

Morphology, Anatomy and Palynology of *Salvia indica* L. (Labiatae)

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Abstract: *Salvia indica* L., which belongs to section *Aethiopsis*, is a perennial herb usually growing on rocky limestone slopes and *Quercus* scrubs in the Southeast Anatolia. In this study, the morphological, anatomical and palynological characteristics of this species using Scanning Electron Microscopy (SEM) have been investigated. In addition, nutlet morphology, emended and expanded description, phenology and ecology of *S. indica* are presented. The stem anatomical structure showed the general features of the family Labiatae. The leaf characters such as the structure of mesophyll and vascular bundles provide important information of taxonomic values. The pollen grains are hexacolpate, radially symmetrical, isopolar and suboblate. Their exine sculpturing is bireticolate-perforate. The nutlets are rounded-trigonal in transverse sections, ovate in shape, glabrous and slightly tuberculate. The species often grows in neutral sandy soils (pH 7.44) with a middle level of organic content (2.62%).

Key words: Labiatae . *Salvia indica* . Morphology . Anatomy . Palynology . Nutlet

INTRODUCTION

The family Labiatae has cosmopolitan distribution and includes over 250 genera and close to 7000 species [1]. The family is known for its fine ornamental or culinary herbs like basil, lavender, mint, oregano, rosemary, sage and thyme and is a rich source of essential oils for the flavouring and perfume industry [2].

Labiatae is the third largest family in Turkey. According to recent literature [3], Labiatae is composed of 45 genera and 574 species, 256 of which are endemic, in Turkey. Its endemism ratio is 44.5%.

The genus *Salvia* (Labiatae: tribe Menthae) represents a cosmopolitan assemblage of nearly 1000 species displaying a remarkable diversity in growth forms, secondary products, floral morphology and pollination biology. *Salvia* has radiated extensively in three regions of the world: Central and South America (500 spp.), Western Asia (200 spp.) and Eastern Asia (100 spp.) [4]. *Salvia* species are used in traditional medicines all around the world, possessing antibacterial, antioxidant, antidiabetic and antitumor properties [5]. In addition, many *Salvia* species are used as herbal tea and for food flavouring, as well as in cosmetics, perfumery and the pharmaceutical industry [6].

Since 2005, as a part of a revision of the genus *Salvia* in Turkey, the authors have carried out extensive field studies and collected a large number of specimens.

Moreover, population size and phenological and ecological properties were noticed in the field. The studies have revealed two new species, namely *S. marashica* A. İlçim, F. Celep & Dogan [7] and *S. ekimiana* F. Celep and Dogan [8] and two new records, namely *S. viscosa* Jacq. [9] and *S. macrosiphon* Boiss. [10].

The anatomical and palynological characteristics of *S. indica* have not been studied before. Therefore, the present study aims to give a detailed account of the morphological, anatomical and palynological features of *S. indica*.

MATERIALS AND METHODS

Plant specimens were collected from the different localities in Turkey; C6 K.Maras: Çaglayanerit, above Erince village, 37° 44' 279'' N 37° 24' 221'' E, 1281 m, 3 v 2008, F.Celep 1419! C9 Siirt: Eruh to Sirmak, 8 km to Sirmak city border, near Yanilmazlar village, 37° 42' 269'' N 42° 15' 804'' E, 1195 m, 5 vi 2008, AKahraman 1539! A distribution map of *S. indica* is given (Fig. 1). The specimens have been stored in the Middle East Technical University (METU) Department of Biological Sciences and Ankara University Herbarium (ANK).

Anatomical studies were carried out on specimens kept in 70% alcohol. The paraffin method was used for the transverse sections of the leaves and stems. The specimens were embedded in the paraffin wax and then

