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## **Morphology, Anatomy, Hair and Karyotype Structure of *Salvia blepharoclaena* Hedge and Hub.-Mor. (Lamiaceae), Endemic to Turkey**

Mustafa Özkan and Erhan Soy

Department of Biology, Faculty of Arts and Science, Ahi Evran University, 40100 Kırsehir/Turkey

**Abstract:** *Salvia blepharoclaena* is an endemic species belonging to the family Lamiaceae. In this study, morphology, anatomy, hair properties and chromosome number and morphology of this species were analysed. Chromosome number of *Salvia blepharoclaena* was counted as  $2n = 14$ .

**Key words:** *Salvia blepharoclaena*, morphology, anatomy, karyology, hair, Turkey

### **INTRODUCTION**

The family Lamiaceae has an important role as a source of medicinal and aromatic plants of commercial importance. The genus *Salvia* L. comprises approximately 900 species distributed all around the world. *Salvia* is represented by 88 taxa, of which 43 are endemic in Turkey (Davis, 1982; Dönmez, 2001).

*Salvia* species contain monoterpene with antiseptic characteristics (Nakipoğlu, 1993a). In recent studies on this species, it has been observed that the compounds decrease DNA synthesis in the cell (Nakipoğlu, 1993a). *Salvia* species are used in folk medicine all around the world, they possess antibacterial, antioxidant (Dobrynin *et al.*, 1976), antidiabetic and antitumor (Hanson and Hocking, 1957). The morphological, anatomical and karyological properties of *Salvia blepharoclaena* has not been studied.

Therefore, the purpose of this research was to investigate the morphological, anatomical and karyological properties of *Salvia blepharoclaena*.

### **MATERIAL AND METHODS**

Samples were collected from different localities in Turkey. The specimens kept in the herbarium of Ahi Evran University, Arts and Sciences Faculty, Department of Biology and numbered as 14 and 15. Samples were collected from the following locations: B4, Kırsehir, Çiçek dağı, Ahmet Veli Turbe, Forested area, 1500 m, 28 V 2004 and B4, Kırsehir, Sofular village, 1500 m, 7 VI 2004. Analyses of populations were made using 20 morphological characters. Each value is the average of ten measurements from different specimens. Anatomical studies were carried out on fresh samples or samples kept in alcohol. The paraffin method was used for preparing a

cross section of plants root, stem, leaves, petiole and flowers. For study of somatic chromosomes, root tips were obtained from seeds germinated in petri dishes. They were pre-treated in  $\alpha$  monobromonaphthalene overnight and then fixed in alcohol: acetic acid (3:1). Roots were hydrolyzed in 1 N HCl at 60°C for 15 min and stained in Feulgen. Squashes were made in 45% acetic acid. Chromosome measurements were based on five metaphase plates. Chromosomes were classified using the nomenclature of Levan *et al.* (1964). Photographs were taken with Leica DM LB microscopy. For SEM studies, clean seed, leaf, petiole and stem (without fixation) were glued on aluminium stubs using double-sided adhesive and coated with gold palladium to a thickness of 40-50 nm. The specimens were viewed in scanning electron microscope and photographed at different magnifications.

### **RESULTS**

**Morphological properties:** Perennial herbs, woody at base. Root is 25-72 cm length. Pale-brown hard is present on the root. The stem is 16.5-38.5 cm long and rectangular. Stem is erect and ascending. Stem is covered by glandular and eglandular hair (glandular villous). Leaves are pinnatisect and oblong-ovate, mostly basal. Glandular and eglandular hair is present on both upper and lower epidermis of leaves. The edge of leaves are crenate. flowers are zygomorphic. The flowers are arranged verticillately and 2-16 flowers are present at verticillaters. The shape of the calyx is broadly infundibular. Calyx is 18-24 mm, which corolla 28-36 mm. The upper lip of corolla has two lobules. Corolla white with a pale yellow, upper lip violet veined labellum, tube to 30 mm, broad. The corolla tube is straight (not squamulate). Stamens are A type (Fig. 1a and b). The filament is 7-10 mm and anther 2-3 mm in length. The



Fig. 1a,b: General appearance of *Salvia blepharoclaena*, a: plant, b: corolla, c: dissected calyx, d: calyx, e: stamen, f: pistil, g: leaf Scale bar: 5 cm

