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Original scientific article

PALYNOLOGICAL ANALYSIS SOME SPECIES OF CHENOPODIACEAE AND ITS SYSTEMATIC IMPLICATIONS USING SCANNING ELECTRON MICROSCOPY

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Jalilzadeh A., S. M. M. Hamdi, Y. Asri, M. Assadi, A. Iranbakhsh (2022). *Palynological analysis and genetic variations some species of Chenopodiaceae and its systematic implications using scanning electron microscopy*. - Genetika, Vol 54, No.2, 539-552.
Chenopodiaceae is a large, diverse and cosmopolitan family within the order Carophyllales. The majority of Chenopodiaceae species are adapted to saline, hypersaline, xerophytic and xerohalophytic communities or ruderal habitats. The family Chenopodiaceae is stenopolinous the pollen grains are monad and exclusively polypantoporate with tiny spinules on both tectum and operculum. Pollen morphology of six genera of Chenopodiaceae (*Seidlitzia*, *Atriplex*, *Bassia*, *Salsola*, *Krascheninnikovia* and *Spinacia*) have been studied in details. These plant species were collected from different phytogeographical regions of Iran. The palynological investigation was done using scanning electron microscopy (SEM) techniques. Pollen characters studied in this study include pollen and pore diameters, number and density of apertures, interporal distance (chord), chord/pollen diameter ratio, pore diameter/pollen diameter ratio as well as spinule density on tectum and operculum. We used different multivariate statistical methods to reveal the species relationships. Ward clustering analyses have been done to check out the relationship among the species. The shapes of pollen grains were radially symmetrical, isopolar, pantopolyporate and spheroidal. Their exine structure is similar. In the Chenopodiaceae, three pollen types have been defined, mainly on the basis of pollen grain size, pore number, number of microechinae on pore membrane, the density of spinules, and pore edge shape.
Key words: Chenopodiaceae, pollen morphology, systematics, phytogeographical regions

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INTRODUCTION

Members of the Chenopodiaceae are widely distributed in the steppe, desert, and saline–alkaline areas in southern Africa, Central Asia, South America, and Oceania, and along the coast of the Mediterranean, Caspian, and Red seas (KADEREIT *et al*., 2004; PERVEEN and QAISER, 2012; PUNSALPAAMUU *et al*., 2012). Chenopodiaceae is a large, diverse and cosmopolitan family within the order Carophyllales. The majority of Chenopodiaceae species are adapted to saline, hypersaline, xerophytic and xerohalophytic communities or ruderal habitats. Chenopodiaceae s.s., includes 105 genera and 1400 to 1700 species (HERNÁNDEZ-LEDESMA *et al*., 2015; AKHANI *et al*., 2016). The family has been subjected to extensive molecular studies during the past two decades, representing one of the most phylogenetically well-known families in Caryophyllales with seven distinct subfamilies including 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). The inclusion of Chenopodiaceae in its closely related family Amaranthaceae has also been suggested by many morphological, serological and molecular studies (e.g. MALLIGSON, 1922; JUDD *et al*., 2002; APG IV, 2016) although there are some recently published studies considering them as two separate families with quite different global distributions and habitat preferences (HERNÁNDEZ-LEDESMA *et al*., 2015). The family Chenopodiaceae is stenopolinous (ERDTMAN, 1969; LU *et al*., 2019), the pollen grains are monad and exclusively polypantoporate with tiny spinules on both tectum and operculum (NOWICKE, 1975). There are a few comprehensive pollen morphological studies in Chenopodiaceae treating the whole family (MONOSZON, 1951; DAMBACH, 1993), Suaedoideae (DEHGHANI and AKHANI, 2009) and Salsoloideae subfamilies (TODERICH *et al*., 2010) and Atripliceae tribe (OLVERA *et al*., 2006), resulting in important taxonomic, ecological and biological applications. Local studies focusing on pollen morphology of single or few genera of the family are also available (MCANDREWS and SWANSON, 1967; UOTILA, 1974; PERVEEN and QAISER, 2012). HAO *et al*. (1989) proposed a subdivision of Chinese Chenopodiaceae into five types (*Salicornia europaea* type, *Ceratoides latens* type, *Salsola collina* type, *Anabasis* type, and *Chenopodium album* type) based on pollen grain size, pore number, number of microechinae on pore membrane, the density of spinules, and pore edge shape.

