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PALYNOLOGICAL STUDY OF SOME IRANIAN SPECIES OF *SCABIOSA* L. (CAPRIFOLIACEAE)

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Abstract

The pollen morphology of six species of *Scabiosa* L. (Caprifoliaceae) from Iran has been examined by scanning electron microscopy (SEM). Pollen grains were tricolpate in *S. columbaria* and triporate in the rest studied species. Two types of exine ornamentation were revealed: spinulate and gemmate. The pollen shape in polar view varied from triangular to circular among investigated taxa. Statistical analysis showed that some quantitative morphological features such as polar axis (P), equatorial axis (E) and aperture diameter were main characters in identification of the taxa studied. These taxa were separated from each other using cluster analysis and placed within two clusters. Our result based on UPGMA analysis is in agreement with morphological classification and recent findings on taxonomic position of the *Scabiosa*.

Introduction

Scabiosa L. belonging Caprifoliaceae includes approximately 80 annual or perennial herbs, distributed in Europe and the Mediterranean Basin, southern Africa and eastern Asia (Reveal and Chase, 2011). Some of the species are known as a source of herbal medicine for the treatment of many human diseases (Bonet *et al*., 2007). Few *Scabiosa* species are cultivated as ornamental plants.

Linnaeus (1753) distinguished three genera that include *Scabiosa* L., *Dipsacus* L. and *Knautia* L. Later on, *Scabiosa sensu* Linnaeus was segregated into different genera: *Pterocephalus* Adans, *Succisa* Haller, *Cephalaria* Schrad. *ex* Roem & Schult. After remaining species of *Scabiosa* in different sections, were raised to new genera: *Lomelosia* Raf, *Sixalis* Raf, *Pseudoscabiosa* Devesa, *Pterocephalidium* G. Lopez (Greuter and Raus, 1985; Adanson, 1763; Haller, 1768; Devesa, 1984a, b; Lopez-Gonzales, 1987). Rechinger and Lack (1991) did not accept the nomenclatural changes suggested by Greuter and Raus (1985). Jamzad (1993) maintained a traditional and broad concept of genus *Scabiosa* too.

Taxonomic problems and species complexity are very common in this genus. Hybridization is common and, as a result, the number of reported species (and subspecies) has widely varied (Bobrov, 1957; Grossman, 1975; Jasiewicz, 1976).Since taxonomic position of some species is ambiguous, finding various remarkable characters will be useful to determine the taxonomic status of species. Pollen morphology has been able to reposition several disputed genera and interpret problems related to the origin and evolution of many taxa (Nair, 1980) and to derive classification of angiosperms (Cronquist, 1981).

Pollen morphology of this family has been studied by various authors (Ting, 1949; Nowicke and Skvarla, 1979; Feng *et al*., 2000). Erdtmann (1952) studied 35 genera of Dipsacaceae, and results showed the presence of two types of pollen apertures in this family: porate and colpate.

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Caputo and Cozzolino (1994) divided Dipsacaceae into two major clades based on morphological and palynological characters. Khalik (2010) studied various genera of Dipsacaceae and proved the taxonomic value of pollen characters. Pollen shape, size, exine ornamentation, number and features of apertures represent useful characters for distinguishing among species.

There are 22 species of *Scabiosa* recorded in the Flora Iranica (Rechinger, 1989) that species are divided into two subgenera (*Scabiosa* and *Asterocephalus*) and three sections including *Scabiosa, Asterocephalus* and *Olivierianae*. Greuter and Raus (1985) treated Iranian species of *Scabiosa* into two genera as *Lomelosia* (= *Scabiosa* sect. *Astrocephalus* and sect. *Olivierinae*) and *Scabiosa s. str.* (*Scabiosa s. l* sect. *scaboisa*).

The taxonomic grouping of the genera *Scabiosa* and *Lomelosia* is exactly unknown (Mayer and Ehrendorfer, 1999). For example on the basis of The plant list (2013), situation of many Iranian species is unresolved (*L. flavida*, *L. calocephala*, *L. bicolor*, *L. esfandiarii*, *L. prophyroneura*, *L. schimperiana*, *L. machrochaete*, *L. kermanensis* and *L. leucactis*) and some of them are accepted names as *Scabiosa* (*S. rotata*, *S. micrantha*, *S. argentea*). As well as, *L. caucasica* and *L. olivieri* accepted as genus *Lomelosia* and *S. persica* and *S. columbaria* accepted as genus *Scabiosa*.

As the circumscription of these groups are not obvious completely and it has not been stabilized yet, in this work only the genus *Scabiosa sensu* Rechinger and Lack (1991) has been subject of studies.

