POLLEN MORPHOLOGICAL INVESTIGATIONS OF SALVIA L. IN SOUTHEASTERN OF TURKEY AND ITS TAXONOMIC IMPLICATION

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Keywords: Salvia; Pollen; Light microscopy; Scanning electron microscopy; Turkey.

Abstract

In this study, the pollen morphology and exine structure of nine species of the genus Salvia L. (Lamiaceae) were investigated using light microscopy and scanning electron microscopy (SEM). Six micromorphological characters (pollen shape, polar length, equatorial width, exine and intine thickness, colpus length and colpus width) of pollen grains of Salvia have been identified. The palynological observations revealed that pollen grains of most studied taxa of Salvia were suboblate shape and possess hekzacolpate aperture. Tectal surface sculpture was a good criterion to identify particular taxa from Salvia. The pollen of which is characterized by reticulate, the pollen ornamentation was similar in all studied taxa.

Introduction

The genus Salvia L., the largest genus in the family Lamiaceae, contains about 1000 species worldwide. The genus is distributed principally in three regions, ranging from Central and South America to western Asia, and also into eastern Asia (Walker and Sytsma, 2007). The first revision of Salvia in Turkey was made by Hedge (1982), who recognized 86 species, 1 hybrid and 1 doubtful species. Since 2005, as part of a revisional study of Salvia in Turkey, the authors have carried out extensive field studies and collected a large number of specimens. Population sizes and phenological and ecological properties were also observed in the field. The studies have revealed 2 new species (İlçim et al., 2009; Celep and Doğan, 2010), 2 new varieties (Celep et al., 2009; Celep et al., 2010) and 2 new records (Celep et al., 2009; Kahraman et al., 2009). Pollen morphological characters have long been used to solve taxonomic problems in a number of plant families (Castro et al., 2009). Additionally, the authors have examined morphology, anatomy, trichome, nutlet, and pollen micromorphology of some Turkish Salvia species (Kahraman et al., 2009; Kahraman et al. 2010). Pollen morphological properties are used for identification to place a species in the correct taxonomic rank by the taxonomist (Ahmad et al., 2018). Scanning electron microscopy (SEM) has been used for the dissimilarity of species on the basis of exine ornamentation (Guimaraes et al., 2018). Aktaş et al., 2020 investigated some Turkish Salvia and they reported that palynological characters are important for differences among studied taxa.

Salvia is spreading in the province of Mardin is one of the important genera represented by four sections and nine species. The aim of this study to investigate quantitative and qualitative morphological characters of pollen of genus Salvia in Mardin, which is important for taxonomic identification.

Materials and Methods

Plant specimens were collected (Salvia bracteata Banks & Sol., Salvia macrochlamys Boiss. & Kotschy, Salvia suffruticosa Montbret & Aucher ex Benth., Salvia trichoclada Benth. (sect.

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Salvia); Salvia multicaulis Vahl (sect. Hymenosphace); Salvia montbretii Benth., Salvia palaestina Benth., Salvia syriaca L. (sect. Aethiopis) and Salvia russellii Benth. (sect. Hemisphace)) from different localities in Mardin (Table 1). Voucher samples were deposited at the Mardin Artuklu University herbarium, Turkey. All the pollen grains for light (LM) and (SEM) by the standard methods described by Erdtman (1945). Pollen grains for LM examination were prepared following the standard procedure of Wodehouse (1935). They were observed in glycerin-water using a standard Isolab microscope with D plan 1.00-1.25 160/0.17 oil immersion objective and NFKx3.3 LD 125 lens. Thirty pollen grains per specimen were regarded as sufficient for the palynological analysis. For SEM, pollen were removed by distilled water treatment, the air-dried, pollens were directly mounted on stubs using double-sided adhesive tape and uncoated. The photomicrographs were taken with a FEI Quanta Feg 250 scanning electron microscope.

Pollen shape, size, ornamentation, polar length, equatorial width, exine and intine thickness, colpus length and colpus width for 30 pollen grains were measured under binocular light microscope and Polar/Equatorial ratios were calculated. The terminology of the pollen follows that of Punt et al. (2007). For average, five readings were taken and statistically analyzed by using (IBM SPSS) statistics 24 software. The values are presented as minimum, maximum and standard deviation, that is represented in Table 2.

