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THE MORPHOLOGICAL, ANATOMICAL AND KARYOLOGICAL PROPERTIES OF ENDEMIC *SALVİA HYPARGEİA* FİCH. & MEY. (*LAMİACEAE*) IN TURKEY

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Abstract

In this study, morphological, anatomical and chromosome properties of *Salvia hypargeia* Fich. & Mey. which is an endemic plant of the Irano-Turanien phytogeographic region were investigated. The glandular and eglandular hair of this species were examined and classified. Morphologically, it was observed that the species have a perennial root system, the herbaceous stem is rectangular, leaves are simple type, glandular and eglandular hairs are present on the both surface of leaves. At the same time, inflorescence is raceme, kalix and corolla are bilabiate and also species contain two B type of stamen. Anatomically, the internal morphological properties of root, stem, leaf and petiole were determined. Chromosome morphology of this species was examined and diploid chromosome number was found to be 2n=22.

Introductıon

The genus *Salvia* L., has c. 900 species throughout the world. The two largest centres of the genus are in America and in South-West Asia (Hedge, 1960; 1992). Anatolia is a major centre for *Salvia* L. in Asia where 50.6 % of the 87 species are endemic in Turkey (Hedge, 1982; Davis, 1988; Vural & Adıgüzel, 1996). Some of these are shrubby or subshrubby and perennial. In addition, *Salvia* species are grown in parks and gardens as ornamental plants (Nakipoğlu, 1993). In Turkey, they grow between an altitude of s.1.-3350m and distributed at 500-2000 m height on limestone slopes banks with *Pinus brutia* and fallow fields.

Salvia species are an important group of useful plants. Dried leaves and flowers of some species which have active substances are used in preparation of some drops and treatment of some illnesses. *Salvia* has prosuse glandular hairs (Metcalfe & Chalk, 1950) which certain etheric oil. Essential oils, which have frangrance, is a characteristic feature of many species of *Salvia*. Therefore, it is widely used in perfumery and as a sweetener in the food industry (Kesercioğlu & Nakipoğlu, 1992). *Salvia* species contain monoterpenes with antiseptic characteristics (Nakipoğlu, 1993). In recent studies, it has been observed that the compounds in *Salvia* decrease DNA synthesis in the cell. This feature is important in the diagnosis and treatment of cancer (Nakipoğlu, 1993).The leaves of *Salvia* species are used as tea. The gelatinous substance is produced from the seed that has mucilage. This substance is used as good varnish and sweetener in Mexico (Estilai & Hashemi, 1990).

The studies on the anatomy and karyology of this genus are limited. This may be due to the very small size of the chromosomes. Recent studies have been done on the anatomical structure of the secretory hair of *Salvia* sp., (Werker *et al*., 1985; Kesercioğlu & Nakipoğlu, 1992). Also many authors have mentioned the chromosome number of

different species of *Salvia* (Stewart, 1939; Epling *et al*., 1962; Gill, 1971; Haque, 1981; Nakipoğlu, 1993a&b). The reported chromosome numbers are 2n=14, 15, 16, 22, 32, 42, 44 (Davis, 1988). The chromosome counts of many species of *Salvia* in Turkey are also unknown. Morphological, anatomical and karyological structure of *S. hypargeia* has not been studied before. The present report gives an account of the morphological, anatomical and karyological properties of *S. hypargeia*.

Materıal and Methods

The specimens were collected during the flowering period. *S. hypargeia* were collected from around Amasya, Kayseri, Kastomonu, Konya and Maraş. Herbarium samples were prepared and deposited at the Ondokuz Mayıs University, Amasya Education Faculty. Herbarium and fresh samples were used for morphological features and biometric measurements. A part of the material was fixed in 70% alcohol for anatomical studies of root, stem, leaf and petiole.

The regions where these plants were collected are as follows:

1. A5 Amasya: University district, forest area and road side, 500m, 12.06.2000 Kandemir, 050 (Fig. 1).
2. A4 Kastamonu: Gavur Mountain, forest area, 850m, 15.06.2000 Kandemir, 051 (Fig. 1).
3. B5 Kayseri: Bakır Mountain, rocky area, 1000, 20.06.2000 Kandemir, 052 (Fig. 1).
4. C5 Maraş: Elbistan-Gürün district, forest area, 1100m, 01.07.2000 Kandemir, 053 (Fig. 1).
5. C5 Maraş: Tekir Plateau, forest area and rocky area, 1250 m, 03.07.2000, Kandemir 054 (Fig. 1).
6. C4 Konya: Aydos Mountain, rocky area, 1400m, 07.07.2000, Kandemir, 055 (Fig.1).

Anatomical studies were carried out on fresh samples kept in alcohol. The parraffin method was used for preparing a cross section of root, stem, leaves and petiole (Algan,1981; Özyurt, 1978). The anatomical measurement of various organs (root, stem, leaf and petiole) of *S. hypargeia* are given in Table 1. Squash tecniques were used for karyological analysis (Elçi, 1982). Chromosome morphology was determined according to Levan *et al*., (1964).

Morphologıcal propertıes

S. hypargeia is a perennial herbaceous plant. Morphological properties of its root, stem, leaf, petiol and flower are given below:

Root: The top root of the taxon is 10-15x0.4-0.6 cm in length. Dense-dark brown hard bark surrounds the root (Fig. 2).

Stem: The stem is 25-60 cm long and clearly rectangular in shape. The perennial herbaceous stem is erect and usually unbrached toward the top. The upper part of stem is covered by glandular-pilose hairs which have essential oil. The lower part of stem is covered by eglandular arachnoid to lanata hair. These hairs give grey-white colour to the stem (Fig. 2).

