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ANATOMICAL AND PALYNOLOGICAL STUDIES OF THREE *SALVIA* L. SPECIES IN AND AROUND ESKISEHIR, TURKEY

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Abstract

The anatomical and palynological features of three species *Salvia cryptantha* Montbret & Aucher ex Benth., *S. candidissima* Vahl subsp. *occidentalis* Hedge and *S. forskahlei* L. have been carried that show natural distribution in and around Eskişehir. It was found that *S. cryptantha* and *S. candidissima* subsp. *occidentalis* showed the broader distribution than *S. forskahlei*. In the cross-sections of the root, it was observed that the pith was completely covered by parenchyma cells. In cross sections of the stem from epidermis is covered by more eglandular and glandular hair. The presence of large paranchymatic cells was observed in the pith of the species. The species showed similarities in terms of leaf anatomy. Upper and lower surfaces of leaf are covered by more eglandular and glandular hair. Leaves are amphistomatic and bifacial. Plants have an amaryllis, a mesomorph and an anisocytic type stomata. The central vascular cylinder of leaf is composed of the phloem and the xylem bundle. Vascular bundle is collateral. Vascular bundle is covered by a typical parenchymatic bundle. They were determined as stephanocolpatae type and shaving uboblate-subprolate shape, tectate-reticulate-perforate/tectate-reticulate-granulate/tectate-bireticulate ornamentation of *Salvia* taxa of the pollen morphological studies.

Introduction

The genus *Salvia* L. (Lamiaceae) consists of more than 1000 species which are distributed all over the world. The genus is comprised 500 spp. of which in central and south America 250, in central Asia/Mediterranean and eastern Asia 40 species are found. Turkey is a major diversity centre for *Salvia* in Asia. Since the most recent works of the genus in Turkey, six new species have been described; the total has now reached 97 (Kahraman *et al*. 2010a, Bagherpour *et al*. 2010). Increasing day by day in Turkey with many systematic studies on new *Salvia* species also continue to contribute to the Flora of Turkey. Turkey on *Salvia* species Davis (1982-1988), and in recent years Dönmez (2001), Hamzoğlu *et al*. (2005), Bagherpo *et al*. (2009), Behçet and Avlamaz (2009), İlçim *et al*. (2009), Kahraman *et al*. (2009), Celebi and Doğan (2010) have made systematic studies.

In genus *Salvia* anatomical, morphological and palynological studies have been conducted (Özdemir and Altan 2005, Baran and Özdemir 2006, Kaya *et al*. 2007, Kahraman *et al*. 2009, Kahraman *et al.* 2009b, Kahraman *et al*. 2009c, Koyuncu *et al*. 2009, Bagherpour *et al*. 2010, Eşiz Dereboylu 2010, Kahraman *et al.* 2010a, Kahraman *et al.* 2010b, Kahraman *et al.* 2010c, Kahraman *et al*. 2010d).

Attempt was made to investigate some anatomical and palynological features of the species of *Salvia cryptantha* Montbret & Aucher ex Benth., *S. candidissima* Vahl subsp. *occidentalis* Hedge and *S. forskahlei* L. under light microscope and scanning electron microscope for the first time.

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Materials and Methods

The specimens were collected from Eskişehir and its nearby area. *Salvia cryptantha* Montbret & Aucher ex Benth., B3: Eskişehir-Seyitgazi road, road sides, 39°48'30.3" N- 30°43'3.3" E, 1008m, 15.05.2010, OUFE: 15938; *S. candidissima* Vahl subsp. *occidentalis* Hedge B3: Mihalıççık-Gürleyik Village, creek environment, 39°59'37.1" N- 31°20'22.6" E, 763 m. 01.07.2010, OUFE:15929; *S. forskahlei* L. B3:Bozüyük-Kozpınar Village, open area, 39°51'30.9" N- 29°40'24.2" E, 900 m. 30.06.2010, OUFE:15940. In order to ensure a systematic study of the material, herbarium samples were prepared and kept as voucher specimens at the Eskisehir Osmangazi University Herbarium. For the anatomical study; root, stem and leaf were fixed in 70% alcohol. From the Herbarium sample, the detailed morphological characteristics of the species were established. For the anatomical investigations, samples were taken from alcohol by hand and scalpel. Anatomical sections of the plants were taken from its root, stem and leaves. The photograph dimensions were 100 µm (Metcalfe and Chalk 1950, Esau 1967, Fahn 1967, Yentür 1995).

