A Study of the Anatomy and Pollen Morphology of Two Economically Important Species of Stachys L. (Lamiaceae) in Turkey

İsmühan POTOĞLU ERKARA* Onur KOYUNCU
Eskişehir Osmangazi University, Faculty of Science and Art, Department of Biology, 26480, Meselik, Eskisehir, TURKEY

* Corresponding Author
e-mail: ismuhan@ogu.edu.tr

Received: 11 March 2007
Accepted: 27 April 2007

Abstract

In this study, the detailed anatomical properties and morphological structure of the pollen of the Turkish taxa *Stachys annua* (L). L. subsp. *annua* var. *annua* and *Stachys byzantina* C. Koch were observed by light microscope for the first time. The plants have a compact root anatomy with a full xylem component in pith. Lamiaceae demonstrated a typical four corners in the stem, and under a single sequenced epidermis is to be found 3-4 sequenced layers of the collenchyma cell, while at the innermost we can see a compact parenchyma. In the leaf mesophyll, the palisade and spongy parenchyma cells are of a similar shape. The leaf is amphistomatic. The plant has an amaryllis, a mesomorphic, and an anisocytic type stomata. The results of the light microscope investigation revealed the suboblatae-subprolatae and tricolpatae in the pollen of these two *Stachys* taxa. Upon close investigation of the exine, it was also determined *Stachys annua* subsp. *annua* var. *annua* and *Stachys byzantina* are tectatae-granulatae.

Key words: *Stachys,* anatomy, pollen, Light microscope, Turkey.

INTRODUCTION

The genus *Stachys* L. is one of the largest genera of the Lamiaceae. This genus consists of more than 270 species in the world, and is also presented as 101 taxa in Turkey. *Stachys* species are found in mild regions of the Mediterranean and in south-west Asia. Of the 101 *Stachys* species reported in the Flora of Turkey, 72 are described, and of these 29 are endemic [1]. The level of endemism of this type is 43.4% (33/76), while the level of endemic subspecies is 36.7% (11/30), and the number of varieties 40% (6/15) [1]. The rate of endemism for the *Stachys* genus is 40% [2].

They are known in Anatolia as Adacayi and Dag cayi, and used as sage and in popular medicines to treat genital tumours, sclerosis of the spleen, inflammatory tumours, coughs and ulcers. Teas prepared from the whole plant or leaves are used in phytotherapy, possessing sedative, antispasmodic, diuretic and emmenagogue activities [3, 4]. In addition, extracts or components of the *Stachys* species exert various pharmacological effects: anti-inflammatory [5, 6], antimicrobial [7] antibacterial [8], anxiolitik [9, 10], antioxidant [11-14].

Davis [1] conducted a systematic study on the Stachys species in Turkey. There have not been a sufficient number of anatomical studies on the *Stachys* species done in Turkey, with only a few conducted until experimentation by Uysal [2, 15]. No study into the pollen morphology of the *Stachys* species in Turkey has been encountered in the Literature. In this study, the anatomical characteristics and detailed pollen morphologies of the members of the Lamiaceae family; *Stachys annua* (L). L. subsp. *annua* var. *annua* and *Stachys byzantina* C. Koch; were investigated for the first time under light microscope.

This plant's abundant yield from nature and its resulting high crop, as well as the lack of any dangerous effects has brought about comparison [2]. We began this research after perceiving the need for biological characteristics of plants to be supported by anatomical and palynological research in order to shed more light on evolutionary and systematic relationships.

Therefore, our purpose was to determine the anatomical and palynological characteristics of two *Stachys* taxa collecting from Geyve (Adapazari) and its environs.

MATERIALS AND METHODS

Anatomical Studies

The species *Stachys annua* (L). L. subsp. *annua* var. *annua* and *Stachys byzantina* C. Koch were collected from Geyve (Adapazari) and its environs for this study. In order to ensure a systematic study of the material obtained, herbarium samples were prepared and these samples were protected as herbarium samples at the Eskişehir Osmangazi University Herbarium (OUFE). For the anatomical study; the root, stem and leaves were fixed in 70% alcohol and then kept in the same solution until the acquisition of cross-sections. We also obtained lower and upper cross- sections from the fresh materials. All the crosssections were stained with Sartur (60 ml lactic acid, 45 ml Sudan III (Merck), 2 gr aniline, 0.2 gr I, 1 gr KI, 10 ml alcohol (95 %), 80 ml distilled water) and were thus made permanent preparates by means of glycerine gelatine. From the Herbarium sample, the species' detailed morphological characteristics were established and pollen preparation arrangements were used for the designated species. For the anatomical investigations, samples were taken from the alcohol by hand and scalpel. The Prior marker was investigated under light microscope and microscope photographs were taken with a Spot In-SIGHT Colour Digital camera and an Olympus type microscope. A

Table 1. Pollen Obtained From The Plant Samples.

