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

MEHMET FIRAT1*, BIROL BAŞER2 AND AKIN AZIRET3

1Yüzüncü Yıl University, Faculty of Education, Department of Biology, Van/Turkey
2Bitlis Eren University, Faculty of Arts and Science, Department of Biology, Bitlis/Turkey
3Fırat 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 antidiuretic 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 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 & Quaiser (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 an invasive 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 isopolar. 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.

<table>
<thead>
<tr>
<th>Pollen</th>
<th><em>Salvia kurdica</em></th>
<th><em>S. macrochlamys</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Polar axis (P) (µm)</td>
<td>48.03 ± 3.52</td>
<td>47.77 ± 3.63</td>
</tr>
<tr>
<td>Min-max</td>
<td>44–54</td>
<td>49–62</td>
</tr>
<tr>
<td>Equatorial axis (E) (µm)</td>
<td>55.10±4.35</td>
<td>52.87 ± 5.23</td>
</tr>
<tr>
<td>Min-max</td>
<td>49–62</td>
<td>40.07 ± 3.59</td>
</tr>
<tr>
<td>P/E Pollen shape</td>
<td>0.81, suboblate</td>
<td>0.90, suboblate</td>
</tr>
<tr>
<td>Colpus length (Clg) (µm)</td>
<td>39.10 ± 3.60</td>
<td>40.07 ± 3.59</td>
</tr>
<tr>
<td>Min-max</td>
<td>30–45</td>
<td>7.50–11</td>
</tr>
<tr>
<td>Colpus width (Clw) (µm)</td>
<td>8.71 ± 0.89</td>
<td>6.50–11</td>
</tr>
<tr>
<td>Min-max</td>
<td>7.50–11</td>
<td>6.50–11</td>
</tr>
<tr>
<td>Exine thickness (µm)</td>
<td>1.78 ± 0.24</td>
<td>1.80 ± 0.14</td>
</tr>
<tr>
<td>Min-max</td>
<td>1.50–2.25</td>
<td>1.80 ± 0.14</td>
</tr>
<tr>
<td>Intine thickness (µm)</td>
<td>1.06 ± 0.19</td>
<td>0.80 ± 0.13</td>
</tr>
<tr>
<td>Min-max</td>
<td>0.75–1.50</td>
<td>0.80 ± 0.13</td>
</tr>
<tr>
<td>Ornamentation</td>
<td>Bireticulate</td>
<td>Bireticulate and perforate</td>
</tr>
</tbody>
</table>

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).
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, isopolar, 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 µm² 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. *aucher*, *S. aramensis*, *S. divaricata*, *S. palaestina*, *S. smyrnena*, *S. glutinosa*, *S. cadmica*, *S. macrochlamys*, *S. kurdica*, and *S. hyargeia* (Hedge 1982). In our study, while *S. kurdica* had bireticulate ornamentation, species *S. aramensis* and *S. divaricata* had reticulate ornamentations. While *S. palaestina* and *S. smyrnena* species had 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. glutinosa*. 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 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).
We think that the study of \textit{S. macrochlamys} is a species close to \textit{S. kurdica}. In the study of Kahraman et al. (2010b), the pollen of the species was 6-colporate, radially symmetric and isopolar, pollen shape P/E was 0.90 oblate-spheroidal, polar and equatorial axes were 74.77 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, \textit{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 bireticate (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 \textit{S. sclarea} (sect. \textit{Aethiopis}) are orbicular-triangular 3.00 × 2.00 mm. Marin et al. (1996) observed that the surface ornamentation of the species of this genus has a protuberance. Hedge (1982) measured the nutlets of \textit{S. verticillata} (sect. Hemisphare) as 2.20 × 1.30 mm. Marin et al. (1996) observed the surface ornamentations of this species as reticullar papillae. To date, there have been no studies on \textit{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 \textit{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). \textit{S. kurdica} and \textit{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


### Table 2. Pollen morphological data of \textit{Salvia kurdica} taxon in SEM.

<table>
<thead>
<tr>
<th>Takson</th>
<th>Average number of primer reticule in 5 µm²</th>
<th>Average diameter of primer lumina (µm)</th>
<th>Average thickness of primer muri (µm)</th>
<th>Average diameter of second lumina (µm)</th>
<th>Average thickness of second muri (µm)</th>
<th>Average number of primer reticule in 25 µm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{S. kurdica}</td>
<td>12.71</td>
<td>1.96</td>
<td>0.37</td>
<td>0.19</td>
<td>0.27</td>
<td>5</td>
</tr>
</tbody>
</table>

### Table 3. The comparison of nutlet morphologies of \textit{Salvia kurdica} and \textit{S. macrochlamys}.

<table>
<thead>
<tr>
<th>Nutlet</th>
<th>\textit{Salvia kurdica}</th>
<th>\textit{S. macrochlamys} (Kahraman et al., 2010b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Size</td>
<td>4.70‒3.47 mm</td>
<td>4.70‒3.80 mm</td>
</tr>
<tr>
<td>Surface</td>
<td>Orbicular ovate-oblong</td>
<td>Orbicular-triangular and orbicular-wide ovate</td>
</tr>
<tr>
<td>Ornamentations</td>
<td>Glabrous and protuberance</td>
<td>Glabrous and protuberance</td>
</tr>
<tr>
<td>Colour</td>
<td>Black</td>
<td>Black</td>
</tr>
</tbody>
</table>


(Received for publication 14 January 2016)