There are no reports on pollen morphology of the *Scabiosa* from Iran. The present study aims to survey the pollen morphology of six Iranian species of *Scabiosa* L. (belonging to three sections) as *S. columbaria* L., *S. micrantha* Desf., *S. persica* Boiss., *S. calocephala* Boiss., *S. olivieri* Coult. and *S. flavida* Boiss. & Hausskn. using scanning electron microscopy and evaluating its significance in taxonomy of the genus.

Materials and Methods

The plant samples for the study were collected from natural populations in different regions of Iran during spring and summer of 2013. Four to five individuals were collected randomly from each locality. Details of localities are given in Table 1. The voucher specimens were deposited in Herbarium of Shahid Beheshti University (HSBU).

Table 1. Localities and voucher numbers of the taxa studied.

Genus	Species	Locality	Voucher No.
Scabiosa L.	*S. columbaria* L.	Mazandaran, Siahbisheh	HSBU4004
	S. micrantha Desf.	North Khorasan, 45 km of Bojnurd	HSBU4005
	S. persica Boiss.	West Azarbaijan, Piranshahr	HSBU4000
	S. alocephala Bioss.	Tehran, SorkhHesar Park	HSBU4001
	S. olivieri Coult.	Tehran, Telo	HSBU4003
	S. flavida Boiss. & Hausskn	North Khorasan, 45 km East of Bojnurd	HSBU4002

For SEM, pollen samples were mounted on stubs using double-sided adhesive tape. Macro and microphotographs which showed general view of pollen surface were taken by Phenomprox SEM at an accelerating voltage 10.0 kV. For measurements “Image Tools ver. 3.00” software with high degree of accuracy and confidence was used. Some palynological characteristics, such as

equatorial diameter (*E*), polar axis length (*P*), *P/E*, log *P/E*, exine ornamentation and pore dimensions, were described for each sample.

In order to show relationships of species, we performed a cluster analysis based on Euclidean Distances with un-weighted pair-group method with arithmetical mean (UPGMA) method by using the program PAST ver. 2.17c (Hammer *et al*., 2001).

Results

Palynological characters were randomly measured by using minimum 20 pollen grains. The quantitative and qualitative palynological data of six investigated taxa were shown in Table 2.

Table 2. Evaluated pollen characters in the studied taxa.

	S. columbaria	*S. micrantha*	*S. persica*	*S. calocephala*	*S. olivieri*	*S. flavida*
Pollen type	Tricolpate	Triporate	Triporate	Triporate	Triporate	Triporate
Polar axis (P)	80.71 ± 3.24	28.44 ± 2.86	27.01 ± 2.02	22.04 ± 2.46	93.55 ± 4.12	90.32 ± 3.59
Equatorial axis (E)	58.07 ± 5.23	68.26 ± 3.61	98.42 ± 4.82	87.65 ± 4.46	121.39 ± 6.13	117.44 ± 5.15
(P/E)	1.38	0.41	0.27	0.25	0.77	0.76
Pollen shape	Prolate	Preoblate	Preoblate	Preoblate	Suboblate	Suboblate
Polar view	Circular	Circular-triangular	Triangular	Triangular	Circular-triangular	Circular-triangular
Aperture	Linear	Circular	Elliptic	Elliptic	Circular	Circular
Aperture diameter	40.67 ± 2.96	14.33 ± 1.27	15.69± 1.56	15.19 ± 2.05	15.02 ± 0.92	15.89 ± 1.08
Exine ornamentation	Spinulate-Spinuloid	Spinulate-Spinuloid	Gemmate-Spinuloid	Gemmate-Spinuloid	Spinulate-Spinuloid	Spinulate-Spinuloid

There are two major types of pollen grain apertures. It varies from tricolpate in *S. columbaria* to triporate in the rest studied species (Fig1) as *S.persica* and *S. calocephala* have elliptical pore and *S. micrantha*, *S. flavida* and *S. olivieri* have circular pores (Table 2).

The size of the pollen grain of studied taxa ranged from 28 × 68 µm (*P* × *E*) in *S. micrantha* to 93 × 121 µm (*P* × *E*) in *S. olivieri*. The ratio between the mean polar axis (*P*) and the mean equatorial diameter (*E*) can be used to assign the pollen grains to shape classes as of Erdtmann (1952). The shape of the pollen grain in equatorial view varied from preoblate to prolate among investigated taxa. The pollen shape in polar view varied from triangulate in *S. persica* and *S. calocephala*, circular in *S. colembaria* to circular-triangular in the rest of the species (Fig. 1).

