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Morphological, anatomical and karyological properties of *Salvia cadmica* (*Lamiaceae*) endemic to Anatolia

Abstract

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Salvia cadmica was investigated morphologically, anatomically and karyologically. Glandular and eglandular hairs were determined in the cross sections from the stem, leaf, petiole, flower and pedicel. The hairs were clasified in respect to the numbers of base, stalk and head cells. In karyological studies, chromosome numbers and morphology of the species were determined. Chromosome numbers of Salvia cadmica was counted as $2n = 14$.

Key words: *Salvia cadmica*, morphology, anatomy, karyology.

Introduction

Salvia species are important group of useful plants which have been known and haven't lose their importance since ancient times. The genus is named "Salvia" derived from "Salveo" which means "to save, to recover" in Latin. The antibacterial, antituberculous and antiphlogistic activities of the constituents of *Salvia* species are well-established (Ulubelen & al. 2001). Beside *Salvia* species have medicinal value, they are also grown in parks and gardens as ornamental plants (Nakipoğlu 1993).

The genus *Salvia* L. *is* quite well known for its horticultural and commercial importance (Bhattacharya 1976). The seeds of *Salvia* species often produce mucilage on wetting (Davis 1982). The mucilage of the seeds is used for lacquerware and is mixed with fruit juices to produce pleasant drinks in Mexico (Estilai & al. 1990). In the East countries, that mucilage is used for the treatment of eye diseases (Baytop 1999).

The genus *Salvia* L. with over 900 species is probably the largest member of the family *Lamiaceae* and is found in both subtropical and temperate parts of the world. The two largest centres of the genus are in America and in South-West Asia. Anatolia is a major centre for *Salvia* in Asia (Vural & Adıgüzel 1996). Since the most recent revision of the genus in Turkey, four new species have been described; the total has now reached 90 (Davis 1982, Davis & al. 1988, Güner & al. 2000, Dönmez 2001 Hamzaoğlu & al. 2005). 46 of these *Salvia* species are endemic of Turkey (Davis 1982).

Recent studies have been done on the anatomical structure of the secretory hairs of *Salvia* species (Werker & al. 1985a, b, Kesercioğlu & Nakipoğlu 1992, Serrato-Valenti & al. 1997, Bisio & al. 1999, Özdemir & Şenel 1999, 2001) Any study on *Salvia cadmica* that is the subject of this study hasn't been found except the main knowledge in "Flora of Turkey". Studies on the karyotypes of this genus could not sufficiently be carried out because its chromosome sizes were too small. The aim of the present study was to determine the morphological, anatomical and karyological properties of *Salvia cadmica*.

Materıals and methods

The specimens were collected from the different natural populations in Turkey. Specimens were collected the following locations:

- B4, Ankara, Bala, Beynam national park, 1200m., 14 VI 2004, Soy 12
- A4, Ankara, Ayaş, Ayaş mountain, 1500m, 20 VI 2004, Soy 13.

Anatomical studies were carried out on the fresh samples or samples kept in alcohol. The paraffin method was applied for preparing the cross sections of root, stem, leaves, petiole and flowers. For the study of somatic chromosomes, root tips were obtained from seeds germinated in petri dishes. They were pre-treated in α-monobromonaphtalane over night and then fixed in alcohol: acetic acid (3:1). Roots were hydrolyzed in 1 N HCl at 60 ºC for 15 min and stained in Feulgen dye. Squashes were made within 45 % acetic acid. Chromosome measurements were based on five metaphase plates. Chromosomes were classified using the nomenclature of Levan & 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.

Morphologıcal propertıes

The perennial herb had a woody rootstock**.** Root was 7.5-50cm in length. Pale-brown hard cover was present on the root. The stem was 8-34cm long and rectangular. Stem was erect, ascending and not branched. Stem was covered by glandular and eglandular hairs. Leaves were simple and oblong-ovate, mostly basal. Glandular and eglandular hairs were present on both upper and lower epidermis of the leaves. The edge of leaves were crenate, flowers were zygomorphic. The flowers were arranged in verticillasters and 4-10 flowers were present in each verticillaster. The shape of the calyx was campanulate. Calyx was 15-20mm, corolla was 16-31mm. The upper lip of corolla had two lobules. The corolla tube was straight and not squamulate. Stamens were A type (Fig. 1). The flament was 5-6mm and anther 2-3mm in length. The stigma was bifurcate. The style was 18-28mm long. Fruit type was nutlet. The seeds were colored brown and ovate in shape (Fig. 5).