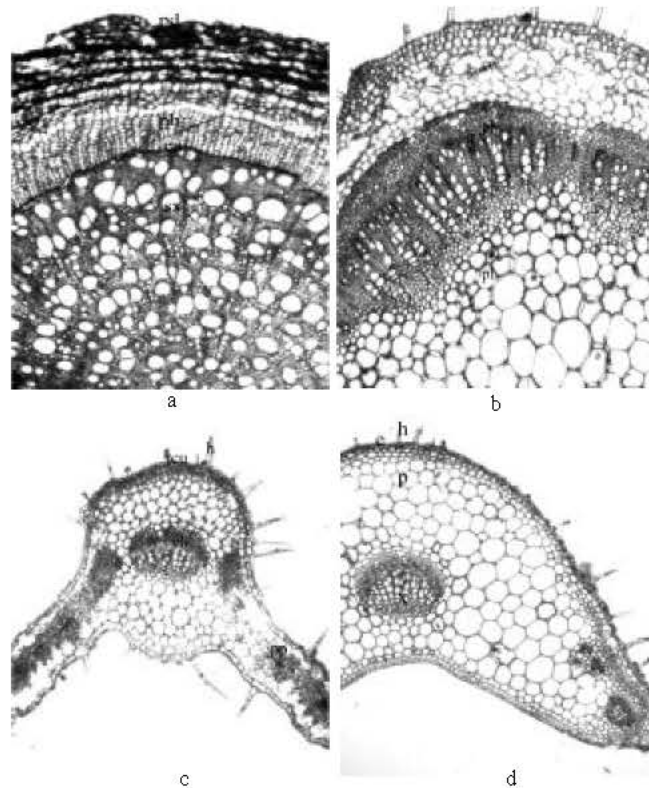


Fig. 2: Cross section of root (a), stem (b), leaf (c), petiole (d) of *S. blepharoclaena* p: peridermis, ph: phloem, c: cambium, x: xylem, co: cortex, s: sclerenchyma, pi: pith, e: epidermis, pa: parenchyma, cu: cuticle, h: hair, pp: palisade parenchyma

stigma is bifurcate. The style is 21-41 mm long. Fruit type is nutlet. Seeds are brown colored and very rounded trigonous in shape.

**Anatomical properties:**

**Root anatomy:** *Salvia blepharoclaena* is a perennial endemic species. Periderm is present on other most layer as a protective tissue. Below it lies a cortex tissue. Cortex is multilayered and parenchymatic. Cambium cells are 1-2 layered, flat and distinguishable. Phloem take places a small part in the bundles. Primary xylem rays lie in the cortex. It is 1-2 layered and homogenous. In the pith, primary xylem tissue is present (Fig. 2a).

**Stem anatomy:** Epidermis is single layered. Shape of this cell is hexagonal or ovoidal (Fig. 2b). There are glandular and eglandular hairs on epidermis. Hairs are unicellular or multicellular. Collenchyma is 5-8 layered and located under epidermis. Cambium is distinguishable. The pith is large and consist of parenchymatic cells.

**Leaf anatomy:** Leaf is with a thick cuticle layer on outermost (Fig. 2c), followed by single layered epidermis which has glandular and eglandular hairs. Thickness of cuticle is 5-12.5  $\mu$ . The upper epidermis cells are bigger than the lower epidermis cells. Type of the vascular bundle is collateral. Palisade parenchyma cells are 2-3 layered. Stomata type is diacytic. The differences of leaves are shown in Table 1.

**Petiole anatomy:** Petiole is covered by ovoidal and hexagonal epidermal cells. There are a lot of glandular and eglandular hairs on epidermal cells (Fig. 2d). Parenchymatic cortex is present under epidermis. There are one large bundle on median region of petiole. A small bundle is also present near these bundle. Type of vascular bundle is collateral.