Taxa	Herbarium Number, Collector	Collecting Area
S. annua (L). L. subsp. *annua* var. *annua*	OUFE: 12635, O. Koyuncu	A3 Sakarya: Geyve, Balaban Köyü, hillside, 05.07.2003 N:40° 38 16.2-E: 030° 19' 48.6'', 256m
S. byzantina C. Koch	OUFE: 12912, O. Koyuncu	A3 Sakarya: Geyve, Örencik Köyü, road sides, 14.06.2005, N: 40°34'50.9"-E 030°20'08.2", 110m.

variety of foundation anatomical books and conducted studies were used as sources for identification of the plants [2, 15-18].

Pollen Morphology Studies

The pollen samples were obtained from dried plants at the Osmangazi University Science Faculty Department of Biology Herbarium (OUFE) (Table 1).

The pollen morphology of the taxa in the study was investigated through light microscope. Faegri and Iversen's terminology for the names of the exine layers were used. In the light microscope investigations, the pollen acquired from the samples were obtained set by the method of preparation described by Wodehouse [19] and Erdtman [20].

Identifications and counts of pollens were obtained. For the counts a x 10 ocullar, and x 10 and x 40 plan objectives were used; for the purpose of identification a x 100 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. When prepared according Wodehouse's [19] and Erdtman's [20] methods, the exine and intine thickness pertaining to each pollen taxon is to be measured a minimum of 20 and a maximum of 50 times. From these obtained measurements, a natural mathematical mean is calculated. Microphotographs were taken at the Osmangazi University Science Faculty, Department of Biology by Spot In-SIGHT Color Digital camera and an Olympus type microscope. The photograph dimensions were 10 µm, 25 µm and 100 µm. A variety of foundation palynological books and studies conducted were drawn from for identification of the pollens, [19-24].

RESULTS

Anatomical Studies

For the anatomical investigations, sections were taken from the plants root, stem, and leaves.

Root

Stachys annua* (L). L. subsp. *annua* var. *annua

The plant has an external periderm (Figure 1), followed by 2-3 layers and flat and wide shaped cells, after which the phelloderm can be found. The cortex covers a very small area, and underneath is the endodermis, which is composed of a ring-shaped dense cell wall. Beneath this is situated the pericycle, which is composed of a much thinner cell wall. After the pericycle, 3-4 layers constitute the dense cell wall, from which we can locate the ring-shaped scleranchyma. Following this, the bundle sheath is situated from the xylem to the root and is filled with root xylem components. On the outside of the xylem is an even thinner cell wall which is made up of small, internal denser wall and larger xylem components. Scattered between the scleranchyma rings and the xylem, we can indistinctly observe the phloem constituted by 1-2 layer cells.

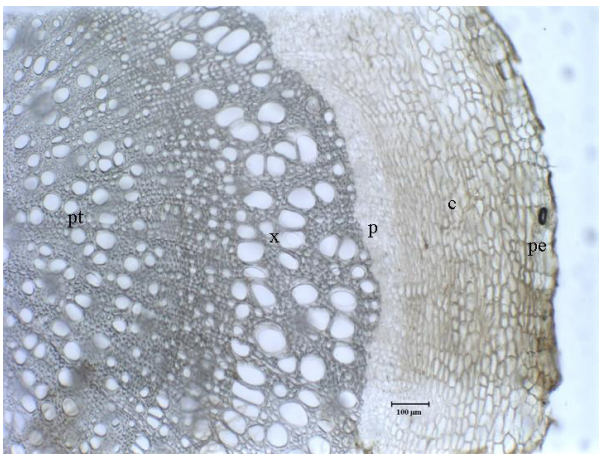

Figure 1. Cross-section of the root of *Stachys annua* (L). L. subsp. *annua* var. *annua*. Key: pe: periderm, c: cortex, x: xylem, p: phloem, pt: pith.

***Stachys byzantina* C. Koch**

The plant has external periderm. The protective tissue demonstrated good development in the periderm, and supplemented the protective duty of the narrow cortex (Figure 2). Following this, after 2-3 layers of flat and wide shaped cells, the phelloderm is observable. The cortex covers a very small area, and underneath is the endodermis, which is composed of a ring-shaped dense cell wall. Beneath this is situated the pericycle which is composed of a much thinner cell wall. After the pericycle are 3-4 layers constituting the dense cell wall, and here we can locate the ring-shaped scleranchyma. After the periskl, 2-3 layers, constituting the dense cell wall, we can locate the ring-shaped scleranchyma and this encompasses the entire transporter bunch to the scleranchyma. Following this, the bundle sheath is situated from the xylem to the root and is filled with root xylem components. Again, on the outside of the xylem is an even thinner cell wall and is made up of small, internal denser wall and larger xylem components. Scattered between the scleranchyma rings and the xylem, we can indistinctly isolate the phloem constituted by 2-3 layer cells.

