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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	Kalınşalba	*Salvia*	C8 Mardin: Artuklu, 37°23’37’’N-40°40’42’’E	F Mungan Kılıç 226
S. trichoclada	Meşeşalbası	*Salvia*	C8 Mardin; Artuklu, 37°24’41’’N-40°41’13’’E	F Mungan Kılıç 225
S. multicaulis	Kürtreyhanı	*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.

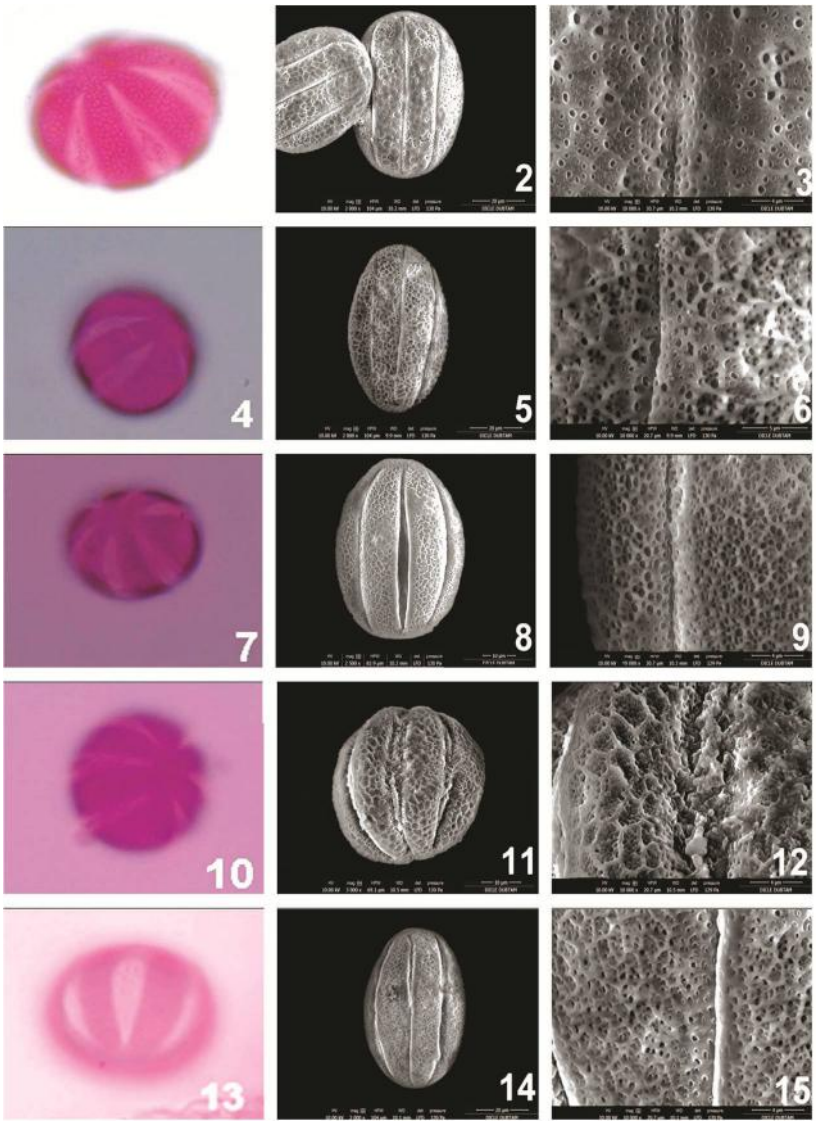

Fig. 1. Pollen grains of *Salvia* using light microscope (LM) and a scanning electron microscope (SEM); 1-3) *Salvia bracteata*; 4-6) *S. macrochlamys* 7-9); *S. suffruticosa*; 10-12) *S. trichoclada*; 13-15) *S. multicaulis*; 1, 4, 7, 10, 13) LM overview; 2, 5, 8, 11, 14) SEM overview; 3, 6, 9, 12,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

