DOI: 10.22092/BOTANY.2021.355379.1261

Pollen micromorphological study of some *Chenopodiaceae* species and their taxonomical relationships in Iran

Received: 07.08.2021 / Accepted: 11.09.2021

Afsaneh Jalilzadeh: PhD Student, Department of Biology, Islamic Azad University, Science and Research Branch, Tehran, Iran

Seyed Mohammad Mahdi Hamdi✉: Associate Prof., Department of Biology, Islamic Azad University, Central Tehran Branch, Tehran, Iran (m.hamdi@iauctb.ac.ir)

Younes Asri: Research Associate Prof., Research Institute of Forests and Rangelands, Agricultural Research Education, and Extension Organization (AREEO), Tehran, Iran

Mostafa Assadi: Research Prof., Research Institute of Forests and Rangelands, Agricultural Research Education, and Extension Organization (AREEO), Tehran, Iran

Alireza Iranbakhsh: Prof., Department of Biology, Islamic Azad University, Science and Research Branch, Tehran, Iran

Abstract

Pollen grains micromorphological characteristics in nine genera of *Chenopodiaceae* (*Atriplex*, *Bassia*, *Bienertia*, *Chenopodium*, *Dysphania*, *Haloxylon*, *Kochia*, *Salsola*, and *Suaeda*) that tolerate more difficult ecological conditions in Iran were examined by Light Microscopy (LM) and Scanning Electron Microscopy (SEM). The obtained results showed that, the pollen grains quantitative and qualitative micromorphological characters were different in the studied species. A dendrogram obtained from the pollen grain characters in our research placed these taxa on two groups and three pollen types. Although, pollen group 1 had not taxonomical values. Pollen group 2 partly was in agreement with taxonomical level based on recently taxonomical and phylogenetic works including type A (*Atriplex*), type C (*Chenopodium*), and type D (*Dysphania*). Pore number had a major role to construct group 2. The number of echinate on pore membrane and on 10 μm^2 area of exine had major roles in constructing of group 1. The other pollen grains characters were important for the delimitation of both groups.

Keywords: Exine, phylogenetic relationships, pollen grain, pollen type, taxonomy

ریزریخت‌شناسی دانه گرده در برخی گونه‌های تیره اسفناجیان و روابط آرایه‌شناختی آن‌ها*

دریافت: ۱۴۰۰/۰۵/۱۶ / پذیرش: ۱۴۰۰/۰۶/۲۰

افسانه جلیل‌زاده: دانشجوی دکتری گروه زیست‌شناسی، دانشگاه آزاد اسلامی، واحد علوم و تحقیقات، تهران، ایران

سید محمد مهدی حمدی✉: دانشیار گروه زیست‌شناسی، دانشکده علوم، دانشگاه آزاد اسلامی، واحد تهران مرکز، تهران، ایران (m.hamdi@iauctb.ac.ir)

یونس عصری: دانشیار پژوهش مؤسسه تحقیقات جنگل‌ها و مراتع کشور، سازمان تحقیقات، آموزش و ترویج کشاورزی، تهران، ایران

مصطفی اسدی: استاد پژوهش مؤسسه تحقیقات جنگل‌ها و مراتع کشور، سازمان تحقیقات، آموزش و ترویج کشاورزی، تهران، ایران

علیرضا ایرانبخش: استاد گروه زیست‌شناسی، دانشگاه آزاد اسلامی، واحد علوم و تحقیقات، تهران، ایران

خلاصه

ویژگی‌های ریزریخت‌شناسی دانه گرده نُه جنس از تیره اسفناجیان (*Atriplex*، *Bassia*، *Bienertia*، *Chenopodium*، *Dysphania*، *Haloxylon*، *Kochia*، *Salsola* و *Suaeda*) که قادر به تحمل شرایط بسیار حاد بوم‌شناختی در ایران هستند، به وسیله میکروسکوپ نوری (LM) و میکروسکوپ الکترونی نگاره (SEM) مورد بررسی قرار گرفت. نتایج این تحقیق نشان داد که صفات کمّی و کیفی ریزریخت‌شناسی دانه گرده در جنس‌های مورد بررسی متفاوت بودند. دندروگرام به دست آمده از این بررسی، جنس‌های مورد بررسی را در دو گروه و سه تیپ قرار داد. اگر چه گروه اول از لحاظ آرایه‌شناسی و فیلوژنی مفید واقع نشد، اما گروه دوم تا حدودی مطابق با سطوح آرایه شناسی و فیلوژنی براساس بررسی‌های اخیر مربوط به جنس‌های مورد مطالعه بود که شامل تیپ‌های A (*Atriplex*)، C (*Chenopodium*) و D (*Dysphania*) می‌شد. تعداد حفره‌ها، نقش مهمی در ایجاد گروه دوم داشت. تعداد echinus ها در حفره‌ها و در سطح ۱۰ میکرومتر مربع اگزین نقش مهمی در ایجاد گروه اول داشتند و بقیه صفات دانه گرده در تعیین هر دو گروه دارای اهمیت بودند.

واژه‌های کلیدی: اگزین، تاکسونومی، تیپ گرده، دانه گرده، روابط فیلوژنتیکی

* مستخرج از رساله دکتری نگارنده نخست به راهنمایی دکتر سید محمد مهدی حمدی و دکتر یونس عصری ارایه شده به دانشگاه آزاد اسلامی، واحد علوم و تحقیقات

Introduction

Chenopodiaceae is one of the families of *Caryophyllales* that has adapted to saline regions or agricultural habitats with plenty of varieties in Iran (Kuhn *et al*. 1993, Hedge *et al*. 1997, Cue´noud *et al*. 2002, Toderich 2010, Ghahremaninejad *et al.* 2017). Based on Flora Iranica (Rechinger 1973), this family, embraces two subfamilies including 44 genera and 10 tribes in Iran. Molecular studies have separated *Chenopodiaceae* in seven subfamilies viz., *Chenopodioideae*, *Betoideae*, *Corispermoideae*, *Salicornioideae*, *Suaedoideae*, *Camphorosmoideae*, and *Salsoloideae* (Kadereit *et al*. 2003, Akhani *et al*. 2007, Kadereit *et al*. 2010, Wen *et al*. 2010, Fuentes-Bazan *et al*. 2012, Dehghani *et al.* 2020). This monophyletic family, is closely related to *Amaranthaceae* in some molecular traits, but based on some taxonomical issues, is recently combined in *Chenopodiaceae* (Cuénod *et al*. 2002, Kadereit *et al*. 2003, Müller & Borsch 2005, Judd *et al*. 2008, Ghahremaninejad *et al.* 2017, APG IV 2016). Based on APG IV, Ghahremaninejad *et al.* (2017) comprising both flora of Iran and Afghanistan, concluded that, the family *Amaranthaceae* had 54 genera in Iran with the combination of *Chenopodiaceae* in this family.