Polen samples were obtained from dried flower specimens from Eskisehir Osmangazi University Science and Art Faculty Department of Biology Herbarium (OUFE). At the palynological study, pollens of 10-15 different herb's flowers were used for each specimen which were taken from different areas. The pollen morphology of taxa was investigated using light microscopy and scanning electron microscopy (SEM). Faegri and Iversen's terminology for the names of the exine layers was used (Faegri and Iversen 1975). In the light microscope investigation, the pollen grains acquired from the samples were obtained using the preparation method described by Wodehouse (1935) and Erdtman (1969). Identifications and counts at 10x and 40x plan objectives were used; for the purpose of identification, a 100x plan oil-immersion objective was used. Pollen identifications and counts were obtained by Prior binocular microscope. The spacing between each ocular micrometer was 0.98 µm. According to Wodehouse's (1935) and Erdtman's (1969) methods, the exine ansd intine thickness of the taxa was measured.

For SEM investigations, non-accetolyzed pollen grains were directly placed onto stubs, coated with gold, and examined with a Jeol 5600 LV-SEM (Walker, 1974a-b) Terminologies for pollen morphology were used (Wodehouse 1935, Pokrovskaia 1958, Kuprianova 1967, Erdtman 1966, Erdtman 1969, Faegri and Iversen 1975).

Results and Discussion

There is a peridem layer on the outer surface of the root. Its cells are crushed, broken up and sometimes worn out. Parenchymatous cortex is present under the periderma. The breadth of its cells is bigger than the length. These cells have regular layers. Phloem occupies a narrow area. Cambium cells are distinguishable. Pith region is narrow because of xylem that occupies much of the pith region (Fig. I, 1A; Fig. II, 1B; Fig. III, 1C).

It is of typical four-cornered type and possesses a thick cuticle with small-celled epidermis on the outside. A large amount of blanketing and secreting down erupt from the epidermis. Epidermal cells are surrounded by a thin and slightly undulated cuticle. There are multicellular hairs on the epidermis and most of them are glandular. The glandular hairs are capitate with distinct head. Cortex is 2-5-layered and parenchymatous. Cells of the cortex are ovoid. There is a 5-6-layered collenchymatous tissue. (Fahn 1967). Dense collenchymatous cells located at the corners of the stem is a distinguishing anatomical characteristic of the family Lamiaceae (Metcalfe and Chalk 1950, Fahn 1967, Baran and Özdemir 2006, Kaya *et al.* 2007, Kahraman *et al.* 2009b). There is a sclerenchymatous sheath on the phloem tissue which occupies a small region. Cambium is not

distinguishable and the pith region is large and parencymatic. Starch grains were found in some cells of the pith (Fig. I, 2A; Fig. II, 2B; Fig. III, 2C).