**Hair properties:** Results shows that *S. blepharoclaena* has the various glandular and eglandular hair at stem, leaf, petiole, pedicel and flower. The glandular hair is more variable at flowers and leaves than the other parts of plant (Table 2). Plant has capitate hairs (Fig. 3 and 4). There are

Table 1: Anatomical measurement of *S. blepharoclaena*

		Broad ( $\mu$ )	Length ( $\mu$ )
Root	Periderm cell	22,5	40
	Diameter of trachea	25	80
Stem	Cuticle	5	12,5
	Epidermis cell	10	30
	Diameter of pith cell	45	125
	Diameter of trachea	15	40
Leaf	cuticle	5	12,5
	Upper epidermis cell	10-25	30-60
	Lower epidermis cell	10-15	20-27,5
	Palisade parenchyma cell	120	185
Petiole	Cuticle	5	7,5
	Epidermis cell	12,5	27,5
	Parenchyma cell	52,5	100
	Diameter of trachea	10	25
Flower	Cuticle	5	10
	Adaxial cell	25	30
	Abaxial cell	10	22,5

Table 2: Hair type of various organs of *S. blepharoclaena*

	Glandular hair						Eglandular hair	
	I. Type			II. Type				
	Head cells	Stalk cells	Base cells	Head cells	Stalk cells	Base cells	Base cells	Hair cells
Stem	1	-	1	1	1	3	1	1
	1	1	2	1	2	4	2	2
	2	2	1				3	3
							4	4
								5
								6
Leaf	1	1	1	1	2	1	1	1
	2	2	2		3	2	2	2
		3	3		4		3	3
			4				4	
Petiole	1	-	1	1	2	1	1	1
	2	1	2		3	2	2	2
		2			4		3	3
								5
Flower								9
	1	1	1	1	3	1	1	1
	2	2			4	2	2	2
						3	3	3
								4
								5
Pedicel	1	1	1	1	2	1	1	1
	2	2			3	2	2	2
								3
								4
								5

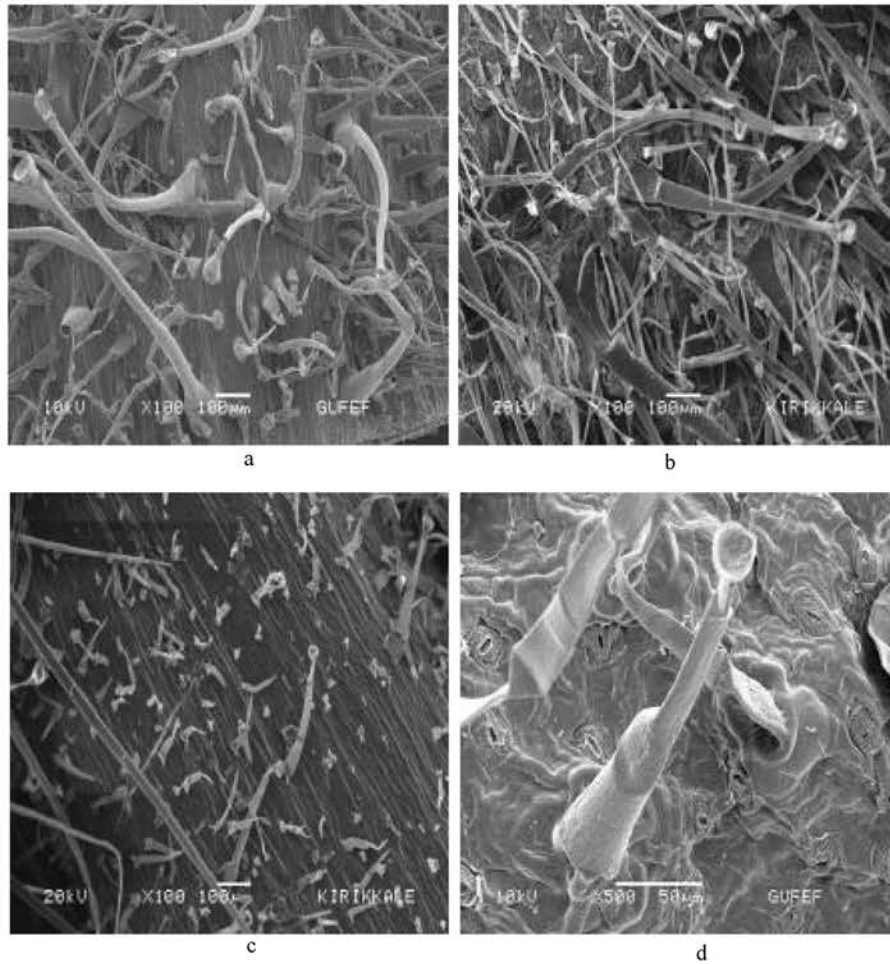


Fig. 3: Glandular and eglandular hair different parts of *S. blepharoclaena* (SEM) a. stem, b. leaf, c. petiole, d. flower