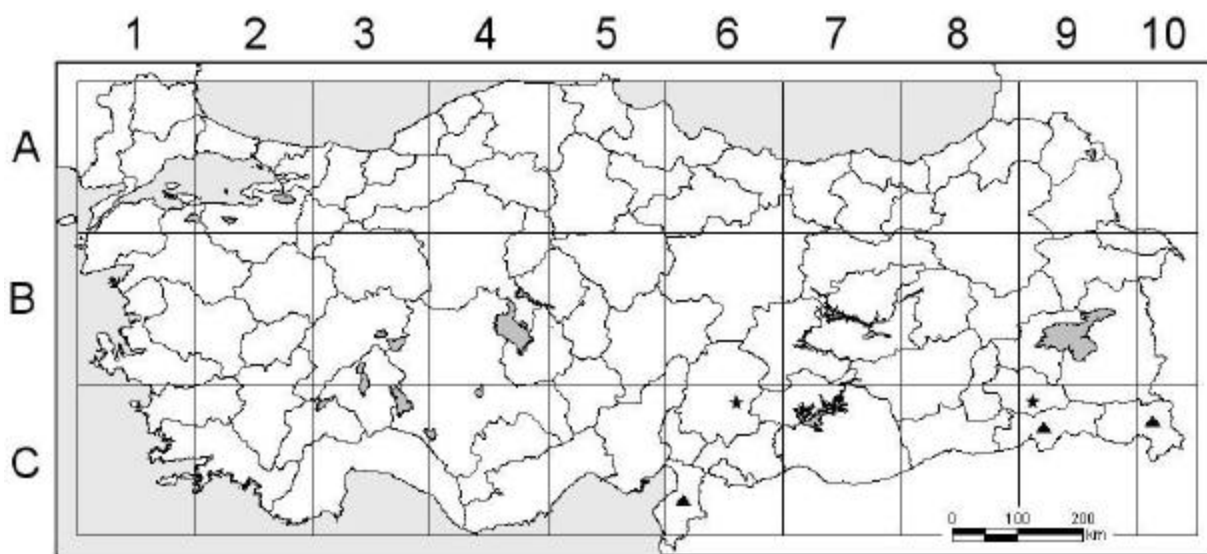


Fig. 1: Distribution map of *S. indica* collected by us (★) and cited in the Flora of Turkey (▲).

sectioned with a Leica RM2125RT rotary microtome. All sections were stained with Safranin and Fast Green and then mounted with Canada Balsam or Entellan [11]. Measurements and photographs were taken using a Leica DM1000 binocular light microscope with a Leica DFC280 camera.

For palynological investigations, pollen material was obtained from herbarium samples. The pollen slides were prepared according to Wodehouse [12] technique. Measurements and observations were made using the Leica DM1000 binocular light microscope with the Leica DFC280 camera. The polar length (P), the equatorial length (E), the colpus length (CLG), the exine and the intine thickness for 30 pollen grains were measured under the light microscope (x 1000) and P/E ratios were calculated. The pollen grains were observed and photographed with a JEOL-6060 scanning electron microscope to determine their exine ornamentation. Pollen terminology of Faegri and Iverson [13] has been used.

The nutlets were first examined using a Leica S8AP0 stereomicroscope to ensure that they were of normal size and maturity. In order to determine the average seed size, 30 mature nutlets were measured.

For ecological studies, soil samples were taken from suitable habitats of *S. indica*. All soil samples were analyzed at the Soil, Fertilizer and Water Resources Central Research Institute, Ankara. Soil texture, pH, CaCO₃, N, P, K and organic matter analysis were made using standard techniques [14] and the results have been evaluated [15].

RESULTS

Morphological characteristics: As presented in Fig. 2-3, *S. indica* plants are perennial herbs. Stems 80-150 cm, erect, branched above, sparsely eglandular-pilose below, densely or sparsely glandular or eglandular pilose above. Leaves simple, 15-30 x 10-24 cm, broadly ovate, truncate, reticulate, erose-dentate, glaucous or eglandular-pilose especially on the veins, cordate; cauline leaves ± sessile. Petiole 2-12 cm. Inflorescence paniculate. Verticillasters 48-flowered, clearly distant. Bracts 8-10 x 6-8 mm, ovate-acuminate. Pedicels 2-5 (-7) mm, erect. Calyx campanulate, 10-12 x 7-9 mm, truncate at apex, densely glandular-villous and sparsely eglandular, slightly broadening and up to 15 mm in fruit; upper lip shortly tridentate; calyx teeth mucronate, connivent. Corolla upper lip lilac, lower lip dark violet, spotted with purple, 25-32 mm, strongly compressed laterally; tube 9-13 mm, whitish, short, squamulate within, abruptly ventricose above. Stamens 2. Upper thecae clearly longer than filaments; filaments 3-4 mm, fertile anther 4-5 mm, upper thecae 17-21 mm. Style glabrous, 38-45 mm, exerted from corolla lips and divided into two parts at apex.