Pollen morphology of 22 specimens of 9 species belonging to six genera in the subfamily Salsoloideae and Chenopodioideae (Chenopodiaceae), was studied using Light (LM) and Scanning Electron Microscopy (SEM) to evaluate the taxonomic importance of pollen characters. Pollen grains were characteristically pantopolyporate with 14-70 evenly distributed pores on the surface. The significant differences in pollen characters among populations of subfamily Salsoloideae and Chenopodioideae may indicate genetic diversity or polyploidy in different populations. In this study, we aim to evaluate the taxonomic importance of pollen morphology in reflecting phylogenetic relationships and delimiting genera and species of the subfamily Salsoloideae and Chenopodioideae from Iran.

MATERIAL AND METHODS

Pollen morphology of 22 specimens of 9 species belonging to six genera under the subfamily Salsoloideae and Chenopodioideae was studied using Light (LM) and Scanning

Electron Microscopy (SEM) (Table 1). Pollen morphological characters used in this study include pollen diameter, pore number, pore diameter, operculum diameter, chord distance, exine thickness, plus number and characteristics of exine spinules and operculum spinules. 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) (Table 1). The name of genera and species is based on latest accepted morpho-molecular concepts (KADEREIT *et al*., 2003; HERNÁNDEZ LEDESMA *et al*., 2015). In order to prepare the samples for SEM studies, pollen grains were mounted on carbon pasted stubs and covered with 10 nm gold using a Magnetron Sputter Coater. At least three micrographs of the whole pollen grains and a surface close up micrograph at ×10000 were taken for each sample with a Hitachi Su 3500 electron microscope (Central Laboratory at University of Shahid Beheshti, Tehran). The average density of spinules on 2µm2 of tectum area and the average number as well as density of spinules on operculum were counted and calculated based on three to five SEM observations. The visibility of perforations at ×10000 magnification was also documented (Table2). The nomenclature for description of the pollen morphology follows that of ERDTMAN (1969).

Table 1. List of the investigated taxa including origin of voucher specimens

Taxa	Locality	Subfamily	Collector & Voucher
Seidlitzia rosmarinus Bunge ex Boiss.	North Khorasan; Jajarm	Salsoloideae	Joharchi & Zangooei, FUMH, 41363
Spinacia turkestanica Iljin b	Razavi Khorasan; Sarakhs	Chenopodioideae	Ayatollahi & Zangooei, FUMH, 13908
Salsola arbusculiformis Drobow n	Razavi Khorasan; Kashmar	Salsoloideae	Joharchi, FUMH, 46172
Salsola kali L.	Semnan; Garmsar	Salsoloideae	Hamdi; IAUGH,20171
Atriplex leucoclada Boiss.	Semnan; Garmsar	Chenopodioideae	Hamdi; IAUGH,201109
Bassia scoparia (L.) A.J.Scott	Semnan; Garmsar	Chenopodioideae	Hamdi; IAUGH,20198
Atriplex griffithii Moq.	South Khorasan; Qaen	Chenopodioideae	Joharchi, FUMH, 34456
Atriplex moneta Bunge ex Boiss.	South Khorasan; Ferdows	Chenopodioideae	Raafei & Zangooei FUMH, 30665
Krascheninnikovia ceratoides (L.) *Gueldenst.*	Semnan; Garmsar	Chenopodioideae	Hamdi; IAUGH,21986

Pollen morphology of 22 specimens of 9 species belonging to six genera under the subfamily Salsoloideae and Chenopodioideae was studied using Light (LM) and Scanning Electron Microscopy (SEM) (Table 1). Pollen morphological characters used in this study include pollen diameter, pore number, pore diameter, operculum diameter, chord distance, exine thickness, plus number and characteristics of exine spinules and operculum spinules. 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) (Table 1). The name of genera and species is based on latest accepted morpho-molecular concepts (KADEREIT *et al*., 2003; HERNÁNDEZ LEDESMA *et al*., 2015). In order to prepare the samples for SEM studies, pollen grains were mounted on carbon pasted stubs and covered with 10 nm gold using a Magnetron Sputter Coater. At least three micrographs of the whole pollen grains and a surface close up micrograph at ×10000 were taken for each sample with a Hitachi Su 3500 electron microscope (Central Laboratory at University of Shahid Beheshti, Tehran). The average density of spinules on 2μm2 of tectum area and the average number as well as density of spinules on operculum were counted and calculated based on three to five SEM observations. The visibility of perforations at ×10000 magnification was also documented (Table2). The nomenclature for description of the pollen morphology follows that of ERDTMAN (1969).

