 subsidiary cells)	Ad	0	0	-	
16	Min (Mean \pm SD) Max	Ab	0	0 (4.6 \pm 5.5) 16.7	0.0031 ^(**)	
17	% Polycytic (5 subsidiary cells)	Ad	0 (0.6 \pm 1.6) 4.8	0 (16.2 \pm 28.9) 100	0.0456 ^(*)	
18	Min (Mean \pm SD) Max	Ab	0 (3.7 \pm 4) 12.5	0 (4.6 \pm 5.5) 16.7	0.0004 ^(***)	
19	% Tetracytic	Ad	93.3 (99 \pm 2.2) 100	0 (70.4 \pm 40.1) 100	0.0103 ^(*)	
20	Min (Mean \pm SD) Max	Ab	87.5 (95.8 \pm 4) 100	63.6 (80.4 \pm 12.2) 100	< 0.0001 ^(****)	
21	% Anisocytic	Ad	0 (0.4 \pm 1.7) 6.7	0	0.3259	
22	Min (Mean \pm SD) Max	Ab	0 (0.6 \pm 2.2) 8.3	0	0.3259	
23	Guard cell length (μm)	Ad	29.7 (33.4 \pm 2.8) 38.5	27.5 (35.7 \pm 5.3) 43.9	0.1500	
24	Min (Mean \pm SD) Max	Ab	23.4 (33.1 \pm 5.7) 43.3	36.6 (42 \pm 4.4) 50	< 0.0001 ^(****)	
25	Guard cell width (μm)	Ad	7.4 (10.7 \pm 1.7) 14	9.3 (15.8 \pm 2.8) 20.7	< 0.0001 ^(****)	
26	Min (Mean \pm SD) Max	Ab	10.7 (12.6 \pm 1.2) 15.1	11.9 (14 \pm 1) 15.7	0.0013 ^(*)	
27	Guard cells Size (μm^2)	Ad	227.4 (358.2 \pm 74) 480.6	390.3 (566 \pm 142.4) 839.1	< 0.0001 ^(****)	
28	Min (Mean \pm SD) Max	Ab	250 (418.6 \pm 100.9) 594.4	457 (590.2 \pm 81.5) 713	< 0.0001 ^(****)	
29	Guard cell area (μm^2)	Ad	178.6 (281.3 \pm 58.1) 377.4	306.5(444.4 \pm 111.8) 658.9	< 0.0001 ^(****)	
30	Min (Mean \pm SD) Max	Ab	196.4(328.7 \pm 79.2) 466.8	358.8(463.5 \pm 64) 559.9	< 0.0001 ^(****)	
31	Stomata length (μm)	Ad	29.7 (33.4 \pm 2.8) 38.5	34.3 (43.2 \pm 6.3) 55.1	< 0.0001 ^(****)	
32	Min (Mean \pm SD) Max	Ab	23.2 (33.1 \pm 5.7) 43.3	44.7 (49.8 \pm 4.8) 61.6	< 0.0001 ^(****)	
33	Stomata width (μm)	Ad	22.2 (28.4 \pm 3.6) 35.4	24.4(34.3 \pm 5.3) 45.9	0.0012 ^(*)	
34	Min (Mean \pm SD) Max	Ab	24.1(28.5 \pm 3.2) 33.5	27.1(31.2 \pm 2.8) 36.4	0.0183 ^(*)	
35	Size of stomata (μm^2)	Ad	704.6 (953.3 \pm 172.9) 1217.2	992(1502.2 \pm 420.8) 2528.2	< 0.0001 ^(****)	
36	Min (Mean \pm SD) Max	Ab	561.7 (956.4 \pm 260.2) 1416.2	1223.1(1556 \pm 225.1) 1947.9	< 0.0001 ^(****)	

Ad: Adaxial surface; Ab: Abaxial surface; (****) Extremely significant; (**) Highly significant and (*) & (†) Significant.

However, the adaxial surface of *S. multiflorus* displays the highest size of epidermal cells (Table 4) with a mean size of about $12643.6 \mu\text{m}^2$

tailed by the adaxial surface of *C. album* and the abaxial surface of *S. multiflorus* (7200.1 and $6251.7 \mu\text{m}^2$ correspondingly), whereas the

