

Comparative Anatomy and Histology of Leaf and Stem in *S. aegyptiaca*, *S. deserti* and *S. spinosa*

Abdullah R. Doaigey and Hesham A. Gawad

Department of Botany, College of Science, King Saud University, Riyadh, Saudi
Arabia

ABSTRACT. Leaves and stems of *Salvia aegyptiaca* L., *S. deserti* Decne. and *S. spinosa* L. show similar frequencies of diacytic stomata and of eglandular and glandular trichomes, but differ as to size and distribution of trichomes. Studies of transverse sections of the stems indicate that the cortex is made of collenchymatous tissue in the corners immediately below the epidermis interchanged with 3 or 4 layers of well developed palisade, chlorenchymatous cells in *S. aegyptiaca* and *S. deserti*, and with 2 to 4 layers of small rounded, chlorenchymatous cells in *S. spinosa*. The remainder of the cortex is a distinct single layer of large parenchymatous cells in *S. aegyptiaca* and *S. deserti*, while in *S. spinosa* it consists of 4 to 7 layers of parenchymatous tissue. Transverse section of the leaves show that the three species are characterized by the presence of collenchymatous tissue on the adaxial and abaxial sides of the midrib regions and the presence of only palisade tissue in the intercostal regions. This study indicates that while these three species of *Salvia* share several common anatomical characters, *S. aegyptiaca* and *S. deserti* are closely related to each other and are quite distinct from *S. spinosa*.

On the basis of reduction in leaf size, increased development of palisade tissues in the leaf and stem, and increased leaf pubescence, it is suggested that *S. deserti* is most adapted to aridity while *S. spinosa* is the least.

The genus *Salvia* (Lamiaceae) is represented in the flora of Saudi Arabia by 5 species. Out of a total of 3 species growing in the vicinity of Riyadh, two, *S. aegyptiaca* and *S. deserti*, contrast sharply in both stature and morphology from the third, *S. spinosa*. *S. aegyptiaca* and *S. deserti* are dwarf shrubs, 15-20 cm high at maturity, bearing few small leaves, 1-2 cm long, described respectively as canescent and white hispid (Migahid 1978), whereas *S. spinosa* has large leaves, up to 20 cm long, and is roughly twice as big as the other two species. The present study compares and contrasts the anatomical and histological features of leaves and

stems in the three species. Anatomy of the genus *Salvia* was reviewed in 1950 by Metcalfe and Chalk. More recently, Singh *et al.* (1974) studied the trichomes in 12 species of *Salvia* that included only one species, *S. aegyptiaca*, of the above mentioned 3 species. Bokhari and Hedge (1977) studied both epidermal and internal anatomical characters of 8 species of *Salvia* that included *S. aegyptiaca* and *S. deserti*, but not *S. spinosa*. This has prompted us to initiate this study on the above mentioned three species which were collected from the same location in their natural habitat.

Material and Methods

Stems and leaves were excised from flowering specimens of *Salvia aegyptiaca*, *S. deserti* and *S. spinosa*, growing in their natural habitats near Riyadh, and preserved in 70% v/v ethanol. For examination of epidermal characteristics, epidermal strips were removed from the preserved materials and mounted in 50% v/v glycerol. For histology, pieces of tissue were dehydrated in the usual way and embedded in wax. Sections (20-30 μm) were cut on a rotary microtome, stained in safranin and light green and mounted in Canada Balsam. Other sections were treated with phloroglucinol/HCl to test for lignin, or with iodine solution to test for starch. Drawings were made with the help of a Reichard screen microprojector.

Results

Common Characters to the Three Species

The three species have the following common epidermal features, (i) ovate, diacytic stomata distributed on the stem and upper and lower surfaces of the leaves, (ii) uniseriate multicellular eglandular trichomes on both stems and leaves, and (iii) glandular trichomes on the leaves (Tables 1, 3, Fig. 1, 3, 5). In addition, they share certain common histological features in both stems and leaves. Thus, in the leaves, there is no spongy mesophyll, this tissue being represented by layers of palisade-like photosynthetic cells (Fig. 2, 4, 6). This lack of spongy mesophyll is considered to be related to the xerophytic habit (Metcalfe and Chalk 1950, Bokhari and Hedge 1977). The midrib region is strengthened in all three species by subepidermal collenchyma on both the upper and lower sides of the vascular bundle. The palisade proper extends from the intercostal region along the flanks of the midrib, meeting with the collenchyma in the midrib region (Fig. 2, 4).