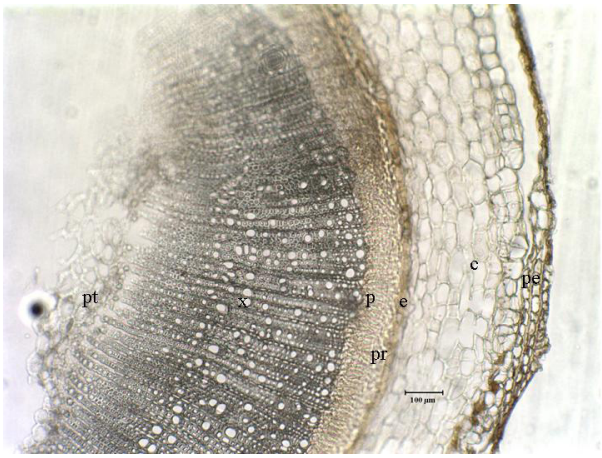

Figure 2. Cross-section of the root of *S. byzantina* C. Koch. Key: pe: periderm, c: cortex, e: endodermis, pr: pericycle, x: xylem, p: phloem, pt: pith.

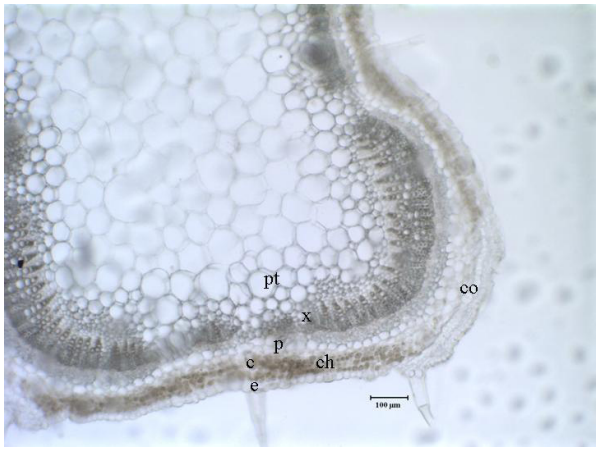

Figure 3. Cross-section of the stem of *Stachys annua* (L). L. subsp. *annua* var. *annua*. Key: e: epidermis, ch: chloranchyma, c: cortex, co: collenchma, x: xylem, p: phloem, pt: pith.

Stem

Stachys annua* (L). L. subsp. *annua* var. *annua

The plant is of the typical four corner type, with a thick cuticle with small cell epidermis beginning on the outside. A large amount of blanketing and secreting down erupt from the epidermis. Following the epidermis, the 5-6 cells that make up the collenchyma can be seen in line leading up to the cortex, and immediately under the 2-3 layer of the rigid cell wall is the chloranchyma. After the chloranchyma, bulk cells and ring-shaped starch sheaves are observable and at this stem plate we encounter the endodermis. Schlerancymatic cell clusters are situated in patches beneath the starch sheath, and between these scleranchyma cells and the xylem, covering a small area, are 1-2 layers of phloem. Following this, there is a wide space covered by trache, tracheid, and xylem parenchyma and between these, in the interior, is the xylem scleranchyma. In the pith, there is a covering with wide space parencymatic cells (Figure 3).

***Stachys byzantina* C. Koch**

On the most outward thick cuticle and underside of the plant lies the epidermis (Figure 4). Blanketing and secreting down erupt from the epidermis. Following the epidermis, we find the 5-6 cells that make up the collenchyma in line leading up to the cortex, and immediately under the 2-3 layer of the rigid cell wall is the chloranchyma. After the chloranchyma, it can be seen bulk cells and ring shaped starch sheath are seen and at this stem plate, encountered the endodermis. Beneath the starch sheath, schlerancymatic cell clusters are situated in patches. Located between the scleranchyma cells and the xylem are a few bulky phloem. Following this, there is a wide space covered by trache, tracheid, and xylem parenchyma and between these, in the insular space, we can find xylem scleranchyma. In the pith, there is a covering with a wide space parencymatic cells (Figure 4).