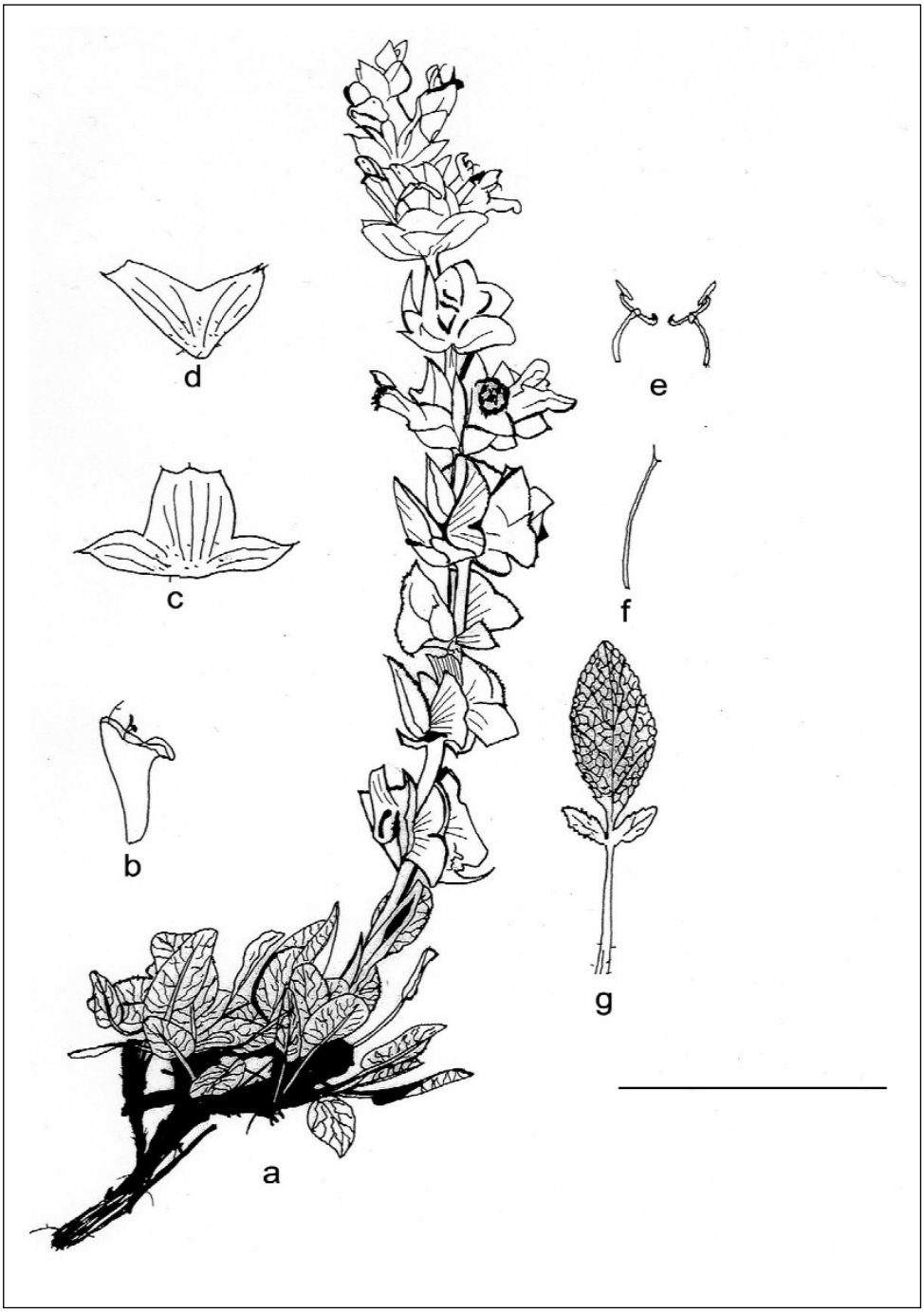

Fig. 1. *Salvia cadmica* Boiss.: **a,** habit; **b,** corolla; **c,** dissected calyx; **d,** calyx; **e,** stamen; **f,** pistil; **g,** leaf. Scale bar: 5 cm.

