Pak. J. Bot., 49(2): 617-621, 2017.

POLLEN AND NUTLET MICROMORPHOLOGY OF A RARE SPECIES *SALVIA KURDICA* (LAMIACEAE) FROM TURKEY

MEHMET FIRAT[1*], BIROL BAŞER[2] AND AKIN AZIRET[3]

[1]*Yüzüncü Yıl University, Faculty of Education, Department of Biology, Van/Turkey*
[2] *Bitlis Eren University, Faculty of Arts and Science, Department of Biology, Bitlis/Turkey*
[3]*Fırat University, Keban Vocational School, Department of Environmental Protection, Elazığ/Turkey*
[*]*Corresponding author's email: kuyucak65@yahoo.com*

Abstract

Salvia kurdica Boss & Hohen. ex. Benth. is known from only 2 localities in the territory of southeastern Turkey and northern Iraq. As a part of the fieldwork, this species was collected from Şırnak Province. In this study, the micromorphological characteristics of its pollen grains and nutlets have been investigated using scanning electron microscopy and a light microscope. The pollen grains are hexacolpate, radially symmetrical, isopolar, and suboblate. Its exine sculpturing is bireticulate-perforate. The nutlets are rounded-trigonous in transverse sections, orbicular ovate-oblong in shape, glabrous and slightly tuberculate. This is an invasive species in Turkey.

Key words: Morphology, Nutlet, Pollen, *Salvia kurdica*, Turkey.

Introduction

Lamiaceae, with its more than 250 genera and approximately 7000 species, has a cosmopolitan distribution (Thorne 1992). It is the third largest family in Turkey, with 45 genera and 574 species, 256 of which are endemic. The rate of endemism is 44.5% in this family (Davis 1965–1985, Güner *et al*., 2000). *Salvia* L., which belongs to Lamiaceae and has also a cosmopolitan distribution with approximately 1000 species (Harley *et al*., 2004). Many species of *Salvia* are used in medicine and few of them are grown as garden plants. The genus is distributed extensively in 3 regions of the world; of the 1000 species of *Salvia* L., 500 are located in Central and South America, 200 in Western Asia, and 200 in Eastern Asia (Walker & Systhma 2007). *Salvia* species are used in medicine, perfumery, and cosmetics industries as tonic, antibacterial, carminative, antiseptic, and antihidrotic agents. In addition, since they contain essential oils, they are also used in the food industry as flavoring and aromatic agents (Demirci *et al*., 2003).

Although Lamiaceae is considered cosmopolitan, it is absent in coldest regions. Erdtman (1945) divided the pollen of Lamiaceae into 2 main groups based on their aperture number; the first group has tricolpate pollen grains and comprises the subfamily Lamioideae, while the second group has hexacolpate pollen grains and comprises the sub-family Nepetoideae (Cantino & Sanders 1986). Henderson *et al*. (1968) gave brief descriptions of the pollen morphology of 59 *Salvia* taxa, 20 of which grow in Turkey. The first revision of *Salvia* L. in Turkey was made by Hedge (1982), who recognized 86 species, 1 hybrid, and 1 doubtful species. Since then, 6 more new species and 3 new records have been described from Turkey. The genus *Salvia* has been subjected to a number of studies, mainly based on morphology (Hedge, 1982). Recently, Perveen & Qaiser (2003) mentioned that the pollen morphology of the family Lamiaceae from Pakistan does not support the sub-family level classification, while it may be used in the identification of the species. Moon *et al*. (2008) studied the pollen morphology and ultrastructure of 32 taxa of *Salvia* (subtribe Salviinae). Moreover, Jafari & Nikian (2008) reported that the pollen characters of 4 desert species of *Salvia* may be used for their identification.(Kahraman *et al*., 2009a-b, 2010a-b, 2010c, 2012) and Kahraman & Dogan (2010) reported that the pollen size, shape and exine ornamentation, and nutlet micromorphology in the genus *Salvia* were important in distinguishing between the species. Ozkan *et al*. (2009), suggested that the nutlet character combinations were correlated to the stamen type, for each taxon in *Salvia*, based on selected species from Turkey. For instance, type A stamen have spherical-foveate mericarps or taxa, while type B stamen have spherical-reticulate mericarps (Ozkan *et al*., 2009). Ozler *et al*. (2011) studied the pollen grains of 30 taxa of *Salvia*, belonging to sections *Salvia*, *Horminum*, *Drymosphace*, *Plethiosphace*, and *Hemisphace*, using light microscopy (LM) and scanning electron microscopy (SEM). Ozler *et al*. (2013), in their study on *Hymenosphace* and *Aethiopis* sections of the genus *Salvia*, found that the pollen features of closely related species indicate some differences that can be used for their identification. In a study by Salimpour *et al*. (2014), the nutlet morphology of 12 *Salvia* L. (Lamiaceae, Mentheae) species was examined using SEM. Their results from the nutlet data did not support the correlation between the nutlet sculpture and the stamen type in these *Salvia* species. To date, the palynological characteristics and nutlet morphology of *S. kurdica* have not been studied. Therefore, the present study aims to give a detailed account of the palynological features and nutlet morphology of *S. kurdica*.This is invisive species species in Turkey,. It has been reported that *S. kurdica* shows distribution only in N. Iraq and S.E. Anatolia (Davis 1982). However, it has been determined that *S. kurdica* also grows in a narrow area of Şırnak Province in Turkey.