Table 2. Details of SEM measurements (mean and standard deviation) in examined taxa of Chenopodiaceae

Species	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
Seidlitzia rosmarinus	17.9	3.62	3.08	19.3	14.3	14.1	0.16	3.4	0.80	0.20	0.85	6.27	0.17	0	0
Spinacia turkestanica	34.34	3.62	2.31	4.8	70.5	6.4	0.29	3.4	2.05	0.11	0.64	2.08	0.07	0	1
Salsola arbusculiformis	22.9	4.17	3.05	21.6	14.4	10.5	0.25	3.2	0.63	0.18	0.73	7.08	0.13	0	0
Salsola kali	17.06	2.9	2.09	16.2	37.8	18.4	0.15	4.2	2.22	0.17	0.72	7.75	0.12	0	1
Atriplex leucoclada	19.81	2.22	1.53	12.6	51.7	14.9	0.19	7.8	2.61	0.11	0.69	8.24	0.08	0	1
Bassia scoparia	20.31	2.81	3.7	9	64	12.5	0.19	7.2	3.15	0.14	1.32	2.43	0.18	0	1
Atriplex griffithii	20.38	3.86	1.71	10.7	47.8	14.7	0.2	4.8	2.35	0.19	0.44	6.26	0.08	0	1
Atriplex moneta	18.88	2.79	1.44	7.5	33.3	12.9	0.25	3.42	1.76	0.15	0.52	5.21	0.08	0	2
Krascheninnikovia ceratoides	29.04	3.11	2.02	10.8	65	15.6	0.2	4.4	2.24	0.11	0.65	5.35	0.07	0	0

Abbreviation: A (pollen size. μm); B (interporal distance. μm); C (pore diameter. μm); D (NMPM: number of microechinate on pore membrane); E (pore number); F (number of microechinate on 9μm^2 area of exine); G (microechinate height on exine surface. μm); H (pore number in 100μm^2 of exine); I (pore number/ pollen size); J (interporal distance/ pollen size); K (pore diameter/interporal distance); L (NMPM/pore diameter); M (pore diameter/pollen size); N (microechinate distribution: 0=separate, 1=fused); O (pore edge shape: 0=highly sunken, 1=sunken, 2=prominent).

Statistical analyses

The means and standard deviations of all data were calculated using Microsoft Excel software. Pore number estimation was obtained according to the formula provided by Campbell (1992) in which the number of pore or any other evenly distributed objects on a spherical surface equals: N = 540/[arcsin (C/D)] where N= number of pores, C= the distance between two adjacent pores and D= pollen diameter (Table 2).

The evaluated and measured characters of the species studied of subfamily Salsoloideae and Chenopodioideae are summarized in Tables 2. To detect significant differences in the studied characters among the various studied species, an analysis of variance (ANOVA) was performed. For multivariate analysis, the mean of the quantitative characters was used. Principal Components Analysis (PCA) was performed among the species to determine palynological characters useful for separating the species. In order to group the species, cluster analysis using WARD (Minimum spherical variance) methods and PCA ordination plot were performed by PAST software (HAMMER *et al*., 2001), using Euclidean and taxonomic distance among the species was calculated (PODANI, 2000). The qualitative traits were coded as binary or multistate. Variables were systematized for multivariate statistical analysis. Average taxonomic distances and squared Euclidean distances were applied as dissimilarity coefficients in the pollen data cluster analysis. Image Tool Version 3.0 (http://ddsdx.uthscsa.edu/dig/itdesc.html) was used to carry out the required measurements.

RESULTS

Infrageneric variation

We showed that *Salsola arbusculiformis* and *Seidlitzia rosmarinus* from subfamily Salsoloideae possess type I pollen grains. *Atriplex leucoclada, Atriplex griffithii*, *Atriplex moneta* had type II pollens. Species, i.e., *Bassia scoparia*, *Krascheninnikovia ceratoides* and *Spinacia turkestanica* belonging to subfamily Chenopodioideae showed the type III of pollen grains (Fig1).