In the stem also, the outer cortex is composed in all three species of photosynthetic cells alternating with layers of collenchyma cells which form the characteristic angles of the lamiaceae stem, whereas the inner cortex is composed of parenchyma cells without chloroplasts (Fig. 2, 4, 6). The phloem and xylem are fused with groups of fibres to form a continuous vascular cylinder surrounding a medulla of large vacuolate parenchyma cells.

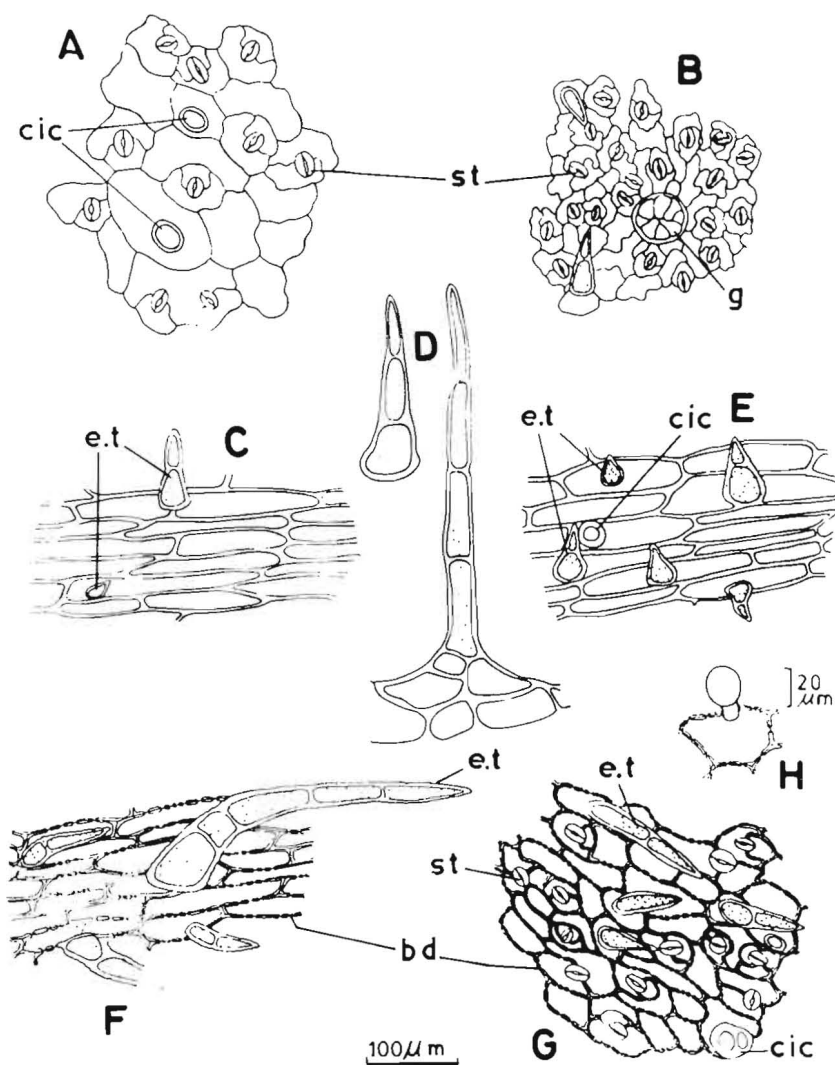


Fig. 1. Epidermal and trichome characters of *S. aegyptiaca*. **A**, leaf, intercostal region, upper epidermis. **B**, leaf, intercostal region, lower epidermis. **C**, leaf, midrib, upper epidermis. **D** and **E**, leaf, midrib, lower epidermis. **F** and **G**, stem epidermis. **H**, leaf, lower epidermis, glandular trichomes.