Materials and Methods

Samples were collected between 2011 and 2013 from Şırnak Province in Turkey, which is located in the C9 square of the grid system, and used in the 'Flora of Turkey'. The collected samples were pressed, the locality information and population details were recorded carefully, and photographs were taken. Diagnosis was prepared by referring to the 'Flora of Turkey' and 'East Aegean Islands' (Davis, 1965–1985). The morphological

limits were updated based on the measurements of the collected samples. For the palynological investigations, pollen material was obtained from the herbarium samples. The pollen slides were prepared according to the method of Wodehouse (1935). Measurements and observations were made using the Olympus BX41 binocular light microscope (LM). The polar length, equatorial length, colpus length, and exine and intine thickness for 30 pollen grains were measured under the LM (magnification of 1000×). The nutlets were examined using the Leica MZ5 stereomicroscope to ensure their size and maturity. In order to determine the average nutlet sizes, 10 mature nutlets were measured.

During the SEM, selected dry samples of pollens and nutlets were placed on aluminum stabs with the help of double-sided adhesive tape and coated in gold with a vacuum.After that, they were observed and photographed with a Jeol JSM 7001-F SEM. The descriptive terms used are according to Punt *et al*. (2007).

Results

The morphological measurements of *S. kurdica* were taken and compared with the ones that were readily available, and the differences were recorded. Pollen of *S. kurdica* are usually 6-colpate, tectate, oblate, radial symmetric, and ispolar. Their polar and equatorial appearance was also determined (Table 1, Figs. 2a-b). According to the SEM, the exine sculpture was bireticulate (Fig. 1a-b). Average number of primer reticule in 5 µm2, diameter of primer lumina, thickness of primer muri, diameter of seconder lumina, thickness of seconder muri and number of lumina in primer reticule in 25 µm2 were measured with SEM (Table 2).

Table 1.The comparison of pollen morphologies of *Salvia kurdica* and *S. macrochlamys*in LM.

Pollen	*Salvia kurdica*	*S. macrochlamys* (Karaman *et al*., 2010b)
Polar axis (P) (µm) Min-max	48.03 ± 3.52 44–54	47.77 ± 3.63
Equatorial axis (E) (µm) Min-max	55.10±4.35 49–62	52.87 ± 5.23
P/E Pollen shape	0.81, suboblate	0.90, suboblate
Colpus lenght (Clg) (µm) Min-max	39.10 ± 3.60 30–45	40.07 ± 3.59
Colpus width (Clt) (µm) Min-max	8.71 ± 0.89 7.50–11	------
Exine thickness (µm) Min-max	1.78 ± 0.24 1.50–2.25	1.80 ± 0.14
Intine thickness (µm) Min-max	1.06 ± 0.19 0.75–1.50	0.80 ± 0.13
Ornamentation	Bireticulate	Bireticulate and perforate