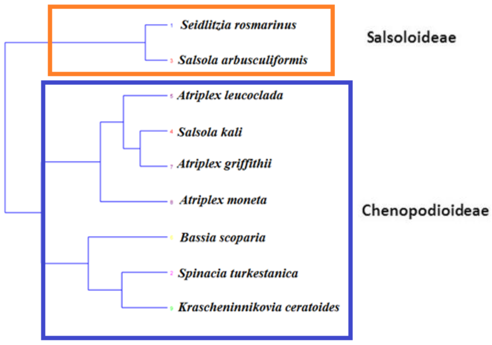

Figure 1. Ward clustering of Chenopodiaceae species.

Factor analysis shows three factors explained more than 81% of the total observed variation in studied pollen grains. The first factor revealed pollen diameter, pore number, pore diameter, operculum diameter, chord distance, exine thickness, plus number and characteristics of exine spinules and operculum spinules illustrated more than 43% of the observed variation in the second factor. Principal component analysis (PCA) based on pollen grain qualitative and quantitative traits confirms the cluster analysis results by Ward’s method (Fig2).

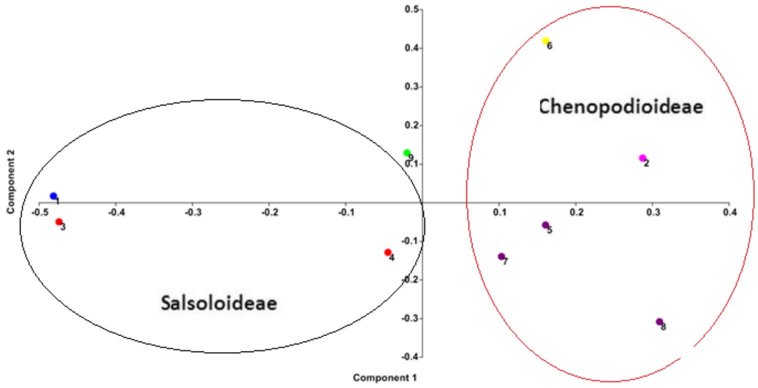

Figure 2. Principal component analysis (PCA) of Chenopodiaceae species based on observed pollen data.

General pollen grain features

The SEM micrographs of six genera and 9 species of Chenopodioideae and Salsoloideae subfamily from Iran are presented in Figs 3-4. Table 1 represents all studied species/populations in this study and distribution ranges. Pollen grains in all studied samples of the subfamily are monad, pantopolyporate, tectate, spheroidal in shape, with pores distributed evenly on the tectum. Tectum and operculum are covered with spinules, Pollen size averages of different species range from 17.06 to 34.34 µm in diameter and average pore numbers vary from 14 to 70 in examined species of these subfamily. The means and standard deviations of pollen character measurements from electron microscopy are presented in Tables 2. A summary of pollen morphological results and important findings for each genus is also provided in the same table.

Seidlitzia rosmarinus Bunge ex Boiss. (Fig. 3 A1-A2).

Pollen morphology of five accessions of the Iran, *Seidlitzia rosmarinus* studied by electron microscopy, showed a continuous variation in terms of size, pore number and the distance between adjacent pores. Pollen diameter ranges from 17.2-17.9 µm, pore number varies from 14.1-14.5 and chord distance is between 3.2 and 3.9 µm in five examined accessions (Table 2). According to electron microscopy images of the sample number of echinate on 3 μm^2 area of exine for this species are 14.1 respectively, which are the lowest numbers of pore compared to all other studied species in the subfamily (Table 2).

Spinacia turkestanica Iljin (Fig. 3 B1-B2).

Six representatives of the widely distributed species *Spinacia turkestanica* were examined in this study. Pollen diameter in populations of this species ranges from 33 to 34.87 µm, pore number varies from 70-71, and chord distance is 3-3.9 µm (Table 2). The sample collected from Razavi Khorasan; Sarakhs (Ayatollahi & Zangooei, FUMH, 13908) shows larger pollen with bigger pores, longer chord distance and thicker exine compared with the other genus that show insignificant variations in pollen traits among them (Table 2). Pollen in this genus represents the biggest pollen and pore sizes and the highest pore number on the tectum comparing to all other studied samples in the subfamily.