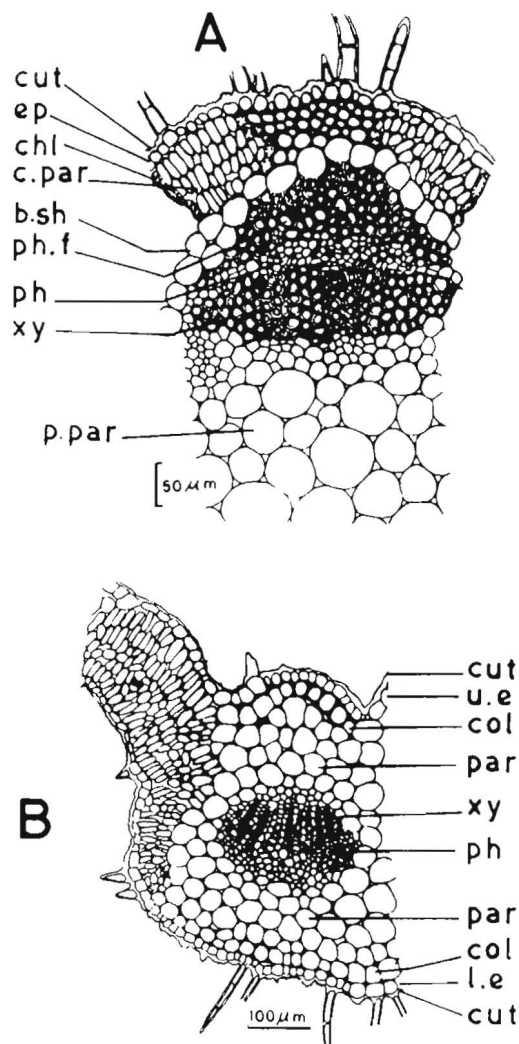


Fig. 2. Transverse sections of stem (**A**) and leaf (**B**) in *S. aegyptiaca*.

Differences between S. aegyptiaca and S. deserti

The two dwarf species have many features in common (Tables 1-4) but are distinguishable readily by the size and lignification of the eglandular trichomes. In *S. aegyptiaca*, the trichomes are from 1-5 cells long and lack lignin in their walls (Table 1), whereas in *S. deserti* there are up to 11 cells and the walls are lignified.

Table 1. Leaf epidermal characters.

Character	Upper (U) or lower (L) epidermis	Species		
		<i>S. aegyptiaca</i>	<i>S. deserti</i>	<i>S. spinosa</i>
Cuticle	U L	thin, smooth similar	thin, striated similar	thin, smooth similar
Epidermal cells	U L	thin or thick, sinuous or straight anti- clinal walls similar	thick, sinuous or straight beaded anti- clinal walls similar	thin, sinuous or straight anticlinal walls similar
Stomata	U L	ovate, diacytic, frequent similar, more frequent	as for <i>S. aegyptiaca</i> similar	as for <i>S. aegyptiaca</i> similar
Glandular trichomes	U L	Type I, occasional Type I, occasional Type II, frequent	absent Type I, II frequent	Type III, occasional Type III, occasional
Eglandular trichomes	U L	1-5 cells long, thick warty cellulosic walls, frequent similar	1-11 cells long, acute, tip, thick lignified warty walls similar but more frequent	2-5 cells long, thick warty walls, frequent similar

Type I. unicellular head, unicellular stalk.

Type II. multicellular head, unicellular stalk.

Type III. unicellular head, multicellular stalk with thick smooth or occasionally warty walls.

In addition, glandular trichomes are present on both upper and lower sides of the leaves of *S. aegyptiaca*, whereas in *S. deserti* they are present only on the lower side of the leaves. The size of the vascular bundle in the midrib of the leaves varies between the two species. It is smaller in *S. deserti* and appears more spherical in section as opposed to ovate in *S. aegyptiaca* (Fig. 2, 4). Differences in the vascular tissues of the stem exist between the two species. In *S. aegyptiaca* the xylem vessels