In several families of *Caryophyllales*, pollen grains have evolved complex architectures and ultrastructures, based on tricolpate pollen of the eudicots (Hernández-Ledesma *et al.* 2015, Hamdi *et al*. 2009) with several *Amaranthaceae* exhibiting strongly derived metareticulate pollens with the highest number of apertures known in angiosperms (Borsch 1998, Borsch & Barthlott 1998). Also, pollen morphology of *Chenopodiaceae* appears to vary most in the number of apertures and number, size, and frequency of spinules on, and punctae in the ectexine (Hernández-Ledesma *et al*. 2015, Dehghani & Akhani 2009). In this way, palynological investigation of *Chenopodiaceae* has received considerable attention from several research (Pinar & Inceoglu 1998, Olvera *et al*. 2006, Dehghani & Akhani 2009, Hamdi *et al*. 2009, Malekloo *et al*. 2010, Toderich *et al*. 2010, Perveen & Qaiser 2012, Lu *et al*. 2018, Dehghani *et al*. 2020). Moreover, recently Ghazali (2021) showed that, pollen morphology was valuable in two subfamilies of *Amaranthaceae* (*Amaranthoideae* and *Gomphrenoideae*). The rest of the subfamilies in *Amaranthaceae* (including *Chenopodiaceae*) have not the taxonomical value based on pollen morphology (Ghazali 2021). Therefore, the aim of the present study was to evaluate the taxonomic value of some genera in two subfamilies (*Chenopodioideae* and *Salsoloideae*) in this family from Iran based on pollen micro-morphological characters.

Materials and Methods

In this research, the pollen grains were prepared based on materials deposited in some Herbaria samples from Iran including Garmsar Herbarium (IAUGH), the Herbarium of Ferdowsi University of Mashhad (FUMH), and Research Institute of Forests and Rangelands (TARI) (Table 1). The pollen grains were subsequently examined by light (LM) and scanning electron microscopy (SEM). Samples for light microscopy were mounted on slides by using glycerol jelly, then sealed with nail polish, and observed under Zeiss Axioscope microscope. For SEM, the protocol explained by Davies (1999) was used with some modifications. The specimens were mounted on 12.5 mm diameter stubs and attached with sticky tabs, and then coated in a sputter coater with approximately 25 μm of Gold-Palladium. Pollen grains were examined and photographed by Philips scanning electron microscope (model XL). The qualitative and quantitative characters were defined based on the terminology used by Erdtman (1952), Punt *et al*. (2007), and Hesse *et al*. (2009). The MVSP software (Multi Variate Statistical Package) along with the UPGMA method (Unweighted Pair-Group Analysis) based on Euclidean distances was applied for constructing the dendrogram from the aforementioned pollen grain characters. In addition, principal component analysis (PCA) by MVSP software was used for the studied species (as PCA case scores) and pollen grain characters (as PCA variable loadings) (Kovach 1999).

Table 1. Taxa examined in this research along with related data

Taxon	Locality, date and voucher number
Atriplex tatarica L.	Tehran prov.: Lavasan, 17 Aug. 1999, Malekloo 5707 (IAUGH)
Bassia hyssopifolia (Pall.) Kuntze	Khorasan prov.: Sarakhs, Sazagan river, Jun. 1994, Akhani & Zangouee 2457517 (FUMH)
B. prostrata (L.) Beck. Syn.: *Kochia prostrata* (L.) Schrad.	Khorasan prov.: Torbat-e Heidarieh, 15 Jul. 2007, Jouharchi & Zangouee 39158 (FUMH)
Bienertia cycloptera Bunge ex Boiss.	Khorasan prov.: Kashmar, 13 May 1991, Faghihnia & Zangouee, 21161 (FUMH)
Chenopodium album L. *Ch. album*. subsp. *album*	Tehran prov.: EN Tehran, Sorkheh Hesar, 17 Aug. 1999, Malekloo 5702 (IAUGH)
Ch. foliosum Asch.	Mazandaran prov.: Kiasar forest, 13 Jun. 1994, Mozaffarian & Norouzi 34481 (TARI)
Ch. glaucum L.	Tehran prov.: Lavasan, 14 Aug. 1996, Assadi 64449, TARI
Ch. novopokrovskyanum (Aellen) Uotila	Mazandaran prov.: Sari to Semnan, 17 Aug. 1999, Malekloo 2799 (IAUGH)
Ch. opulifolium Schrad. ex W.D.J. Koch & Ziz.	Tehran prov.: Between Tehran to Karaj, Botanical Garden, 9 Jul. 1997, Assadi 76802 (TARI)
Ch. rubrum L.	Semnan prov.: 17 Jun. 1996, Sharif 40284 (TARI)
Dysphania botrys (L.) Mosyakin & Clemants.	Tehran prov.: 18 Aug. 1999, Malekloo 2800 (IAUGH)
Haloxylon ammodendron (C.A. Mey.) Bunge ex Fenzl.	Khorasan prov.: Gonabad, 8 Apr. 1999, Faghihnia & Zangouee 19256 (FUMH)
Salsola florida (M.Bieb.) Poir.	Azerbaijan prov.: Salmas, 18 Apr. 1996, Assadi & Salehi 31998 (TARI)
Suaeda microsperma Fenzl.	Khorasan prov.: Sabzevar to Esfaraien, 4 Sept. 1990, Faghihnia & Zangouee 19145 (FUMH)

Results

In the present study, 14 taxa from nine genera of *Chenopodiaceae* that are distributed in the desert areas of Iran which are environmentally important (Assadi 2021) were selected. These taxa were investigated based on the pollen morphology characteristics. Quantitative pollen morphology characters were also evaluated (Table 2).

These micromorphological characters were different among the studied species (Table 2). Major qualitative characters were examined such as pollen type, pore size, exine ornamentation, and density of elements of exine ornamentation on exine surface, pore membrane ornamentation, pore membrane ornamentation of pollen grains, and pore edge shape (Table 3).

Pollen type was constant among these genera but *Bassia hyssopifolia* (Pall.) Kuntze and *B. prostrata* (L.) Beck with medium pollen type was different in this character (Table 3). Size of pore as the pollen qualitative characters were detected and three ranges were detected as follows: small (*Atriplex tatarica* Auct., *Chenopodium foliosum* Asch., *Ch. glaucum* L., *Ch. novopokrovskyanum* (Aellen) Uotila, and *Ch. rubrum* Forssk. ex Steud.), medium (*Ch. album* Bosc. ex Moq., *Ch. opulifolium* Schrad. ex DC., *Dysphania botrys* (L.) Mosyakin & Clemants, and *Suaeda microsperma* Fenzl.), and large (the other studied species) (Table 3). The density of echinate exine ornamentation was varied among all studied species. In this way, *Bienertia cycloptera* Bunge and *Ch. foliosum*, had high dense of echinate in exine surface ornamentation, *Haloxylon ammodendron* Bunge had high dense of microechinate in exine surface ornamentation, and the other studied species had low dense echinate exine ornamentation (Sparse) in this region (Table 3, Figs 1 & 2). Pore membrane ornamentation as the fourth qualitative character was dense echinate in *A. tatarica*, *B. hyssopifolia*, *B. prostrata*, *B. cycloptera*, *Ch. foliosum*, *H. ammodendron*, *D. botrys*, and *S. florida* (Table 3, Figs 1B, 1D, 1F, 1H, 1L, 2H, 2F, and 2G). Moreover, *Ch. glaucum* had dense microechinate (Table 3, Fig. 1N). The other studied species was sparse echinate in this region (low density) (Table 3, Figs 1 & 2). Three kinds of pore edge shapes were detected among the studied species as follows: prominent (*S. microsperma*), highly sunken (*B. cycloptera*, *Ch. foliosum*, and *H. ammodendron*), and sunken (the rest of studied species) (Table 3, Figs 1 & 2).

