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POLLEN MORPHOLOGY OF IRANIAN *DRACOCEPHALUM* L. (LAMIACEAE) AND ITS TAXONOMIC SIGNIFICANCE

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Abstract

Pollen morphology of 11 Iranian *Dracocephalum* L. species was investigated using light and scanning electron microscopy to evaluate their taxonomic significance for the infrageneric classification of the genus. Pollen grains of all examined taxa were isopolar, hexacolpate, circular in polar view and spheroidal to prolate in equatorial view (P/E = 1.0−2.0). The smallest pollen grains were observed in *D. aucheri* (P = 29.7 μm, E = 22.6 μm), while the largest pollen was found in *D. lindbergii* (P = 45.1 μm, E = 33.7 μm). The highest and lowest apocolpium index (AI) were measured in *D. aucheri* (AI = 0.27) and *D. surmandinum* (AI = 0.08), respectively. Colpus membrane was egranulate in all examined species except for *D. multicaule* and *D. ghahremanii*. The main exine ornamentation type was characterized as bireticulate including five different subtypes. The results revealed that the exine ornamentation is a diagnostic character useful for the classification of *Dracocephalum.*

Introduction

The genus *Dracocephalum* L. is the second largest genus in the subtribe Nepetinae, tribe Mentheae of Lamiaceae family. The genus is primarily of the Old World and consists of 71 species worldwide, of which 69 species are native to Eurasia (Budantsev, 1987, 1993; Kadereit, 2004), and North Africa and North America have one species each. Morphologically, *Dracocephalum* is a heterogeneous genus comprising species with calyx glabrous within and not gibbous at base; sinuses between lobes of calyx with swollen folds at base, bracteoles aristately toothed, nutlets elliptic to oblong and areole not curved. The genus *Dracocephalum* was divided into three subgenera by Budantsev (1987), *viz*., subg. *Dracocephalum* including seven sections (stamens included, anthers glabrous), *Fedtschenkiella* (Kudr.) Schischk. (stamens exserted, anthers glabrous), and *Ruyschiana* (Mill.) Briq. (stamens included, anthers pubescent). The members of this genus are well-known as medicinal plants with several uses, such as anti-hyperlipidemic, analgesic, antimicrobial, antioxidant, anticancer and oxidative stress protective activity (Sajjadi *et al.*, 1998; Jahaniani *et al.*, 2005; Sonboli *et al.*, 2008).

Rechinger (1982) in his treatment for Flora Iranica area, recognized 18 *Dracocephalum* species of which eight species are growing in Iran. Since the publication of the Flora Iranica, one new species (*D. ghahremanii* Jamzad), a new record (*D. lindbergii* Rech.f.), and a resurrected species (*D. oligadenium* Bornm. & Gauba) have been added to the genus (Esfandiari, 1985; Jamzad, 2012). Considering new findings, the number of species now stands 11, of which five species (*D. ghahremanii* Jamzad, *D. kotschyi* Boiss., *D. oligadenium* Bornm. & Gauba, *D. polychaetum* Bornm. and *D. surmandinum* Rech. f.) are endemic.

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According to the aperture number and number of nuclei, Erdtman (1945) divided the Lamiaceae into two subfamilies. While Lamioideae subfamily comprised tricolpate and bi-nucleate pollen grains, Nepetoideae subfamily is characterized by hexacolpate and tri-nucleate pollen grains. Investigations of pollen morphology in the Lamiaceae have been essential as an aid to classification within this family (Erdtman, 1945; Harley *et al.*, 1992; Abu-Asab and Cantino, 1994).

Pollen morphology of different members of Lamiaceae and other plant families have been frequently investigated by several authors (Moon *et al.*, 2008 ; Ozler *et al.*, 2011; Badamtsetsg *et al*., 2012; Sarwar and Takahashi, 2012, 2013; Jamzad and Hasani-Nejad, 2014) that show its taxonomic and phylogenetic importance. As far as our literature survey could ascertain, there are only a few publications on pollen morphology in *Dracocephalum.* Moon *et al*. (2008) investigated pollen morphology of 12 genera belonging to the tribe Mentheae, subtribe Nepetineae, including 13 species of *Dracocephalum*, one of which, *D. subcapitatum* (Kuntze) Lipsky, grows in northeast of Iran. Pollen morphology of three *Dracocephalum* species, *viz. D. foetidum* Bge., *D. grandiflorum* L. and *D. ruyschiana* L. from Mongolia have already been investigated (Badamtsetsg *et al*., 2012).