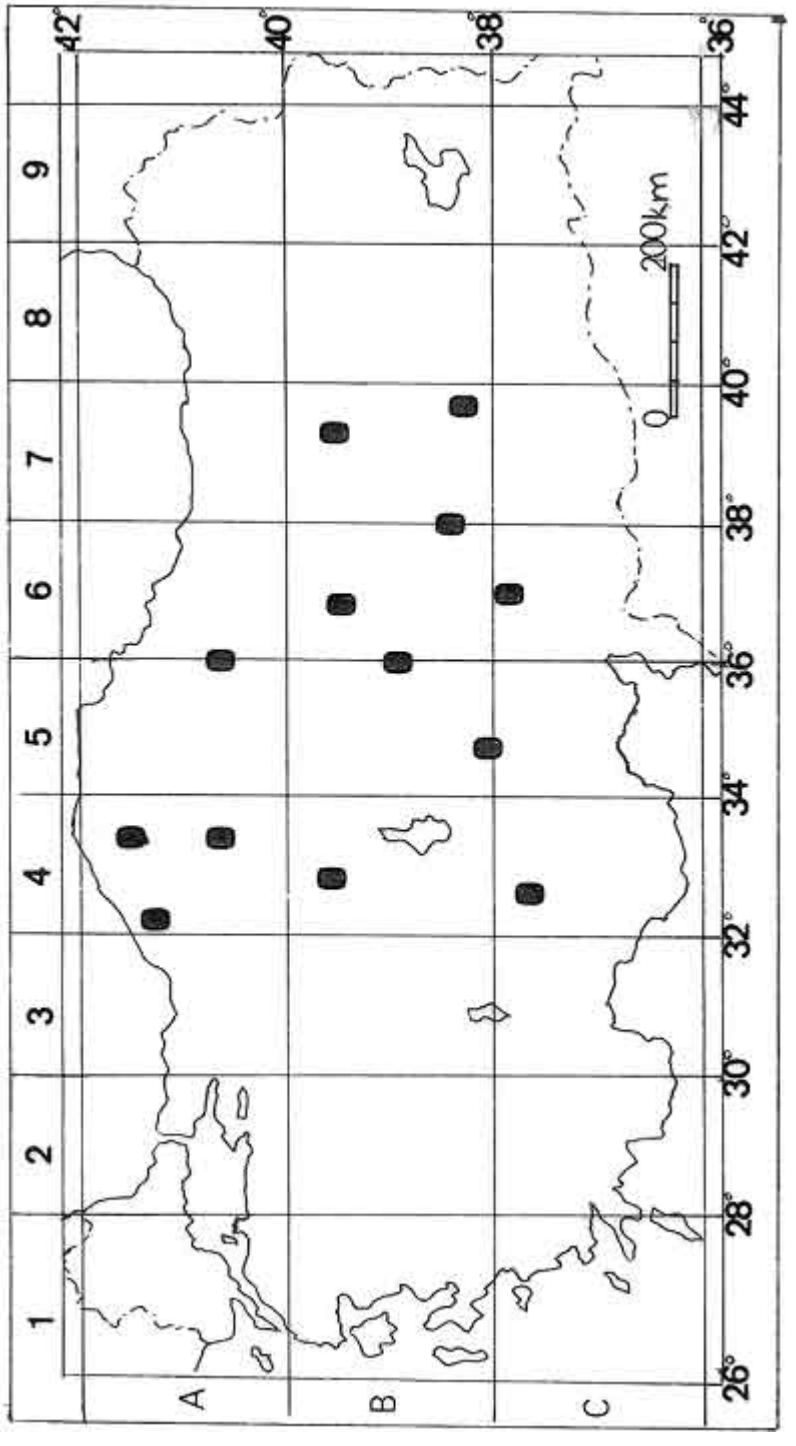

Fig. 1. Distribution of *Salvia hypargeia* in Turkey.
■ *Salvia hypargeia*

Table1. Mean values of germination, seedling height, root length and emergence following gamma radiation.

Variety	Treatments (Gy)	Germination (%)		Seedling height (cm)		Root length (cm)		Emergence (No.)		Correlation (r) dose x emergence
		Actual	% of control	Actual	% of control	Actual	% of control	Actual	% of control	
Basmati-370	Control	100	100	9.37	100	13.05	100	86.6	100	-0.825^{NS}
	150	96.2	96.2	8.29	88.4	10.29	78.8	82.6	95.3	
	200	100	100	6.85	73.1	9.17	70.2	78.2	90.3	
	250	97.5	97.5	6.33	67.5	8.44	64.6	73.3	84.6	
	300	96.2	96.2	4.81	51.5	7.59	58.1	52.4	60.5	
Basmati-Pak	Control	100	100	10.48	100	12.80	100	86.2	100	-0.862*
	150	97.5	97.5	10.68	101.9	9.02	70.4	85.1	98.7	
	200	98.7	98.7	7.59	72.4	7.76	60.6	75.5	87.5	
	250	97.5	97.5	7.05	67.2	6.37	49.7	58.2	67.5	
	300	96.0	90.6	5.32	50.7	5.94	46.4	46.8	54.2	
Super-Basmati	Control	98.7	100	9.97	100	15.86	100	90.4	100	-0.852^{NS}
	150	96.2	97.4	8.68	87.0	11.14	70.2	84.0	92.9	
	200	97.5	98.7	8.67	86.9	10.42	65.6	82.8	91.5	
	250	100	101.3	6.87	68.9	8.81	55.5	58.2	64.3	
	300	96.2	97.4	5.11	51.2	6.32	39.8	44.0	48.6	
LSD values 0.05		3.66		3.72		3.94				* significant at P=0.05 level ^{NS} Non-significant

Leaf: Leaves are simple and mostly basal. They are linear to linear-oblong. Glandular and eglandular hairs are present on both the upper and lower epidermis. Leaves have white lanata lower surface and greenish upper surface. The venation is clear at the leaf. There is a single vein at the middle of leaf. The edge of leaves are subentire. Leaves are 5.5-9x0.8-1 cm in length. The petiole is 0.5-0.9 cm long. Glandular and aglandular hairs are present an the surface of petiole (Fig. 2).

Flower: Inflorescence is raceme. Flowers are zygomorpfic and symmetric (Fig. 3a). The flowers are arranged verticillatelly and 4-8 (mostly 6) flowers are present at verticelles. Flowers are at the base of bracts (Fig. 2). Pedicel is 2-3 mm long. Bracts are 14-17x15-20 mm., broadly ovate, lower surface lanate and upper surface green coloured. The shape of the calyx is tubular-ovate. The upper lip of calyx is truncate and lower lip is bidentate. Calyx has hard lanate and glandular hair. Calyx is 10-12 mm long (Fig. 3b-c), which corolla 25-30 mm (Fig. 3d-e). Upper lip is lavender to purplish-blue and lower lip is lavender to cream. The upper lip of corolla has two lobules and is falcate in shape. The corolla tube is straight and slightly ventricose above. Corolla tube is 13-15 mm length. Stamens are B type (Fig. 3f). The filament is 16-18 mm long and anther 3-4 mm in length. Anther dark or dark purple in colour. The stigma is bifurcate, dark purple coloured (Fig. 3g). The style is 15-25 mm long. Fruit type is nutlet. Seeds are brown coloured and rounded as trigonous.