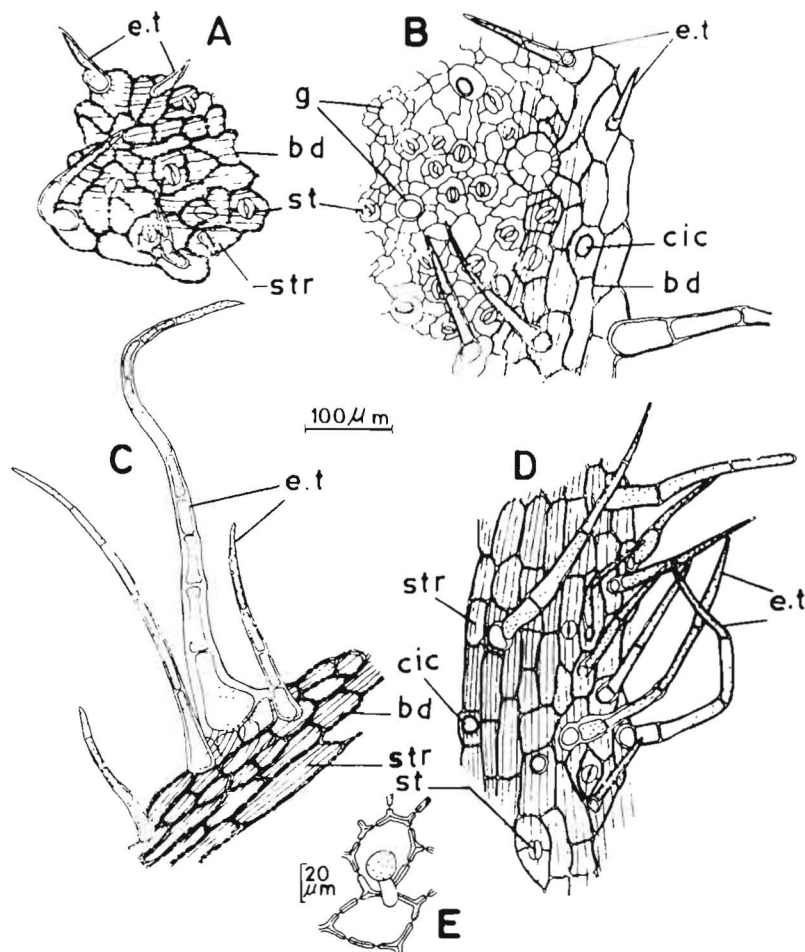


Fig. 3. Epidermal and trichome characters of *S. deserti*. **A**, leaf, intercostal region, upper epidermis. **B**, leaf, intercostal region and midrib, lower epidermis. **C**, leaf, midrib, upper epidermis. **D**, stem epidermis. **E**, leaf, intercostal region, upper epidermis, glandular trichomes.

are regularly arranged and having narrow as opposed to irregular arrangement and wide lumina in *S. deserti*. Furthermore, the primary phloem fibres are lignified in *S. deserti*, whereas it is not lignified in *S. aegyptiaca*. The ground tissue surrounding the vascular bundle is also more extensive in *S. aegyptiaca* than in *S. deserti* (Fig. 2, 4).

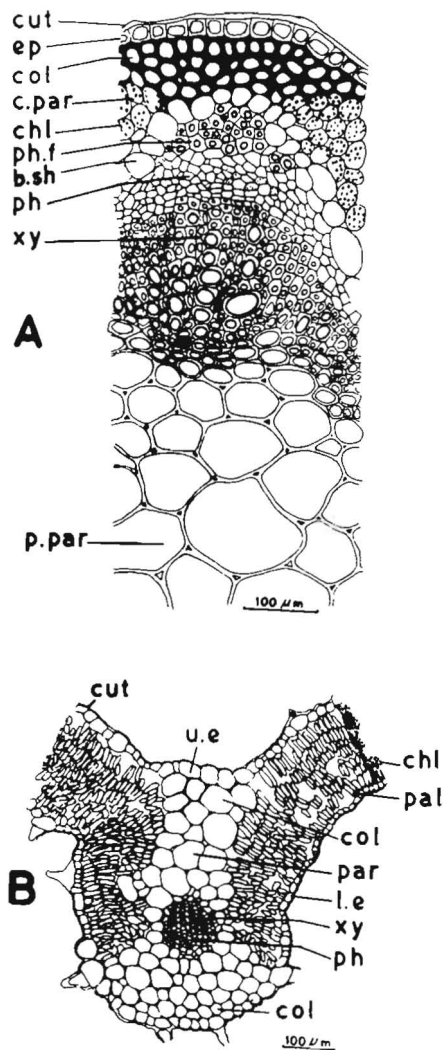


Fig. 4. Transverse sections of stem (A) and leaf (B) in *S. deserti*.

Specific Characters of S. spinosa

S. spinosa is not only larger than the other two species and has larger leaves, but also has certain specific characters not seen in the dwarf species (Tables 1-4, Fig. 7). The outer cortex is more extensive, comprising some 7-9 layers of collenchyma cells in the stem angles as distinct from only 4-5 layers in the dwarf species.