Based on pollen characters, two following groups were constructed: Group 1: *S. florida*, *H. ammodendron*, *S. microsperma*, *B. cycloptera* and *B. prostrata*; and group 2: type D (*D. botrys*), type C (*Ch. album, Ch. foliosum*, *Ch. glaucum*, *Ch. novopokrovskyanum*, *Ch. opulifolium* and *Ch. rubrum*); and type A (*A. tatarica*) and *B. hyssopifolia* (Table 4, Fig. 3).

The obtained results from the PCA including eigenvalues, percentages, and cumulative percentages for both axes, PCA case scores for all genera, and their species, and PCA variable loadings for all pollen characters are shown in table 5. There were two groups in both axes (Fig. 4): taxa of group 1 (negative in axis 2, negative and positive in axis 1) and taxa of group 2 (negative and positive in both axes). Group 2 is segregated in to three types; type D (genus *Dysphania*): positive in both axes; type C (genus *Chenopodium*): negative and positive in axis 1 and positive in axis 2; type A (*Atriplex tatarica*): negative in axis 1 and positive in axis 2, and one taxon (genus *Bassia*: negative in both axes) (Fig. 4). The results of PCA analysis based on the studied species and the pollen grain characters overlapping showed that, pollen character Pn (pore number) had a major role in the statement of group 2 especially type A (Fig. 4). In this way, characters Np (number of echinate on pore membrane) and Ne (number of echinate on 10 μm^2 area of exine) had major roles in the statement of group 1 (Ne: especially *Salsola florida* and Np: the other taxa in group 1) (Fig. 4). The other pollen grain characters, which included group A were important in the determination of all studied taxa (Fig. 4).

Table 2. Pollen grains quantitative characters of SEM (Mean ± SE) in studied taxa

Taxon	A	B	C	D	E	F	G	H	I	J	K	L
Atriplex tatarica L.	22.74±3.25	2.66±0.63	1.24±0.23	5.10±1.04	75	9.50±1.10	0.2±0.04	3.30	0.12	0.47	4.11	0.05
Bassia hyssopifolia (Pall.) Kuntze	27.83±1.12	3.06±0.48	2.26±0.22	7.20±1.33	40	5.10±1.14	0.1±0.03	1.44	0.11	0.74	3.19	0.08
B. prostrata (L.) Beck.	25.62±3.71	3.11±0.57	2.68±0.64	21.7±2.00	16	8.90±1.04	0.05±0.02	0.62	0.12	0.86	8.10	0.1
Bienertia cycloptera Bunge ex Boiss.	20.12±1.58	3.50±0.34	2.86±0.35	19.00±2.37	25	8.30±1.68	0.07±0.02	1.24	0.17	0.82	6.64	0.14
Chenopodium album L.	24.81±1.58	1.41±0.18	2.60±0.32	6.00±0.89	60	5.10±1.70	0.14±0.05	2.42	0.06	1.84	1.65	0.1
Ch. foliosum Asch.	17.22±1.78	2.17±0.26	1.02±0.21	5.70±0.46	45	20.90±3.94	0.16±0.04	2.61	0.13	0.47	4.51	0.13
Ch. glaucum (L.)	21.02±0.98	1.45±0.26	1.85±0.40	4.60±0.67	55	10.60±1.43	0.14±0.04	2.62	0.07	1.28	1.95	0.09
Ch. novopokrovskyanum (Aellen) Uotila	19.64±1.36	1.45±0.28	0.76±0.13	3.60±0.49	65	8.50±2.11	0.14±0.03	3.31	0.07	0.52	5.26	0.04
Ch. opulifolium Schrad. ex W.D.J. Koch & Ziz.	22.79±1.62	1.71±0.18	1.15±0.19	4.00±0.63	55	8.50±1.12	0.16±0.07	2.41	0.08	0.67	4.09	0.05
Ch. rubrum (L.)	19.30±1.33	1.53±0.24	0.91±0.12	4.70±0.90	50	7.40±1.43	0.22±0.05	2.59	0.08	0.59	5.16	0.05
Dysphania botrys (L.) Mosyakin & Clemants.	15.93±0.68	0.87±0.30	2.03±0.32	4.30±1.10	45	9.40±2.00	0.18±0.06	2.82	0.05	2.33	2.77	0.07
Haloxylon ammodendron (C.A. Mey.) Bunge ex Fenzl.	24.74±1.80	3.63±0.43	3.46±0.73	26.50±1.96	20	29.90±2.16	0.07±0.02	0.81	0.15	0.95	7.66	0.14
Salsola florida (M. Bieb.) Boiss.	16.59±2.12	2.96±0.37	2.06±0.37	32.80±2.04	12	17.90±1.51	0.12±0.03	0.72	0.18	0.70	15.92	0.12
Suaeda microsperma Fenzl.	21.64±1.04	2.40±0.42	1.58±0.26	21.60±4.20	35	6.30±1.62	0.12±0.02	1.62	0.11	0.66	3.16	0.07

Abbreviation: A (pollen size, µm), B (interporal distance, µm), C (pore diameter, µm), D (number of microechinate on pore membrane), E (pore number), F (number of microechinate on 10 μm^2 area of exine), G (microechinate height on exine surface, µm), H (pore number/pollen size), I (interporal distance/pollen size), J (pore diameter/interporal distance), K (number of microechinate on pore membrane/pore diameter), and L (pore diameter/pollen size).