The pollen grain morphology of *Dracocephalum* species growing in Iran have not been studied yet. Therefore, the main objectives of the present study are to provide a detailed account of the pollen morphology of 11 *Dracocephalum* species in Iran by light microscopy (LM) and scanning electron microscopy (SEM), and to evaluate its taxonomic significance for the classification of the genus.

Materials and Methods

Plant specimens of 11 *Dracocephalum* species were collected from their natural habitats between 2007 and 2013 and the voucher specimens were deposited in Medicinal Plants and Drugs Research Institute Herbarium of Shahid Beheshti University (MPH), Tehran (Table 1). For the LM studies, pollen grains were prepared following the method of Erdtman (1960). Pollen characteristics, including pollen shape, polar axis (P), equatorial axis (E), colpus length (Cl), exine thickness (Et), mesocolpium diameter (Me), and apocolpium index (AI), were measured from at least 20 mature pollen grains per sample under an Olympus BX–51 microscope. Results are provided as minimum, maximum and mean ± standard deviations.

For the SEM studies, pollen grains were transferred directly to stubs with double-sided adhesive tape and micrographs were obtained using KYKY–EM 3200 SEM at an accelerating voltage of 25 kV. Some characters, such as number of primary lumina per 100 μm^2, shape of primary lumina, diameter of primary lumina, number of perforation in primary lumina, presence or absence of granule on colpus membrane, presence or absence of 1−2 central large hole per primary lumina, and sculpturing type were measured and characterized. The pollen terminology follows Faegri and Iversen (1989), Harley *et al.* (1992) and Punt *et al.* (2007).

Results

The diagnostic pollen grain characters of 11 species of *Dracocephalum* investigated are presented in Tables 2 and 3. Photographs of some *Dracocephalum* species in their native habitats are presented in Figure 1. The LM and SEM micrographs of the studied pollen grains are illustrated in Figures 2–4. The measured parameters of the pollen grains are described below.

Size: The pollen grains were dispersed as monads. The mean size of the polar axis (P) ranges from 29.7 µm in *Dracocephalum aucheri* to 45.1 µm in *D. lindbergii*; the mean size of the equatorial axis (E) ranges from 22.6 µm in *D. aucheri* to 33.7 µm in *D. lindbergii* (Table 2).

Shape: The shape of the pollen grains in equatorial view varies from prolate to spheroidal, whereas their shape in polar view is more or less circular (Table 2; Figs 2–4). Often grain forms vary and co-exist between prolate and subprolate.

Table 1. Collection data and voucher information of *Dracocephalum* species studied.

No.	Species	Collection data
1.	*Dracocephalum aucheri* Boiss.	Tehran: Tuchal mountain, 24.06.2008. Gholipour 1281 (MPH)
2.	*D. moldavica* L.	Mazandaran: Sari, Cultivated, 10.06.2009. Gholipour 1693 (MPH)
3.	*D. thymiflorum* L.	Mazandaran: Siah Bishe, Karaj toward Chalus, Allamol, 30.06.2009. Sonboli & Gholipour 1643 (MPH)
4.	*D. multicaule* Montbr. & Auch. *ex* Benth.	West Azarbayejan: Khoy, Qotor, Habash, Arvin mountain, 01.07.2012. Gholipour 824 (SPNH)
5.	*D. kotschyi* Boiss. *	Tehran: Fasham, Shemshak toward Dizin, 30.06.2010. Sonboli, Hadian & Moridi 1631 (MPH)
6.	*D. lindbergii* Rech. f.	North Khorasan: Bojnourd, Rein, Aladagh mountain, 21.05.2007. Sonboli, Kanani & Gholipour 1737 (MPH)
7.	*D. oligadenium* Bornm. & Gauba *	Mazandaran: Siah Bishe, chalus road, Allamol village, 09.07.2009. Sonboli, Hadian & Moridi 1627 (MPH)
8.	*D. surmandinum* Rech. f. *	Esfahane: Semirom toward Shahreza, Surmand mountain, 18.06.2007. Sonboli, Kanani & Gholipour 1179 (MPH)
9.	*D. polychaetum* Bornm. *	Kerman: Babini village, Hazar mountain, 07.05.2008. Kanani, Gholipour & Mirtajadini 1276 (MPH)
10.	*D. subcapitatum* (Kuntze) Lipsky	Khorasan-e Razavi: Mashhad, Kalat, after Sandugh shekan pass, 10.06.2009. Sonboli & Gholipour 1626 (MPH)
11.	*D. ghahremanii* Jamzad *	Semnan: Shahmirzad, Chashm, Nizva mountain, 12.07.2007. Sonboli & Gholipour 1219 (MPH)