Two types of exine ornamentation found in studied taxa. Gemmate type was observed in *S. persica* and *S. calocephala* as so that, between the gemmate are numerous low irregularly spaced spinuloid (Fig 2). Spinulate type was observed in *S. colembaria*, *S. flavida*, *S. micrantha* and *S. olivieri* as beset with numerous similar small conical spinuloid (Fig. 2).

The taxa investigated were separated from each other in a UPGMA tree (Fig. 3). Cluster analysis showed that species placed in two clusters. *S. olivieri* and *S. flavida* placed in one cluster and the remained taxa placed in other cluster. This cluster divided to two sub-clusters: subcluster I included *S. micrantha*, *S. persica* and *S. calocephala* and subcluster II contained *S. columbaria*.

Principal component analysis (PCA) shows that the polar axis (P), equatorial axis (E) and aperture diameter are main characters in grouping of species (Fig. 4).

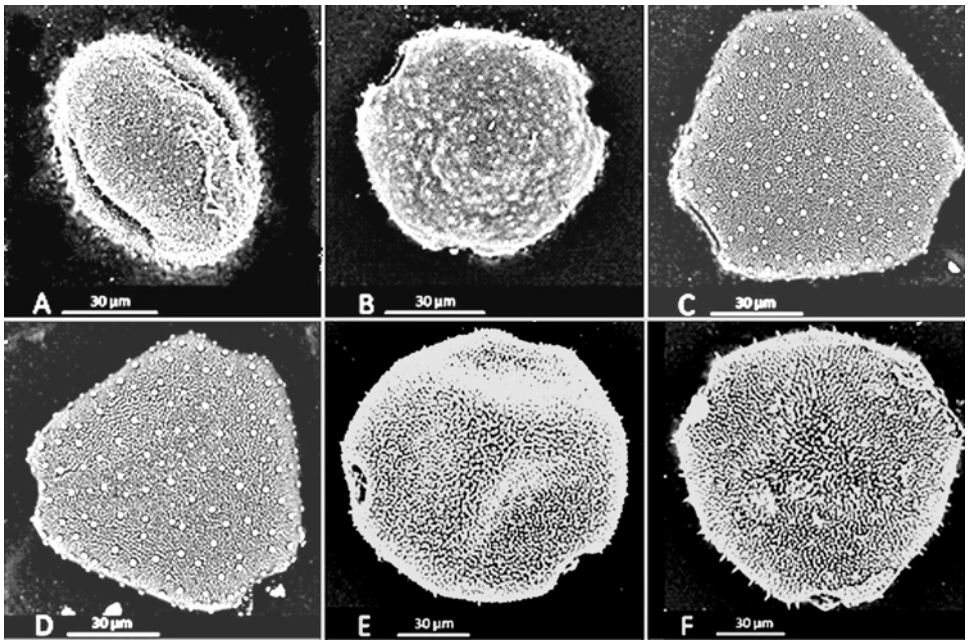

Fig. 1. Pollen electron micrograph of studied taxa. A: *S. columbaria*, B: *S. micrantha*, C: *S. persica*, D: *S. calocephala*, E: *S. olivieri* and F: *S. flavida*.