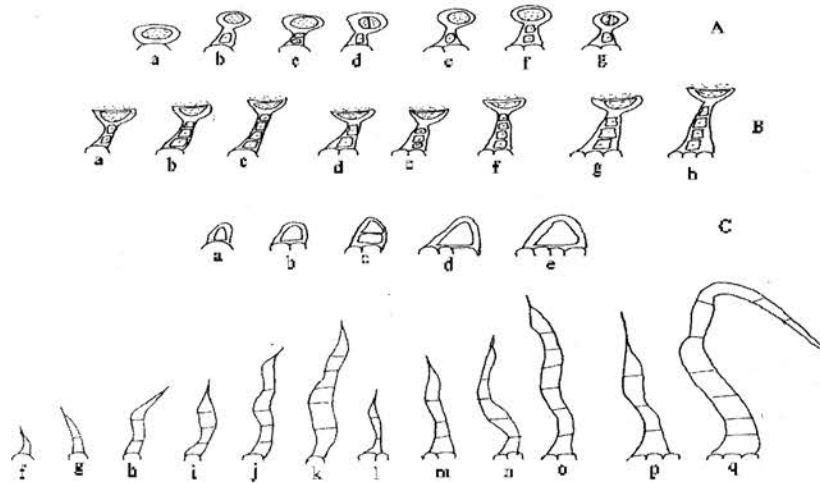


Fig. 4: Glandular and eglandular hair different parts of *S. blepharoclaena* A, B capitate hairs C, D: eglandular hair (A: type I hair, B: type II hair)

capitate glandular hairs which have a cu-like head cell. Capitate glandular hair has various numbers of base cells and stalk cells.

**Karyological properties:** The chromosome number of this species was  $2n = 14$  (Fig. 5). The karyotype of this species consisted of 3 pairs of submedian (sm), others submedian (sm), 1 st, 2nd and 4th chromosomes are submedian region (sm) and the others are subterminal (st). Chromosomes vary from 0.5-1.34  $\mu\text{m}$ . The longest arm is 1.04  $\mu\text{m}$  and the shortest arm is 0.4  $\mu\text{m}$ . No satellite was observed in the karyotype of this species (Fig. 6). Karyotype details, are shown Table 3.

Table 3: Karyotype details of somatic chromosomes of *S. blepharoclaena*

Chromosome pairs	C ( $\mu\text{m}$ )	L ( $\mu\text{m}$ )	S ( $\mu\text{m}$ )	L/S	I (S/C) 100	Centromeric position
1	1.40	0.96	0.44	2.18	31	Submedian (sm)
2	1.34	0.87	0.47	1.85	35	Submedian (sm)
3	1.25	1.04	0.21	4.95	16	Subterminal (st)
4	1.24	0.83	0.41	2.02	33	Submedian (sm)
5	1.13	0.88	0.25	3.52	22	Subterminal (st)
6	1	0.77	0.23	3.34	23	Subterminal (st)
7	0.50	0.4	0.1	4	20	Subterminal (st)

C: Total chromosome length, S: Short arm length, SAT: Satellite, I: Centromeric index L: Long arm length, L/S: Arm ratio