* Endemic to Iran

Apertures: The examined pollen grains were isopolar and hexacolpate. Colpi with their acute ends were distributed symmetrically. The colpus length ranged from 25.5 µm in *D. aucheri* to 40.2 µm in *D. lindbergii*, but mesocolpium varied from 7.6 µm in *D. ghahremanii* to 11.5 µm in *D. lindbergii* (Table 2). The highest apocolpium index (AI) was measured in *D. aucheri* (AI = 0.27), whereas the lowest AI was found in *D. surmandinum* (AI = 0.08) (Table 2). Colpus membrane was egranulate in all examined taxa except for *D. multicaule* and *D. ghahremanii* that were granulate (Table 3; Figs 3 & 4).

Exine sculpturing: The exine thickness varied from 0.8 µm in *D. kotschyi* and *D. thymiflorum* to 1.1 µm in *D. multicaule*, *D. oligadenium, D. surmandinum* and *D. ghahremanii* (Table 2). The main and common exine ornamentation type, examined with SEM in Iranian *Dracocephalum* species studied, was found to be bireticulate. A bireticulum consists of a non-congruent, two-layered reticulum. The main reticulum is referred to as the primary reticulum and the substratum as the secondary reticulum. It can be divided into five subtypes based on shape of primary lumen, the presence or absence of at least one large hole in each primary lumen and the number of secondary lumina per primary lumen (Table 3).

Table 2. Pollen morphological data on LM micrographs of *Dracocephalum* species studied.

Species	P (µm)	E (µm)	Shape				Cl (µm)	Et (µm)	Me (µm)	AI	LM figures
			P	Sp	Ps	S					
Dracocephalum aucheri	24.0 (29.7 ± 2.8) 33.4	19.0 (22.6 ± 2.0) 25.0	++	+	±	-	20.0 (25.5 ± 2.8) 29.0	0.4 (0.9±0.2) 1.2	6.0 (7.9 ± 1.3) 9.6	0.16 (0.20 ± 0.05) 0.27	2A, B
D. moldavica	29.0 (38.8 ± 4.0) 44.0	20.0 (27.5 ± 4.4) 36.0	++	+	±	-	20.2 (32.5 ± 5.3) 39.0	0.4 (0.9±0.3) 1.4	6.0 (8.1 ± 1.6) 11.0	0.15 (0.19 ± 0.04) 0.23	2C, D
D. thymiflorum	28.6 (36.9 ± 5.0) 44.0	19.2 (27.5 ± 5.8) 36.0	+	++	±	-	20.0 (29.1 ± 4.8) 35.0	0.4 (0.8±0.2) 1.2	7.2 (10.0 ± 1.6) 11.6	0.14 (0.20 ± 0.04) 0.25	2E, F
D. multicaule	35.2 (38.9 ± 2.5) 44.2	25.0 (31.4 ± 3.7) 38.4	++	+	±	+	29.0 (32.8 ± 2.7) 39.0	0.8 (1.1±0.3) 1.6	6.0 (7.8 ± 1.8) 13.6	0.12 (0.16 ± 0.03) 0.19	2G–I
D. kotschyi	36.4 (43.7 ± 3.7) 50.8	25.0 (33.5 ± 3.3) 38.6	+	++	±	-	30.0 (38.1 ± 3.6) 44.8	0.6 (0.8±0.1) 1.0	6.0 (11.1 ± 2.0) 13.6	0.10 (0.14 ± 0.02) 0.17	2J –L
D. lindbergii	37.0 (45.1 ± 4.1) 53.8	25.2 (33.7 ± 5.3) 39.2	+	++	-	-	33.0 (40.2 ± 3.8) 48.0	0.6 (0.9±0.3) 1.6	7.0 (11.5 ± 2.8) 16.0	0.14 (0.15 ± 0.01) 0.18	2M, N
D. oligadenium	31.0 (38.8 ± 3.7) 45.6	26.0 (31.3 ± 3.0) 36.0	+	++	+	±	22.0 (30.5 ± 4.3) 38.0	0.8 (1.1±0.2) 1.7	5.6 (8.5 ± 2.2) 13.0	0.12 (0.15 ± 0.02) 0.18	2O, P
D. surmandinum	27.0 (35.8 ± 4.8) 44.0	23.0 (29.5 ± 4.1) 38.0	+	++	+	±	24.0 (30.3 ± 3.8) 38.4	0.8 (1.1±0.2) 1.6	5.0 (7.7 ± 2.0) 12.0	0.08 (0.14 ± 0.05) 0.21	2Q, R
D. polychaetum	33.2 (43.1 ± 4.3) 49.2	21.0 (28.7 ± 5.2) 43.6	++	+	±	-	28.0 (36.9 ± 4.8) 43.0	0.6 (0.9±0.2) 1.4	6.0 (7.7 ± 1.1) 10.0	0.15 (0.18 ± 0.02) 0.20	2S, T
D. subcapitatum	35.0 (38.7 ± 2.2) 42.0	26.0 (30.3 ± 2.7) 36.0	+	++	±	-	25.0 (30.8 ± 3.7) 38.8	0.6 (0.9±0.1) 1.0	5.0 (7.9 ± 2.3) 13.0	0.11 (0.13 ± 0.02) 0.16	2U, V
D. ghahremanii	28.4 (34.0 ± 4.3) 43.0	20.0 (25.4 ± 4.0) 33.0	+	++	+	-	21.0 (27.4 ± 4.5) 36.0	0.8 (1.1±0.3) 1.7	5.0 (7.6 ± 1.7) 11.2	0.16 (0.19 ± 0.03) 0.23	2W, X