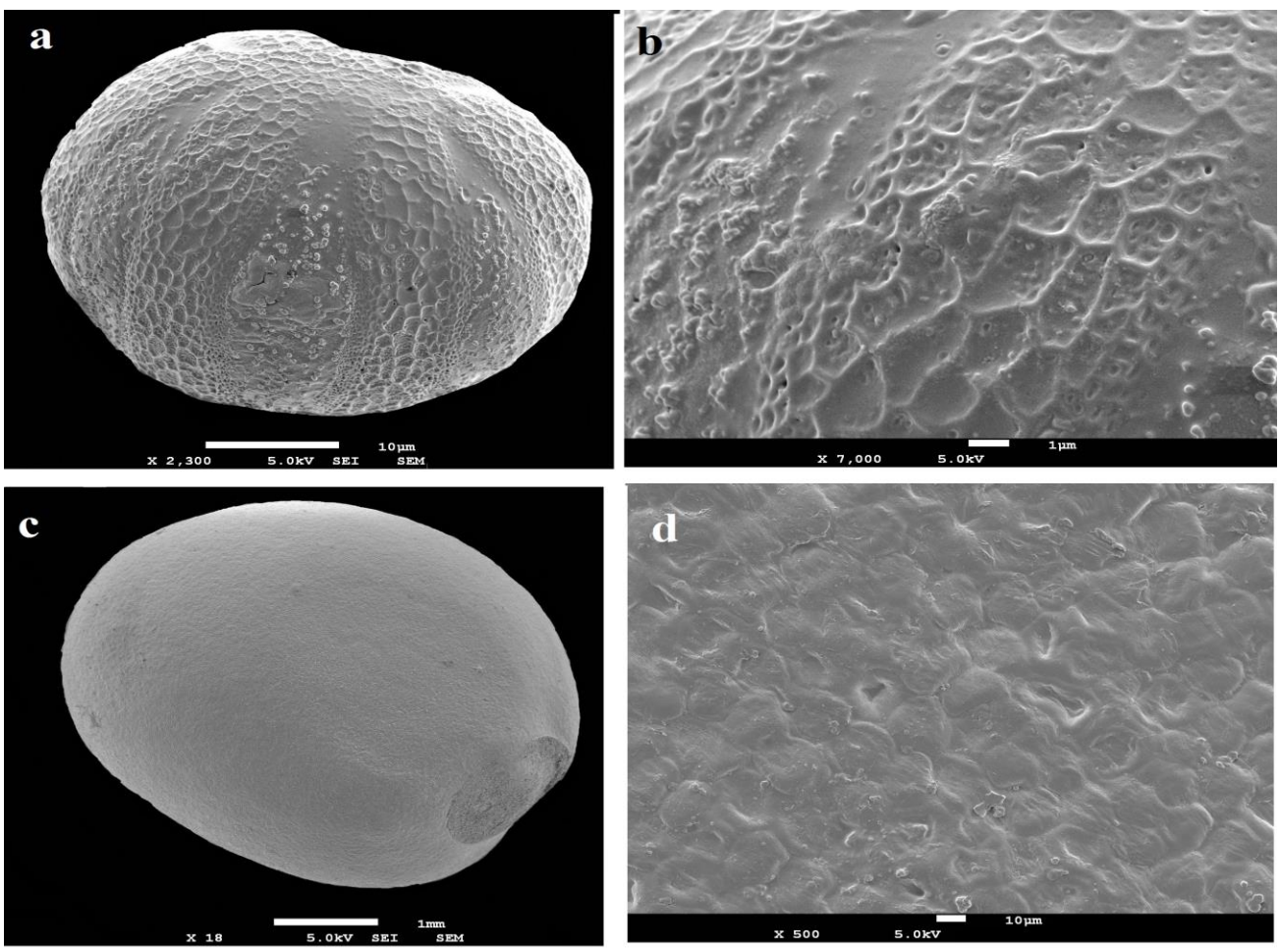

Fig. 1. SEM microphotography of the nutlet and pollen *Salvia kurdica* pollen; **a**- Equatorial view of the pollen with an aperture (X2300), **b**- Pollen surface in detail (X7000), **c**- General appearance of the nutlet (X18), **d**-Nutlet surface in detail (X500).