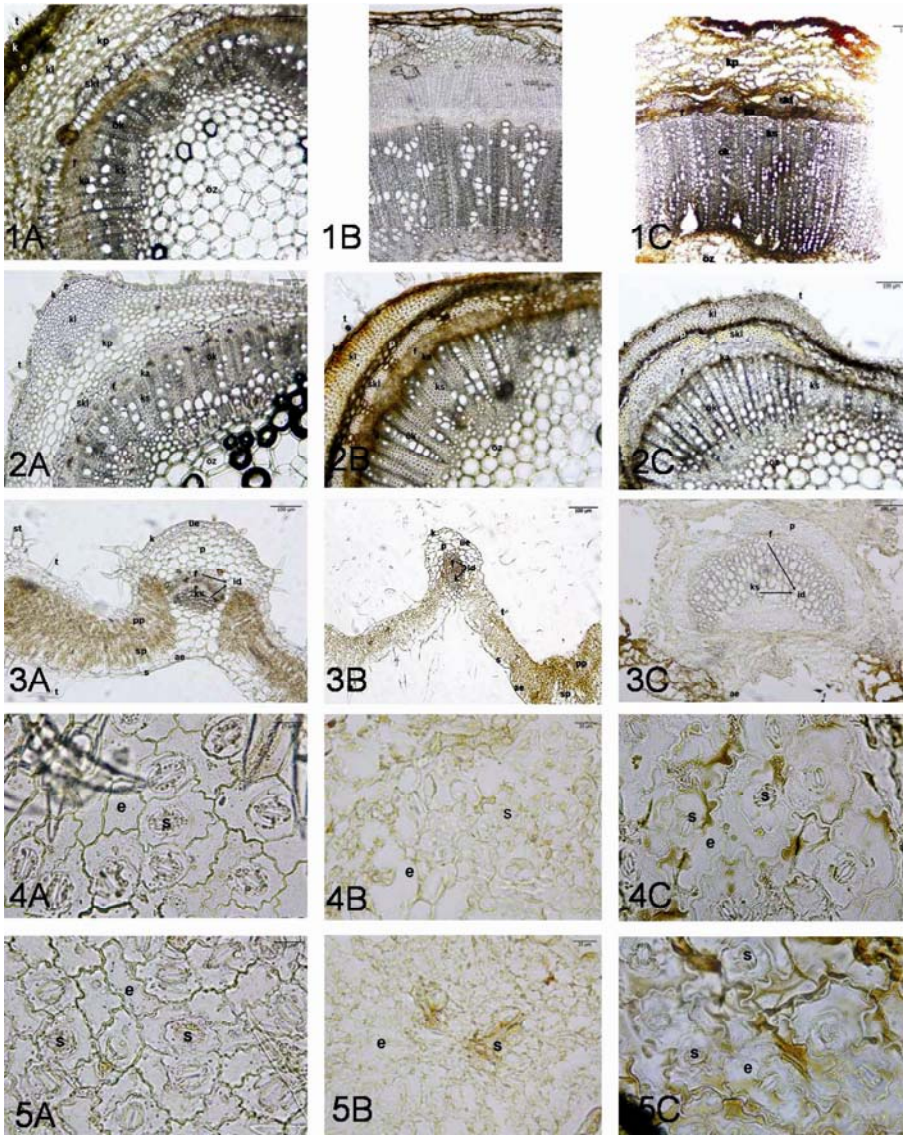

Fig. I **Fig. II** **Fig. III**

Figs I - III. The transverse sections of the root, stem and leaf of *Salvia* species.

Fig. I. 1A. Cross-section of the root, 2A. Cross-section of the stem, 3A. Cross-section of leaf, 4A. Upper surface section of leaf, 5A. Lower surface section of leaf of *Salvia cryptantha.* Key: e: epidermis, c: cortex, x: xylem, p: phloem, pp: palisade parenchyma, sp: spongy parenchyma, s: stomata, pt: pith. Fig. II. 1B. Cross-section of the root, 2B. Cross-section of the stem, 3B. Cross-section of leaf, 4B. Upper surface section of leaf, 5B. Lower surface section of leaf of *Salvia candidissima* subsp. *occidentalis.* Key: e: epidermis, c: cortex, x: xylem, p: phloem, pp: palisade parenchyma, sp: spongy parenchyma, s: stomata, pt: pith. Fig. III. 1C. Cross-section of the root, 2C. Cross-section of the stem, 3C. Cross-section of leaf, 4C. Upper surface section of leaf, 5C. Lower surface section of leaf of *Salvia forskahlei*. Key: e: epidermis, c: cortex, x: xylem, p: phloem, pp: palisade parenchyma, sp: spongy parenchyma, s: stomata, pt: pith.

Table 1. Morphometrical parameters of *Salvia* species (µm).

Taxa	P			E			P/E	L			clg		
	M	S	V	M	S	V		M	S	V	M	S	V
Salvia cryptantha (N)	39,13	2,57	13-33	50,87	4,1	58-44	0,76	42	3,51	48-34	30,6	1,9	35-27
S. cryptantha (A)	41,43	2,44	46-36	50,23	5,03	56-35	0,82	39,03	2,95	46-31	32,64	3,25	39-25
S. candidissima subsp. *occidentalis* (N)	37,93	2,45	42-31	54,43	2,87	61-48	0,69	38,92	2,5	43-33	29,73	2,03	33-25
S. candidissima subsp. *Occidentalis* (A)	40,1	2,79	44-29	51,97	3,49	58-42	0,77	39,7	2,25	45-36	30,73	3,78	36-22
S. forskahlei (N)	37,13	3,93	49-33	45,7	4,02	53-34	0,81	37,78	3,33	44-30	27,47	2,84	35-24
S. forskahlei (A)	36,83	1,88	39-33	48	1,98	50-43	0,76	36,73	3,3	43-26	28,6	2,55	32-23

Table contd. (right side)

clt			t			Exine			intine		
M	S	V	M	S	V	M	S	V	M	S	V
7,9	1,42	11-5	11,63	1,97	15,8	1,48	0,36	2-1	0,83	0,24	1-0,5
10,24	3,2	19-5	11,13	2,1	15-7	1,32	0,38	2-1	-	-	-
10,33	2,20	14-7	11,5	1,96	15-7	1,37	0,37	2-1	0,83	0,24	1-0,5
9,3	2,26	13-5	10,43	1,38	14-8	1,5	0,39	2-1	-	-	-
9,5	2,21	14-5	12,5	2,71	18-6	1,32	0,38	2-1	0,82	0,24	1-0,5
9,33	2,32	15-6	11,37	2,2	16-8	1,3	0,38	2-1	-	-	-

N: Non acetolysed pollen (LM), A: Acetolysed pollen (LM), P: Polar axis, E: Equatorial axis, L: Equatorial countour diameter, t: Apocolpium, clg: Length of the colpus, clt: Width of the colpus, M: Mean, S: Standard deviations, V: Variation (%).