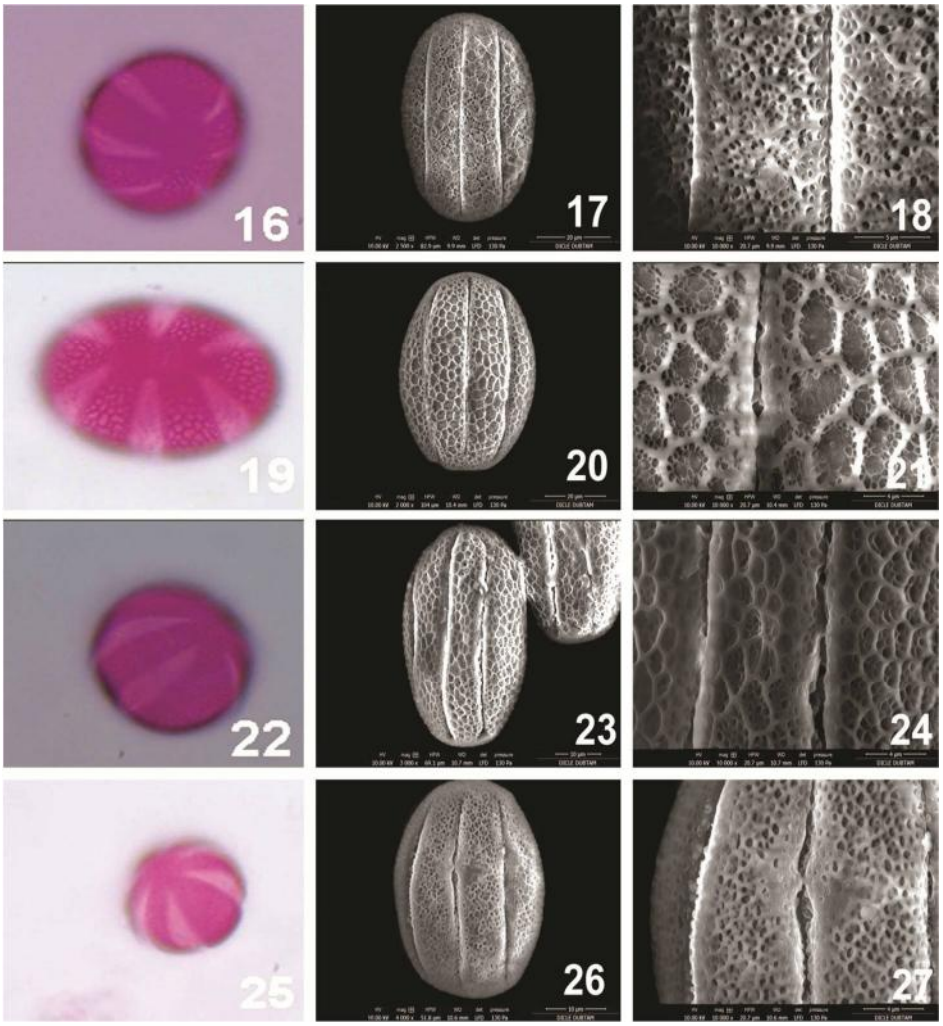

Fig. 1 (Contd.). Pollen grains of *Salvia* using light microscope (LM) and a scanning electron microscope (SEM); 16-18) *Salvia montbretti*; 19-21) *S. palaestina*; 22-24) *S. syriaca*; 25-27) *S. russelli*; 16, 19, 22, 25) LM overview; 17, 20, 23, 26) SEM overview; 18, 21, 24, 27) details of the exine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