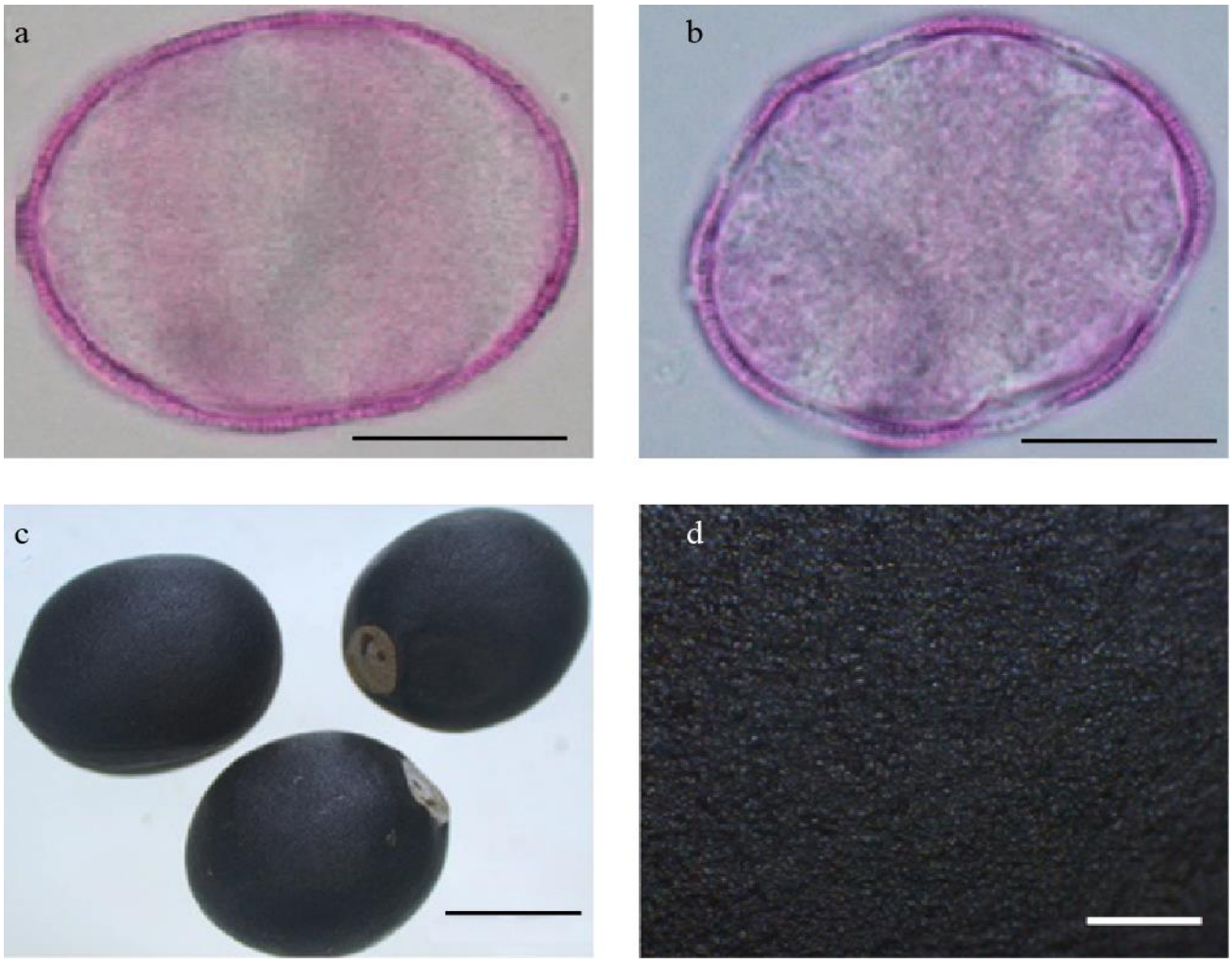

Fig. 2. Light microphotography of the pollen and nutlet of*Salvia kurdica*; a- Polar view of the pollen, b- Equatorial view of the pollen with an aperture (scale 20 µm). c- General appearance of the nutlet, d- Nutlet surface in detail (scale 2 mm).