Table 3. Pollen qualitative characters from SEM measurements in studied taxa

Taxon	A	B	C	D	E	F	G
Atriplex tatarica L.	Small	Small	Sparse	Echinate	Dense	Echinate	Sunken
Bassia hyssopifolia (Pall.) Kuntze	Medium	Large	Sparse	Echinate	Dense	Echinate	Sunken
Bassia prostrata (L.) Beck.	Medium	Large	Sparse	Echinate	Dense	Echinate	Sunken
Bienertia cycloptera Bunge ex Boiss.	Small	Large	Dense	Echinate	Dense	Echinate	Highly sunken
Chenopodium album L.	Small	Medium	Sparse	Echinate	Sparse	Echinate	Sunken
Chenopodium foliosum Asch.	Small	Small	Dense	Echinate	Dense	Echinate	Highly sunken
Chenopodium glaucum (L.)	Small	Small	Sparse	Echinate	Dense	Macroechinate	Sunken
Chenopodium novopokrovskyanum (Aellen) Uotila	Small	Small	Sparse	Echinate	Sparse	Echinate	Sunken
Chenopodium opulifolium Schrad. ex W.D.J. Koch & Ziz.	Small	Medium	Sparse	Echinate	Sparse	Echinate	Sunken
Chenopodium rubrum (L.)	Small	Small	Sparse	Echinate	Sparse	Sparse echinate	Sunken
Dysphania botrys (L.) Mosyakin & Clemants.	Small	Medium	Sparse	Echinate	Dense	Echinate	Sunken
Haloxylon ammodendron (C.A. Mey.) Bunge ex Fenzl.	Small	Large	Dense	Microechinate	Dense	Echinate	Highly sunken
Salsola florida (M. Bieb.) Boiss.	Small	Large	Sparse	Echinate	Dense	Echinate	Sunken
Suaeda microsperma Fenzl.	Small	Medium	Sparse	Echinate	Sparse	Echinate	Prominent

Abbreviation: A (pollen type), B (size of pore), C (density of echinate exine ornamentation on the exine surface), D (exine ornamentation), E (density of pore membrane ornamentation), F (pore membrane ornamentation), and G (pore edge shape: 0= highly sunken, 1= sunken, and 2= prominent).

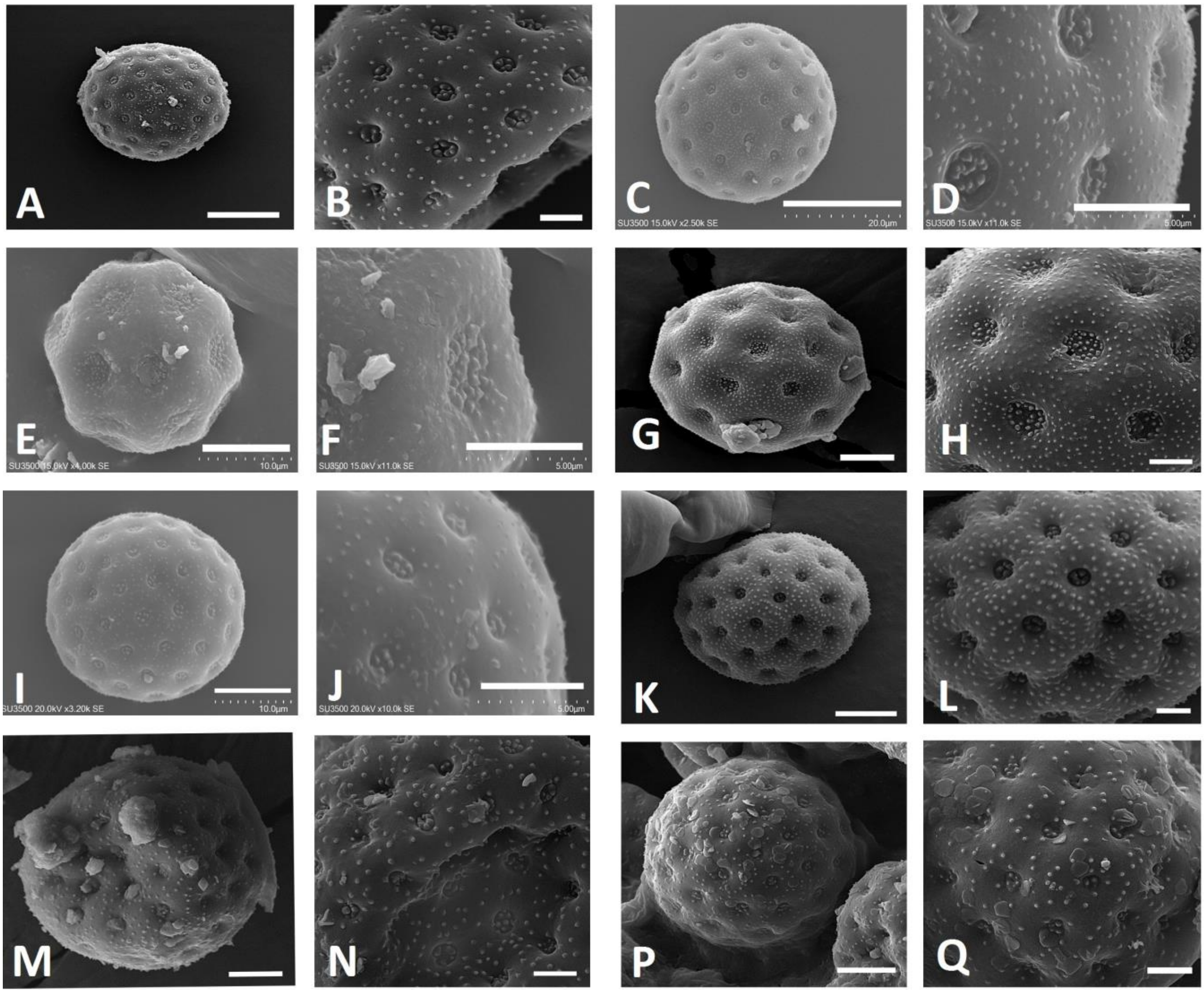

Fig. 1. A, B. Scanning electron microscopic micrographs of the pollen grains in *Atriplex tatarica*, C, D. *Bassia hyssopifolia*, E, F. *Bassia prostrata*, G, H. *Bienertia cycloptera*, I, J. *Chenopodium album* subsp. *album*, K, L. *Ch. foliosum*, M, N. *Ch. glaucum*, and P, Q. *Ch. novopokrovskyanum*). A, C, E, G, I, K, M, and P (Bars: 15 µm), B, D F, H, J, L, N, and Q (Bars: 5 µm).