Phenology, Habitat and Ecology: *S. indica* flowers in April and May and fruits in June. This species grows on rocky limestone slopes and moist places in *Quercus* scrubs between 100 and 1500 m. Other species in its habitat are *S. trichoclada* Benth., *S. macrochlamys* Boiss. & Kotschy, *S. multicaulis* Vahl., *S. pilifera* Montbret & Aucher ex Benth., *Cardaria draba* L., *Alyssum* sp., *Convolvulus* sp., *Hordeum* sp., *Scabiosa* sp., *Astragalus* sp., *Onobrychis* sp., *Centaurea* sp.,

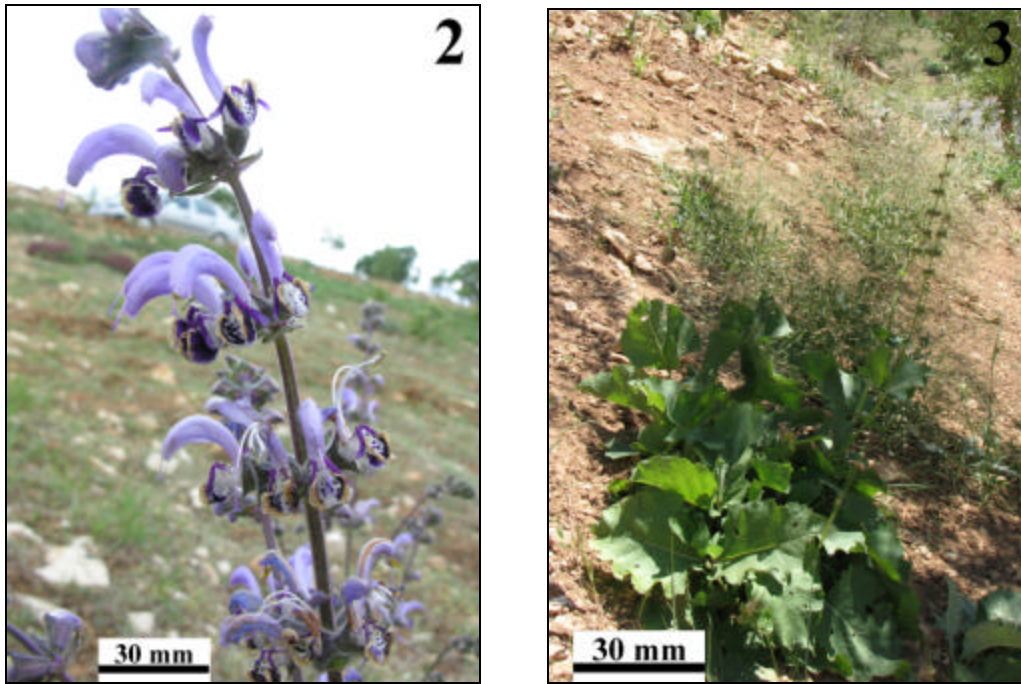


Fig. 2-3: General appearance of *S. indica* in the flowering and the fruiting time.