All size are in µm, except for AI. Numbers refer to minimum, mean±standard deviation and maximum; P = Polar axis, E = Equatorial diameter, Shape = based on P/E ratio. Cl = Colpus length, Et = Exine thickness, Me = Mesocolpium diameter, AI = Apocolpium index, P = Prolate, Sp = Subprolate, Ps = Prolate sphroidal, S = Sphroidal; (+) Presence, (-) Absence, (++) dominant, (±) rare.

Table 3. Pollen morphological data on SEM micrographs of *Dracocephalum* species studied.

Species	Number of primary lumina per 100 μm^2	Diameter of primary lumina (μm)	Thickness of primary muri (μm)	Colpus membrane	Sculpturing subtype*	SEM figures
D. aucheri	20	2.81	< 0.5	Egranulate	I - 1	3A, B
D. moldavica	5	3.29	< 0.5	Egranulate	I - 2	3C, D
D. thymiflorum	24	3.11	> 0.5	Egranulate	I - 3	3E–H
D. polychaetum	26	4.00	> 0.5	Egranulate	I - 4	3I, J
D. multicaule	30	2.37	< 0.5	Granulate	I - 5	3K, L
D. kotschyi	17	3.06	< 0.5	Egranulate	I - 5	4A, B
D. lindbergii	12	2.82	< 0.5	Egranulate	I - 5	4C, D
D. oligadenium	20	3.45	< 0.5	Egranulate	I - 5	4E, F
D. surmandinum	15	3.10	< 0.5	Egranulate	I - 5	4G, H
D. subcapitatum	11	4.00	> 0.5	Egranulate	I - 5	4I, J
D. ghahremanii	24	2.97	< 0.5	Granulate	I - 5	4K, L

*Sculpturing type = Bireticulate.

Fig. 1. *Dracocephalum* species in native habitats. A: *D. aucheri*; B: *D. surmandinum*; C: *D. kotschyi*; D: *D. ghahremanii*; E: *D. multicaule*; F: *D. polychaetum*.

Type I – 1: The exine ornamentation of *D. aucheri* is characterized by polygonal primary lumina shape, absence of large hole and the number of secondary lumina is less than ten per primary lumen (Fig. 3A, B).

Type I – 2: The shape of primary lumina is polygonal-elongate; without large hole and the number of secondary lumina are over ten per primary lumen which was observed in *D. moldavica* (Fig. 3C, D).