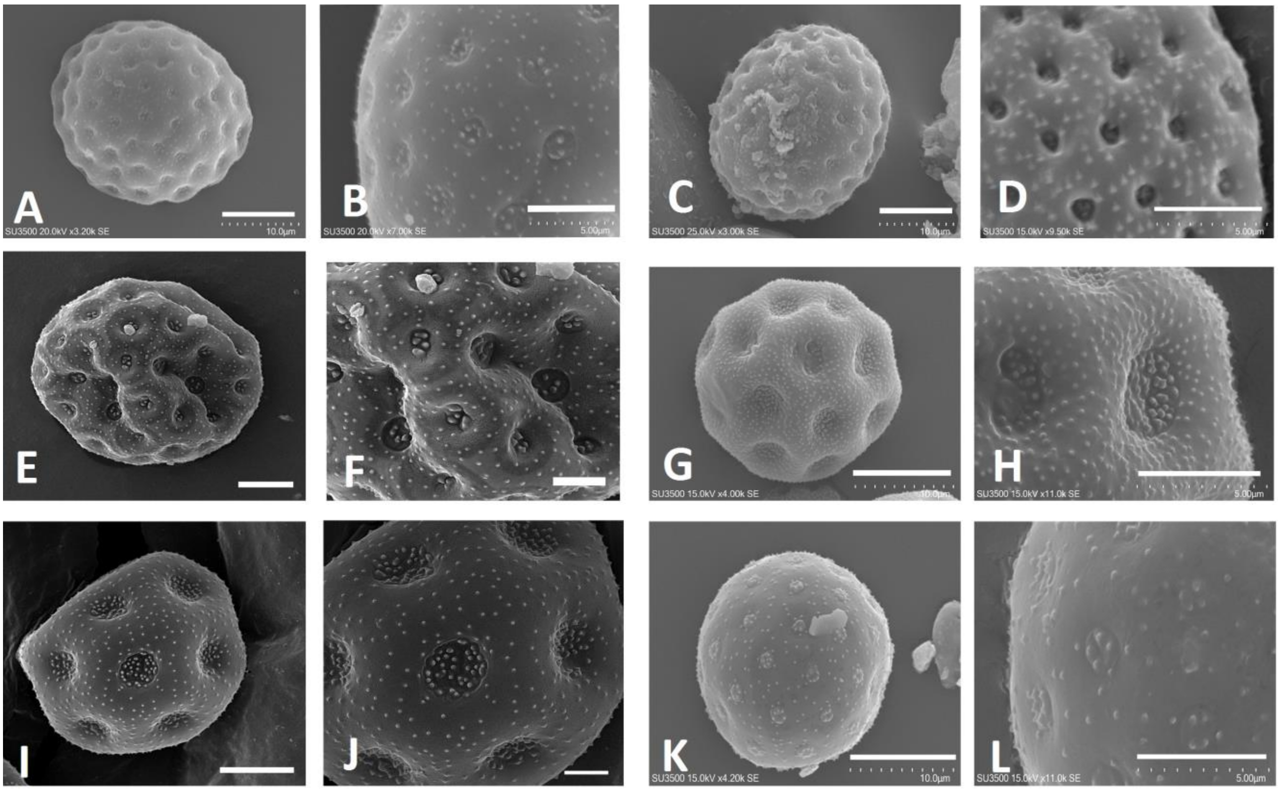

Fig. 2. A, B. Scanning electron microscopic micrographs of the pollen grains in *Chenopodium opulifolium*, C, D. *Ch. rubrum*, E, F. *Dysphania botrys*, G, H. *Haloxylon ammodendron*, I, J. *Salsola florida*, and K, L. *Suaeda microsperma*. A, C, E, G, I, and K (Bars: 15 µm); B, D, F, H, J, and L (Bars: 5 µm).

Table 4. The pollen grains micromorphological characters (variables) analyzed with the UPGMA method for the construction of dendrogram of the studied species (cases), DS (Dissimilarity)

Cluster analysis

Analyzing 25 variables × 14 cases

Euclidean

Node	Group 1	Group 2	Dissimilarity	Objects in group
1	*Chenopodium glaucum*	*Dysphania botrys*	4.474	2
2	Node 1	*Chenopodium rubrum*	6.931	3
3	*Ch. album*. subsp. *album*	*Ch. novopokrovskyanum*	9.457	2
4	Node 2	*Ch. opulifolium*	10.499	4
5	*Bassia prostrata*	*Bienertia cycloptera*	11.525	2
6	Node 3	Node 4	13.086	6
7	Node 5	*Suaeda microsperma*	16.215	3
8	Node 6	*Chenopodium foliosum*	17.954	7
9	*B. hyssopifolia*	Node 8	18.745	8
10	*Atriplex tatarica*	Node 9	24.172	9
11	Node 7	*Haloxylon ammodendron*	25.133	4
12	Node 11	*Salsola florida*	28.645	5
13	Node 10	Node 12	37.866	14

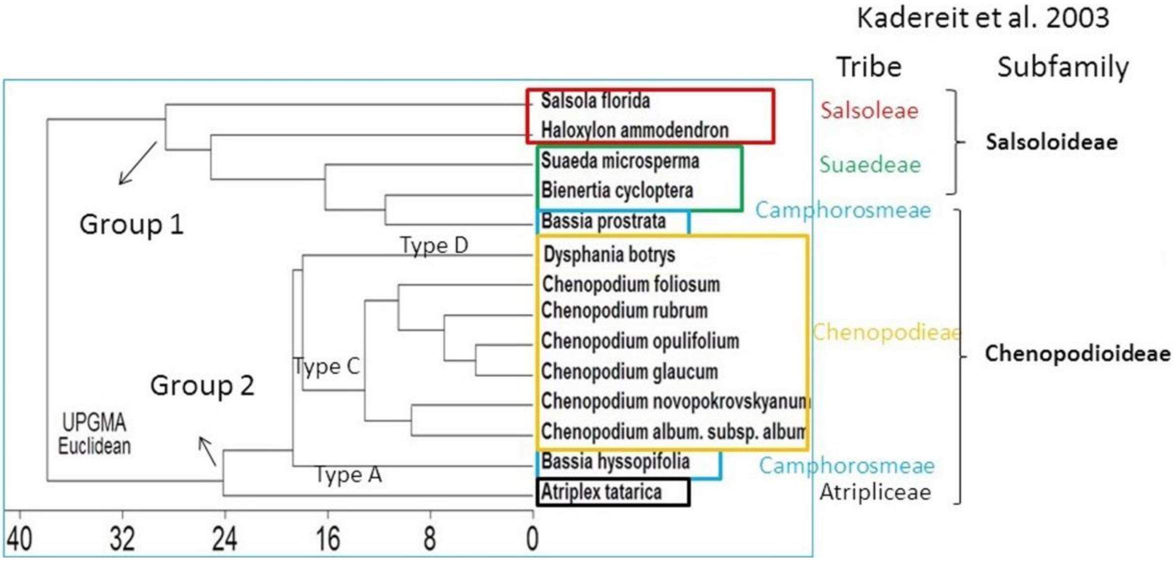

Fig. 3. Dendrogram of the studied species that analyzed by MVSP software based on UPGMA method from pollen data and their taxonomic relationships.