Discussion

In this study, the aim was to determine the micromorphological differences in the pollen and nutlets of *Salvia kurdica* growing naturally in Turkey. The characteristics of the pollen of *Salvia* L. are usually 6-colpate, rarely 8-colpate, tectate, suboblate-oblate, spheroidal (P/E: 0.81–1.08), radial symmetric, and isopolar. Pollen grains are orbicular at polar sight and orbicular-elliptical at equatorial sight. Regarding the LM study, *S. kurdica* pollen are 6-colpate, tectate suboblate, radial symmetric, isoplar, and the exine ornamentations are bireticulate. The pollen measurements of *S. kurdica* are given in Table 1. Polar axis (P) 44.00–48.03–54.00 µm and equatorial axis (E) 49.00–55.10–62.00 µm. Polar axis/equatorial axis (P/E) ratio is suboblate. Colpus length 30.00–39.10–45.00 µm, colpus width 7.50–8.71–11.00 µm, exine thickness 1.50–1.78–2.25 µm, intine thickness 0.75–1.06–1.50 µm (Table 1). The number of primary reticulations at 5 μm^2 was 7–22, the diameter of the primary reticulates was 1.25–3.33 µm, and the flat primer muri and primer muri thicknesses were 0.20–0.41 µm. The number of secondary reticulations was 3–25, the diameter of the secondary reticulations was 0.25–0.60 µm, and secondary muri thicknesses was 0.16–0.33 µm (Table 2). The colpi were wide and long, the tips of the colpi were orbicular, and the colpus membrane was granulate-gemmate (Fig. 1a-b). Hedge (1982) divided *Salvia* species of Turkey into 7 groups (A, B, C, D, E, F, G). *S. kurdica* falls in group E. Group E taxa are *S. aucheri* var. *aucheri*, *S. aramiensis*, *S. divaricata*, *S. palaestina*, *S. smyrnae*, *S. glutinosa*, *S. cadmica*, *S. macrochlamys*, *S. kurdica*,and *S. hypargeia* (Hedge 1982). In our study, while *S. kurdica* had bireticulate ornementation, species *S. aramiensis* and *S. divaricata* had reticulate ornamentations. While *S. palaestina* and *S. smyrnaea* specieshad 13–18 secondary lumina, *S. kurdica* had 7–21 secondary lumina. The primary lumina of *S. kurdica* was flat, similar to the primary lumina of *S. aucheri* var. *aucher* and *S. glutinosais*.The diameter of the secondary lumina of *S. kurdica* was smaller than 1.00 µm. The diameters of *S. cadmica*, *S. macrochlamys*, and *S. hyargeia*were larger than 1.00 µm. According to palynological studies in the literature, the pollen size, characteristics, and surface the ornamentations are not significant for the classification of the genus. As morphologically distant species have similar pollen structures, close species, and even the subspecies and variations of the same species, have different pollen structures. Moreover, some species break away from their close relatives and have unique pollen characteristics (Ozler *et al*., 2013).

Table 2.Pollen morphological data of *Salvia kurdica* taxon in SEM.

Takson	Average number of primer reticule in 5 µm^2	Average diameter of primer lumina (µm)	Average thickness of primer muri (µm)	Average diameter of seconder lumina (µm)	Average thickness of seconder muri (µm)	Average number of lumina in primer reticule in 25 µm^2
S. kurdica	12.71	1.96	0.37	0.19	0.27	5

Table 3. The comparison of nutlet morphologies of *Salvia kurdica* and *S. macrochlamys*.

Nutlet	*Salvia kurdica*	*S. macrochlamys* (Karaman *et al*., 2010b)
Size	4.70–3.47 mm	4.70–3.80 mm
Surface	Orbicular ovate- oblong	Orbicular-triangular and orbicular-wide ovate
Ornamentations	Glabrous and protuberance	Glabrous and protuberance
Colour	Black	Black

We think that *S. macrochlamys* is a species close to *S. kurdica*. In the study of Kahraman *et al*. (2010b), the pollen of this species was 6-colpate, radially symmetric and isopolar, pollen shape P/E was 0.90 oblate-spheroidal, polar and equatorial axes were 47.77 µm and 52.87 µm. Colpus length was 40.07 µm, exine thickness was 1.80 µm an, intine thickness was 0.80 µm and the exine structure was bi-reticulate and perforate (Kahraman *et al*., 2010b; Table 1). In our study, *S. kurdica* had a polar axis of 48.03 µm, an equatorial axis of 55.10 µm, the pollen shape was suboblate, colpus length was 39.10 µm, exine thickness was 1.50 µm, intine thickness was 1.06 µm and the exine structure was bireticulate (Fig. 1a-b; Table 1). Hedge (1970) found that the micromorphology of the nutlet surface has a systematical importance at the level of the genus and species. Hedge (1982) observed that the nutlets of *S. sclarea L.* (*sect. Aethiopis*)are orbicular-triangular 3.00 × 2.00 mm. Marin *et al*. (1996) observed that the surface of the nutlets of this species have a protuberance. Hedge (1982) measured the nutlets of *S. verticillata* L. (sect. Hemisphace) as 2.20 × 1.30 mm. Marin *et al*. (1996) observed the surface ornamentations of this this species as reticular papillae.