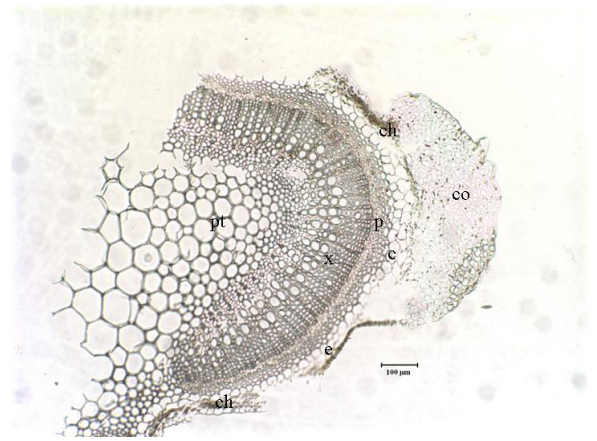

Figure 4. Cross-section of the stem of *S. byzantina* C. Koch. Key: e: epidermis, ch: chloranchyma, c: cortex, co: collenchma, x: xylem, p: phloem, pt: pith.

Leaf

Stachys annua* (L). L. subsp. *annua* var. *annua

The most outward dense cuticle; a range of epidermis can be found on the underside (Figure 5). Epidermis cells can be observed of different sizes. The contour of the palisade and spongy parenchyma cells cannot be easily distinguished in the mesophyll tissue. The underside epidermis cells generally more fully demonstrate the leaf's bifacial characteristic. Both the upper surface of leaves and lower surface of leaves are covered by copious amounts of blanketing and secreting down (Figure 6-7). The leaf is amphistomatic. The plant has an amaryllis, a mesomorphic and anisocytic type stomata. The leaf's central vascular transporter is composed of the phloem bundles and the xylem. A typical parencymatic bundle is surrounded by the bundle sheath cells.

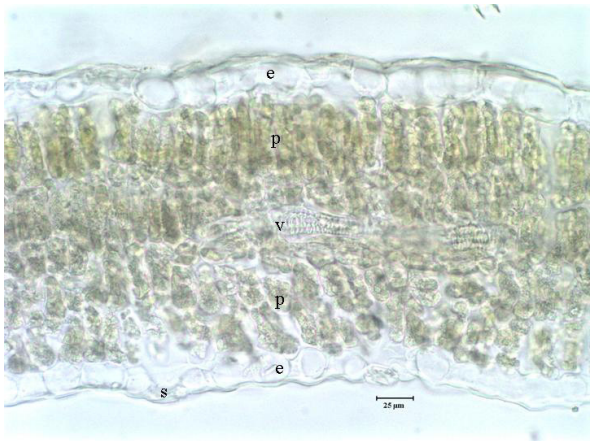

Figure 5. Cross-section of leaf of *Stachys annua* (L). L. subsp. *annua* var. *annua*. Key: e: epidermis, p: parenchyma, v: vascular bundle, s: stoma.

Figure 6. Upper surface section of leaf of *Stachys annua* (L). L. subsp. *annua* var. *annua*. Key: e: epidermis, s: stoma.

Figure 7. Lower surface section of leaf of *Stachys annua* (L). L. subsp. *annua* var. *annua*. Key: e: epidermis, s: stoma.

***Stachys byzantina* C. Koch**

The most outward dense cuticle; a range of epidermis can be found on the underside (Figure 8). Epidermis cells can be observed at different sizes. The contour of the palisade and spongy parenchyma cells cannot be easily distinguished in the mesophyll tissue. The underside epidermis cells generally more fully demonstrate the leaf's bifacial characteristic. The leaf's upper surface and lowersurface are covered by copious amounts of blanketing and secreting down (Figure 9-10). The leaf is amphistomatic.The plant has an amaryllis, a mesomorph, and anisocytic type stomata. The leaf's central vascular transporter is composed from the phloem bundle and the xylem. A typical parencymatic bundle is surrounded by the bundle sheath cells.

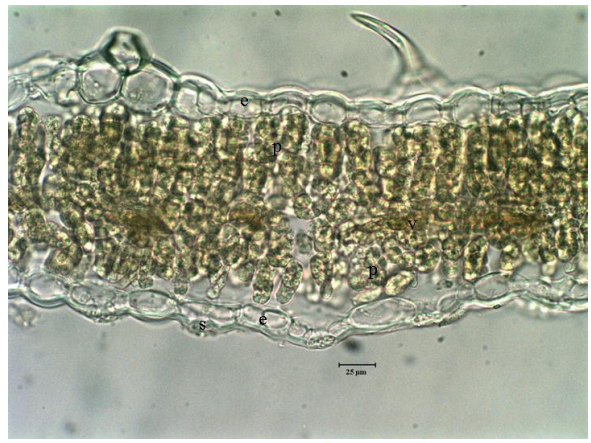

Figure 8. Cross-section of leaf of *S. byzantina* C. Koch. Key: e: epidermis, p: parenchyma, v: vascular bundle, s: stoma.