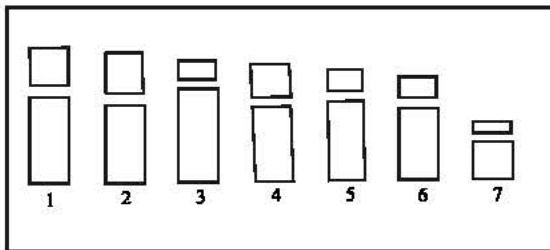


Fig. 5: Ideogram of chromosome complement of *S. blepharoclaena*

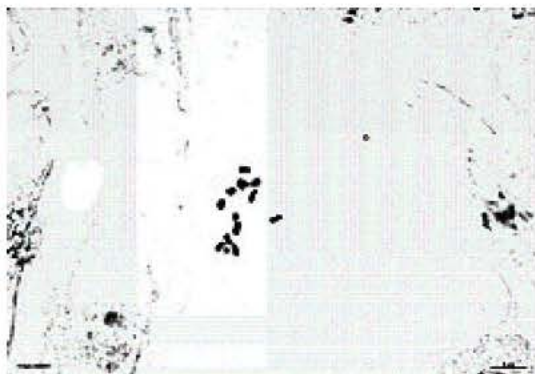


Fig. 6: Mitotic metaphase chromosomes of *S. blepharoclaena*

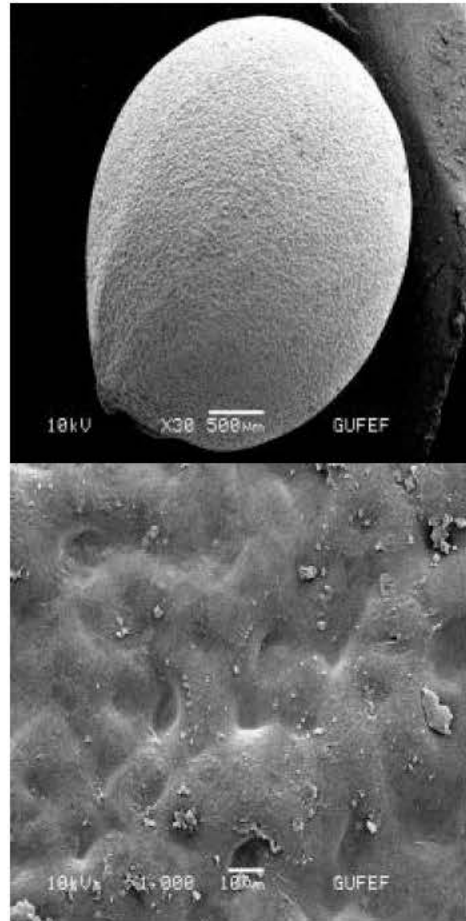


Fig. 7: SEM micrographs of seed surface at high magnification

## DISCUSSION

The present study, showed that morphological characters such as the number of fertile stamen, type of stamen, properties of glandular and eglandular hairs, shape of corolla and calyx, flowers at verticil lasters have taxonomic value. This species is endemic for Turkey. Endemic species have great importance in plant geography. The karyotype analysis obtained in this study will support the taxonomical study. Furthermore, determination of chromosomal characters will provide a better identification and taxonomical position for a taxon that has been examined morphologically.

Metcalf and Chalk gave information about general anatomical characteristics of *Salvia* genus. The same researchers stated that the rays consist of 2-12 or more lines of cells in this family. In this study it was discovered that these rays consist of 1-2 line cells.

Özdemir and Senel (1999) observed that *Salvia sclerea* has bifacial type of leaves and diacytic

type stomata. But leaves of *Salvia blepharoclaena* is isolateral and diacytic stomata. Metcalfe and Chalk (1972) pointed out in the Lamiaceae family, the structure of vascular bundles in petiole is important in terms of taxonomy. In our study, there are one large bundle in centre of petiole. There are two small bundles which located on one side and one on the other side. Seeds are mostly ellipsoid and brown colored. Seed surface is foveolate (Fig 7a and b). Hair which secretes etheric oil, characteristic of the lamiaceae family. In this study, it was observed that *S. blepharoclaena* had capitate hair on the stem, petiole, leaves and pedicel. Nakipoğlu *et al.* (1993b and c) observed that *Salvia* species had B type chromosomes but in our study we could not observe any B type chromosome. In our study we examined that *Salvia blepharoclaena*  $2n = 14$ . Estilai *et al.* (1990) determined that the chromosomes numbers of *Salvia* species could vary between  $2n = 14$  and  $2n = 64$ . It shows an agreement with our results. Some researcher observed that the Mediterranean group seems to be characterized by  $x = 7$  (Afzal-Rafii, 1976), those in Europe and Russia by  $x = 11$  (Patudin *et al.*, 1974), those in California by  $x = 16$  (Epling *et al.*, 1962). We observed similar results such as *Salvia blepharoclaena*  $2n = 14$ .

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