Anatomıcal properties

Root: Periderm and on outer surface of the root is 4-8 layered. There are flattened parenchymatous cells of primary cortex under periderm. Cortex is multilayered and parenchymatous. Parenchyma cells are 10-25x13-55 µ. Cell size is larger in primary cortex than in secondary cortex. Cambium is 1-3 layered and 8-17x13-28 µ and distinguishable. In the pith there is a primary xylem tissue. Secondary xylem rays are 1-3 layered but sometimes 8 layered and heterogenous. Primary xylem rays are 1-2 layered (Fig. 4).

Woody stem: Epidermis is single layered and consists of flat ovoidal cell. These cells are sometimes crushed. There are glandular and eglandular hairs on epidermis. Peridermis is present under epidermis. Periderm is 1-3 layered. Its cells are hexagonal in shape. There are sclerenchymatous rings between cortex and vascular bundle region. Parenchymatous cortceal cells are 10-13 layered and 20-40x25-80 µ in diameter. Phloem elements are present under primary cortex. Cambium is 2-4 layered and cell size is 13-18x20-24 µ and sometimes crushed. The pith is large 38-125 µ (Fig. 5).

Herbaceous stem: Epidermis is single layered. The cells are ovoid. There are glandular and aglandular hairs on epidermis. The glandular hairs are 1-2 layered, while eglandular hair consist of tall multilayered cells. Collenchyma is 3-5 layered and located under epidermis. These cells are signboard collenchyma and 5-7 µ. Collenchyma is present particulary at the corner of stem. Cortex is 5-7 layered and paranchymatous. Cells of cortex are angular or ovoidal 5-30x12-45 µ. There is a sclerenchymatous sheath on the phloem. There is a cambium between phloem and xylem. Cambium cells are 1-2 layered, 5-7 µ tall and regular hexagonal in shape. The pith is large and consists of paranchymatous cells and 20-120 µ (Fig. 6).

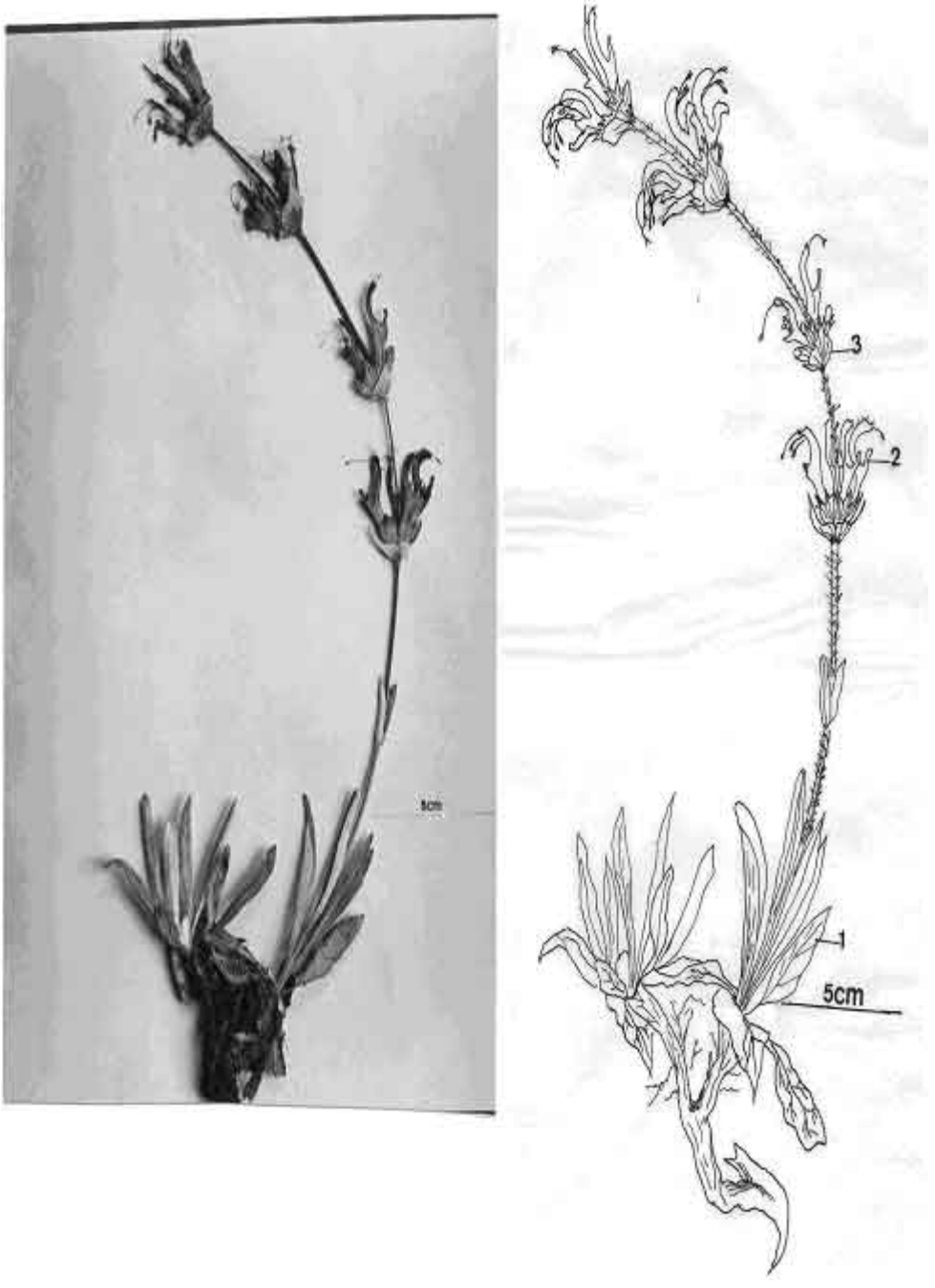

Fig. 2. General appearance of *S. hypargeia* (Kandemir 051)
1. Leaf, 2. Flower, 3. Bract