Figure 9. Upper surface section of leaf of *S. byzantina* C. Koch. Key: e: epidermis, s: stoma.

Figure 10. Lower surface section of leaf of *S. byzantina* C. Koch. Key: e: epidermis, s: stoma.

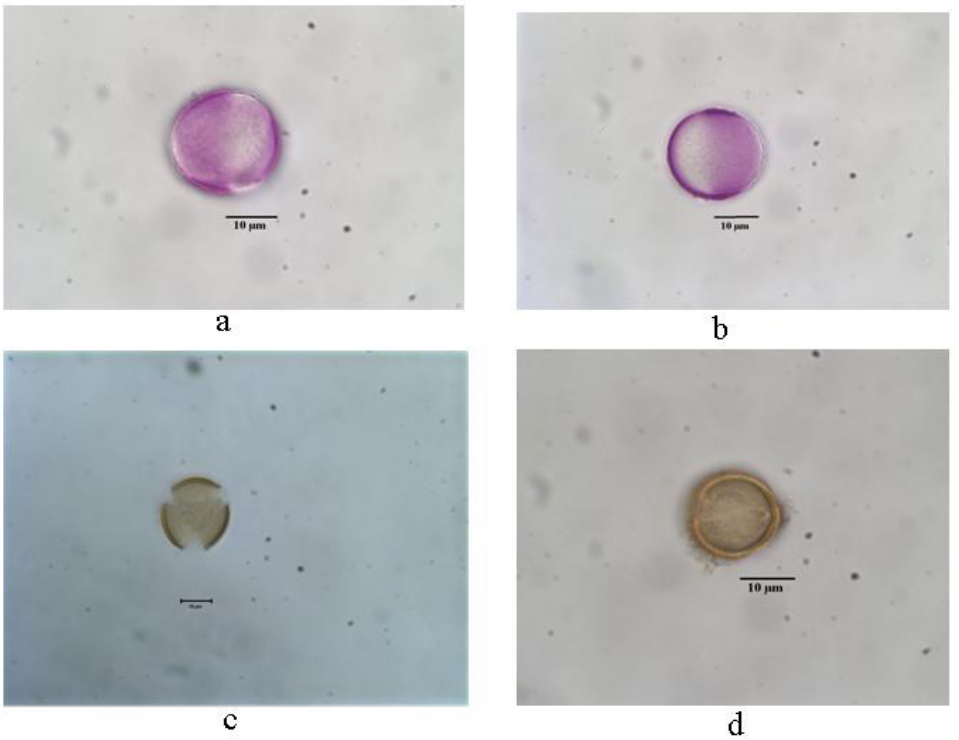

Figure 11 a-d. Pollen microphotography of *Stachys annua* (L). L. subsp. *annua* var. *annua*, a) Polar view of a non acetolysed pollen in Light microscope, b) Equatorial view of a non acetolysed pollen in Light microscope, c) Polar view of an acetolysed pollen in Light microscope, d) Equatorial view of an acetolysed pollen in Light microscope.

Pollen Morphology Studies

Stachys annua* (L). L. subsp. *annua* var. *annua

Pollen grains are suboblatae-subprolatae, tricolpatae P/E=0.96 (W), 0.94 (E). Ornamentation is tectatae-reticulatae. Exine 1.05 µ (W), 1.27 µ (E), Tectum granule (Figure 11, Table 2).

***Stachys byzantina* C. Koch**

Pollen grains are suboblatae-subprolatae, tricolpatae P/E=1.00 (W), 0.91 (E). Ornamentation is tectatae-reticulatae. Exine 1.13 µ (W), 1.25 µ (E), Tectum granule (Figure 12, Table 2).

Abbreviations: 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, Var: Variation.