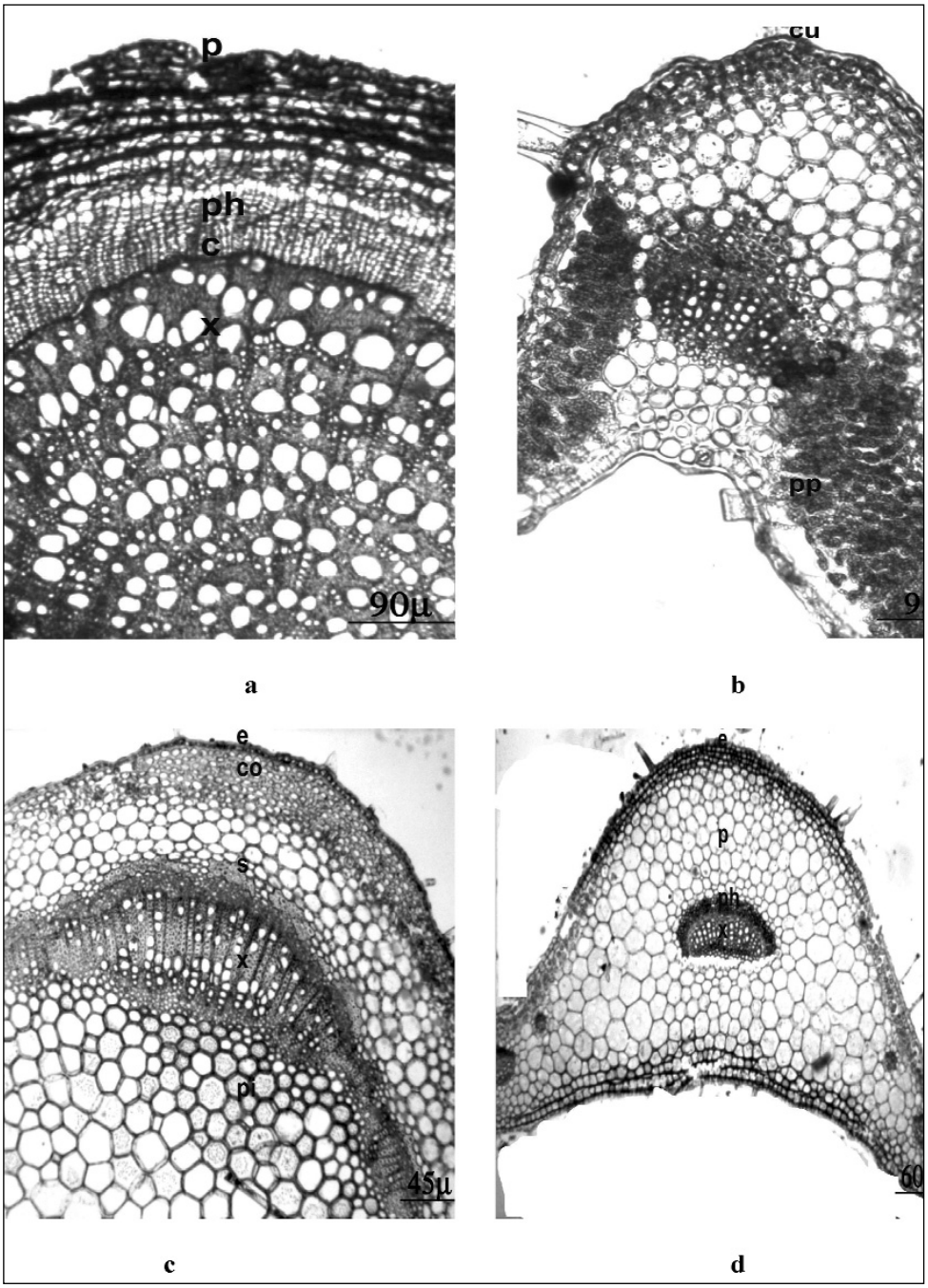

Fig. 2. *Salvia cadmica* Boiss.: **a,** Cross-section of root; **b,** leaf; **c,** stem and **d,** petiole.
p – peridermis, ph – phloem, c – cambium, x– xylem, co – cortex, s – sclerenchyma, pi – pith, e – epidermis, cu – cuticle, h – hair, pp - palisade parenchyma.

Anatomıcal properties

Root: *Salvia cadmica* was a perennial endemic species. Periderm was present on the outermost layer as a protective tissue. Cortex under periderm was multilayered and consisted of parenchymatous cells sized 25-40µm. Cambium cells were 1-4 layered, flattened and distinquable. Phloem took place in a small part in the vascular bundles. Primary xylem rays were 1-2 layered and homogenous. The pith region was filled with primary xylem tissue (Fig.2a).

Stem: Epidermis was single layered and consisted of hexagonal or ovoidal cells. There were glandular and eglandular hairs on epidermis. Hairs were unicellular or multicellular. Collenchyma was 8-9 layered and located under epidermis. Cambium was not distinquable. The pith was large and consisted of parenchymatous cells (Fig.2b).

Leaf: Leaf had a thick cuticle on the outermost layer, followed by a single layered epidermis which had glandular and eglandular hairs. Thickness of cuticle was 5-12,5µm. There were more hairs on the adaxial surface than the abaxial. The adaxial epidermis cells were larger than the abaxial. Vascular bundles were regularly ordered in the leaves. Type of the vascular bundle was collateral. Palisade parenchyma were 2-3 layered. Stoma type was diacytic (Fig. 2c).