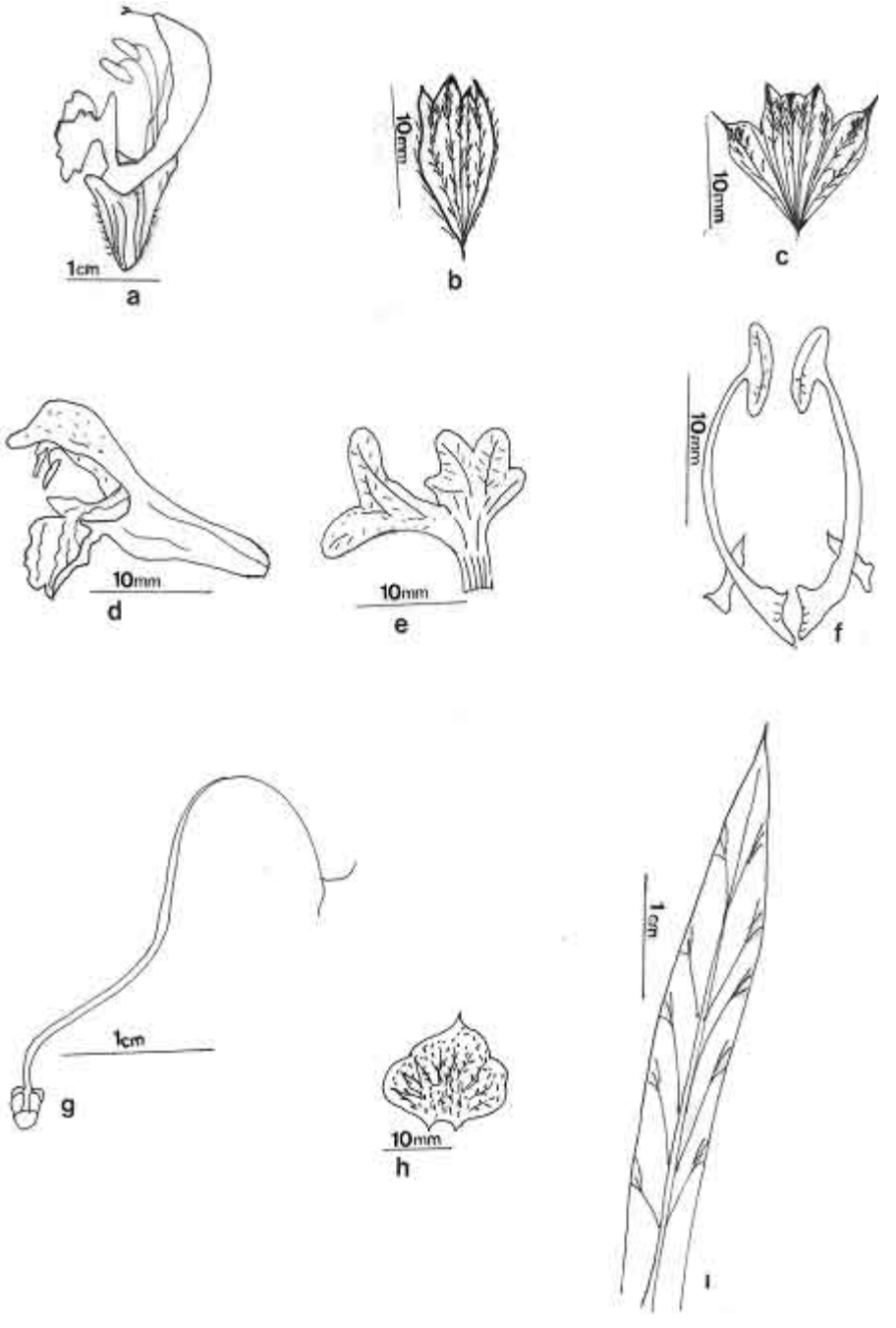

Fig. 3. (a-1) The flower segments of *S. hypargeia* (Kandemir 051)

a. The lontitudinal appearance of flower, b-c. Calyx, d-e. Corolla, f. Stamen, g. Pistil,

h. Bract, i, Leaf

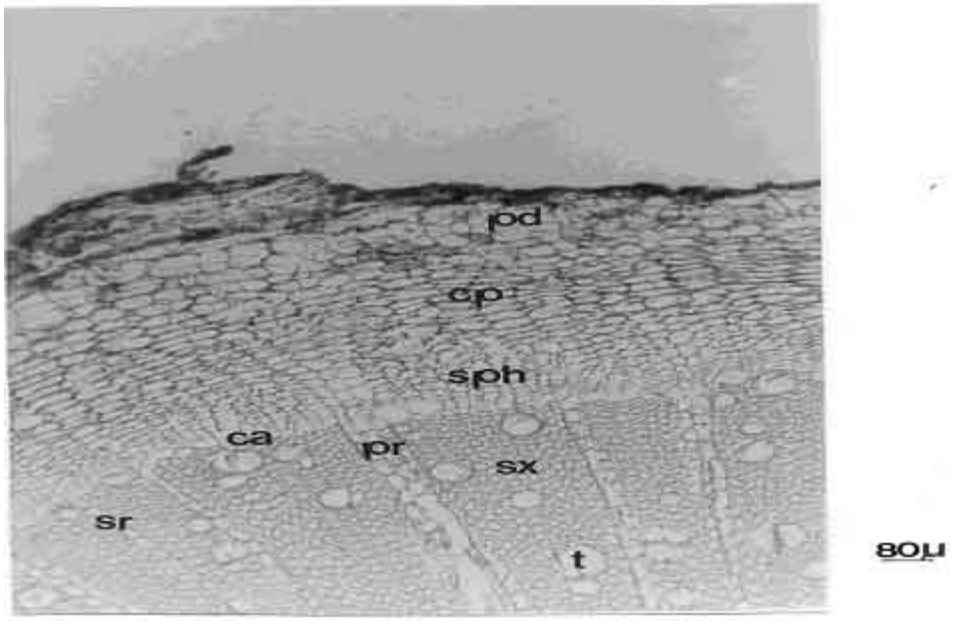

Fig. 4. Cross-section of root of *S. hypargeia* (Kandemir 051).
pd. Peridermis, cp. Cortex parenchyma, sph. Secondary phloem, ca. Cambium, sx. Secondary xylem, pr. Primer pith ray, t. Trache, sr. Secondary pith ray (10x10).