abaxial surface of *C. album* illustrate the lowest size of epidermal cells (with a mean size of $4829.7\mu\text{m}^2$). Moreover, the adaxial surface of *C. album* shows the highest wall thickness of the epidermal cell with a mean wall thickness of about $5.3\mu\text{m}$ (Table 4), followed by the abaxial surface of *C. album* ($4.7\mu\text{m}$), while the adaxial and the abaxial *S. multiflorus* have a mean wall thickness of about 3.5 and $2.9\mu\text{m}$, respectively. On the other hand, the abaxial surface of *C. album* exhibit the highest mean number of Stomata (27 stomata) in a view field of $\times 250$ tracked by the adaxial surface of *C. album* and abaxial surface of *S. multiflorus* with a mean number of stomata 23 and 14 in a view field of $\times 250$ sequentially, while the adaxial surface of *S. multiflorus* showed the lowest mean number of Stomata (three stomata) in a view field of $\times 250$ (Table 4). Moreover, Table 4, illustrate that the abaxial surface of *S. multiflorus* exhibit the highest Guard cells area with a mean Guard cells area of about $463.5\mu\text{m}^2$ followed by the adaxial surface of *S. multiflorus* and the abaxial surface *C. album* with a mean Guard cells area of about 444.4 and $328.7\mu\text{m}^2$ respectively, whereas the adaxial surface of *C. album* shows the lowest Guard cells area (with a mean of guard cells area of about $281.3\mu\text{m}^2$). Furthermore, Table 4, demonstrates that the abaxial surface of *S. multiflorus* displays the highest Size of stomata with a mean size of Stomata of about $1556\mu\text{m}^2$ tailed by the adaxial surface of *S. multiflorus* and the adaxial surface of *C. album* with a mean Size of stomata of about 1502.2 and $956.4\mu\text{m}^2$ correspondingly, while the adaxial surface of the *C. album* exhibited the lowest Size of stomata (with a mean of Size of stomata of about $953.3\mu\text{m}^2$). In addition, Table 4 reveals that twelve quantitative characters show high significance in distinguishing between the two Amaryllidaceae taxa (*C. album* and *S. multiflorus*). These properties includes eight quantitative properties observed on both the adaxial and abaxial surfaces (Number of epidermal cells per $\times 250$ field of view, Width of epidermal cells, Size of the epidermal cells, Wall thickness of the epidermal cells, Number of stomata per $\times 250$ view, Stomata Index, Guard cells area and the Size of stomata), two quantitative properties specific to the adaxial surface (Size of epidermal

cell, and Guard cell width), and two quantitative properties particular to the abaxial surface (Percentage of Tetracytic stomata and Guard cell length).

Two-Way Cluster Analysis was used to evaluate the association between *C. album* and *S. multiflorus* based on the investigated epidermal features illustrated in Tables 3 and 4. The similarity matrix derived from this analysis was utilized to create a dendrogram (Figure 5), which distinguished the two Amaryllidaceae taxa, *C. album* and *S. multiflorus*, into two groups at a relative similarity level of 87.92%. Group I comprised *C. album*, while Group II comprised *S. multiflorus*.

DISCUSSION

Based on previous observations, the epidermal cells of *C. album* are linear and elongated, with straight ends that wrap around the cell wall. In contrast, the epidermal cells of *S. multiflorus* are rectangular, featuring a sinuous pattern in their cell walls. Those findings are consistent with the work of Awasthi et al. (1984), where they noted that the epidermal cells in all studied Amaryllidaceae, including three species of *Crinum* L. and *S. multiflorus*, are elongated in the direction of the cell axis and generally, their anticlinal walls are either straight or obliquely oriented; while, in *S. multiflorus*, the walls were sinuous. Furthermore, Awasthi et al. (1984) documented two types of stomatal abnormalities observed in the epidermis of *Crinum* species: stomata connected by cytoplasmic strands and stomata with a single guard cell. They also recorded a stomata connected by cytoplasmic strands in the epidermis of *S. multiflorus*. Moreover, several studies have indicated that within certain plant families, low genetic diversity and divergence exist among specific genera. For instance, Vieira and Charlesworth (2001) reported low genetic divergence between the genera *Antirrhinum* L. (tribe: Antirrhineae, family: Scrophulariaceae) and *Digitalis* L. (tribe: Digitalideae, family: Scrophulariaceae). Similarly, Teklemariam et al. (2023) discovered that multiple species within the genera *Santiria* Blume and *Dacryodes* Vahl (tribe: Bursereae, family: Bursereaceae) share identical *rbcL*

sequences, indicating no interspecific divergence. These findings suggest a high degree of genetic similarity among genera within the same family. This aligns with our previous results, which demonstrate that, the relative

similarity between *C. album* which belongs to the genus *Crinum* L., tribe Amaryllideae, and *S. multiflorus* which belongs to the genus *Scadoxus* Raf., tribe Haemantheae, according to Takhtajan, (2009) is 87.92%.