Table 2. Leaf internal structure.

Region	Character	Species		
		<i>S. aegyptiaca</i>	<i>S. deserti</i>	<i>S. spinosa</i>
Intercostal	Mesophyll	palisade-type cells, no spongy-type cells	as for <i>S. aegyptiaca</i>	as for <i>S. aegyptiaca</i>
Midrib	Chlorenchyma	palisade-type, extends on the full length of the flanks reaching collenchyma on lower side	as for <i>S. aegyptiaca</i>	as for <i>S. aegyptiaca</i> but it extends partially on the flanks
	Collenchyma	one subepidermal layer on upper side, two layers on lower side	as for <i>S. aegyptiaca</i>	2-3 subepidermal layers on upper side, 2-3 layers on lower side that extend partially on the flanks
	Parenchyma	several layers	as for <i>S. aegyptiaca</i>	several layers forming wide area, large intercellular spaces
	Vascular bundle	ovate, xylem vessels in rows	circular, small xylem vessels in rows	ovate, large, xylem vessels in rows

The photosynthetic tissue alternating with the collenchyma of the outer cortex also has rounded chlorenchyma, whereas it is of palisade-like chlorenchyma in the other two species (Table 4, Fig. 2, 4, 6). The parenchyma of the inner cortex, bundle sheath, in the two dwarf species is of one layer of large parenchyma and is distinct from that of *S. spinosa* which is represented by several layers of thin-walled smaller parenchyma cells with large intercellular spaces. A striking difference between *S. spinosa* and the other two species is in the morphology and distribution

Table 3. Stem epidermal characters.

Character	Species		
	<i>S. aegyptiaca</i>	<i>S. deserti</i>	<i>S. spinosa</i>
Cuticle	thin, smooth	thin, striated longitudinally	thin, striated longitudinally
Epidermal cells	polygonal, thick slightly sinuous and beaded anticlinal walls <i>at stem angles:</i> cells rectangular or polygonal, elongate, thick, straight and beaded anticlinal walls	as for <i>S. aegyptiaca</i> as for <i>S. aegyptiaca</i>	polygonal, elongate, straight anticlinal walls as above
Stomata	ovate, diacytic absent at stem angles	as for <i>S. aegyptiaca</i>	ovate, diacytic frequent over entire stem surface
Glandular trichomes	absent	absent	unicellular heads, 2-5 celled stalks, thick smooth walls, occasionally warty in basal cells, frequent but more so towards the stem tip
Eglandular trichomes	1-5 cells long thick warty walls, frequent but less so at stem angles	1-10 cells long thick warty walls, acute tips, frequent	2-8 cells long thick warty walls, acute tips, frequent

of the trichomes. Unicellular eglandular trichomes are for instance, missing in *S. spinosa*, where glandular trichomes are not present on the stems of the dwarf species. In addition, all glandular trichomes observed on *S. spinosa* are with multicellular stalk, whereas those observed on the other two species are with unicellular stalk.

Table 4. Stem internal structure.

Character	Species		
	<i>S. aegyptiaca</i>	<i>S. deserti</i>	<i>S. spinosa</i>
Cortex	3-4 layers of palisade-like chlorenchyma cells interspersed by 4-5 layers of collenchyma <i>at stem angles</i>	as for <i>S. aegyptiaca</i>	2-4 layers of more rounded chlorenchyma cells interspersed by 7-9 layers of collenchyma <i>at angles</i>
Bundle sheath	one layer of large parenchyma cells, no chloroplasts or starch	as for <i>S. aegyptiaca</i>	5-7 layers of thin-walled parenchyma cells, no chloroplasts or starch, large intercellular spaces
Vascular tissues	phloem and xylem in continuous cylinder, primary phloem fibres <i>at stem angles</i> no lignin	as for <i>S. aegyptiaca</i> but xylem vessels irregularly arranged and having wide lumina, primary phloem fibres <i>at stem angles</i> have narrow lumina and are lignified	as for <i>S. deserti</i> but vessel elements radially arranged, phloem fibres small in diameter, thick walls, no lignin
Medulla	thin-walled parenchyma cells, large intercellular spaces	thick-walled parenchyma	as for <i>S. aegyptiaca</i>