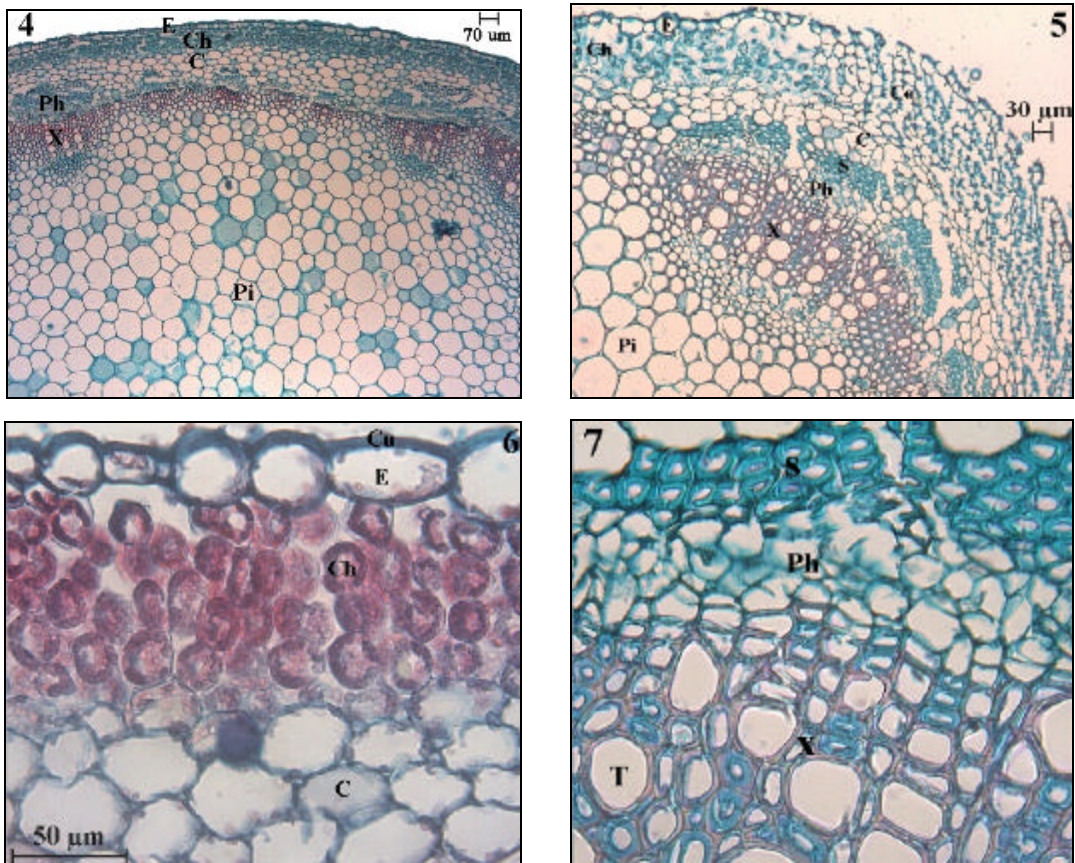


Fig. 4-7: The transverse section the stem of *S. indica*. Cu, cuticle; E, epidermis; Ch, chlorenchyma; Co, collenchyma; C, cortex; S, sclerenchyma; Ph, phloem; X, xylem; T, trachea; Pi, pith region.