Table 1. The location and habitats of studied specimens in Salvia.

| Species         | Local name      | Section     | Collection areas and coordinates | Voucher specimen no |
|-----------------|-----------------|-------------|----------------------------------|--------------------|
| S. bracteata    | Çobanşalbası    | Salvia C8   | Mardin; Artuklu, 37°24'41''N-40°41'13''E | F Mungan Kılıç. 222 |
| S. macrochlamys | Çölşalbası      | Salvia C8   | Mardin; Artuklu, 37°19'22''N-40°46'04''E | F Mungan Kılıç 204 |
| S. suffruticosa | Kalmışalba      | Salvia C8   | Mardin; Artuklu, 37°23'37''N-40°40'42''E | F Mungan Kılıç 226 |
| S. trichocladá | Meşeşalbası     | Salvia C8   | Mardin; Artuklu, 37°24'41''N-40°41'13''E | F Mungan Kılıç 225 |
| S. multicaulis | Kürşreyhani     | Hymenosphace C8 | Mardin: Mazıdağı, 37°27'41''N-40°26'55''E | F Mungan Kılıç 212 |
| S. montbretii   | Kabaşalba       | Aethiopis C8 | Mardin: Midyat, 37°26'13''N-41°21'06''E | F Mungan Kılıç 209 |
| S. palaestina   | Sürmelişalba    | Aethiopis C8 | Mardin: Kızıltepe, 37°18'09''N-40°37'23''E | F Mungan Kılıç 201 |
| S. syriaca      | Çevlikotu       | Aethiopis C8 | Mardin: Mazıdağı, 37°28'00''N-40°26'42''E | F Mungan Kılıç 211 |
| S. russellii    | Kurdeşk        | Hemisphace C8 | Mardin: Midyat, 37°25'45''N-41°21'30''E | F Mungan Kılıç 210 |
Results and Discussion

Pollen morphological analysis

The pollen properties of nine species were studied by using LM and SEM (Fig. 1). The pollen grain characters of the taxa studied are presented in Table 2. Pollen grains are characterized by radiosymmetric monads and isopolar.

Fig. 1. Pollen grains of Salvia using light microscope (LM) and a scanning electron microscope (SEM); 1-3) Salvia bracteata; 4-6) S. macrochlamys 7-9); S. suffruticosa; 10-12) S. trichocladu; 13-15) S. multicaulis; 1, 4, 7, 10, 13) LM overview; 2, 5, 8, 11, 14) SEM overview; 3, 6, 9, 12, 15) details of the exine and colpi using an SEM.
Pollen size

The average size of pollen grains was from 24.8 to 46.2 μm polar length and 30.5 to 56.5 μm in equatorial width (Table 2). *S. palaestina* pollen grains with polar length 46.2 μm and 56.5 μm equatorial width is biggest pollen. *S. russellii* pollen grains with polar length 24.8 μm and equatorial width 30.5 μm is smallest pollen (Table 2, Fig. 2).

Fig. 1 (Contd.). Pollen grains of *Salvia* using light microscope (LM) and a scanning electron microscope (SEM); 16-18) *Salvia montbretii*; 19-21) *S. palaestina*; 22-24) *S. syriaca*; 25-27) *S. russelli*; 16, 19, 22, 25) LM overview; 17, 20, 23, 26) SEM overview; 18, 21, 24, 27) details of the exine and colpi using an SEM.

Pollen shape

Three types of pollen shapes were determined according to P/E ratio result.

**Type 1:** Oblate (P/E ratio 0.50-0.75 μm); This type is represented by *S. suffruticosa* with P/E ratio 0.71 μm (Figs 1, 3).
Table 2. Pollen morphological data of the *Salvia* species (values in μm).