Discussion

The present study emphasizes anatomical and histological features characteristic of the genus *Salvia* and pinpoints the particular features by which three species inhabiting Saudi Arabia can be distinguished from each other. The two dwarf species, *S. aegyptiaca* and *S. deserti*, are closely related anatomically and distinct from the larger species, *S. spinosa*. Similarities and differences are summarized in Fig. 7. Our findings agree with those of Metcalfe and Chalk (1950), Singh, *et al.* (1974) and Bokhari and Hedge (1977). However, in agreement with Singh *et al.* (1974), on *S. aegyptiaca*, and Bokhari and Hedge (1977), on *S. aegyptiaca* and *S.*

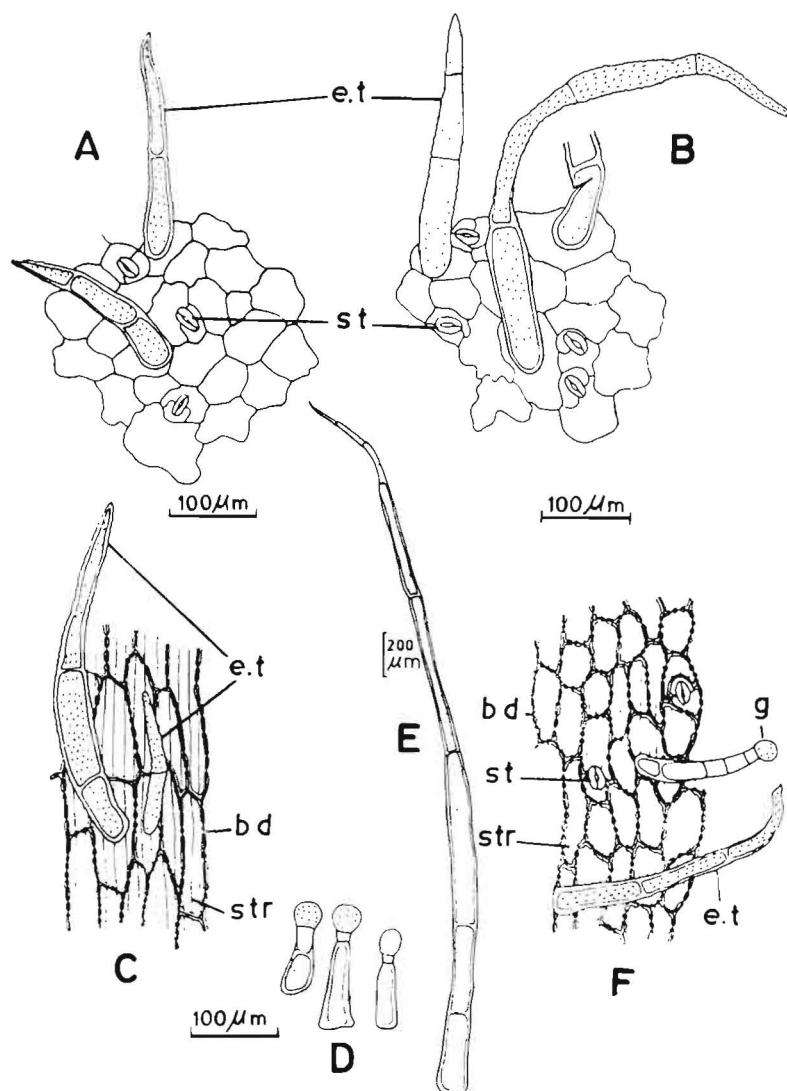


Fig. 5. Epidermal and trichome characters of *S. spinosa*. **A**, leaf, intercostal region, upper epidermis. **B**, leaf, intercostal region, lower epidermis. **C**, leaf, midrib, upper epidermis. **D**, leaf, intercostal region, upper epidermis, glandular trichomes. **E**, stem, epidermal glandular trichomes. **F**, stem, epidermis.