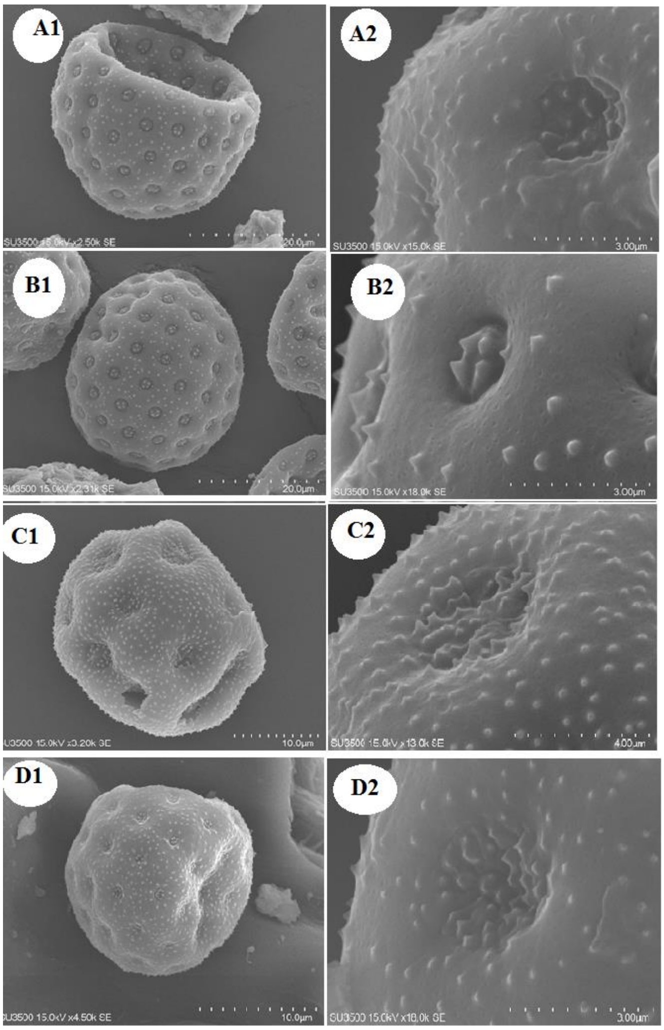

Fig. 3. Scanning electron microscopic micrographs of the pollen grains Chenopodiaceae :A1-A2: *Seidlitzia rosmarinus*, B1-B2: *Spinacia turkestanica*, C1-C2: *Salsola arbusculiformis*, D1-D2: *Salsola kali*

Salsola arbusculiformis Drobow (Fig. 3 C1-C2).

Pollen diameter in *Salsola arbusculiformis* ranges from 21.11- 23.14 μm, pore number varies from 13 to 14, and chord distance is 4.1-4.3 μm (Table 2). The average number of echinate on 3 μm^2 area of exine for this species vary from 10 to 10.8, respectively (Table 2). Pollen in this species shows the lowest numbers of pore in comparison with other studied species (Table 2).

Salsola kali L. (Fig. 3 D1-D2).

Pollen grains radial symmetrical, isopolar, pantopolyporate, spheroidal, pollen diameter (A) 17.06 mm. Pores 2.09 mm (C) in diameter and circular. 16.02 conical spinules on operculum. Distance between the centers of the adjacent pores (B) 2.9 mm. Pore number 37.8. Ornamentation scabrate; The average number of echinate on 3 μm^2 area of exine for this species vary from 17 to 18.4, tectal spinules conical, 0.15 mm high, 0.2 mm wide. Pollen in this species shows the lowest size of pollen and microechinate high on exine surface in comparison with other studied species (Table 2).

Atriplex leucoclada Boiss. (Fig. 4 E1-E2).

Pollen grains radial symmetrical, isopolar, pantopolyporate, spheroidal, pollen diameter (A) 19.81 mm. Pores 1.53 mm (C) in diameter and circular. Operculum 2.3 mm (E) wide; 0.38 mm high, 12.6 conical spinules on operculum. Distance between the centers of the adjacent pores (B) 2.22 mm. Pore number 51.7. Ornamentation scabrate; The average number of echinate on 3 μm^2 area of exine for this species vary from 14.2 to 14.9, tectal spinules conical, 0.19 mm high, 0.2 mm wide. Pollen in this species shows the the smallest size of pore in comparison with other studied species (Table 2).

Bassia scoparia (L.) A.J.Scott (Fig. 4 F1-F2).

Pollen grains radial symmetrical, isopolar, pantopolyporate, spheroidal, pollen diameter (A) 20.31 mm. Pores 3.7 mm (C) in diameter and circular. Operculum 2.2 mm (E) wide; 0.32 mm high, 9 conical spinules on operculum. Distance between the centers of the adjacent pores (B) 2.81 mm. Pore number 64. Ornamentation scabrate; The average number of echinate on 3 μm^2 area of exine for this species vary from 12 to 12.5, tectal spinules conical, 0.19 mm high, 0.2 mm wide. Pollen in this species shows the highest numbers of pore in comparison with other studied species (Table 2).