Petiole: Petiole was covered by ovoidal and hexagonal epidermis cells. There were a lot of glandular and eglandular hairs on epidermis. Parenchymatous cortex was present under epidermis. There were only one large bundle on the median region of petiole and two small bundles at both end. Bundle type was collateral (Fig. 2d).

Hair properties: As shown in Fig. 3 and Fig. 4, *S. cadmica* had the various glandular and eglandular hairs at the stem, leaf, petiole, pedicel and flower. The glandular hairs were more variable on petiol and leaves than those on other parts of the plant. The species had Type I, II and III capitate glandular hairs. Peltate glandular hairs were not observed on the investigated species. Eglandular hairs were widespread on all aerial organs of the plant. The stem and leaf were the richest organs in point of cell numbers of eglandular hairs (Fig. 3, Fig. 4a-d, Tab. 1) .

Karyological properties

The chromosome number of this species was $2n$=14 (Fıg. 6). The karyotype of this species consisted of 2 pairs of subterminal (st), 2 pairs of median (m), and 3 pairs of submedian (sm) chromosomes. 3th and 5th chromosomes were subterminal (st), 4th and 6th chromosomes were median (m), 1st 2nd and 5th chromosomes were submedian (st). Chromosome sizes varied from 2.52-1.37µm. The longest arm was 1.74µm and the shortest arm was 0.28µm. No satellite was observed in the karyotype of this species (Fig.7). K

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 verticillasters had taxonomic value. This species is endemic for Turkey.