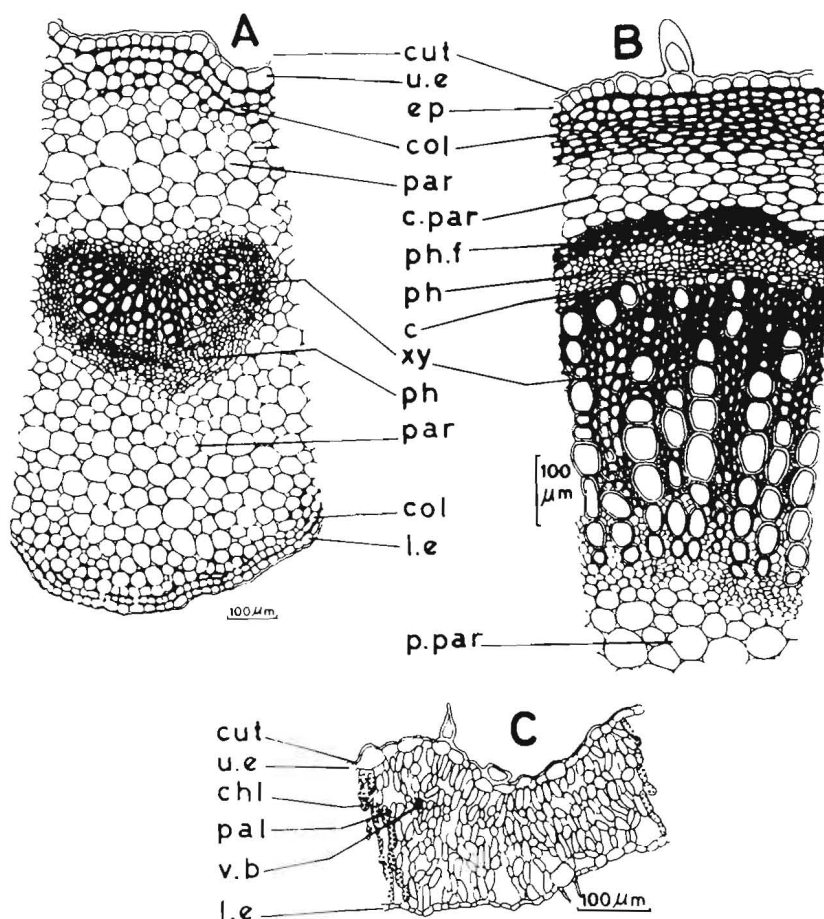


Fig. 6. Transverse sections of leaf midrib (A), stem (B) and leaf intercostal region (C) in *S. spinosa*.

deserti, we have observed the presence of unicellular eglandular trichomes, which has not been recognized for all members of Lamiaceae by Metcalfe and Chalk (1950). Although our findings of the lignification of the eglandular trichomes of *S. deserti* has not been reported previously. In addition, while this study indicates the adaptive features of these species to aridity, it demonstrates the presence of some variation in this regard between *S. aegyptiaca* and *S. deserti* on one hand and *S. spinosa* on the other hand. Reduction in leaf surface (Weaver and Clementis 1938, and Begg 1980) and cell size (Nobel 1980) and increased development of palisade tissue (Shield 1950 and Bokhari and Hedge 1977) and leaf pubescence (Ehleringer 1980) are xeromorphic characters that have been suggested to enhance the ability

Salvia L.

1. Eglandular and glandular trichomes present.
2. Cuticle thin, smooth or striated.
3. Stomata diacytic.
4. Stem cortex has collenchyma, chlorenchyma and parenchyma.
5. Stem, vascular tissue forming a continuous cylinder.
6. Leaf, mesophyll, palisade type, no spongy mesophyll.
7. Midrib region has collenchyma on the adaxial and abaxial sides.



- | | |
|---|--|
| 1. Unicellular eglandular trichomes present. | 1. Unicellular eglandular trichomes absent. |
| 2. Leaf, some glandular trichomes with multicellular heads. | 2. Leaf, glandular trichomes with multicellular heads absent. |
| 3. Leaf, photosynthetic tissue extends in full length on the midrib flanks. | 3. Leaf, photosynthetic tissue extends partially on the midrib flanks. |
| 4. Cortex, chlorenchymatous cells, palisade type. | 4. Cortex, chlorenchymatous cells, isodiametric type. |
| 5. Cortex, collenchyma, 4 to 5 layers. | 5. Cortex, collenchyma, 7 to 9 layers. |
| 6. Cortex, parenchyma, one layer. | 6. Cortex parenchyma, 5 to 7 layers. |
| 7. Stem, glandular trichomes absent. | 7. Stem glandular trichomes present. |