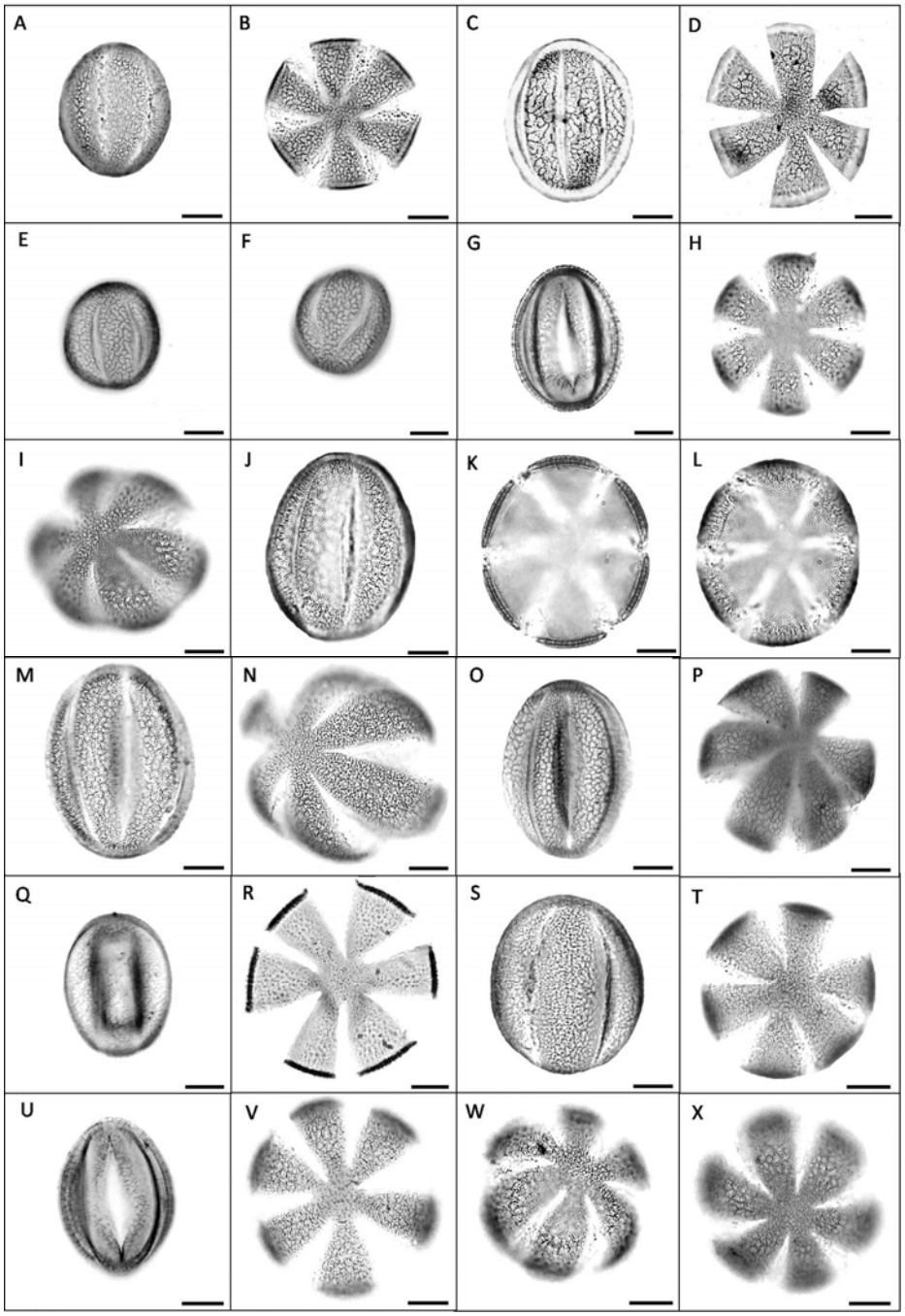

Fig. 2. LM micrographs of pollen grains of *Dracocephalum* L. A,B: *D. aucheri*; C,D: *D. moldavica*; E,F: *D. thymiflorum*; G–I: *D. multicaule*; J–L: *D. kotschyi*; M,N: *D. lindbergii*; O,P: *D. oligadenium*; Q,R: *D. surmandinum*; S,T: *D. polychaetum*; U,V: *D. subcapitatum*; W,X: *D. ghahremanii*. Scale bars = 10 μm.