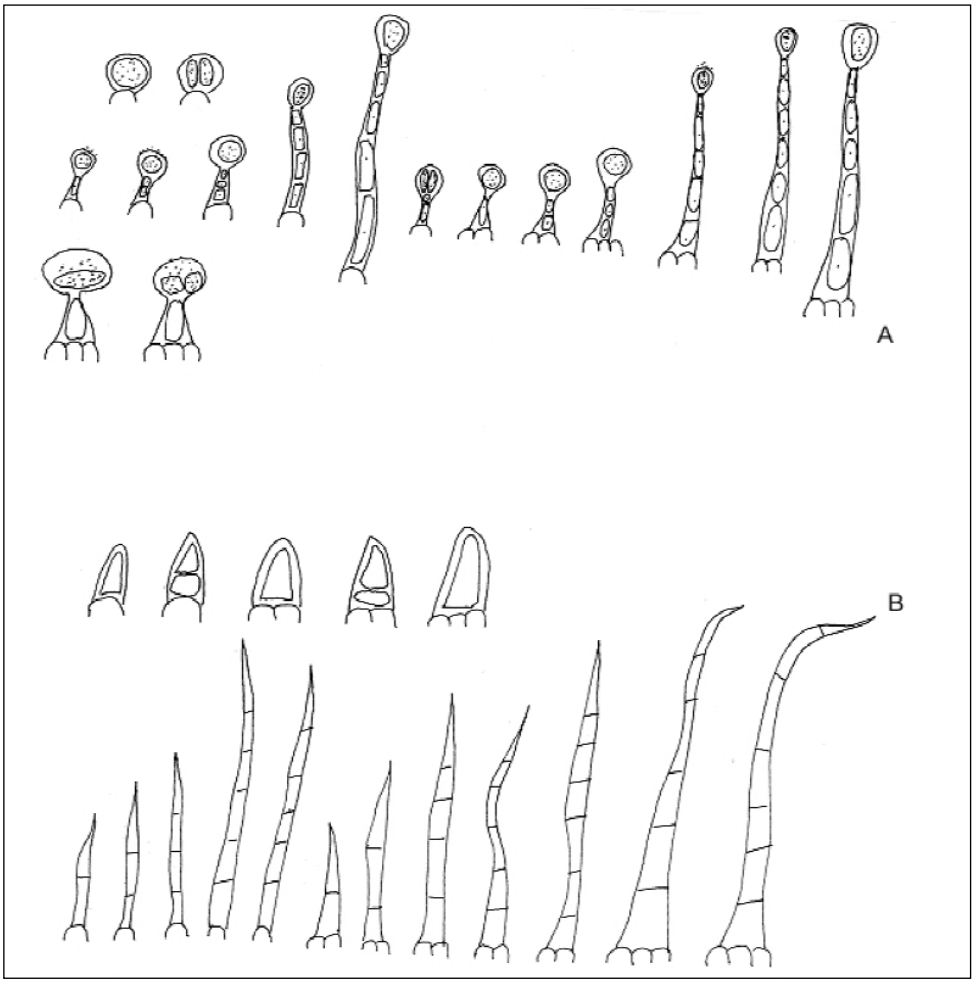

Fig. 3. *Salvia cadmica* Boiss.: Some glandular and eglandular hairs from the different parts: **A,** capitate glandular hairs; **B,** eglandular hairs.

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 the taxon that has been examined morphologically.

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

Özdemir & Şenel (1999) observed that *Salvia sclarea* L. had bifacial type of leaves and diacytic type stomata. But leaves of *Salvia cadmica* was isolateral and diacytic stomata.

Table 1. Glandular and eglandular hair type of various organs of *Salvia cadmica* Boiss.

	Glandular Hair									Eglandular Hair	
	Capitate glandular hairs										
	Type I			Type II			Type III				
	Head cells	Stalk cells	Base cells	Head cells	Stalk cells	Base cells	Head cells	Stalk cells	Base cells	Base cells	Hair cells
STEM	1	1	1	1	2	1				1	1
	2	2	2	1	1	1				1	2
	1	3	3							2	3
	1	5	1							1	4
										3	5
										1	6
										2	7
PETIOLE	1	1	1	1	1	1	1	2	1	1	1
	1	2	2	1	2	1	1	3	1	1	2
	1	3	1							3	3
										2	7
LEAF	1	1	1	1	2	1	1	2	1	1	1
	1	2	2	1	3	1	1	3	1	2	2
	1	3	3				1	4	1	4	3
	1	5	3							3	4
										4	5
										1	6
										2	7
PEDICEL	1	1	1	1	1	1					
	2	2	1	1	1	1					
	1	3	1								
FLOWER	1	-	1	1	2	1				1	1
	2	1	2							2	2
	1	2	1							1	3
										1	5

Metcalfe and Chalk (1972) pointed out that the structure of vascular bundles in petiole was important in terms of taxonomy for the family *Lamiaceae*. In our study, there was only one large bundle in the centre of petiole. The two small bundles were located at both end of the petiole. Seeds were mostly ovate in shape, colored brown and had reticulate surface type of sculpture (Fig. 5).