Table 5. The pollen grain morphological characters analyzed by principal components analysis, E. values: Eigen values and Cum. percentage (Cumulative percentage)

Principal components analysis Analyzing 25 variables × 14 cases		
	Axis 1	**Axis 2**
Eigen values	377.191	51.722
Percentage	78.148	10.716
Cum. percentage	78.148	88.864
PCA variable loading		
	Axis 1	**Axis 2**
Sp	-0.036	-0.005
De	-0.014	0.025
Ex	-0.006	-0.008
Dm	-0.003	-0.04
Pm	-0.007	0.09
Pt	-0.006	-0.012
Pz	-0.007	-0.416
Sps	-0.004	-0.046
Id	-0.032	-0.024
Sid	-0.002	-0.007
Pd	-0.03	-0.02
Spd	-0.006	-0.007
Np	-0.314	-0.799
Snp	-0.025	-0.104
Pn	0.931	-0.24
Ne	-0.161	0.26
Sne	-0.005	0.053
Eh	0.002	0.001
She	0.001	0
P	0.042	0.022

Table 5 (contd)

I	-0.002	0.002
D	0.001	0.007
N	-0.046	-0.181
S	-0.001	0.002
Pe	0.006	-0.049
PCA case score		
	Axis 1	**Axis 2**
Atriplex tatarica 1	8.68	-1.443
Bassia hyssopifolia 2	-0.402	-0.438
B. prostrata 3	-8.11	-1.844
Bienertia cycloptera 4	-5.498	-1.116
Chenopodium album. subsp. *album* 5	4.933	-1.031
Ch. foliosum 6	0.37	1.962
Ch. glaucum 7	3.537	0.486
Ch. novopokrovskyanum 8	6.284	-0.138
Ch. opulifolium 9	3.648	0.104
Ch. rubrum 10	2.352	0.557
Dysphania botrys 11	1.03	1.633
Haloxylon ammodendron 12	-8.438	-1.467
Salsola florida 13	-5.407	5.288
Suaeda microsperma 14	-2.98	-2.555

Sp (Size of pore), De (Density of echinate exine ornamentation on the exine surface), Ex (Exine ornamentation), Dm (Density of pore membrane ornamentation), Pm (Pore membrane ornamentation), Pt (Pollen type), Pz (pollen size), Sps (SD of polen size), Id (interporal distance), Sid (Sd of interporal distance), Pd (pore diameter), Spd (SD of pore diameter), Np (number of echinate on pore membrane), Snp (SD of number of echinate on pore membrane), Pn (pore number), Ne (number of echinate on 10 µm^2 area of exine), Sne (SD of number of echinate on 10 µm^2 area of exine), Eh (echinate height on exine surface), She (SD of echinate height on exine surface), P (pore number/pollen size), I (interporal distance/pollen size), D (pore diameter/interporal distance), N (N/pore diameter), S (pore diameter/pollen size), and Pe (pore edge shape).

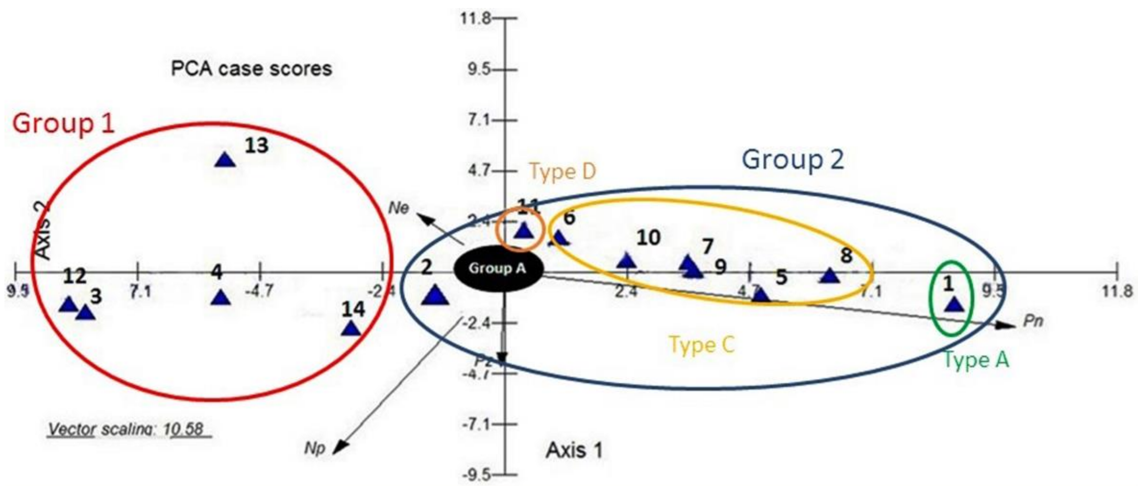

Fig. 4. Principal components analysis scatterplot obtained from the overlapping of species (case scores) and the pollen grain characters (variable loadings). Variable loading (pollen grain characters): Ne (number of echinate on 10 µm^2 area of exine); Np (number of echinate on pore membrane); Pn (pore number) and character group A (the other pollen morphological characters). Case scores (species): *Atriplex tatarica* (1); *Bassia hyssopifolia* (2); *B. prostrata* (3); *Bienertia cycloptera* (4); *Chenopodium* album. subsp. *album* (5); *Ch. foliosum* (6); *Ch. glaucum* (7); *Ch. novopokrovskyanum* (8); *Ch. opulifolium* (9); *Ch. rubrum* (10); *Dysphania botrys* (11); *Haloxylon ammodendron* (12); *Salsola florida* (13); and *Suaeda microsperma* (14).

Discussion

Results derived from the present study revealed some valuable quantitative and qualitative pollen morphological characters used to better view the taxonomical level of the studied genera. Besides, the results of our research were partly in accordance with the previous studies in this field (Pinar & Inceoglu 1998, Olvera *et al*. 2006, Dehghani & Akhani 2009, Hamdi *et al*. 2009, Malekloo *et al*. 2010, Toderich *et al*. 2010, Perveen & Qaiser 2012, Lu *et al*. 2018, Dehghani *et al*. 2020, Ghazali 2021). In the present investigation, palynological dendrogram separated the studied specimens in to two groups that were partly in agreement with their taxonomical issues. Although, group 1 was heterogeneous and had not any type based on pollen morphology. Group 2 was partly suitable and segregated three types. In this way, *Atriplex tatarica* was placed in type A. This species belongs to the tribe *Atripliceae* and subfamily *Chenopodioideae* (Kadereit *et al*. 2003). Recently, palynological work had done in this tribe (Olvera *et al*. 2006). The major results of this research showed that, pollen grains were pantoporate, spheroidal, or subspheroidal shape. Some pollen quantitative characters were important and had taxonomical values such as pollen diameter, pore number, pore diameter, interporal distance, spinule and puncta density, number of ectexinous bodies, and their spinules. This research showed that, pollen morphological data support the exclusion of *Suckleya* A.Gray from this tribe and pollen morphology does not support generic recognition of *Atriplex*, *Neopreissia* Ulbr., and *Obione* Gaertn. In addition, pollen morphological characters supported their generic status of genera *Axyris* L., *Ceratocarpus* Buxb. ex L., *Endolepis* Torr., *Krascheninnikovia* Gueldenst., *Microgynoecium* Hook.f., *Proatriplex* (W.A. Weber) Stutz & G.L. Chu, and *Spinacia* L. (Olvera *et al*. 2006). Additionally, *Atriplex* pollen types were seen in some recent works (Perveen & Qaiser 2012, Lu *et al*. 2018). In our research, the studied species of this tribe segregated the other genera. Therefore, these results indicated that, pollen data are potentially useful in the classification of the tribe.