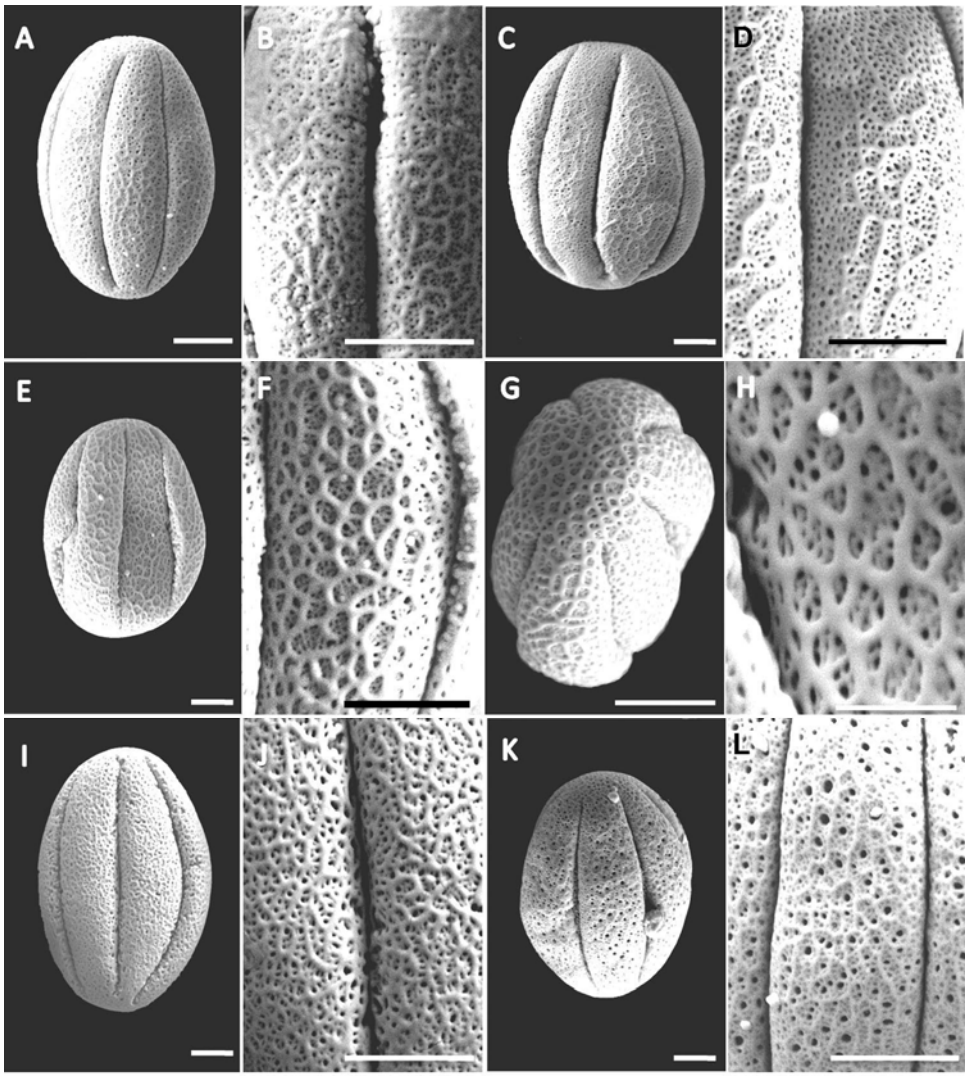

Fig. 3. SEM micrographs of pollen grains of *Dracocephalum* L.. A,B: *D. aucheri*; C,D: *D. moldavica*; E−H: *D. thymiflorum*; I,J: *D. polychaetum*; K,L: *D. multicaule*. Scale bars: A−G, I−L = 10 μm; H = 5 μm.

Type I – 3: In *D. thymiflorum* the shape of primary lumina was polygonal-rounded with strongly thickened primary muri. The number of secondary lumina is more than ten per primary lumen (Fig. 3E−H).

Type I – 4: The shape of primary lumen was polygonal-elongate, without large hole and the number of secondary lumina are less than ten per primary lumen which was observed in *D. polychaetum* (Fig. 3I, J).

Type I – 5: This subtype is frequent and common in a group of species, which are morphologically similar too. The primary lumen is polygonal and each primary lumen contains more than 10 secondary lumina and at least one large hole, which was observed in *D. ghahremanii*, *D. kotschyi*, *D. lindbergii*, *D. multicaule*, *D. oligadenium*, *D. subcapitatum* and *D. surmandinum* (Fig. 3K, L, 4A−L).