The upper epidermis consists of flat-ovoidal cells and the lower epidermis is made up of cells having same length and breadth. Stoma cells are present both in the upper and in the lower epidermis. Palisade parenchyma cells are two-layered. Glandular and aglandular hairs are present on both upper and lower epidermis. Most of the glandular hairs were capitate and they had two head cells. There are a lot of glandular and eglandular hairs on the epidermal cells. The leaf is amphistomatic (Fig. I, 3A-5A; Fig. II, 3B-5B; Fig. III, 3C-5C). The plant has an amaryllis type, a mesomorphic and anisocytic type of stomata. The leaf's central vascular cylinder is composed of the phloem and the xylem and is situated in a position above the external 1 - 2 cell layers and the xylem. The xylem is composed of trachea and tracheids and covers a wide space. After the xylem, 1 - 2 layers of schlerancymatic cells and the parencymatic tissue can be seen.

In transverse sections of the upper root, it was seen from the composition of the cortex structure and periderm that secondary growth is the result of the plant's long-existence. This has been stressed in the literature related to the subject (Fahn 1967, Metcalfe and Chalk 1972, Yentür 1995, Özdemir and Senel 1999, 2001, Kandemir 2003, Özdemir and Altan 2005, Baran and Özdemir 2006). The plant profits in terms of protection, durability, and resistance against external effects in the ring-shaped vascular bundles of the surrounding scleranchyma.

Metcalfe and Chalk (1972) gave information about general anatomical characteristics of the genus *Salvia*. They also remarked that there were collenchyma in each corner. They also stated that the rays may consist of 2 - 12 or more layers of cells in this family. In the present study, it was seen that these rays consist of 1 - 7 layers cells. Since the number of rays is different in every species, this can be used as an identifying character of the species. Similar observation has been reported in the root anatomy of *Salvia sclarea, S. hypargeia, S. huberi* and *S. napifolia* (Özdemir and Senel 1999, 2001, Kandemir 2003, Özdemir and Altan 2005, Baran and Özdemir 2006). The lignifiying cell walls are sourrended by xylem. This can be seen particularly in *Salvia sclarea, S. hypargeia, S. huberi* and *S. napifolia*. This results development of secondary growth. The typical stem is four-cornered. Under the epidermis there are 5 - 6 cortex cell layers and below these the 2 - 3 layers of the dense cell wall are situated in the chlorenchyma. The existence of chlorenchyma may be considered as a typical response to the photosynthetic ability of the stem. Thus, the effect of photosynthesis of the leaf and also the stem is increased (Fahn 1967). The typical four-cornered stem with dense collenchymatic cell walls located in the corners is a distinguishing anatomical characteristic of the Lamiaceae (Fahn 1967, Metcalfe and Chalk 1972). The existence of the stem's parencymatic pith is observable in the stems of *Salvia sclarea, S. hypargeia, S. huberi* and *S. napifolia* (Özdemir and Senel 1999, 2001, Kandemir 2003, Özdemir and Altan 2005, Baran and Özdemir 2006). In the leaf anatomy, epidermal cells of different sizes can be observed with larger epidermal cells occurring on the underside (Yentür 1995). The leaves are bifacial. The occurrence of the leaf being bifacial has been reported in *Salvia sclarea, S. hypargeia, S. huberi* and *S. napifolia* (Özdemir and Senel 1999, 2001, Kandemir 2003, Özdemir and Altan 2005, Baran and Özdemir 2006). There are amaryllis type and anisosytic type of stomata on both surfaces of the leaf. Thus, the leaf is amphistomatic. Same as repeated in the leaves of *Salvia sclarea, S. hypargeia, S. huberi* and *S. napifolia* (Özdemir and Senel 1999, 2001, Kandemir 2003, Özdemir and Altan 2005, Baran and Özdemir 2006).