Nakipoğlu & al. (1993a, b) observed that *Salvia* species had B type chromosomes but in our study we could not observed any B type chromosome. Estilai & al. (1990) determined that the chromosomes numbers of *Salvia* species could vary between $2n = 14$ and $2n = 64$. Some researchers observed that the Mediterranean group of *Salvia* seemed to be characterized by $x = 7$ (Afzal-Rafii 1976), those in Europe and Russia by $x = 11$ (Patudin & al. 1975), those in California by $x = 16$ (Epling & al. 1962). On the other hand, Afzal-Rafii (1988) counted chromosome number of *Salvia cadmica* species from different two localities,

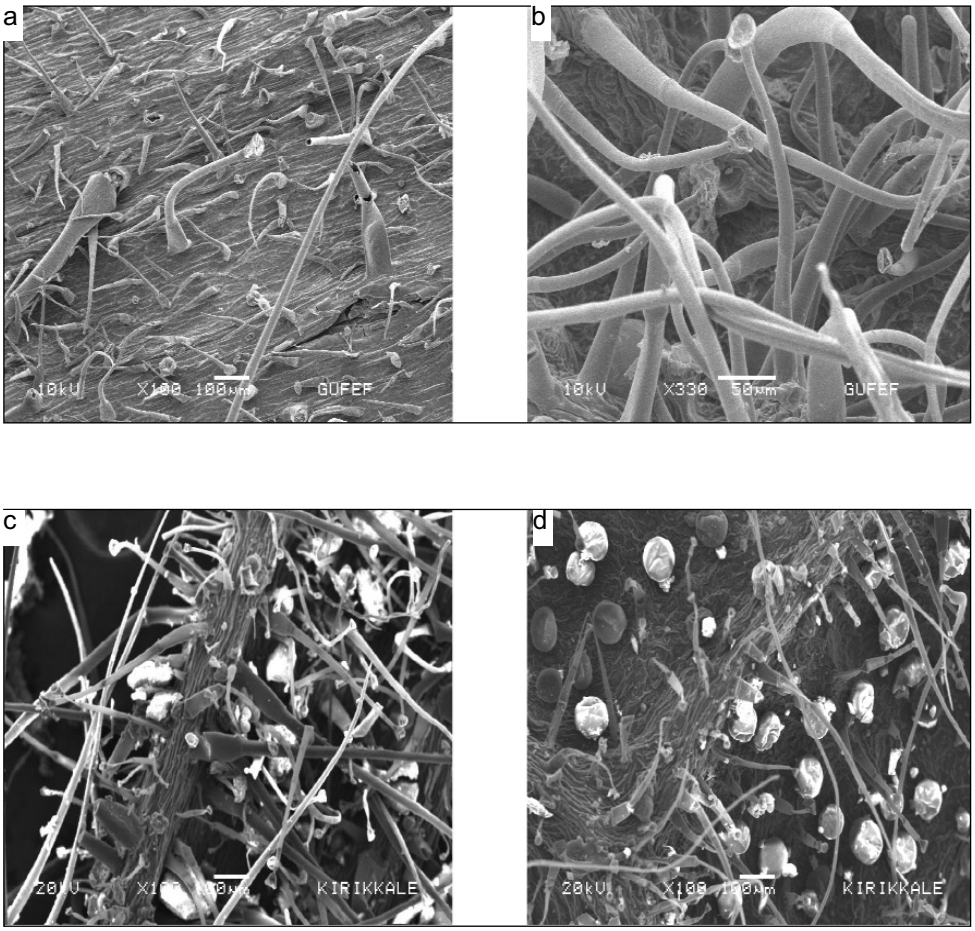

Fig. 4. *Salvia cadmica* Boiss.: Glandular and eglandular hairs from the different parts (SEM): **a,** stem; **b,** leaf; **c,** petiole; **d,** corolla.