S. spinosa

- | | |
|---|--|
| 1. Eglandular trichomes 1 to 5 cells long, unlignified. | 1. Eglandular trichomes 1 to 11 cells long, lignified. |
| 2. Leaf, vascular bundle large, ovate. | 2. Leaf, vascular bundle small, circular. |
| 3. Midrib, ground tissue wide. | 3. Midrib, ground tissue narrow. |

*S. aegyptiaca**S. deserti*

Fig. 7. Anatomical histological relationships of *S. aegyptiaca*, *S. deserti*, and *S. spinosa*.

of plants to survive water stress and high solar radiation encountered in arid habitat. It is interesting to note that while all 3 species studied show high degree of leaf pubescence which would reduce leaf absorption of solar radiation (Ehleringer 1980), *S. aegyptiaca* and *S. deserti* may be regarded as having better adaptability to aridity than *S. spinosa* since they show much reduced leaf surface and cell size and much increased development of palisade tissues in both of leaves and stems. These 2 dwarf species are also characterized by the presence of one layer of bundle sheath made of large parenchyma cells while in *S. spinosa* it is of 5-7 layers of small parenchyma cells, which may suggest that this character may have a bearing on plant adaptation to aridity. Since *S. deserti* shows more leaf pubescence than *S. aegyptiaca*, it is suggested that *S. deserti* is more adapted to aridity.

Abbreviations

bd, beading; **b. sh**, bundle sheath; **c**, cambium; **chl**, chloroplasts; **cic**, cicatrix; **c. par**, cortical parenchyma; **e. t**, englandular trichomes; **cut**, cuticle; **ep**, epidermis; **l. e**, lower epidermis; **m**, mesophyll; **pal**, palisade tissue; **par**, parenchyma; **ph**, phloem; **ph. f**, primary phloem fibres; **p. par**, pith parenchyma; **st**, stomata; **str**, cuticular striations; **v. b**, vascular bundle; **u. e**, upper epidermis; **xy**, xylem.

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دراسة تشريحية وهستولوجية مقارنة لورقة وساق كل من سلفيا إيجبتياكا و سلفيا ديسيرتى و سلفيا سبينوزا

عبد الله رشيد الدعيجى و هشام عبد الجواد
قسم النبات، كلية العلوم، جامعة الملك سعود، الرياض، المملكة
العربية السعودية

تشابه أوراق وسيقان هذه الأنواع الثلاثة من السلفيا من حيث وجود الثغور متعامدة الخلايا المساعدة وتكررها والشعيرات الغدية واللاغدية، إلا أنها تختلف بالنسبة لحجم وتوزيع الشعيرات. وتوضح دراسات القطاعات العرضية للسيقان أن القشرة تتكون من نسيج كولنشيىمى يوجد فى أضلع السيقان تحت البشرة مباشرة ويكون متبادلا مع ٣ أو ٤ طبقات من نسيج عمادى كلورنشيىمى وذلك فى سلفيا إيجبتياكا و سلفيا ديسيرتى أما فى سيقان سلفيا سبينوزا فإنه يتبادل مع ٢ إلى ٤ طبقات ذوات خلايا كلورنشيىمية مستديرة صغيرة الحجم. وتتكون باقى القشرة من طبقة واحدة مميزة من خلايا برنشيىمية كبيرة الحجم وذلك فى سلفيا إيجبتياكا و سلفيا ديسيرتى بينما فى سلفيا سبينوزا تكون من ٤ إلى ٧ طبقات من خلايا برنشيىمية. وتوضح القطاعات العرضية للأوراق وجود نسيج كولنشيىمى تحت كل من البشرة العليا والبشرة السفلى فى منطقة العرق الوسطى ووجود نسيج عمادى فقط

فى منطقة مابين العروق للأنواع الثلاثة . وبناء على هذه الدراسة فإنه يتضح أن هذه الأنواع الثلاثة من السلفيا تشترك فى عدة صفات تشريحية إلا أن سلفيا إيجبتياكا وسلفيا ديسيرتى أكثر تقارباً لبعضهما البعض ومتميزين عن سلفيا سبينوزا .

بناء على اختزال سطح الورقة وزيادة تكون النسيج العمادى فى الورقة والساق وزيادة شعر الأوراق فقد اقترح أن سلفيا ديسيرتى هى الأكثر تأقلاً للظروف الصحراوية الجفافية بينما أن سلفيا سبينوزا هى الأقل .