Based on Pinar & Inceoglu (1998), five pollen types were determined with pollen morphological characters (especially pollen size) in the species of *Chenopodium* L. The examined species had radially symmetrical, isopolar, pantopolyporate, and spheroidal shape. Also, the sporoderm structures were similar in all studied species (Pinar & Inceoglu 1998). In recent research, palynomorphological study of 14 species of genus *Chenopodium* was evaluated and two type pollens based on pore on the surface (foveate and perforate) were constructed. This result partly confirmed the taxonomy of the studied species of this genus during this research (Hamdi *et al*. 2009). Malekloo *et al*. (2010) revised the taxonomical level of *Ch. album* in Iran and pollen morphology helps this revision. Palynological characters issues confirmed this revised along with some other morphological characters (seed ornamentation and stem anatomy) during this research. Moreover, *Chenopodium album*-type was detected in Flora of Pakistan by Perveen & Qaiser (2012) based on pollen morphological characters. In our research, the studied species of genus *Chenopodium* were placed in type C based on all pollen characters (quantitative and qualitative characters). As a whole, pollen morphology was a valuable character in the classification of this genus.

Based on our dendrogram, *Dysphania botrys* as type D was placed near type C in group 2. This situation was verified by the phylogram of Kadereit *et al*. (2003) that showed both genera were placed on one clade based on *rbcl* sequences. The other studied species including group 1 and *Bassia hyssopifolia* from group 2 were not constructed any pollen type.

In a recent investigation, Dehghani & Akhani (2009) had done pollen morphological research in genera *Bienertia* and *Suaeda* and their results showed that, quantitative pollen characters such as pollen diameter, pore number, pore diameter, operculum diameter, chord distance, exine thickness, pore number, and qualitative characteristics of exine spinules and operculum spinules had variation and valuable in taxonomical issues. In this way, *Bienertia* was clearly distinguished from *Suaeda* (Dehghani & Akhani 2009). Also, our results verified the segregation of both genera. *Bienertia cycloptera* was placed together in *Kochia prostrata* (Syn.: *Bassia prostrata*) based on our results. *Kochia prostrata*, and *B. hyssopifolia* from subfamily *Chenopodioideae*, and tribe *Camphorosmeae* were investigated in our research. Our palynological dendrogram of both genera is not matched with the taxonomical levels.

Although, *Haloxylon ammodendron* and *Salsola florida* in group 1 were not placed in any pollen type in our pollen dendrogram. Some palynological studies had good supported the taxonomical level such as Toderich *et al*. (2010), Perveen & Qaiser (2012), and Lu *et al*. (2018). In some species of the genus *Salsola*, the pollens are radially symmetrical isopolar, pantopolyporate, spherical or subspheroid. The pollen characters like size, pore number, chord (C/D ratio), pore diameter, exine thickness, level of the sinking of the pore, convexness of mesoporial exine, spinule, and minute-hole densities, and the number of spines on the pore membrane appeared to be useful characters in distinguishing the species. Among these pollen characters, the C/D and P/E ratios had intraspecific variations. Based on the pollen dendrogram, three pollen types were recognized. These pollen types partly agreed with taxonomical levels in *Salsola* (Toderich *et al*. 2010). Pollen morphology of 30 genera of *Chenopodiaceae* was detected in Pakistan. Examined pollen was seen with morphological character including: radially symmetrical, apolar pantoporate, spheroidal, sexine slightly thicker or thinner than nexine and tectum sparsely to densely punctate and rarely spinulose. Moreover, two pollen characters were important as taxonomical values, and this family was divided in to four pollen types (*Arthrocnemun indicum*-type, *Atriplex stocksii*-type, *Chenopodium album*-type, and *Haloxylon persicum*-type). Consequently, pollen types supported specific and generic taxonomical levels (Perveen & Qaiser 2012). Lu *et al*. (2018) provide a new pollen classification of family *Chenopodiaceae* with six pollen types and link them to those plant communities includes, for example, temperate dwarf semi-arboreal desert (*Haloxylon* type), temperate succulent halophytic dwarf semi-shrubby desert (*Suaeda*, *Kalidium*, and *Atriplex* types), temperate annual graminoid desert (*Kalidium* type), temperate semi-shrubby and dwarf semi-shrubby desert (*Kalidium*, *Iljini*, and *Haloxylon* types), and alpine cushion dwarf semi-shrubby desert (*Krascheninnikovia* type).

Conclusion

Dehghani *et al.* (2020) showed pollen morphology of subfamily *Salicornioideae* had taxonomic value and supported phylogenetic relationships in this subfamily. Pollen morphology characters were diverse in *Amaranthaceae* (including *Chenopodiaceae*) such as *Betoideae*, *Camphrosomoideae*, *Chenopodioideae*, *Corispermoideae*, *Salicornioideae*, *Salsoloideae*, *Suaedoideae*, and *Polycnemoideae* subfamilies (Ghazali 2021). Our palynological research partly supported the phylogenetic relationships of *Chenopodioideae* and *Salsoloideae* subfamilies.

Acknowledgements

Authors thank Engr. M.R. Joharchi (Ferdowsi University of Mashhad, Mashhad, Iran) for preparing some of the specimens and also Mr. S. Javadi Anaghizi (Central Laboratory of Shahid Beheshti University, Tehran, Iran) for providing SEM photographs.

References

Akhani, H., Edwards, G. & Roalson, E.H. 2007. Diversification of the old world Salsoleaes l. (Chenopodiaceae): molecular phylogenetic analysis of nuclear and chloroplast datasets and a revised classification. International Journal of Plant Sciences 168(6): 931–956.

APG, I.V. 2016. An update of the angiosperm phylogeny group classification for the orders and families of flowering plants. Botanical Journal of the Linnean Society 181(1): 1–20.

Assadi, M. 2021. Chenopodiaceae family in Iran, the plants tolerant to harshy and unfavorable habitats. Journal of Iran Nature 5(25): 27–35.

Borsch, T. 1998. Pollen types in the Amaranthaceae, Morphology and Evolutionary Significance. Grana 37: 129–142.

Borsch, T. & Barthlott, W. 1998. Structure and evolution of misarticulate pollen. Grana 37: 68–78.

Cue´noud, P., Savolainen, V., Chatrou, L.W., Powell, M., Grayer, R.J. & Chase, M.W. 2002. Molecular phylogenetics of Caryophyllales based on nuclear 18S rDNA and plastid rbcL, atpB, and matK DNA sequences. American Journal of Botany 89: 132–144.

Davies, H.A. 1999. General preparation of material and staining of sections. Methods in Molecular Biology 117: 1–11.