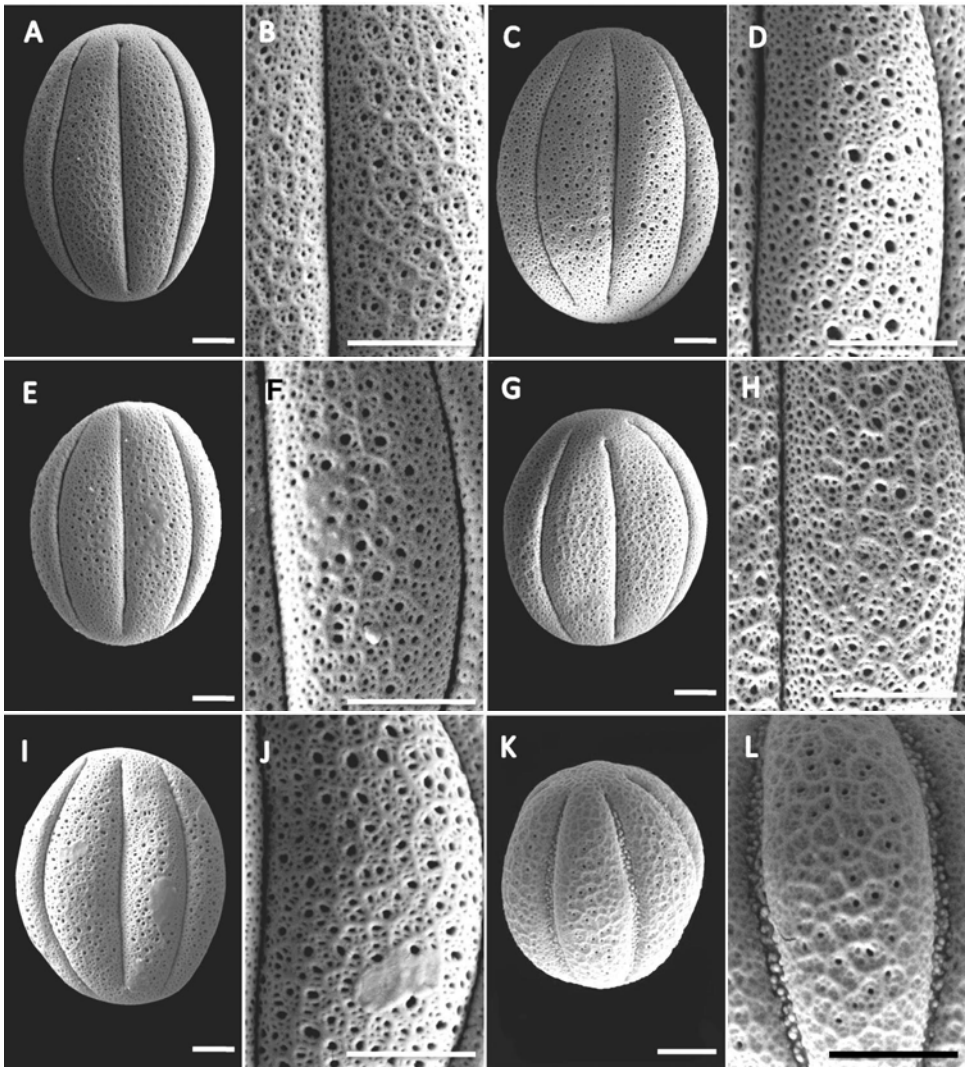

Fig. 4. SEM micrographs of pollen grains of *Dracocephalum* L. A,B: *D. kotschyi*; C,D: *D. lindbergii*; E,F: *D. oligadenium*; G,H: *D. surmandinum*; I,J: *D. subcapitatum*; K,L: *D. ghahremanii*. Scale bar = 10 μm.

Discussion

The present study revealed that all of the examined taxa had isopolar and hexacolpate pollen grains. Moon *et al*. (2008) examined the pollen morphology of 13 *Dracocephalum* species distributed mainly in Central Asia. Among them *D. imberbe* (P = 31.7 μm, E = 24.7 μm) and *D. grandiflorum* (P = 54.6 μm, E = 44.0 μm) were found as the smallest and largest taxa, respectively. In another study, size of the pollen grains of *D. grandiflorum* was found to be large (P = 52.63 μm, E = 29.88 μm), whilst *D. foetidum* and *D. ruyschiana* were characterized to be medium (Badamtsetsg *et al*., 2012).