Table 2. Morphometrical Parameters Of Some Stachys Taxa

Taxa	P			E			P/E	L			clg			clt			t			Exine			intine		
	M	S	Var.	M	S	Var.		M	S	Var.	M	S	Var.	M	S	Var.	M	S	Var.	M	S	Var.	M	S	Var.
S. annua (L). L. subsp. *annua* var. *annua* **(N)**	27.53	0.37	40-24	28.43	0.34	41-25	0.96	28.72	0.32	32-26	20.48	0.30	25-15	9.36	0.39	14-3	4.65	0.24	10-2	1.05	0.01	1.5-1	0.5	0.03	0.75-0.25
S. annua (L). L. subsp. *annua* var. *annua* **(A)**	22.26	0.26	28-19	23.52	0.27	31-20	0.94	24.39	0.58	32-20	17.5	0.76	20-15	11.16	094	14-8	4.97	0.25	10-3	1.27	0.03	2-1	--	-	--
S. byzantina C. Koch **(N)**	26.09	0.34	33-22	26	0.27	30-22	1.00	25.71	0.50	30-20	20.62	0.41	29-16	8.04	0.30	14-3	5.29	0.43	13-2	1.13	0.02	1.75-1	0.52	0.02	0.75-0.25
S. byzantina C. Koch **(A)**	18.5	0.34	24-14	20.26	0.31	25-14	0.91	19.90	0.49	24-14	13.70	0.32	17-10	9.59	0.24	13-7	9.06	0.22	12-5	1.25	0.04	2-1	--	--	--

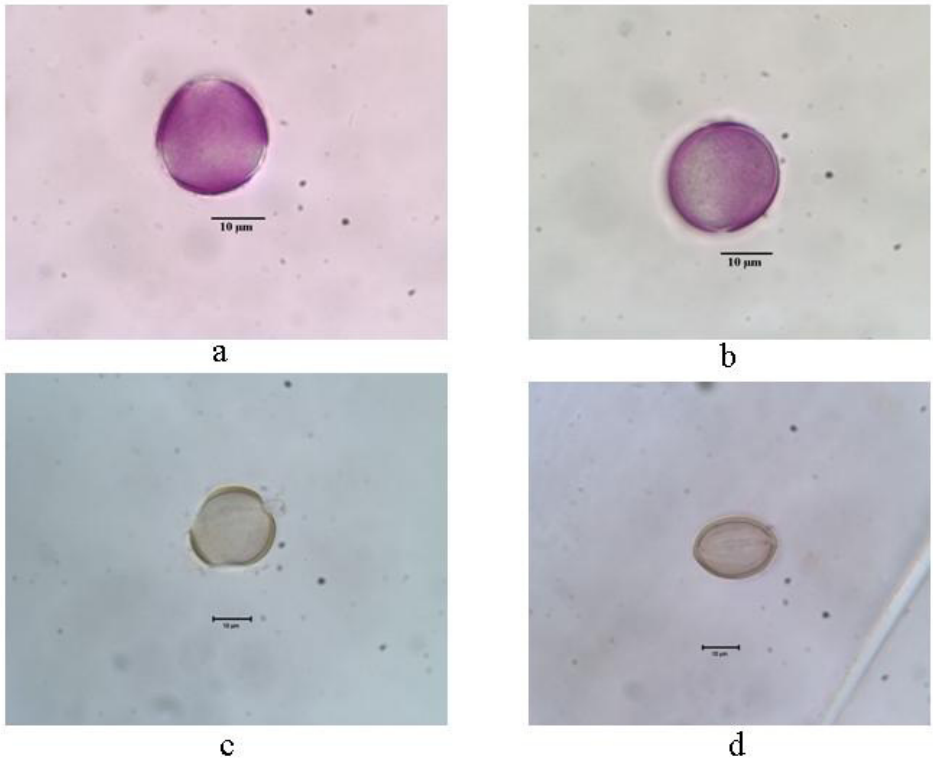

Figure 12 a-d. Pollen microphotography of *S. byzantina* C. Koch, a) Polar view of a non acetolysed pollen in Light microscope, b) Equatorial view of a non acetolysed pollen in Light microscope, c) Polar view of an acetolysed pollen in Light microscope, d) Equatorial view of an acetolysed pollen in Light microscope.