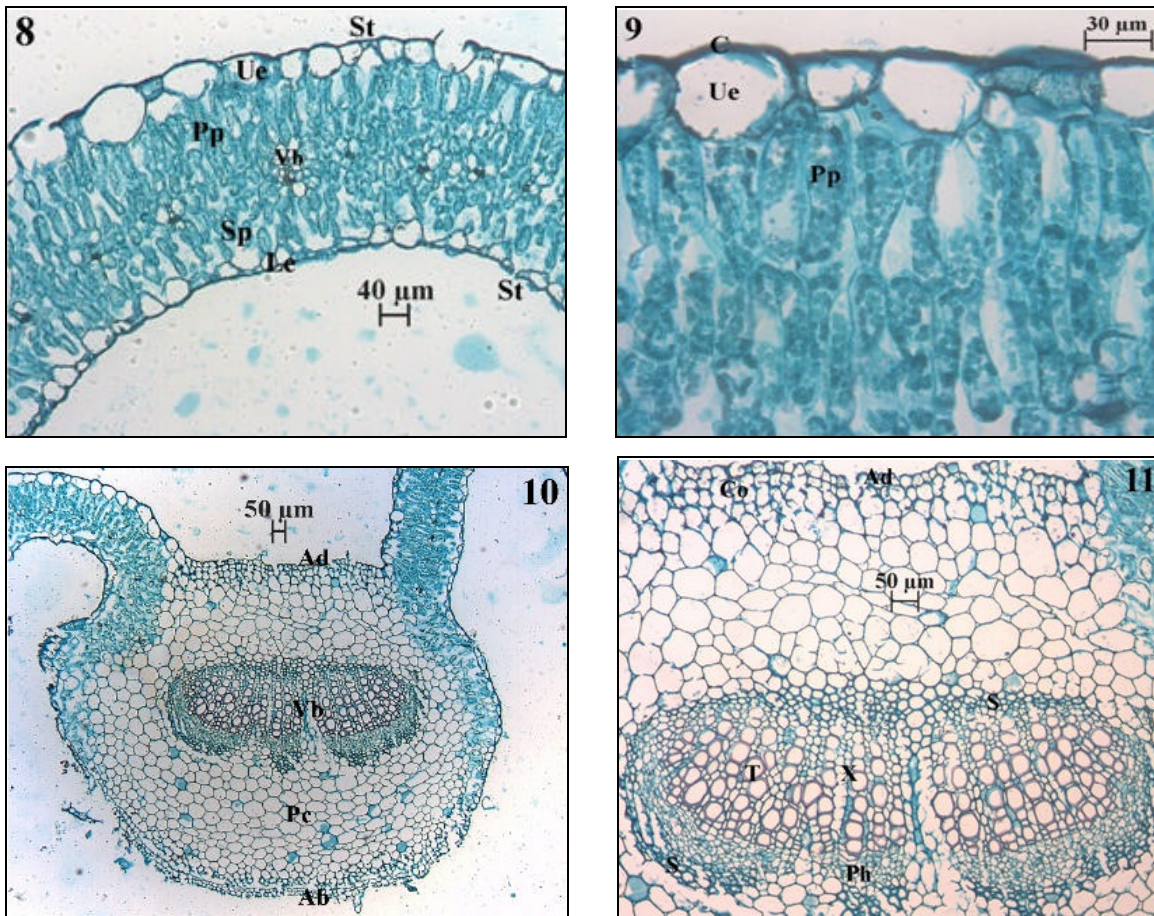


Fig. 8-11: The transverse section of the leaf of *S. indica*. Cu, cuticle; Ue, upper epidermis; Le, lower epidermis; Co, collenchyma; Pc, parenchymatic cell; Vb, vascular bundle; S, sclerenchyma; X, xylem; Ph, phloem; Pp, palisade parenchyma; Sp, spongy parenchyma; St, stomata; Ad, adaxial epidermis; Ab, abaxial epidermis.

Silene sp., *Galium* sp., *Vicia* sp. and *Anchusa* sp. According to the soil samples collected from the suitable habitats of *S. indica*, the species often prefers sandy soils which are neutral (pH 7.44) with a middle level of organic content (2.62%). The N content of the soil is 0.13%. The amounts of P and K present are 7.44 and 215.23 ppm, respectively. The CaCO₃ content in the soil is 1.06%.

Anatomical characteristics

Stem: In the transverse section of the stem, epidermis is covered by a thin layer of cuticle. This layer contains sparsely or densely eglandular or glandular trichomes. The epidermis consists of uniseriate oval or rectangular cells. The collenchyma tissue, which is located immediately under the epidermis, is composed of regular cells. There are 3-5 layers of chlorenchymatous cortex with a lot of chloroplasts below the epidermis in between the corners (Fig. 4-5). The cortex (30-150 µm) consists of 2-6 layers of squashed oval or almost rectangular parenchymatous cells with intercellular

spaces (Fig. 6). Vascular bundles are often separated by paranchymatous cells. Sizes of the vascular bundles at the corners are more or slightly larger than the others. Sclerenchymatic sheat above the phloem comprises of 6-30 cells in between the corners which are more in the corners (Fig. 7). Cambium is not distinguishable. The phloem and the xylem members are clear. The xylem elements (90-350 µm) occupy a larger region than the phloem (60-100 µm). The pith is wide and consists of hexagonal or orbicular parenchymatous cells with intercellular spaces (Table 1).

Leaf: The upper and lower epidermal layers are covered with a thin cuticle layer. Thickness of the cuticle of both epidermal layers is almost equal and ranges between 3.3 and 5.0 µm. Both epidermises consist of uniseriate, oval or rectangular cells. Size of the upper epidermis cells is two times larger than that of lower epidermis cells. The upper and lower walls of both epidermis cells are thicker than the lateral walls. All trichomes on both surfaces are of eglandular type.