| Pollen       | Polar length(P) | Equatorial width (E) | P/E ratio | Colpus length(Cl) | Colpus width(Cwd) | Exine thickness | Intine thickness | Polen shape | Apertural condition | Ornamentation          |
|--------------|-----------------|----------------------|-----------|-------------------|-------------------|-----------------|-----------------|-------------|-------------------|------------------------|
|              | Min-max Mean (SD) | Min-max Mean (SD)   |           | Min-max Mean (SD) | Min-max Mean (SD) | Min-max Mean (SD) | Min-max Mean (SD) |             |                   |                        |
| *S. bracteata* | 38.4-65.6       | 44.8-62.9            | 0.88      | 27.2-44.2         | 5.7-12.9          | 1.7-3.3         | 0.7-1.4        | Suboblate    | Hexacolpate       | Reticulate-perforate   |
|              | 47.5 (6.79)     | 53.2 (5.25)          |           | 35.8 (3.54)       | 9.5 (2.12)        | 2.30 (0.47)     | 1.0 (0.24)     |             |                   |                        |
| *S. macrochlamys* | 33.5-47.6      | 37.1-50.0            | 1.0       | 17.8-42.7         | 3.8-9.9           | 1.1-2.2        | 0.4-1.3        | Spheroidal   | Hexacolpate       | Reticulate-perforate   |
|              | 41.7 (4.85)     | 39.9 (4.88)          |           | 34.3 (6.3)        | 6.8 (1.7)         | 1.60 (0.29)     | 0.8 (0.19)     |             |                   |                        |
| *S. suffruticosa* | 25.90-36.97    | 36.64-47.41          | 0.71      | 21.01-34.03       | 6.03-11.60        | 1.23-2.20      | 0.5-0.97       | Oblate       | Hexacolpate       | Bireticulate            |
|              | 30.7 (3.34)     | 42.71 (3.27)         |           | 26.14 (3.44)      | 8.83 (1.56)       | 1.71 (0.28)     | 0.74 (0.12)    |             |                   |                        |
| *S. trichocladia* | 31.19-43.35    | 41.31-52.51          | 0.81      | 26.23-37.52       | 7.45-13.73        | 1.49-2.61      | 0.54-1.45      | Suboblate    | Hexacolpate       | Reticulate-perforate   |
|              | 38.5 (3.45)     | 47.3 (3.05)          |           | 31.03 (2.74)      | 9.25 (1.61)       | 1.92 (0.33)     | 0.86 (0.18)    |             |                   |                        |
| *S. multicaulis* | 36.73-44.89    | 41.52-59.67          | 0.85      | 28.64-39.51       | 6.62-10.85        | 1.68-2.92      | 0.64-1.26      | Suboblate    | Hexacolpate       | Reticulate-perforate   |
|              | 40.1 (2.25)     | 47.11 (4.07)         |           | 33.20 (2.74)      | 8.56 (1.28)       | 2.29 (0.32)     | 0.99 (0.15)    |             |                   |                        |
| *S. montbretii* | 31.79-44.40    | 40.92-53.55          | 0.81      | 25.56-36.50       | 5.20-12.09        | 1.36-2.22      | 0.77-1.10      | Suboblate    | Hexacolpate       | Reticulate-perforate   |
|              | 38.3 (3.79)     | 47.25 (3.63)         |           | 30.04 (3.83)      | 8.04 (1.67)       | 1.88 (0.25)     | 0.90 (0.10)    |             |                   |                        |
| *S. palessina* | 36.3-57.9       | 41.2-67.2            | 0.81      | 31.7-52.9         | 6.52-11.6         | 1.4-2.9        | 0.8-1.3        | Suboblate    | Hexacolpate       | Bireticulate            |
|              | 46.2 (5.4)      | 56.56 (6.4)          |           | 42.0 (6.7)        | 8.9 (1.9)         | 2.10 (0.4)     | 1.0 (0.1)     |             |                   |                        |
| *S. syriaca*  | 28.28-41.67     | 25.53-46.70          | 0.87      | 21.30-31.37       | 6.57-12.49        | 1.06-2.56      | 0.55-0.99      | Suboblate    | Hexacolpate       | Reticulate-perforate   |
|              | 33.6 (4.04)     | 34.47 (5.74)         |           | 26.18 (3.13)      | 9.12 (1.88)       | 1.78 (0.35)     | 0.81 (0.12)    |             |                   |                        |
| *S. russelli* | 20.6-33.7       | 23.8-37.3            | 0.80      | 15.8-25.3         | 3.7-7.7           | 1.3-1.8        | 0.3-0.9        | Suboblate    | Hexacolpate       | Reticulate-perforate   |
|              | 24.8 (3.0)      | 30.5 (3.1)           |           | 20.0 (2.6)        | 5.6 (1.2)         | 1.54 (0.15)     | 0.7 (0.16)     |             |                   |                        |

(Min: minimum. Max: maximum. SD: standard deviation).
Type 2: Suboblate (P/E ratio 0.76-0.88 μm); This type is represented by seven species *S. bracteata, S. trichoclada, S. multicaulis, S. montbretti, S. palaestina, S. syriaca, S. russelli* with a range of P/E ratio from 0.81 μm to 0.88 μm (Figs 1, 3).