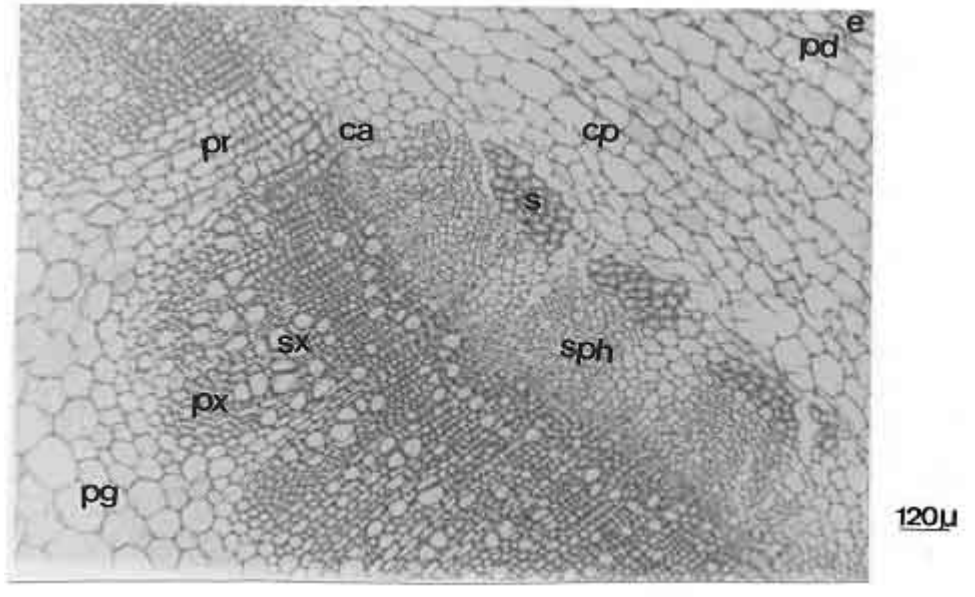

Fig. 5. Cross section of woody stem of *S. hypargeia* (Kandemir 051).
e. Epidermis, pd. Peridermis, cp. Cortex parenchyma, s. Sclerenchyma, sph. Secondary phloem, ca. Cambium, sx. Secondary xylem, px. Primer xylem, pr. Primary pith ray, pg. Pith region (10x10).

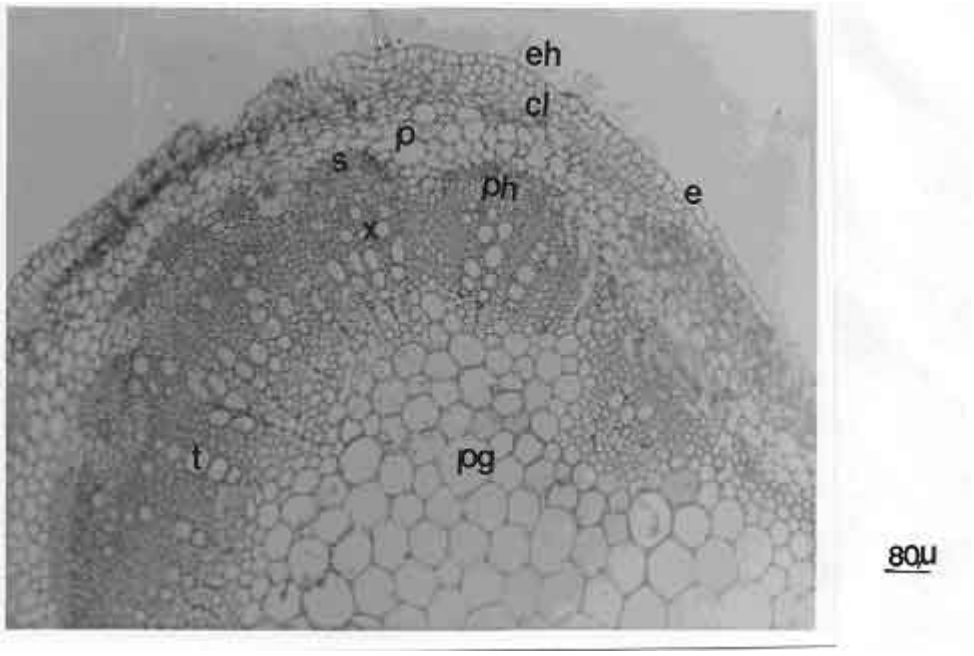

Fig. 6. Cross-section of herbaceous stem of *S. hypargeia* (Kandemir 051).
e. Epidermis, cl. Collenchyma, s. Sclerenchyma, p. Parenchyma, ph. Phoem, x. Xylem, pg. Pith region, t. Trachea, eh. Eglandular hair (10x10).

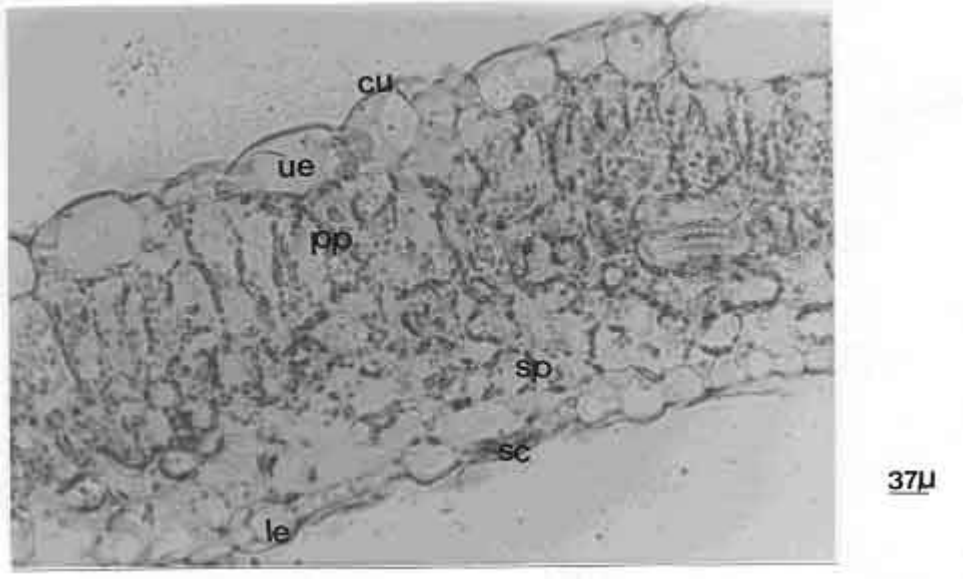

Fig. 7. Cross-section of leaf of *S. hypargeia* (Kandemir 051).
cu. Cuticle, ue. Upper epidermis, pp. Palisade parenchyma, sp. Spongy parenchyma, le. Lower epidermis, sc. Stoma cell (10x40).