The results of the light microscopical investigation of pollen morphology revealed the suboblata-subprolatae and hexazonocolpate in *S. cryptantha*, *S. candidissima* subsp. *occidentalis* and *S. forskahlei*. It was also determined upon scrutiny of the exine *S. cryptantha*, *S. candidissima* subsp. *occidentalis* and *S. forskahlei* are tectate-reticulate-perforate/tectate-reticulate-granulate/tectate-bireticulate, respectively (Figs IV-VI). The essential criteria for the determination of the phylogenetic relationship of the characteristics of the aperture and exine function of this species has been reported Kuprinova 1967, Cronquist 1968, Walker 1974a-b,

Takhtajan 1980, Celenk *et al*. 2008, and Özler *et al.* 2011. In our analysis of these taxa, we observed that genetic distinctions encompassed differences in the measurements, raising objections to the possession of a morphological characteristic passing to the pollen structure of these species (Cronquist 1968, Celenk *et al.* 2008, Koyuncu *et al.* 2009, Bagherpour *et al.* 2010, Kahraman *et al.* 2010a, Kahraman *et al.* 2010b, Kahraman *et al.* 2010c, Özler *et al*. 2011).

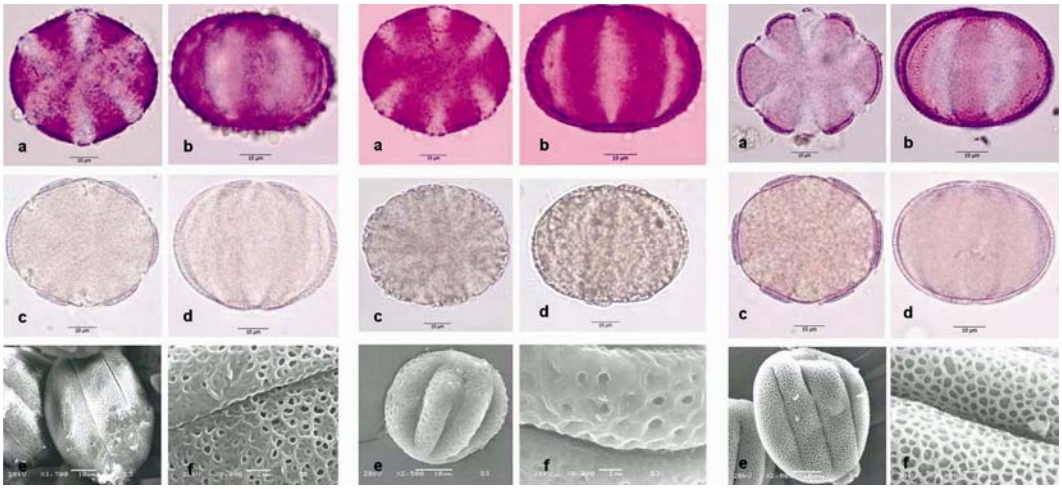

Fig. IV **Fig. V** **Fig. VI**

Figs IV-VI. LM and SEM microphotographs of the pollen of *Salvia cryptantha*, *S. candidissima* subsp. *occidentalis* and *S. forskahlei*.

Fig. IV. a-f. Pollen microphotography of *Salvia cryptantha*, (a) polar view of a non acetolysed pollen under Light microscope, (b) equatorial view of a non acetolysed pollen under light microscope, c) polar view of an acetolysed pollen under light microscope, (d) equatorial view of an acetolysed pollen under light microscope, (e) equatorial view of a non acetolysed pollen in SEM, (f) close up of a non acetolysed pollen grains in SEM. Fig. V. 7a-f. pollen microphotography of *Salvia candidissima* subsp. *occidentalis*, (a) polar view of a non acetolysed pollen under light microscope, (b) equatorial view of a non acetolysed pollen under light microscope, (c) polar view of an acetolysed pollen under light microscope, (d) equatorial view of an acetolysed pollen under light microscope. (e) equatorial view of a non acetolysed pollen in SEM, (f) close up of a non acetolysed pollen grains in SEM. Fig. VI. a-f. pollen microphotography of *Salvia forskahlei* L., (a) polar view of a non acetolysed pollen under light microscope, (b) equatorial view of a non acetolysed pollen under light microscope, (c) polar view of an acetolysed pollen under light microscope, (d) equatorial view of an acetolysed pollen under light microscope, (e) equatorial view of a non acetolysed pollen in SEM, (f) close up of a non acetolysed pollen grains in SEM.

From the above studies it may be said that this investigation has shed light on the exposed systematic-phylogenetic relationship of investigated taxa. The determination of the taxa' pollen morphological structure has led us to consider the usefulness of pollen studies in distinguishing the characteristics possessed by taxa.

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