To date, there have been no studies on *S. kurdica* nutlets. In our study, the nutlets are ovate-oblong, 4.70 mm × 3.47 mm in diameter. It was also glabrous. The nutlets of *S. macrochlamys*, which is close to Turkey's flora, in our opinion, has orbicular-triangular and orbicular-wide ovate shape, and has dimensions of 4.70 mm × 3.80 mm. The surface is glabrous, has a protuberance, and is rough (Kahraman *et al*., 2010b). *S. kurdica* and *S. macrochlamys* have similar dimensions, shape, and surface ornamentations (Table 3). Some researchers have found that the nutlet micromorphology is an important taxonomic character in flowering plants, as well as in Lamiaceae (Ryding, 1994; Jamzad, 2000; Salmaki, 2008). According to this study, the size, characteristics, and surface ornamentation of the pollen and the nutlets play an important role in the distinction of species.

References

Cantino, P.D. and R.W. Sanders. 1986. Sub-familial classification of Labiatae. *Syst., Bot*., 11: 163-85.

Da Cheng, H. *et al*. 2016. Mutational, phylogeny and evolution analyses of Salvia copalyl diphosphate synthase. *Pak. J. Bot*., 48(1): 231-239.

Davis, P.H. (ed.). 1965-1985. *Flora of Turkey and the east Aegean islands*, 1–9. Edinburg University Press, Edinburg.

Davis, P.H. 1982. *Salvia* L. In: (Ed.): Davis, P.H. *Flora of Turkey and the East Aegean Islands*. Edinburgh University Press. 7: 36-463 pp.

Demirci, B., K.H.C. Baser, B. Yildiz and Z. Bahcecioglu. 2003. Composition of the essential oils of six endemic *Salvia* spp. from Turkey. *Flavour and Fragrance Journal*, 18: 116-121.

Erdtman, G. 1945. Pollen morphology and plant taxonomy. IV. Labiatae, Verbanaceae and Avicenniaceae. *Svensk Bot. Tidskr*., 39: 279-285.

Güner, A. and *et al*. 2000. *Flora of Turkey and the East Aegean Island*, 11(Supplement): Edinburgh Press.

Harley, R.M., S. Atkins and A.L. Budantsey. 2004. Labiatae. In: (Ed.): Kadereit, J.W. *The Families and Genera of Vascular Plants*, 7: Berlin: Springer, 167-275.

Hedge, I.C. 1982. *Salvia* L. In: (Ed.): Davis, P.H. *Flora of Turkey and the East Aegean Islands*, 7: Edinburgh: Edinburgh University Press, 400-461.

Hedge, J.C. 1970. Observations on the mucilage of Salvia fruits. *Notes from the Royal Botanic Garden Edinburgh*, 30: 79-95.

Henderson, D.M., H. Prentice and I.C. Hedge. 1968. Pollen morphology of Salvia and some related taxa. *Grana Palynol*., 8: 70-85.

Jafari, A. and M. Nikian. 2008. Micromorphological, anatomical and pollen sculpturing study on four desert species of *Salvia* in center of Iran. *Asian J Plant Sci*., 7: 736-741.

Jamzad, Z., M.M. Harley, M. Ingrouille, M.S.J. Simmomds and A. Jalili. 2000. Pollen exine and nutlet surface morphology of the annual species of *Nepeta* L. (Lamiaceae) in Iran. In: (Eds.): Harley, M.M., G. M. Morton and S. Blackmore. *Pollen and Spores: Morphology and Biology, Royal Botanical Gardens, Kew*, 385-397.

Kahraman, A. and M. Dogan. 2010. Comparative study of *Salvia limbata* C.A. and *S. palaestina* Bentham (sect. Aethiopis Bentham, Labiatae) from East Anatolia, Turkey. *Acta. Bot. Croat*., 69: 47-64.