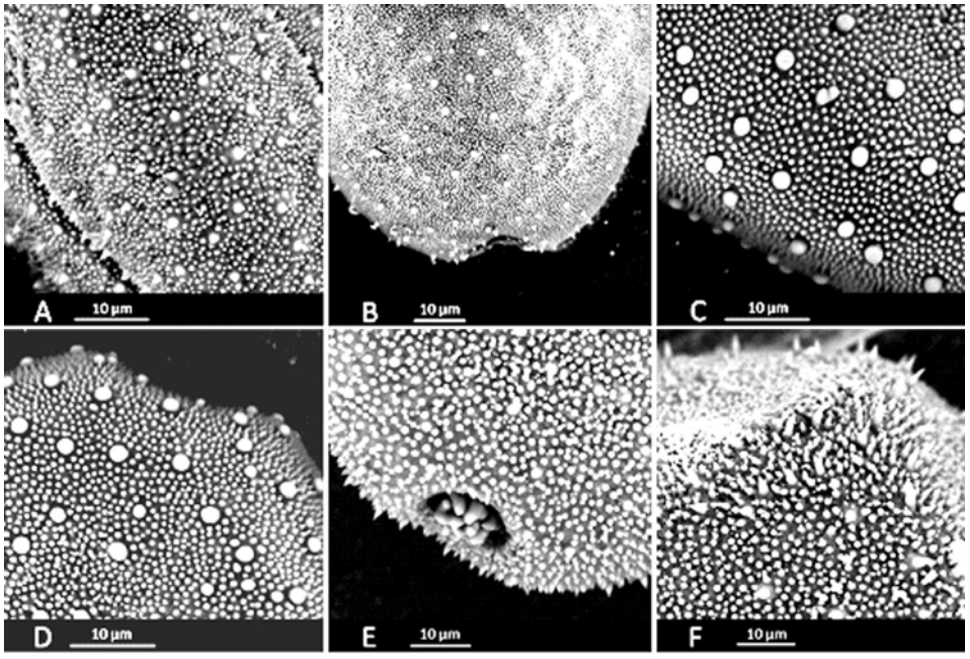

Fig. 2. Pollen surface ornamentations in studied taxa. A: *S. columbaria*, B: *S. micrantha*, C: *S. persica*, D: *S. calocephala*, E: *S. olivieri* and F: *S. flavida*.

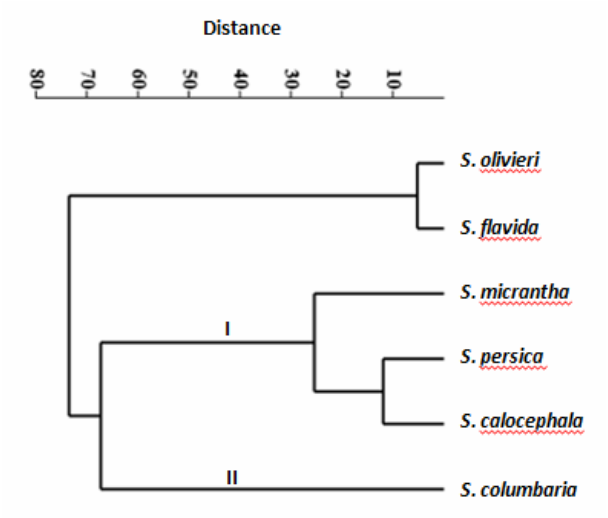

Fig. 3. UPGMA dendrogram showing the relationship among studied *Scabiosa* species based on palynological characters.

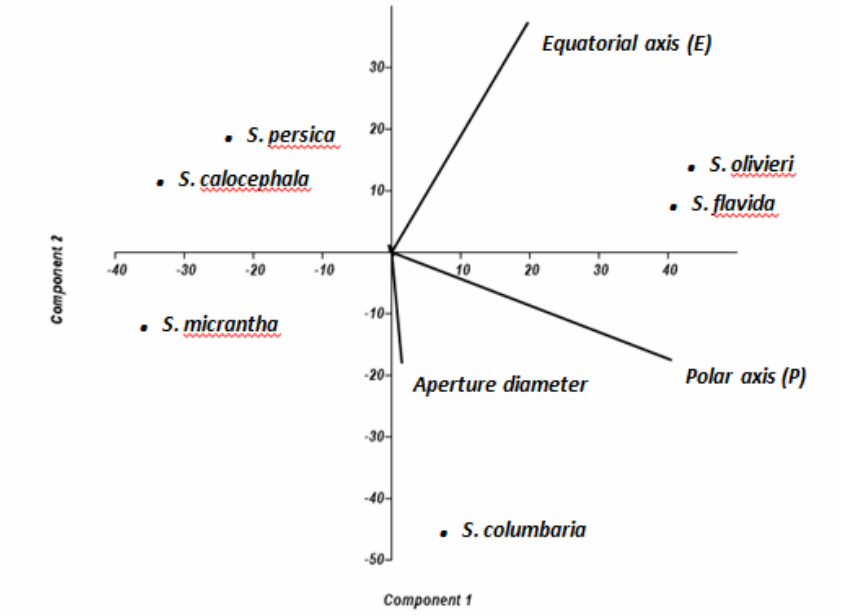

Fig. 4. Principal Component Analysis (PCA) among the studied *Scabiosa* species based on palynological characters.

Discussion

There are lots of debates on taxonomy of the genus *Scabiosa* and undoubtedly the genus has been the subject of the various taxonomic studies. Taxonomic classification of *Scabiosa* species is still unresolved. Because of morphological similarities between *Scabiosa* species are very high, so it seems that the application of pollen morphology can help us to resolve the taxonomical

problems between the species. Previous studies show that pollen characters can be used for solving taxonomic problems (Khalik, 2010). In this study, palynological characters varied among the investigated taxa and were useful in identification of taxa. For example, *S. columbaria* can be distinguished from the other taxa based on aperture types. *P*/*E* ratio was useful in identification of *S. micrantha* from *S. olivieri* and *S. flavida*, where their exine ornamentation is very similar.