Pollen	Polar length(P) Min-max Mean (SD)	Equatorial width (E) Min-max Mean (SD)	P/E ratio	Colpus length(Clg) Min-max Mean(SD)	Colpus width(Cwd) Min-max Mean(SD)	Exine thickness Min-max Mean (SD)	İntine thickness Min-max Mean (SD)	Polen shape	Apertural condition	Ornamentation
S. bracteata	38.4-65.6 47.5 (6.79)	44.8-62.9 53.2 (5.25)	0.88	27.2-44.2 35.8 (3.54)	5.7-12.9 9.5 (2.12)	1.7-3.3 2.30 (0.47)	0.7-1.4 1.0 (0.24)	Suboblate	Hexacolpate	Reticulate-perforate
S. macrochlamys	33.5-47.6 41.7 (4.85)	31.7-50.0 39.9 (4.88)	1.0	17.8-42.7 34.3 (6.3)	3.8-9.9 6.8 (1.7)	1.1-2.2 1.60 (0.29)	0.4-1.3 0.8 (0.19)	Spheroidal	Hexacolpate	Reticulate-perforate
S. suffruticosa	25.90-36.97 30.7 (3.34)	36.64-47.41 42.71 (3.27)	0.71	21.01-34.03 26.14 (3.44)	6.03-11.60 8.83 (1.56)	1.23-2.20 1.71 (0.28)	0.5-0.97 0.74 (0.12)	Oblate	Hexacolpate	Bireticulate
S. trichoclada	31.19-43.35 38.5 (3.45)	41.31-52.51 47.3 (3.05)	0.81	26.23-37.52 31.03 (2.74)	7.45-13.73 9.25 (1.61)	1.49-2.61 1.92 (0.33)	0.54-1.45 0.86 (0.18)	Suboblate	Hexacolpate	Reticulate-perforate
S. multicaulis	36.73-44.89 40.1 (2.25)	41.52-59.67 47.11 (4.07)	0.85	28.64-39.51 33.20 (2.74)	6.62-10.85 8.56 (1.28)	1.68-2.92 2.29 (0.32)	0.64-1.26 0.99 (0.15)	Suboblate	Hexacolpate	Reticulate-perforate
S. montbretti	31.79-44.40 38.3 (3.79)	40.92-53.55 47.25 (3.63)	0.81	25.56-36.50 30.04 (3.83)	5.20-12.09 8.04 (1.67)	1.36-2.22 1.88 (0.25)	0.77-1.10 0.90 (0.10)	Suboblate	Hexacolpate	Reticulate-perforate
S. palaestina	36.3-57.9 46.2 (5.4)	41.2-67.2 56.5(6.4)	0.81	31.7-52.9 42.0 (6.7)	6.52-11.6 8.9(1.9)	1.4-2.9 2.10 (0.4)	0.8-1.3 1.0 (0.1)	Suboblate	Hexacolpate	Bireticulate
S. syriaca	28.28-41.67 33.6 (4.04)	25.53-46.70 38.47 (5.74)	0.87	21.30-31.37 26.18 (3.13)	6.57-12.49 9.12 (1.88)	1.06-2.56 1.78 (0.35)	0.55-0.99 0.81 (0.12)	Suboblate	Hexacolpate	Reticulate-perforate
S. russelli	20.6-33.7 24.8 (3.0)	23.8-37.3 30.5 (3.1)	0.80	15.8-25.3 20.0 (2.6)	3.7-7.7 5.6 (1.2)	1.3-1.8 1.54 (0.15)	0.3-0.9 0.7 (0.16)	Suboblate	Hexacolpate	Reticulate-perforate

(Min: minumum. 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).

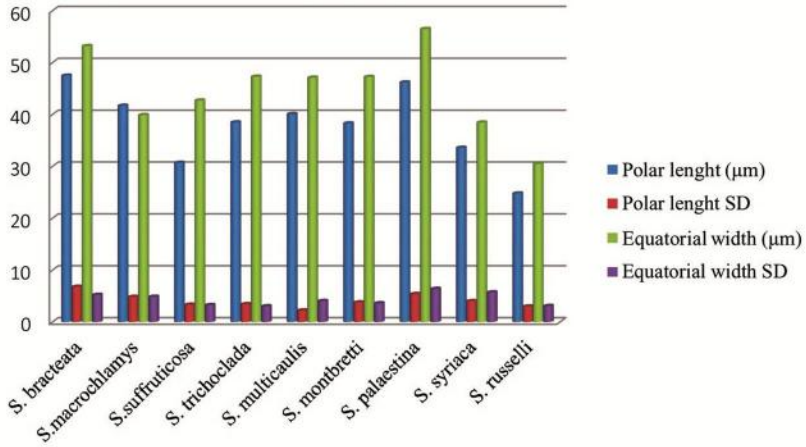

Fig. 2. Polar and Equatorial diameter variations of *Salvia* species.