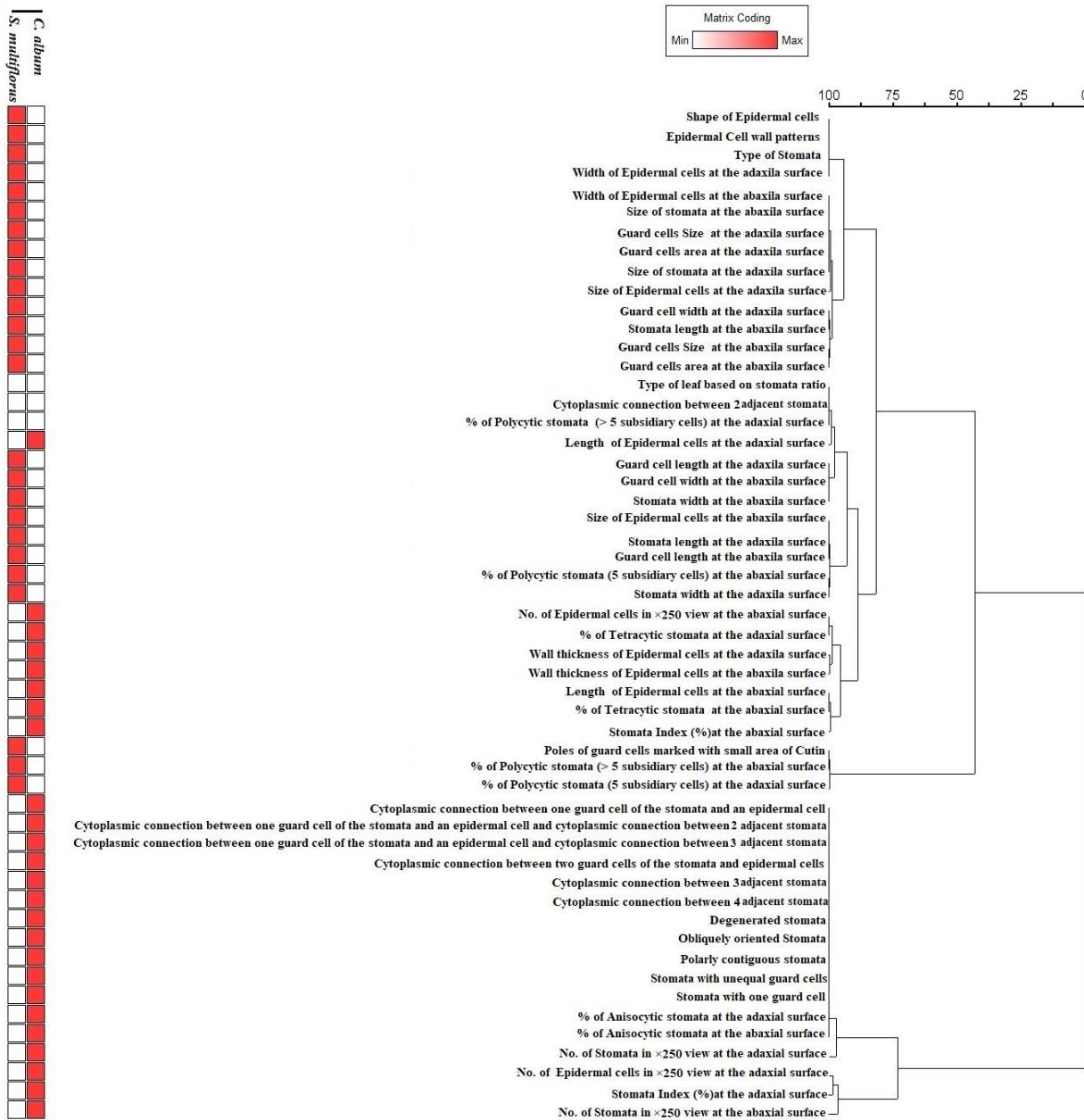


Fig. 5. Cluster analysis illustrates the relationship among the two investigated Amaryllidaceae taxa (*C. album* and *S. multiflorus*) based on the studied leaf epidermal characteristics using the Two-Way Cluster Analysis (TWCA) - Group average.

CONCLUSION

The adaxial and abaxial epidermal cells of *C. album* are linear and elongate, while the epidermal cells of *S. multiflorus* are rectangular. Moreover, the Epidermal cell wall patterns of *C. album* are straight with an end wrap around the cell wall, enclosing adjacent cells, while *S. multiflorus* has sinuous epidermal cell wall patterns. Stomata are present on both surfaces of the leaves, especially in *C. album*, while in the adaxial surface of *S. multiflorus*, they are rare. Furthermore, about twelve quantitative features show high significance in distinguishing between *C. album* and *S. multiflorus*. In addition, based on 53 studied properties, the Two-Way Cluster Analysis illustrate that relative similarity level between *C. album* and *S. multiflorus* is about 87.92%.

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CONFLICT OF INTEREST

Authors hereby declare that they have no conflict of interest.

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