Dehghani, M., Djamali, M. & Akhani, H. 2020. Pollen morphology of the subfamily Salicornioideae (Chenopodiaceae) in Eurasia and north Africa. Palynology 452: 45–258.

Dehghani, M. & Akhani, H. 2009. Pollen morphological studies in subfamily Suaedoideae (Chenopodiaceae). Grana 48: 79–101.

Erdtman, G. 1952. Pollen Morphology and Plant Taxonomy, Angiosperms. Almqvist and Wiksell, Stockholm.

Fuentes-Bazan, S., Uotila, P. & Borsch, T. 2012. A novel phylogeny-based generic classification for *Chenopodium* Sensu Lato, and a tribal rearrangement of Chenopodioideae (Chenopodiaceae). Willdenowia 42: 5–24.

Gamal, E. & El Ghazali, B. 2021. Pollen Morphological Studies in Amaranthaceae s.lat. (incl. Chenopodiaceae) and Their Taxonomic Significance: A Review. Grana. https://doi.org/10.1080/00173134.2021.1950829.

Ghahremaninejad, F., Ataei, N. & Nejad Falatoury, A. 2017. Comparison of angiosperm flora of Afghanistan and Iran in accordance with APGIV system. Nova Biologica Reperta 4(1): 73–97.

Ghazali, G.E.B. 2021. Pollen Morphological Studies in Amaranthaceae s. lat. (incl. Chenopodiaceae) and their Significance. A Review.

Hamdi, S.M.M., Malekloo, M., Assadi, M. & Nejadsatari, T. 2009. Pollen micromorphological studies of the genus *Chenopodium* (Chenopodiaceae) In Iran. Asian Journal of Plant Science 8(2): 129–137.

Hesse, M., Halbritter, H., Zetter, R., Weber, M., Buchner, R., Frosch-Radivo, A. & Ulrich, S. 2009. Pollen Terminology (An Illustrated Handbook). Springer, 266 Pp., New York.

Hedge, I.C., Akhani, H., Freitag, H., Kothe-Heinrich, G., Podlech, D., Rilke, S. & Uotila, P. 1997. Chenopodiaceae, *In*: Flora Iranica (K.H. Rechinger, ed.). Akad D.U.VA, Graz.

Hernández-Ledesma, P., Berendsohn, W.G., Borsch, T.O., Von Mering, S., Akhani, H., Arias, S., Castañeda-Noa, I., Eggli, U.R., Eriksson, O., Flores-Olvera, H., Fuentes-Bazán, S., Kadereit, G., Klak, C., Korotkova, N., Nyffeler, R., Ocampo, G., Ochoterena, H., Oxelman, B., Rabeler, R.K., Sanchez, A., Schlumpberger, B.O. & Uotila, P. 2015. A taxonomic backbone for the global synthesis of species diversity in the angiosperm order Caryophyllales. Willdenowia 45: 281–383.

Judd, W.S., Campbell, C.S., Kellogg, E.A., Stevens, P.F. & Donoghue, M.J. 2008. Plant Systematics: A

Phylogenetic Approach. 3rd ed. MA, Sinauer Assoc, Sunderland.

Kadereit, G., Borsch, T., Weising, K.H. & Freitag. 2003. Phylogeny of Amaranthaceae and Chenopodiaceae and the evolution of c4 photosynthesis. International Journal of Plant Sciences 164(6): 959–986.

Kadereit, G., Mavrodiev, E.V., Zacharias, E.H. & Sukhorukov, A.P. 2010. Molecular phylogeny of *Atripliceae* (Chenopodioideae, Chenopodiaceae) implications for systematics, biogeography, flower and fruit evolution, and the origin of c4 photosynthesis. American Journal of Botany 97(10): 1664–1687.

Kovach, W. 1999. MVSP-a Multivariate Statistical Package for Windows, Ver. 3.1. Kovach Computing Services, Pentraeth, Wales, Great Britain.

Kuhn, U., Bittrich, V., Carolin, R., Freitag, H., Hedge, I.C., Uotila, P. & Wilson, P.G. 1993. Chenopodiaceae. Pp. 253–281. *In*: K. Kubitzki, J.G. Rohwer & V. Bittrich (eds), Families and Genera of Vascular Plants, Vol. II, Flowering Plants, Dicotyledons: Magnoliid, Hamamelid and Caryophyllid families. Springer, Berlin.

Lu, K.Q., Li, M., Wang, G.H., Xu, L.S., Ferguson, D.K., Trivedi, A., Xuan, J., Feng, Y., Li, J.F., Xie, G., Yao, Y.F. & Wang, Y.F. 2018. New pollen classification of chenopodiaceous for exploring and tracing desert vegetation evolution in eastern arid central Asia. Journal of Systematics and Evolution 57(2): 190–199.

Müller, K. & Borsch, T. 2005. Phylogenetics of Amaranthaceae based on Matk/Trnk Sequence Data-evidence from parsimony, likelihood, and Bayesian analyses. Annals Missouri Botanical Garden 92(1): 66–102.

Malekloo, M., Hamdi, S.M.M., Assadi, M. & Nejadsatari, T. 2010. Morphological, micro-morphological and anatomical studies of *Chenopodium album* complex in Iran. Iranian Journal of Botany 6(1): 69–75.

Olvera, H.F., Fuentes-Soriano, S. & Nez Herna Ndez, E.M. 2006. Pollen morphology and systematics of *Atripliceae* (Chenopodiaceae). Grana 45: 175–194.

Perveen, A. & Qaiser, M. 2012. Pollen flora of Pakistan-Lxx: Chenopodiaceae. Pakistan Journal of Botany 4(4): 1325–1333.

Pinar, N.M. & Inceoglu, O. 1998. Pollen morphology of Turkish *Chenopodium* L. (Chenopodiaceae). Turkish Journal of Botany 23: 179–186.

Punt, W., Hoen, P.P., Blackmore, S., Nilsson, S. & Thomas, L. 2007. Glossary of pollen and spore terminology. Review of Paleobotany and Palynology 143: 1–81.

Rechinger, H.K.R. 1997. Chenopodiaceae in Flora Iranica. Graz. No. 172.

Skvarla J.J. & Nowicke J.W. 1976. Ultrastructure of pollen exine in Centrospermous families. Plant Systematic and Evolution 126: 55–78.

Toderich, K.N., Shuyskaya, E.V., Ozturk, M., Juylova, A. & Gismatulina, L. 2010. Pollen morphology of some Asiatic species of genus *Salsola* (Chenopodiaceae) and its taxonomic relationships. Pakistan Journal of Botany 42: 155–174.

Wen, Z.B., Zhang, M.L., Zhu, G.L. & Sanderson, S.C. 2010. Phylogeny of *Salsoleae* S.L. (Chenopodiaceae) based on DNA sequence data from its, Psbb-Psbh, and Rbcl, with emphasis on taxa of northwestern China. Plant Systematic and Evolution 288(1–2): 25–42.