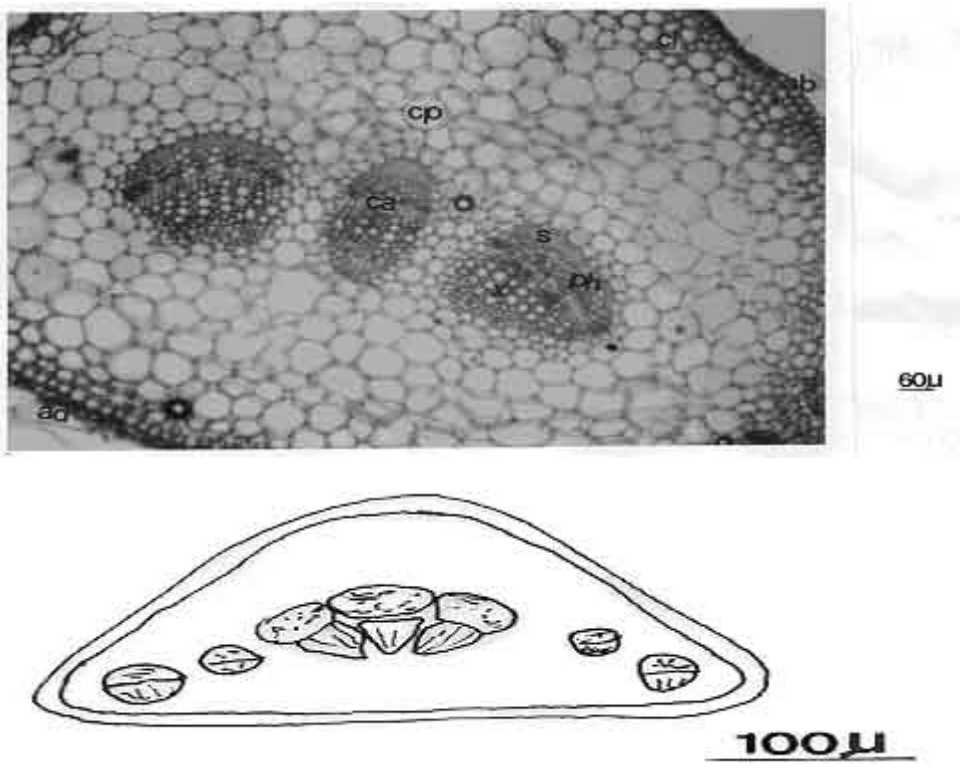

Fig. 8. Cross-section of petiole of *S. hypargeia* (Kandemir 051).
ab. Abaxial epidermis, ad. Adaxial peridermis, cl. Collenchyma, cp. Cortex parenchyma, s. sclerenchyma, ph. Phloem, x. Xylem, ca. Cambium (10x10).

Leaf: Thickness of cuticle is 2-4 µ. There is a single layered epidermis on upper and lower surface of leaf. The shape of epidermal cell is irregular. Upper epidermis cells are 13-45x15-40 µ and lower 8-30x10-30 µ. Stoma cells are present on both upper and lower epidermis. Stoma type is diacytic. Leaf is bifacial. Palisade parenchyma cells are 1-2 layered and 22-55x10-20 µ. Angular collenchyma surrounds the median vein. Spongy parenchyma cells are 1-3 layered and 10-38x1040 µ. Glandular and aglandular hairs are present on both upper and lower epidermis. Most of them are glandular which are small and unicellular. Aglandular hairs are tall and short unicellular (Fig. 7).

Petiole: Petiole is covered by ovoidal and hexagonal epidermal cells. Epidermal cells are 5-35x10-35 µ in adaxial surface and 6-25x10-42 µ in abaxial surface. There are lots of glandular and agulandular hairs on epidermis cells. Most of them are glandular which are short unicellular. Agulandular hairs are multicellular. Parenchymatous cortex is present under collenchyma cells. Cortex cells are 20-25 layered. These cells are 20-58 µ and ovoidal in shape. There are two large vascular bundles on median region of petiole. A small vascular bundle is also present near these bundles. Type of vascular bundle is collateral (Fig. 8). There are lots of sclerenchymat cells on phloem. Collenchyma cells are on corner of petiole and under epidermis.

Haır propertıes

As shown in Fig. 9, *S. hypargeia* have various glandular and egulandular hairs on stem, leaf, petiole and flower. There are lanate hairs, which have head cells on the stem, leaf petiole and flower. The secretion is in outer part of head cell, as this head cell does not break up at some of the lanate glandular hairs. Furthermore, there are the lanate glandular hair which have a cup-like head cell. Lanate glandular hairs have a number of base cells and stalk cells. In addition, stalk cells are not present in some of them. The eglandular hairs have 1-3 base cells and 1-7 hair cell (Table 2).

Karyologıcal propertıes

The chromosome numbers of species was found to be 2n=22 (Fig. 10 and 11). 6^{th} 10^{th} and 11^{th} chromosomes are submedian centromeric and all of the other chromosomes are median centromeric. The chromosome lengths are about 0.30-1.60µ . The longest arm is 1.00µ and the shortest 0.10µ in length. The mean total lenght of chromosomes was found to be 0.86µ. Chromosomes of this species are very small (Table 3). No satellite was observed on karyotypes of this species. There are no B chromosomes in this species.

Dıscussıon

The present study, showed that morphological characters such as the number of fertile stamen, type of stamen, properties of glandular and agulandular hairs, shape of corolla and calyx, structure of bract have taxonomic value. Classification of glandular and aglandular hairs of *S. hypargeia* has been made according to Werker *et al*., (1985). In addition to the previous classification, the number of base cells of glandular and aglandular hairs have been used in this study. *S. hypargeia* has an important feature that brown-green bracts with their thick and broadly-ovate forms like the normal leaves. Because of these features this species can be used as an ornamental plants. Moreover, the bracts possess taxonomical features that are used to determine the species. Stamen type used in the determination of groups of *Salvia* species is of B form. Some researchers have stated that there is mucilage on the seed coat of the family *Lamiaceae*, which cause then to germinate easily, keeping the seeds wet (Fann 1977). In our research, it has been observed that the seeds of *S. hypargeia* are of mucilage, and therefore seeds germinated easily.