Various palynological studies on different species of the Dipsacaceae have confirmed that pollen characters were important to species identification. Perveen and Qaiser (2011) studied palynological characteristic in some species of Dipsacaceae from Pakistan. Their results indicated that pollen characters may be used to delimit the species.

Aperture types are the most significant pollen characters. On the basis of aperture types, *Scabiosa* is divided into two pollen types including tricolpate and triporate. *S. columbaria* was tricolpate while the rest studied taxa were triporate. *Scabiosa* is divided into two subgenera, including *Scabiosa* and *Asterocephalus*, based on morphological characters (such as presence of Groove or pit on epicalyx tube) (Rechinger, 1989) and according to present study, they have tricolpate and triporate apertures, respectively. Perveen and Qaiser (2011) divided the Pakistani species of the family Dipsacaceae into three pollen types on the basis of aperture viz., *Dipsacus inermis*–type, *Pterocephalus gedrosiacus*–type and *Scabiosa candollei*–type. Our results are in agreement with those of Perveen and Qaiser (2011) who have also observed triporate pollen in Pakistani *Scobiosa* species. *S. columbaria* species complex has long posed for a complex taxonomic issue. Hybridization is common and, as a result, the number of reported species (and subspecies) has varied widely (Bobrov, 1957; Grossman, 1975; Jasiewicz, 1976). A revision of the species limits in *S. columbaria* is much needed.

The UPGMA dendrogram based on palynological characters is in concordant with morphological classification. Mayer and Ehrendorfer (1999) investigated differentiation of the epicalyx, the corona, the epi-diaphragm, calyx, pollen, chromosome number and their taxonomic importance in genus *Scabiosa s.l* and concluded that these features could be helpful in circumscribing of this genus.

S. persica and *S. calocephala* have similar characters including: number of corona vein, epicalyx tube pits (oblong shape), presence of sulcus between pits, hidden bristle rather to corona. These two species are different in leaves, leaflets, number of involucre bracts (Jamzad, 1993).

S. olivieri and *S. flavida* have small dipsacaceous- head and thus they are different from other species. These two species have triangle pits, short corona, evident bristles rather to corona, lack of sulcus between pits. There is radiant flower in *S. flavida* but not in *S. olivieri*.

S. micrantha have oval shape dipsacaceous- head in contrast with other species. This specieshas oblong shape pits, sulcus between pits, and evident bristles rather to corona.

Despite other species (five above mentioned species), *S. columbaria* has 8-grooves along epicalyx tube (Jamzad, 1993). Morphological similarity between these taxa is approved by pollen characteristics (Fig 3). *S. micrantha* have some characters of *S. olivieri* group (*S. olivieri* and *S. flavida*) and some of *S. persica* group (*S. persica* and *S. calocephala*). Hence, on the basis of pollen features, *S. micrantha* located between them.

Taxonomic status of *S. calocephala* is unresolved but some data suggest that it is synonymous with *Lomelosia calocephala*. *S. calocephala* and *S. micrantha* based on the presence of pits on epicalyx tube, are placed in sect. *Asterocephalus*. The UPGMA dendrogram showed that this species were very similar to *S. persica*. Therefore, taxonomic status of *S. calocephala* does not change according to pollen morphology.

S. olivieri and *S. flavida* were previously classified within the genus *Scabiosa*, based on the calyx characters (Tackholm, 1974; Rechinger, 1989; Boulos, 2000). Mayer and Ehrendorfer

(1999) separated all species of *Scabiosa* set. *Olivierianae* into *Lomelosia*. In general, our results agree with those of Mayer and Ehrendorfer. Caputo *et al*. (2004) studied phylogenetic relationships among 17 species of Dipsacaceae and they divided Dipsacaceae into two major clades: one including *Lomelosia* and *Pycnocomon*, both in a sister group relationship with a clade including *Scabiosa*, *Sixalix* and *Pterocephalus*, and the other including the rest of species.

Unlike other studies, in this work exine ornamentation was not useful to distinguish among closely related genera such as *Scabiosa* and *Lomelosia*. The exine ornamentation of *S. micrantha* is very similar to *S. olivieri* and *S. flavida* while our results showed that *S. micrantha* was grouped in sect. *Asterocephalus* in accordance with Flora Iranica classification.

Feng *et al*. (2000) studied pollen of 17 species of the genus *Dipsacus* and divided the genus into three pollen types based on exine ornamentation viz., dispinulate-reticulate, spinulate-foveolate and dispinulate or rarely smooth. He further reported that pollen morphology was little helpful at specific level.

The results of this study showed that pollen morphology provided facile and reliable characters for taxonomic studies of *Scabiosa*.

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