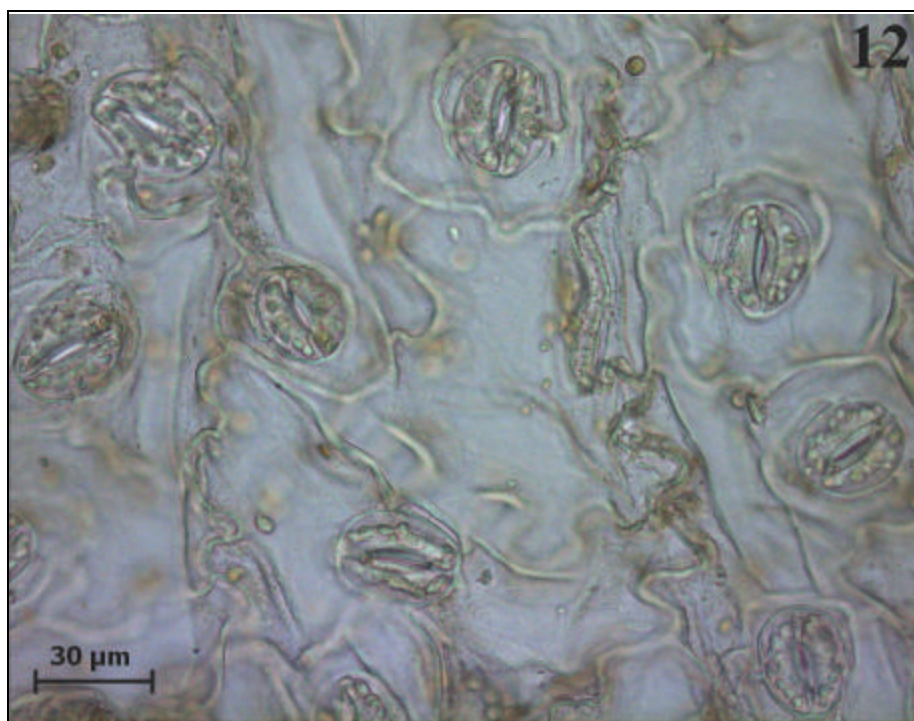


Fig. 12: Surface section of the leaf of *S. indica*.

Table 1: Anatomical measurement of some characters the stem and leaf of *S. indica*

Characters	Width (μm)	Length (μm)
	Min.-Max.	Min.-Max.
Stem		
Cuticle	3.3-7.5	
Epidermis cell	25.1-53.9	12.6-32.2
Chlorenchyma cell	15.3-25.0	17.1-30.0
Cortex cell	24.8-64.4	14.7-48.3
Trachea cell	15.0-54.0	13.0-52.1
Pith cell	28.0-140.0	35.0-157.0
Leaf		
Cuticle	3.3-5.0	
Upper Epidermis Cell	26.4-99.7	20.1-76.4
Lower Epidermis Cell	22.0-42.9	18.0-30.0
Mesophyll Region	160.0-216.0	
Palisade Parenchyma	8.9-21.7	50.3-72.1
Spongy Parenchyma	12.8-21.7	15.8-31.9

Stomata on the lower epidermis are much more than the upper epidermis and they are diacytic (Fig. 12). The leaf is bifacial type. Mesophyll region is composed of 2-3 layers of elongated rectangular palisade cells and 2-4 layers of irregular spongy cells. The palisade and spongy parenchyma cells have a lot of chloroplasts (Fig. 89). The midrib region forms a projecting part (Fig. 10). It is subcircular with abaxial surface convex and adaxial surface flat. Below the uniseriate epidermis, there are collenchymatous cells which provide support.

Arc-shaped vascular bundle is surrounded by sclerenchymatic tissue (Fig. 11). There is single and lobed large vascular bundle in the center (Table 1).

Pollen characteristics: The pollen grains of *S. indica* are radially symmetrical and isopolar. Their shape is suboblate (Fig. 13-14). The dimensions of polar axis and equatorial axis are $44.98 \pm 4.22 \mu\text{m}$ and $52.24 \pm 4.41 \mu\text{m}$, respectively. The ratio of P/E is 0.86. Colpus length is $39.02 \pm 3.49 \mu\text{m}$ and colpus width is $8.64 \pm 1.17 \mu\text{m}$. The aperture is hexacolpate and the colpi are long with acute apices. The exine thickness is $1.49 \pm 0.10 \mu\text{m}$ and the intine thickness is $0.52 \pm 0.05 \mu\text{m}$. The exine sculpturing is bireticate-perforate (Fig. 15-16, Table 2).

Nutlet morphology: The nutlets of *S. indica* are rounded-trigonous in transverse section and ovate in their outline (Fig. 17). Mature nutlets of *S. indica* are 3.6-4.5 mm long and 3.0-3.5 mm wide. Hilum is rounded and whitish and its diameter is 0.4-0.7 mm. The nutlet surface is glabrous, slightly tuberculate and the mature nutlet colour is black (Fig. 18).

DISCUSSION

Morphological characteristics such as leaf size and corolla characteristics are taxonomically significant to identify the species. *S. indica* morphologically differs

Table 2: Morphological measurements (μm) of pollen grains of *S. indica*.