As can be seen from the results presented here, the morphological properties of *S. hypargeia* show some similarities and differences compared with the other findings in the flora of a Turkey. Metcalfe and Chalk (1972) found that the rays consist of 2-12 or more layers of cells in the *Lamiaceae* family. In our research concerning this feature, the cross section of root of *S. hypargeia,* number of cells of primerary rays having taxonomical characters are 1-8. Because the number of rays is different in every species, this can be used as a species-distinguishing feature.

Some researchers have emphasized that the distinguishing feature of the family is a rectangular stem and collenchyma well developed supporting tissue in the corners. Similar observations were made in the present study. Further the collenchyma cells become dense, especially in the corners which belong to lacunar collenchyma type.

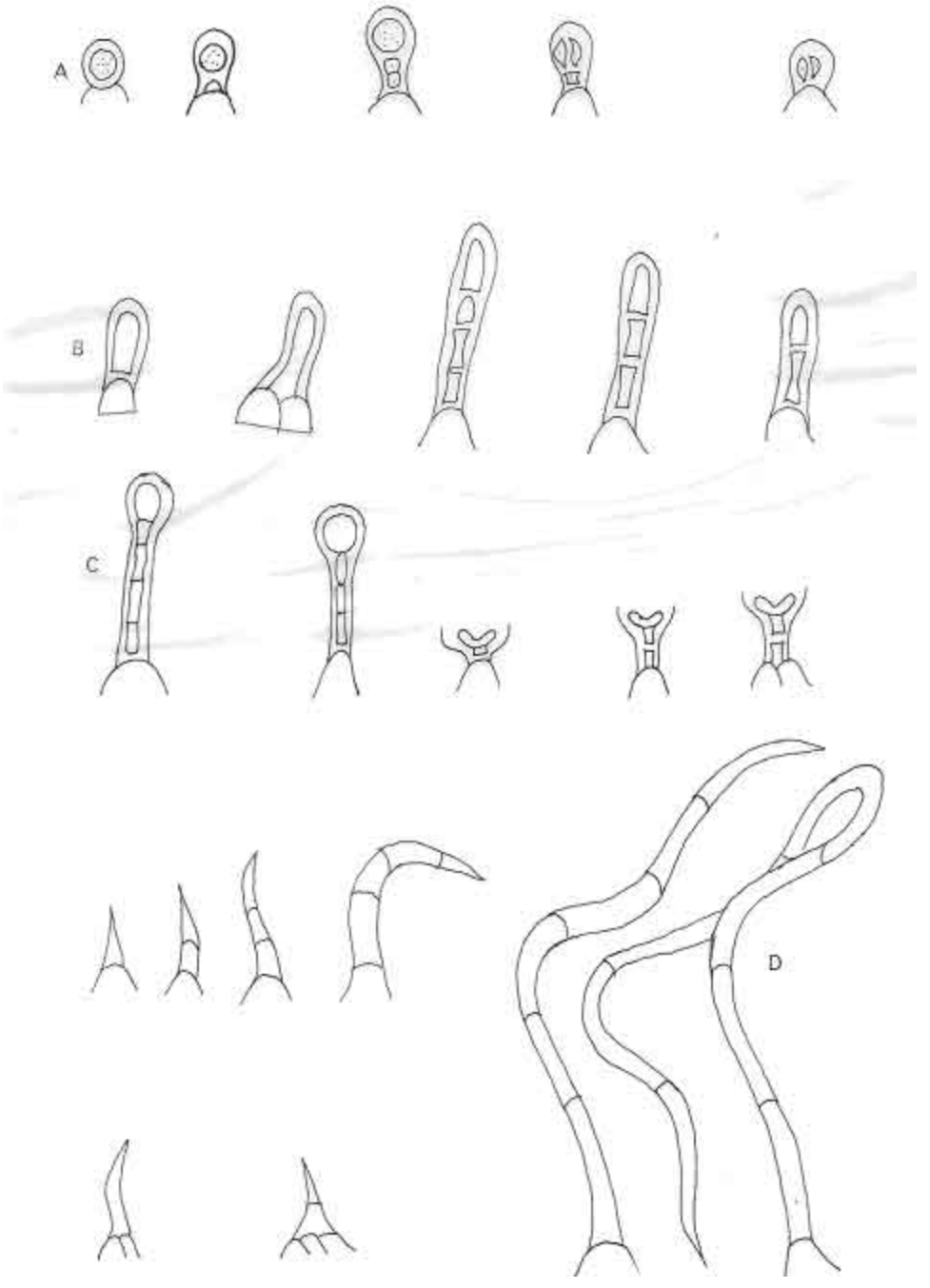

Fig. 9. Glandular and eglandular hair in different part of *S. hypargeia* (Kandemir 051)

A,B,C. Lanate hairs, D. Eglandular hair
(A: type I hair, B: type II hair, C: type III hair)

Table 2. Mean values of plant height, total spikelets per panicle and seed fertility at maturity following gamma irradiation.

Variety	Treatment	Plant height (cm)		Spikelets per panicle		Seed fertility	
	(Gy)	Actual	% of control	Actual	% of control	Actual	% of control
	Control	127.3	100.0	95.7	100.0	92.0	100.0
	150	117.6	92.3	99.5	103.9	56.9	61.8
Basmati-370	200	115.5	90.7	107.6	112.4	56.7	61.6
	250	114.2	89.7	98.5	102.9	42.8	46.5
	300	113.6	89.2	103.5	108.1	28.2	30.6
	Control	117.1	100.0	63.2	100.0	95.1	100.0
	150	108.2	92.3	59.7	94.4	62.6	65.8
Basmati-Pak	200	106.2	90.6	59.4	93.9	58.6	61.6
	250	105.3	89.9	59.2	93.6	41.4	43.5
	300	105.6	90.1	61.2	96.8	26.1	47.4
	Control	94.6	100.0	97.4	100.0	82.5	100.0
	150	84.6	89.4	93.4	95.8	56.2	68.1
Super-Basmati	200	88.8	93.8	100.6	103.2	51.9	62.9
	250	84.3	89.1	92.8	95.2	30.1	36.4
	300	84.5	89.3	92.9	95.3	24.6	29.8
LSD 0.05		5.23		10.52		9.59	

Table 3. Mutagenic effectiveness in M_1 and frequency of chlorophyll mutations in M_2 generation in Basmati rice varieties after gamma irradiations.