DISCUSSION

During anatomic observations of transverse cross-sections of the upper root, it can be seen from the composition of the cortex structure and periderm that secondary growth is a result of the plant's long-existence. This point has been stressed in literature related to the subject [2, 15-18]. The plant profits in terms of protection, durability, and resistance against external effects from the ring-shaped vascular bundles of the surrounding scleranchyma [18]. The same qualities were observed in the root anatomy of Stachys *cretica* L. subsp. *smyrnaea* Rech Fil. and *S. thirkei* [2, 15]. The character of the element xylem along with their fullness, make up the lignified cell wall. This can be seen particularly in *Stachys cretica* L. subsp. *smyrnaea and S. thirke*. This event is the result of the development of secondary growth. The typical stem is four-cornered. Under the epidermis are 5-6 cortex cells layers, and below these in the 2-3 layers of the dense cell wall are situated in the chloranchyma. It is important for the plant that there is the existence of chloranchyma as a typical response to the photosynthetic property of the stem. In this way, the effect of photosynthesis on the leaf and also the stem is increased [18]. The typical four-cornered stem-type with dense collenchymatic cell walls located in the corners is a distinguishing characteristic of the *Lamiaceae* family [16]. After the chloranchyma, a layer of bulk cells and a ring shaped starch sheath are situated. The root of the starch sheath constructs a homologue endodermis and encompasses the surrounding vascular system like a belt [17]. Beneath the starch sheaf, schlerancymatic cell clusters can be seen in patches. In the stems of the species studied, mechanisms for resistance and support against external effects are available. Hardly any selective phloem can be found between the schlerancymatic cell clusters and the xylem. Forming the xylem are trache, tracheids, and the parenchyma and between these, in the insular space, there are scattered scleranchyma clusters. Here in the scleranchyma, support against external effects to the stem is supplied. In the pith, covering a wide area, is situated the parencymatic root. The existence of the stem's parencymatic is observable in the stems of *Stachys cretica* L. subsp. *smyrnaea* and *S. thirkei* [2, 15]. Few stomas are seen in the stem. In the leaf anatomy, there is an external dense cuticle, and on the underside of this is a layer of epidermis. The dense cuticle of the epidermis is positioned alongside the plant's water loss management and this condition is a characteristic peculiar to xerophytic plants [17-18]. Epidermis cells can be observed at different sizes with larger epidermis cells occurring on the underside. The contour of the palisade and spongy parenchyma cells cannot easily be distinguished in the mesophyll tissue. The leaves are bifacial. The occurrence of the leaf being bifacial has been reported in *Stachys cretica* L. subsp. *smyrnaea* and *Stachys thirkei* [2, 15]. Both the upper surface and lower surface of leaves are covered by copious amounts of blanketing and secreting down. There are amaryllis and anisosytic type stomas on both surfaces of the leaf. Thus, the leaf is amphystomatic. This quality of the leaves of *Stachys cretica* L. subsp. *smyrnaea* and *Stachys thirkei* demonstrates concordance [2, 15]. In the transportation bunch of the central vein, 1-2 cell levels occur on the outside of the phloem above the xylem, and the interior of the xylem covers a wider space. 1-2 layers of schlerancymatic cells can be seen in

the xylem, as well as under the parencymatic cells. The bundle encloses a control from the parencymatic cells. In this state, the leaves of Stachys *cretica* L. subsp. *smyrnaea* and *Stachys thirke* showed parallelness [2, 15].

The results of the light microscope investigation revealed the suboblatae-subprolatae and tricolpatae in the pollen of these two *Stachys* taxa. It was also determined upon scrutiny of the exine that *Stachys annua* subsp. *annua* var. *annua* and *Stachys byzantina* are tectatae-granulatae. The essential criteria for the determination of the philogenetic relationship of the characteristics of the aperature and exine function of the *Stachys* species has been reported in the literature [25-29]. In our analysis of these two species, we observed that determined genetic distinctions encompassed differences in the measurements determined, raising objections to the possession of a morphological characteristic passing to the pollen structure of these species [26].

We believe that we may have distinguished a criterion in the pollen morphology of *Stachys*' systematic system which characteristizes ancillary sequence. This study at the same time has also shed light on the exposed systematic-philogenetic relationship of the investigated taxon. The determination of the taxons' pollen morphological structure has led us to better consider the usefulness of pollen studies in distinguishing the characteristics possessed by taxon.

We believe that the important results concluded from the study of pollen anatomy and morphology will lead to a better understanding of the species and provide a contribution to any future studies.

REFERENCES

[1]. Davis, P.H. 1982-1988. Flora of Turkey and the East Aegean Islands, Vol 7, 10, Edinburg University Press.

[2]. Uysal, İ. 2002. *Stachys cretica* L. subsp. smyrnaea Rech Fil. Endemik Taksonunun Morfolojisi, Anatomisi ve Ekolojisi Üzerinde Araştırmalar, Ekoloji, 11 (42): 16-20.

[3]. Yesilada E, Sezik E, Honda G, Takaishi ,Y, Takeda Y, Tanaka, T. 1999. Traditional medicine in Turkey V. Folk medicine in the inner Taurus Mountains. Journal of Ethnopharmacology, 64: 195-210.

[4]. Duke, J.A. 1986. Handbook of Medicinal Herbs. CRC Press, FL, p.457.

[5]. Khanavi, M., Sharifzadeh, M., Hadjiakhoondi, A., Shafiee, A. 2005. Phytochemical investigation and anti-inflammatory activity of aerial parts of Stachys byzanthina C. Koch. Journal of Ethnopharmacy, 97: 463–468.