Polar axis and equatorial diameter of *D. subcapitatum* examined from Turkmenistan were reported to be P = 37.8 μm and E = 37.1 μm (Moon *et al.*, 2008), while in *D. subcapitatum* collected from northeast of Iran and presented in this study, mean polar axis and equatorial diameter are 38.7 μm and 30.3 μm, respectively. Subprolate (Sp) and prolate-spheroidal (Ps) were characterized as the most common pollen shape in the studied species of *Dracocephalum* by Moon *et al*. (2008), but in the Iranian *Dracocephalum* species studied here, subprolate (Sp) was found to be the frequent pollen shape followed by prolate (P) (Table 2). The colpus length (CL) varied from 25.5 μm in *D. aucheri* to 40.2 μm in *D. lindbergii* (Table 2). The mean colpus length in *D. subcapitatum* from Iran was 30.8 μm, whereas mean colpus length in *D. subcapitatum* from Turkmenistan (Moon *et al.*, 2008) was 32.7 μm. Apocolpium index (AI) in the present investigation ranged from 0.08 to 0.27 for Iranian *Dracocephalum* species, while it was found 0.08 to 0.31 for the species studied by Moon *et al.* (2008).

Colpus membranes were granulate in *D. multicaule* and *D. ghahremanii*, whilst the remaining taxa were egranulate (Table 3; Figs 3K,L, 4K,L). Microreticulate exine sculpturing was recorded as the dominant type in *Dracocephalum* species studied by Moon *et al*. (2008) except for *D. nutans*, *D. palmatum* and *D. stamineum*, in which bireticulate type was found as the exine ornamentation type. Bireticulate was also found as the exine ornamentation type in *D. foetidum*, while microreticulate was determined for *D. grandiflorum* and *D. ruyschiana* (Badamtsetsg *et al*., 2012). It is noticeable that the microreticulate exine sculpturing in the taxa studied from Iran was not observed.

Taxonomic implication of pollen data

Our investigation on Iranian species of *Dracocephalum* revealed that several pollen characters can be of taxonomical value. In some cases, these characters supported the specific concept of formerly described species. For example, the separate taxonomic position of *D. oligadenium* is usually controversial. Esfandiari (1985), based on morphological characters, resurrected *D. oligadenium* as a distinct species from *D. kotschyi.* Recently, Jamzad (2012) considered *D. oligadenium* as a synonym of *D. kotschyi* in her treatment of the family Lamiaceae for *Flora of Iran*. Based on pollen data, *D. oligadenium* shows diagnostic features compared to *D. kotschyi* such as: smaller polar axis (38.8 μm vs 43.7 μm), colpus length (30.5 μm vs 38.1 μm), exine thickness (1.1 μm vs 0.8 μm), mesocolpium diameter (8.5 μm vs 11.1 μm) and apocolpium index (0.15 vs 0.14), which are useful for separating it from *D. kotschyi*. Considering the results obtained here from palynological data along with diagnostic morphological characters we may propose that *D. oligadenium* is a distinct species from *D. kotschyi* and could be resurrected as a separate taxon.

According to Flora Iranica (Rechinger, 1982), *D. subcapitatum* is distributed in Khorasan and Semnan provinces of Iran. Based on plants collected from Semnan province, which were previously identified as *D. subcapitatum*, Jamzad (Jamzad, 2012) introduced the new species *D. ghahremanii.* Previous study based on molecular RAPD marker showed that the Semnan population of *D. subcapitatum* is clearly different from other three populations of Khorasan (Sonboli *et al.*, 2011). The pollen morphological characteristics, i.e. number of primary lumina per 100 μm^2 (24 vs 11), diameter of primary lumina (2.97 μm vs 4.0 μm), granulate colpus membrane versus egranulate, provide strong evidence supporting the separate taxonomic position of *D. ghahremanii* from its close relative *D. subcapitatum.* Moreover, some species, *viz*. *D. moldavica, D. thymiflorum* and *D. aucheri* are morphologically well-characterized from the other species of *Dracocephalum* in Iran. These species can also be separated from other species of the genus based on pollen morphology (see Table 3). For example, polygonal-rounded lumen shape in *D. thymiflorum,* the number of primary lumina per 100 μm^2 in *D. moldavica* and exine sculpturing

(Type-I) in *D. aucheri* well-characterized these species from the rest, respectively. Finally, we can conclude that investigation into pollen morphology helps to characterize the taxa of *Dracocephalum*. Studies employing additional taxa of the genus are necessary which might further contribute to utilization of pollen micromorphological characters as significant attributes in classification of the species.

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