Atriplex griffithii Moq. And *Atriplex moneta* Bunge ex Boiss. (Fig. 4 G1-G2; H1-H2).

Three species of this Eurasian genus with four representatives were included in our study. Pollen diameter ranges from 18.88 to 20.38 μm, pore number varies between 33 and 47, and chord distance is 2.79-3.86 μm (Table 2). *Atriplex moneta* shows the smallest size of pore in the whole subfamily. The two studied species are distinguishable based on pollen diameter (18.88 μm in *Atriplex moneta* and 20.38 μm in *Atriplex griffithii)* and pore densities in 10 μm2 of exin (3.4 in *Atriplex moneta* and 4.8 in *Atriplex griffithii*).

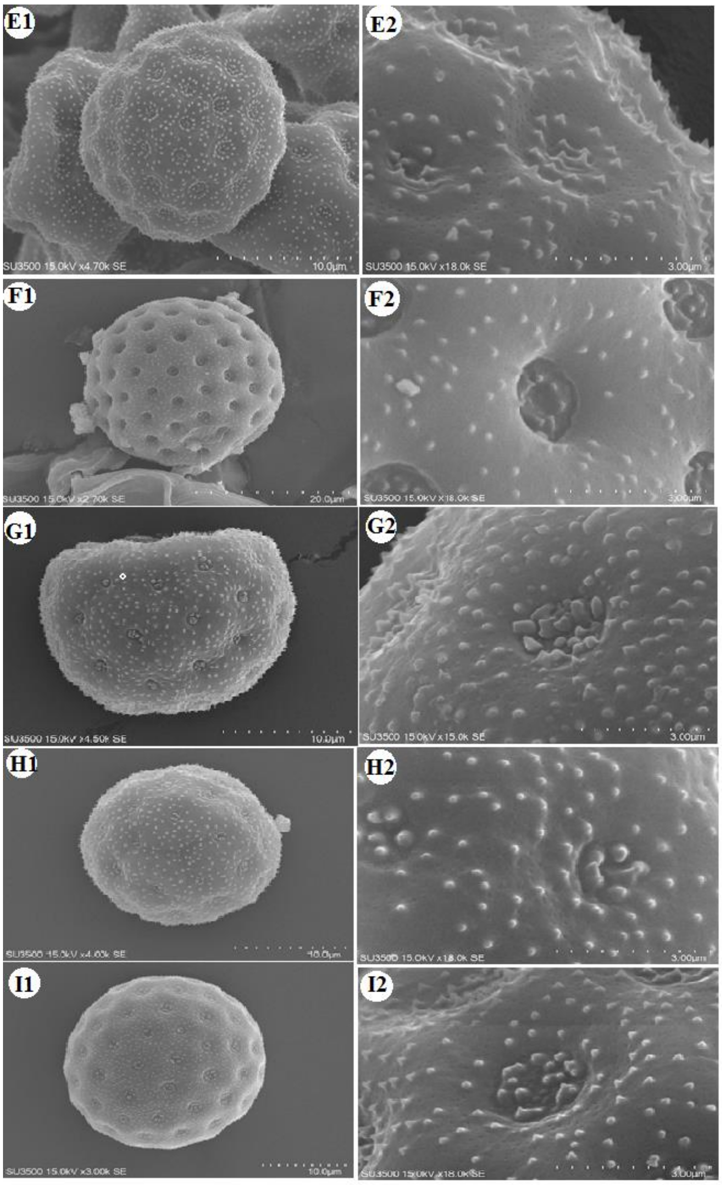

Fig. 4. Scanning electron microscopic micrographs of the pollen grains Chenopodiaceae :E1-E2: *Atriplex leucoclada,* F1-F2: *Bassia scoparia,* G1-G2: *Atriplex griffithii*, H1-H2: *Atriplex moneta*; I1-I2:*Krascheninnikovia ceratoides*

Krascheninnikovia ceratoides (L.) *Gueldenst.* (Fig. 4 I1-I2).