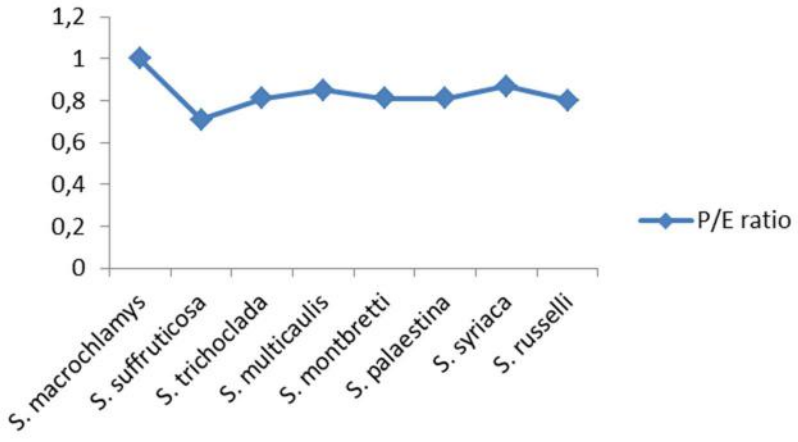

Fig. 3. P/E index of *Salvia* species

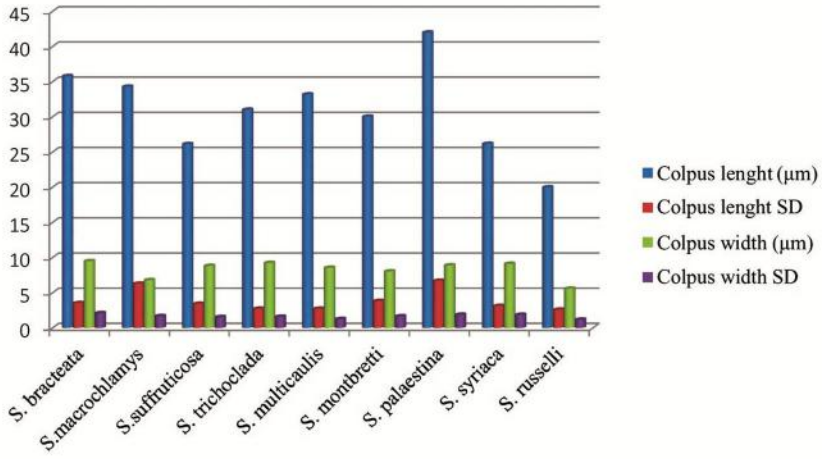

Fig. 4. Colpi length and width variation of *Salvia* species.

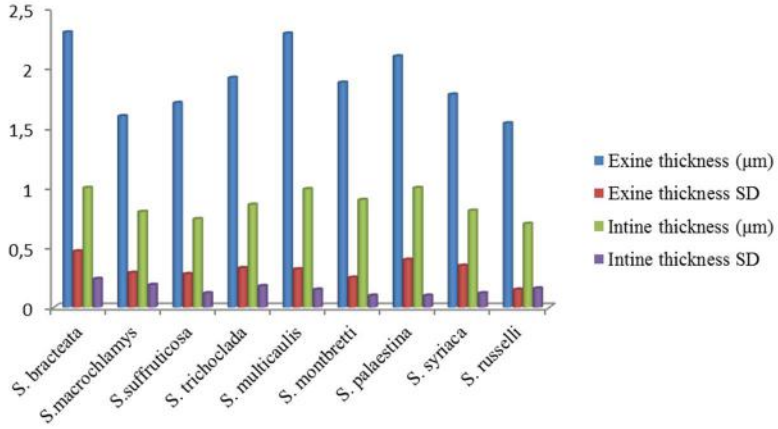

Fig. 5. Variation in exine and intine thickness of *Salvia* species.

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 bireticulate 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 bireticulate. *S. suffruticosa* (sect. *Salvia*) pollen is subprolate and pollen surface bireticulate observed by Aktaş *et al.* (2020).

In this study of *Hymenosphace* 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. Ranjbar *et al*. (2015) indicated that pollen grains of *Hemisphace* were hekzacolpate and reticulate ormanentation. 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 tecniques 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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