Type 1: Spheroidal (P/E ratio 1 μm); This type is represented by *S. macrochlamys* with P/E ratio 1 μm (Figs 1, 3).

Aperture type: All the species of aperture condition is hexacolpate. Colpus length and colpus width mean values were measured 20.0 to 42.0 μm and 5.6 to 9.5 μm, respectively (Table 2, Fig. 4).

Ornamentation: The exine sculpturing studied taxa were reticulate-perforate and bireticulate. The bireticulate tectum type was found *S. palaestina* and *S. suffruticosa*. The other species were reticulate-perforate. The exine thickness is between 1.54-2.30 μm and intine thickness is 0.7-1 μm. (Table 2, Fig. 5).

![Fig. 2. Polar and Equatorial diameter variations of *Salvia* species.](image1)

![Fig. 3. P/E index of *Salvia* species](image2)
In this study the *Salvia* species which distributed in Mardin were investigated for morphological pollen qualitative and quantitative characteristics. The specimens were collected from various localities of Mardin.

In our present study of *Salvia* section: Pollen grains are suboblate, spheroidal and oblate, aperture condition is hexacolpate. Polar length 30.7 µm and 47.5 µm, equatorial width 39.4 to 53.2 µm, and reticulate-perforate and bireticate ornamentation are reported. Özler *et al.* (2011) reported that *Salvia* section’s pollen suboblate to subprolate and aperture condition is hexacolpate and octacolpate, exine structure are reticulate-perforate, reticulate–granulate and bireticate. *S. suffruticosa* (sect. *Salvia*) pollen is subprolate and pollen surface bireticate observed by Aktaş *et al.* (2020).

In this study of *Hymenosphac* section: *S. multicaulis* pollen grain is suboblate, and reticulate-perforate sculpture, Özler *et al.* (2013) and Özler *et al.* 2020 reported that *S. multicaulis* pollen grain is prolate-spheroidal aperture condition is reticulate-perforate.
In our study, Aethiopis section’s species *S. monbretii*, *S. syriaca*, *S. palaestina* pollen are suboblate, and aperture condition *S. monbretii*, *S. syriaca* are reticulate-perforate, *S. palaestina* is bireticulate. Hassan et al. (2009) investigated seven *Salvia* species in Egypt and bireticulate sculpture in *S. palaestina*, pollen grain is suboblate to spheroidal. Moon et al. (2008) reported that bireticulate ornamentation in pollen of *Aethiopis* section. Özler et al. (2013) and Özler et al. 2020 observed that *S. syriaca* characterized by reticulate-perforate, *S. palaestina* is bireticulate exine sculpturing pattern.

In this study of Hemisphace section: *S. russellii* pollen grain is suboblate, and reticulate-perforate sculpture. Ranjar et al. (2015) indicated that pollen grains of *Hemisphace* were hek Zacololate and reticulate ornamentation. Özler et al. (2020) in their study noticed *Hemisphace* species are smaller than the other sections, *S. russellii* pollen grain is oblate-spheroidal, and bireticulate exine ornamentation.

Differences were observed between the results obtained in our study, together with the other related studies about *Salvia*. Özler et al. (2013) and Özler et al. 2020 reported that, different results are obtained when *Salvia* species are examined in terms of shape and size, and they claimed that this differences were due to the techniques used.

In addition, pollen characteristics of the taxa were determined mostly similar each other in our study. There were no significant differences among the palynological properties of the studied taxa. The pollen morphology does not appear to be useful as a taxonomic technique in the identification of *Salvia* species. We believe that the results of the present study are important for taxonomically and evolution in/inter the group concerned. Such results will be the base for future biosystematic studies in *Salvia*.

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*Manuscript received on 12 July 2021; revised on 28 November 2021*