Kahraman, A., F. Celep and M. Dogan. 2009a. Comparative morphology, anatomy and palynology of two *Salvia* L. species (Labiatae) and their taxonomic implications. *Bangl. J. Plant Taxon*, 16: 73-82.

Kahraman, A., F. Celep and M. Dogan. 2009b. Morphology, anatomy and palynology of *Salvia indica* L. (Labiatae). *World Appl. Sci*., 6: 289-296.

Kahraman, A., F. Celep and M. Dogan. 2010a. Anatomy, trichome morphology and palynology of *Salvia chrysophylla* Stapf (Lamiaceae). *S. Afr. J. Bot*., 76: 187-195.

Kahraman, A., F. Celep and M. Dogan. 2010b. Morphology, anatomy, palynology and nutlet micromorphology of *Salvia macrochlamys* (Labiatae) in Turkey. *Biologia*, 65: 219-227.

Kahraman, A., M. Dogan, F. Celep, G. Akaydin and M. Koyuncu. 2010c. Morphology, anatomy, palynology and nutlet micromorphology of the rediscovered Turkish endemic *Salvia ballsiana* (Lamiaceae) and their taxonomic implications. *Nord. J. Bot*., 28: 91-99.

Kahraman, A., S. Bagherpour, E. Karabacak, M. Doğan, H. M. Dogan, I. Uysal and F. Celep. 2012. Species Reassessment of conservation status of *Salvia* L. (Lamiaceae) in Turkey II. *Turk. J. Bot*., 36: 103-124.

Marin, P.D., S. Duletic and B. Petkovic. 1996. Nutlet ornamentation in selected *Salvia* L. species (Lamiaceae). *Flora Mediterranea*, 6: 203-211.

Moon, H.K., S. Vinckier, J.B. Walker, E. Smets and S. Huysmans. 2008. A search for phylogenetically informative pollen characters in the subtribe *Salviinae* (Mentheae: Lamiaceae). *Int. J. Plant. Sci*., 169: 455-471.

Ozkan, M., K. Aktas, C. Ozdemir and G. Guerin. 2009. Nutlet morphology and its taxonomic utility in *Salvia* (Lamiaceae: Mentheae) from Iran. *Acta Bot. Croat*., 68(1): 105-115.

Ozler, H., S. Pehlivan, A. Kahraman, M. Dogan, F. Celep, B. Baser, A. Yavru and S. Bagherpour. 2011. Pollen morphology of the genus *Salvia* L. (Lamiaceae) in Turkey. *Flora*, 206: 316-327.

Ozler, H., S. Pehlivan, A. Kahraman, M. Dogan, F. Celep, B. Baser, A. Yavru and S. Bagherpour. 2013. Pollen morphology of Hymenosphace and Aethiopis sections of the genus *Salvia* (Lamiaceae) in Turkey. *Turk. J. Bot*., 37: 1070-1084.

Perveen, A. and M. Qaiser. 2003. Pollen morphology of the family Labiatae from Pakistan. *Pak. J. Bot*., 35: 671-693.

Punt, W., P.P. Hoen, S. Blackmore, S. Nilsson and A. Le Thomas. 2007. Glossary of pollen and spore terminology. *Rev. Palaeobot. Palynol*., 143: 81pp.

Ryding, O. 1994. Phylogeny of leucas group (Lamiaceae). *Sys. Bot*., 23(2): 235-237.

Salimpour, F., F. Sharifnia and M. Ebrahymiyan. 2014. Nutlet micromorphology in selected species of *Salvia* (Lamiaceae) in Iran. *Scholarly J. Agric. Sci*., 4(2): 97-102.

Salmaki, Y., Sh. Zarre and Z. Jamzad. 2008. Nutlet micromorphology and its systematic implication in *Stachys* L. (Lamiaceae) in Iran. *Feddes Repertorium*, 119(7-8): 631-645.

Thorne, R.F. 1992. Classification and geography of the flowering plants. *Bot. Rev*., 58: 225-348.

Walker, J.B. and K.J. Systhma. 2007. Staminal evolution in the genus *Salvia* (Lamiaceae): Molecular phylogenetic evidence for multiple origins of the staminal lever. *Ann. Bot*., 100: 375-391.

Wodehouse, R.P. 1935. Pollen Grains. New York: McGraw Hill Press. 435 pp.

(Received for publication 14January 2016)