P*	E*	P/E	Clg*	Clw*	Exine*	Intine*
38.69-49.69	45.31-58.12	0.86	33.82-43.51	7.02-10.78	1.28-1.59	0.44-0.60
44.98 \pm 4.22	52.24 \pm 4.41		39.02 \pm 3.49	8.64 \pm 1.17	1.49 \pm 0.10	0.52 \pm 0.05

P, polar axis; E, equatorial axis; Clg, colpus length; Clw, colpus width; *, data are the range, with the mean and standard deviation

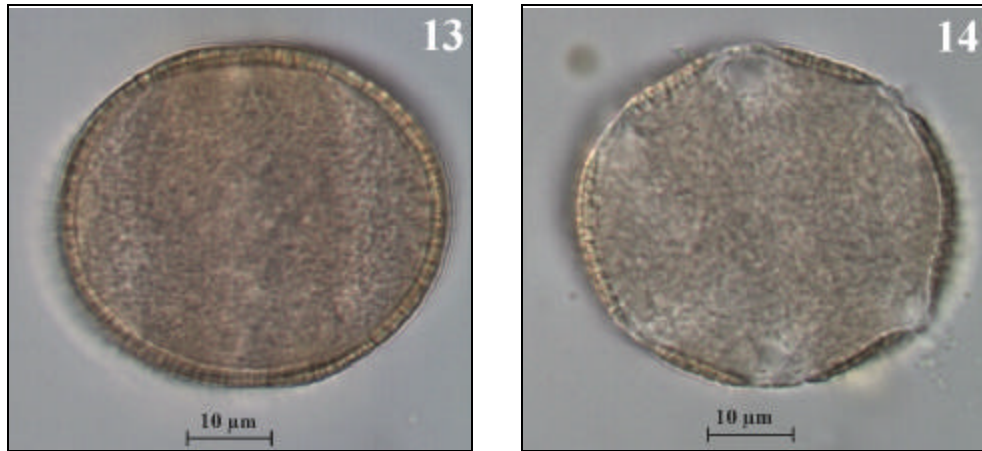


Fig. 13-14: LM micrographs of the pollen of *S. indica* (μm). 13- Equatorial view. 14- Polar view.

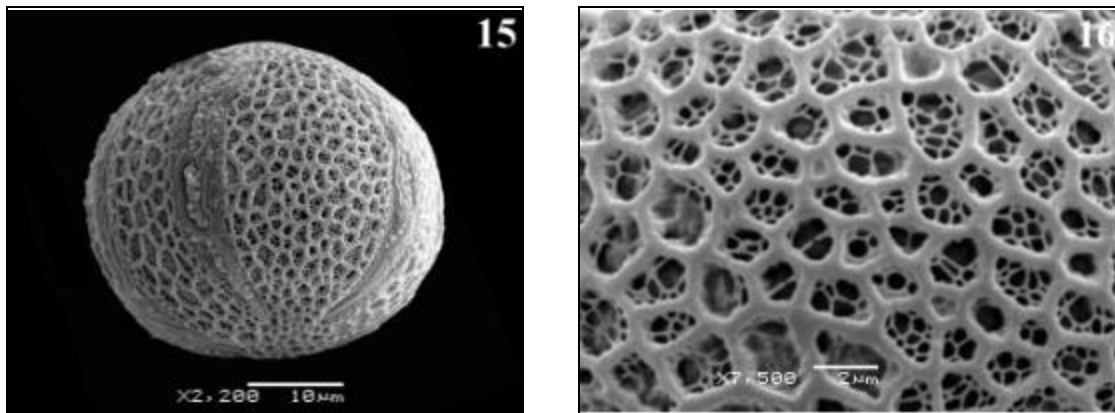


Fig. 15-16: SEM micrographs of the pollen of *S. indica*. 15- Equatorial view. 16- Exine ornamentation

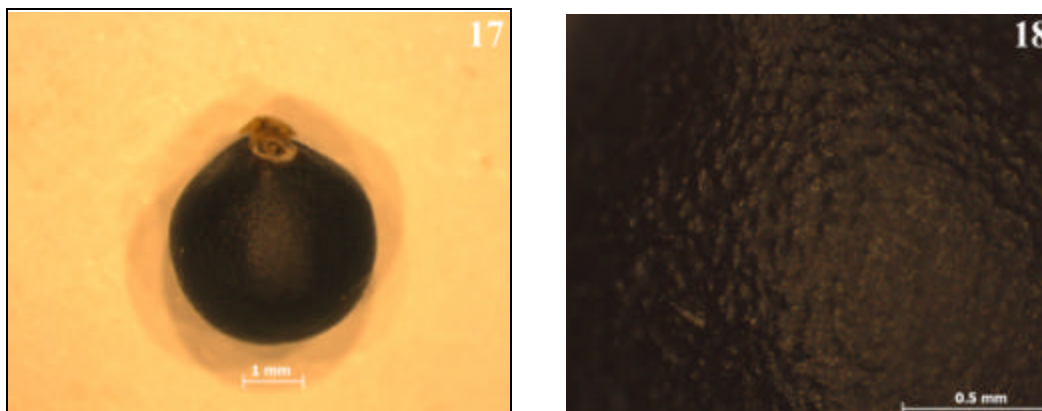


Fig. 17-18: LM micrographs of the nutlet of *S. indica*. 17- General appearance of the nutlet. 18- The nutlet surface.