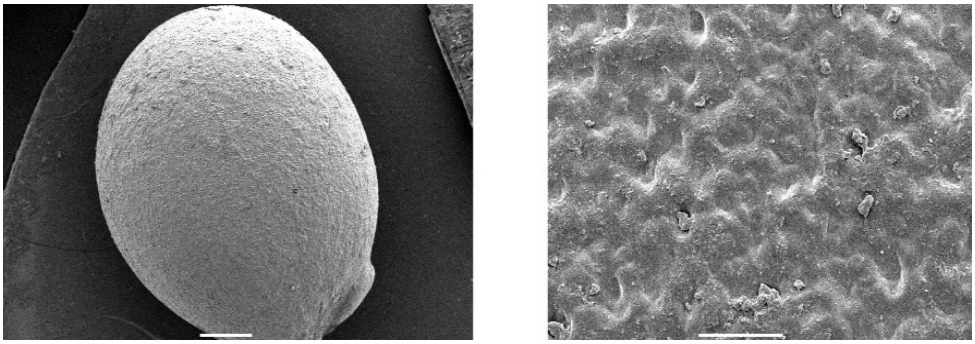

Fig. 5. *Salvia cadmica* Boiss.: SEM micrographs of the seed surface at high magnification.

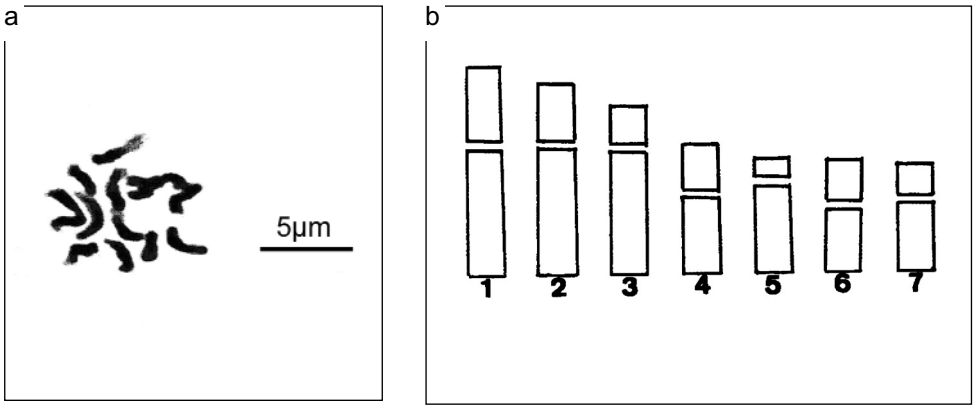

Fig. 6. *Salvia cadmica* Boiss.: **a,** mitotic metaphase chromosomes; **b,** Ideogram of chromosome complement.

Table 2. Karyotype details of somatic chromosomes of *Salvia cadmica* Boiss.

Chromosome pairs	C (µm)	L (µm)	S (µm)	L/S	SAT	I	Centromeric position
1	2.52	1.73	0.79	2.18	-	31	Submedian(sm)
2	2.49	1.74	0.75	2.32	-	30	Submedian(sm)
3	2.25	1.70	0.55	3.09	-	24	Subterminal(st)
4	1.72	1.05	0.67	1.56	-	38	Median(m)
5	1.5	1.22	0.28	4.35	-	18	Subterminal(st)
6	1.44	0.86	0.58	1.48	-	40	Median(m)
7	1.37	0.94	0.43	2.18	-	31	Submedian(sm)

Burdur and Antalya in Turkey and he respectively reported as $2n = 14$ and $2n = 16$. In our study we detected that the chromosome number of *Salvia cadmica* collected from the two localities was the same and $2n = 14$. Our findings corresponded with the literature.

Acknowledgements

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