Variety	Treatment (dose in Gy)	No. of panicles analyzed	No. of panicles mutated	M_1 panicles segregating for chlorophyll mutations (%)	Effectiveness	No. of M_2 seedling analyzed	Chlorophyll mutants	Frequency of chlorophyll mutations (%)	Chlorophyll mutants per 1000 M_2 seedlings
Basmati-370	Control	20	---	----	----	1410	----	----	----
	150	20	6	30	2.OO	1013	22	2.17	21.71
	200	20	15	75	3.75	1115	92	8.25	82.51
	250	20	15	75	3.00	877	70	7.98	79.81
	300	20	12	60	2.00	800	25	3.12	31.25
Basmati-Pak	Control	20	----	----	----	880	----	-----	----
	150	20	4	20	1.33	690	17	2.46	24.63
	200	20	13	65	3.25	584	41	7.02	70.20
	250	20	10	50	2.00	409	24	5.86	58.67
	300	20	6	30	1.00	362	10	2.76	27.62
Super-Basmati	Control	20	----	----	----	1056	----	----	----
	150	20	2	10	0.66	853	16	1.87	18.75
	200	20	6	30	1.50	825	43	5.21	52.12
	250	20	6	30	1.20	566	28	4.94	49.46
	300	20	4	20	0.66	528	17	3.21	32.19

Fig. 10. Mitotic metaphase chromosomes in root tip cells of *S. hypargeia* (Kandemir 051) (10x10)

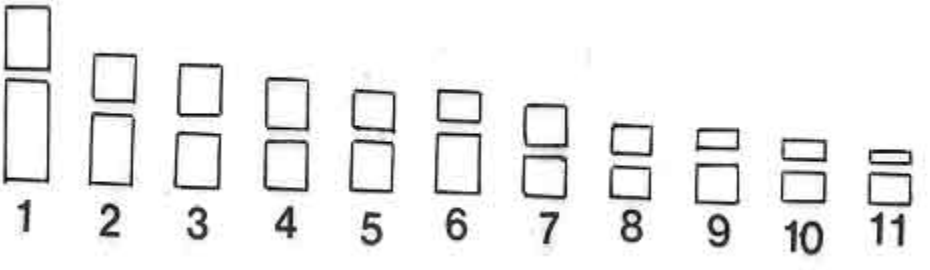

Fig. 11. Idiogram of the chromosome complement of *S. hypargeia* (2n=22).

Çobanoğlu (1988) has stated that there are groups of sclerenchyma on the border of primary and secondary cortex on the stem of *S. palaestina* Bentham. On the other hand, some other researchers have observed cells of sclerenchyma surrounding vessel elements on the herbacous stem of *S. trichoclada* (Çobanoğlu *et al*., 1992). Metcalfe & Chalk (1972), while stating the existence of a well-developed peridermis layer on woody stem, have pointed out that intervascular fibres form intervascular cambium and they separate the continuous xylem at woody stem. In the woody stem, in addition to a sclerenchyma groups on phloem, there is also a sclerenchymatous ring. However, on the herbaceous stem, there are sclerenchyma groups but there is only one sclerenchyma ring. We have observed the same features on the stem of *S. hypargeia.*

The species, having bifacial type of leaves has a diasytic type of stoma. (Metcalfe & Chalk, 1972) observed that mesophyll is completely parenchymatous and there is collenchyma both under and over the median vein in species of *Salvia*. It has been observed that in *S. hypargeia*, mesophyll is parenchymatous and the median vein is

surrounded by collenchyma cells. In addition the number of palisade cells are more in the leaves of this species thus absorbing the sun light efficiently. These leaves have been defined as "sun leaves" by Shields (1950) and Kasaplıgil (1961).

Metcalfe & Chalk, (1972) pointed out that in the *Lamiaceae* the structure of the vascular bundles in petiole is an important taxonomic feature. There are two large and a small vascular bundles in the centre of *S. hypargeia* petiole. In most of the anatomical studies on *Salvia*, it has been found that the order of petiole vascular bundles are different in different species. Cirig & Seçmen (1990) have pointed out that there are three vascular bundles in petiole of *S. kronunburgii,* two of which are on the sides, and the third in the centre of petiole. Nakipoğlu & Oğuz (1990), who studied seven species of *Salvia,* divided vascular bundles of petiole into two types: species with and without basal leaves. *S. hypargeia* is a species with basal leaves, the order of vascular bundles of petiole is the same as the two types mentioned above.

A few studies have been carried out on the chromosomes of species of *Salvia* because they have very small chromosomes (Scheel, 1931; Stewart, 1939; Epling *et al*., 1962; Afzal-Rafıı, 1971; Vij & Kashyap, 1976; Bhattacharya, 1978; Nakipoğlu, 1993a, 1993b). The polyploid ratio of *Salvia* species is 21.7% (Gill, 1971) and the number of chromosome B are different in many species (Hag & Ghoshal, 1980; Afzal-Rafi, 1972). It is faced with B chromosomes especially in the culture forms of *Salvia* genus (Bosemark, 1958). Morever, according to Fröst (1956) the structure of soil and ecological features are important in the observation of B chromosomes. Since the species investigated is not a culture form so no B chromosomes were observed.

Stebbins (1971) and Levan (1964) have reported that species with homogen karyotype belong to old flora (paleoendenic) and those with heterogen karyotype belong to new flora (neoendemic). The chromosome number of *S. hypargeia* has been determined as 2n=22 and the chromosomes types are submedian and median. Therefore, we think this species is an old floristic element (paleoendenic) since it has homogen karyotype. Especially paleoendemics help to evaluate the information about flora origins and old geological area.

These species are found at lower risk-least concern (LR-lc) plant categories (Ekim *et al*., 2000). According to the results obtained in this work, *S. hypargeia* are exhausted and endangered in the distribution localities (Table 4). Therefore, this species should be taken from Lr-lc categories to Lr-nt (lower risk- near threatened or VU (Vulnerable) categories. As a result, this endemic species of our country can be used in maintaning the gene source and cultivation.

Table 4. The population density in localities of *S. hypargeia* where samples were collected .

Locality	The plant numbers in per 100 m^2	
	2000 year	2001 year
Amasya-University district	10	5-6
Kastamonu-Gavur Mountain	13	10
Kayseri-Bakır Mountain	20	10
Maraş-Elbistan-Gürün district	12	8
Maraş-Tekir Plateau	22	15
Konya-Aydos Mountain	16	11

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