Table 3: Morphological characters of *S. indica* based on our study and Flora of Turkey (Hedge 1982a)

Characters	Our measurements	Flora of Turkey
Stems	80-150 cm	to 150 cm
Leaves	15-30 x 10-24 cm	to 30 x 23 cm
Petiole	2-12 cm	c. 10 cm
Verticillasters	4-8-flowered	4-6-flowered
Bracts	8-10 x 6-8 mm	c. 8 x 6 mm
Calyx	10-12 x 7-9 mm	c. 10 x 7 mm
Pedicel	2-5 (-7) mm	2-4 mm
Corolla	25-32 mm	c. 30 mm
Corolla tube	9-13 mm	-
Filaments	3-4 mm	-
Fertile anther	4-5 mm	-
Upper thecae	17-21 mm	-
Style	38-45 mm	-
Nutlets	3.6-4.5 x 3.0-3.5mm	c. 4.5 x 3.5 mm

from the other members of *Salvia* in terms of its large leaves and corolla upper lip lilac, lower lip dark violet, spotted with purple. Although the present results usually correspond with the description recorded in the Flora of Turkey [16] (Table 3), several differences were found here. It was reported that the leaf was to 30 x 23 cm, the bract was c. 8 x 6 mm, the calyx was c. 10 x 7 mm, the nutlet was 4.5 x 3.5 mm in size, the corolla was c. 30 mm, the petiole was c. 10 mm, the pedicel was 2-4 mm in length and number of verticillasters was 4-6-flowered. According to our study, the leaf was to 15-30 x 10-24 cm, the bract was 8-10 x 6-8 mm, the calyx 10-12 x 7-9 mm, the nutlet was 3.6-4.5 x 3.0-3.5 mm in size, the corolla was 25-32 mm, the petiole was 2-12 mm, the pedicel 2-5 (-7) mm was in length and number of verticillasters was 4-8-flowered. We also measured other morphological characters of the species. Research findings reveal that the morphological characteristics of *S. indica* provide some additional information to those data reported in Flora of Turkey [16] (Table 3).

Metcalf and Chalk [17] in their monumental study pointed out that the stems of the family Labiatae species are quadrangular and contain well developed collenchymatous cells in the corners. In addition, they also reported that scleranchymatic tissue surrounds the vascular tissue. We observed same anatomical features in the stems of *S. indica*. Moreover, we observed 3-5 layers of chlorenchyma cells below the epidermis between the corners.

In *Salvia* species, mesophyll region is entirely parenchymatic, the midrib is surrounded by collenchymatous cells and the stomata are of the diacytic type [17]. According to the mesophyll structure, the leaves of *S. indica*, *S. huberi* [18] and

S. sclarea [19] are bifacial, but those of *S. halophila* [20] are monofacial. The structure of vascular bundles in the leaf anatomy of *Salvia* species can be used as a very useful key for distinguishing the species. In the midrib of *S. indica*, there is only one large and lobed vascular bundle. *S. blepharochlaena* as indicated by Ozkan and Soy [21] has one vascular bundle in the middle region and two small bundles on sides while *S. halophila* [20] has two large bundles in the middle of the midrib.

Pollen characteristics of the family Labiatae have been reported to be considerable taxonomic importance [22]. Cantino *et al.* [23] revised the classification of all genera in Labiatae and placed *Salvia* within the subfamily Nepetoideae as the genus *Salvia* has hexacolpate pollen grains. The pollens of *S. indica* are hexacolpate, radially symmetrical and isopolar. Its shape is suboblate. The exine sculpturing is bireticulate-perforate. The pollen in *S. halophila* [20] is hexacolpate, isopolar and prolate. *S. anatolica* Hamzaoglu & A. Duran and *S. bracteata* Banks & Sol. [24] were observed to be oblate-spheroidal shaped pollen grains. While the sculpturing in *S. anatolica* is euryreticulate, sculpturing in *S. bracteata* is suprareticulate. The shape of the pollen and the sculpturing of the exine in the genus *Salvia* may be significant in separating the species.

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