[6]. Maleki,N., A. Garjani, H. Nazemiyeh, N. Nilfouroushan, A.T. Eftekhar Sadat, Z. Allameh, Hasannia, N. 2001. Potent anti-inflammatory activities of hydroalcoholic extract from aerial parts of Stachys inflata on rats. Journal of Ethnopharmacy, 75: 213-218.

[7]. Petrovic, S. M. Ristic, M. Milenkovic, J. Kukic, J. Antic-Stankovic, M. Niketic. 2006. Composition and antimicrobial activity of essential oil of Stachys plumosa Griseb. Flavour and Fragrance Journal, 21:250-252.

[8]. Grujic-Jovanovic, S., Skaltsa, H.D, Marin, P. Sokovic, P. 2004. Composition and antibacterial activityoil of six Stachys species from Serbia. Flavour and Fragrance Journal, 19: 139-144.

[9]. Rabbani, M., S. E. Sajjadi, Zarei H. R. 2003. Anxiolytic effects of Stachys lavandulifolia Vahl on the elevated plus-maze model of anxiety in mice. Journal of Ethnopharmacology, 89: 271-276, 290.

[10]. Rabbani, M., Sajjadi, S.E., Jalali, A. 2005. Hydroalcohol Extract and Fractions of Stachys lavandulifolia Vahl: Effects on Spontaneous Motor Activity and Elevated Plus-maze Behaviour Phytotheraphy Research, 19: 854-858.

[11]. Couladis, M., Tzakou, O., Verykokidou, E., Harvala, C. 2003. Screening of Some Greek Aromatic Plants for Antioxidant Activity. Phytotheraphy Research, 17: 194–195.

[12]. Erdemoglu, N., Turan, N.N., Cakıcı, I., Sener, B., Aydın, A. 2006. Antioxidant Activities of Some Lamiaceae Plant Extracts Phytotheraphy Research, 20: 9-13.

[13]. Haznagy-Radnai, E., Czigle, S.,. Zupko,I., Falkay, G., Mathe, I. 2006. Comparison of antioxidant activity in enzyme-independent system of six Stachys species. Fitoterapia, 77 (7-8): 521-4.

[14]. Matkowski, A., Piotrowska, M. 2006. Antioxidant and free radical scavenging activities of some medicinal plants from the Lamiaceae. Fitoterapia, 77: 346-353.

[15]. Uysal, İ. 1998. Stachys thirkei C. Koch Türünün Morfolojisi, Anatomisi ve Ekolojisi Üzerinde İncelemeler, II. Kızılırmak Uluslar arası Fen Bilimleri Kongresi Bildirileri, Kırıkkale.

[16]. Metcalfe, C. R., Chalk, L. 1950. Anatomy of The Dicotyledons 2, Oxford University Press, London.

[17]. Esau, K. 1967. Plant Anatomy. John Willey & Sons, Inc. New York.

[18]. Fahn, A. 1967. Plant Anatomy, Pergamon Press, New York.

[19]. Wodehouse, R. P. 1935. Pollen Grains. Mc. Grew Hill. New York.

[20]. Erdtman, G. 1969. Handbook of Palynology Morphology, Taxonomy, Ecology. An Introduction to the Study of Pollen Grains and Spores. Hafner Pub. New York.

[21]. Aytuğ, B., Aykut, S., Merev, N., Edis, G. 1971. İstanbul Çevresi Bitkilerinin Polen Atlası, İ. Ü. Yayın No:1650, O. F. Yayın no:174.

[22]. Charpin, J., Surinyach, R., Frankland, A.W. 1974. Atlas of European allergenic pollens. Sandoz Editions, Paris, pp. 20-23.

[23]. Faegri, K., Iversen, J. 1975. Textbook of pollen analysis. 3rd edition. Munksgaard, Copenhagen.

[24]. Pehlivan, S. 1995. Türkiye'nin Alerjen Polenleri Atlası, ÜNAL OFFSET Matbaacılık Sanayi ve Tic. Ltd.Şti., Ankara.

[25]. Kuprianova L.A. 1967. Apertures of pollen grains and their evolution in Angiosperms. Review of Paleobotany and Playnology, 3:73-80.

[26]. Cronquist, A. 1968. The evolution and classification of the flowering plants. Thomas Nelson Ltd. Edinburgh, London.

[27]. Walker, J.W. 1974a. Evolution of exine structure in the pollen of primitive Angiosperms. American Journal of Botany, 61: 891-902.

[28]. Walker, J.W. 1974b. Aperture Evolution in the pollen of primitive Angiosperms. American Journal of Botany, 61: 1112-1137.

[29]. Takhtajan, A.L. 1980. Outline of the classification of flowering plants (Magnoliophyta). Botanical Review, 46: 225-359.