Pollen grains radial symmetrical, isopolar, pantopolyporate, spheroidal, pollen diameter (A) 29.04 mm. Pores 2.02 mm (C) in diameter and circular. Operculum 2.6 mm (E) wide; 0.34 mm high, 10.8 conical spinules on operculum. Distance between the centers of the adjacent pores (B) 3.11 mm. Pore number 65. Ornamentation scabrate; The average number of echinate on 3 μm^2 area of exine for this species vary from 15 to 15.6, tectal spinules conical, 0.2 mm high, 0.2 mm wide.

DISCUSSION

Members of the Chenopodiaceae are the most dominant elements in the central Asian desert. The different genera and species within this family are common in desert vegetation types. Should it prove possible to link pollen types in this family to specific desert vegetation, it would be feasible to trace vegetation successions in the geological past. Nevertheless, the morphological similarity of pollen grains in the Chenopodiaceae rarely permits identification at the generic level. Although some pollen classifications of Chenopodiaceae have been proposed, none of them tried to link pollen types to specific desert vegetation types in order to explore their ecological significance.

Pollen grains of *Chenopodium* species are radially symmetrical, isopolar, pantopolyporate and spheroidal. Their exine structure is similar under LM and TEM. Especially the number of pores and the C/D ratio on periporate pollen have been used as diagnostic character for taxonomic and pollen analytical purposes in the genus *Chenopodium* (MONOSZON, 1951). The C/D ratio is used by MC ANDREWS and SWANSON (1967) when they studied 74 North American sampled of 35 *Chenopodium* species. They found variations in the C/D ratio of the *Chenopodium* samples collected from different localities. UOTILA (1974) pointed out the importance of the C/D ratio and pore number in the *Chenopodium* species. We also gave variation in C/D ratio corresponding pore number according to MC ANDREWS and SWANSON (1967) and polen size in Fig 3-4. The figure shows no correlation between pore number and pollen size of the species examined for the present study. Though the majority of the species have pollen 18-20 μm indiameter. This research suggests that there are significant differences in pollen size and pore number within studies species.

The application of pollen characters is limited in the taxonomy of the highly complicated groups under Salicornioideae (DEHGHANI *et al*., 2020). However, there are hints that indicate some pollen grain traits do correlate with habitat conditions. For example, in a study of pollen characters in the Suaedoideae subfamily it was found that very often higher numbers of pores are positively correlated with wet saline soils. Furthermore, it has been shown that C3 taxa in *Suaeda* Forssk. Ex J.F. Gmel. growing in wetter soils are characterized by higher pore numbers (DEHGHANI and AKHANI, 2009). The most common pollen types in Angiospersms are monocolpate (in monocots) and tricolpate (in dicots). While exine provides protection for the pollen grains, apertures play three major roles including water and gas exchange with the environment, pollen tube germination and harmomegathy (WODEHOUSE, 1935). The last is a process in which pollen shape changes to adapt to the smaller or larger size of cytoplasm caused by dehydration or hydration (WODEHOUSE, 1935; PUNT *et al*., 2007) with apertures playing the most important role (HESLOP-HARRISON, 1979). Phylogenetically, the pantoporate pollen type is a derived form with at least 66 independent origins, occurring in 57 families of angiosperms particularly in the species of Caryophyllales (PRIEU *et al*., 2017). PRIEU *et al*. (2017) argue that pantoporate pollen distribution among angiosperms comes from positive short-term selection (such as accelerating water absorption and hence pollen germination) and negative longterm selection (for example the possibility of pollen desiccation during pollination) since this pollen type occurs frequently but with limited diversification and hardly fixed in large taxonomic groups. Multiple origins of pantoporate pollen is explained by the unique mechanism in which position and number of future apertures are determined by callose deposition (ALBERT *et al*., 2014) but reversion to the ancestral state is almost impossible (MATAMORO-VIDAL *et al*., 2016).

CONCLUSIONS

We conclude that pollen characters are of limited taxonomic value in Salicornioideae subfamily due to high similarity in pollen morphology, however, current taxonomic relationships and delimitations of the genera are relatively shown in multivariate analysis of pollen characters.

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PALINOLOŠKA ANALIZA NEKIH VRSTA CHENOPODIACEAE I NJIHOVE SISTEMATSKE IMPLIKACIJE UTVRĐENE ELEKTRONSKOM MIKROSKOPIJOM

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Izvod

Chenopodiaceae je velika, raznolika i kosmopolitska familija u okviru reda Carophillales. Većina vrsta Chenopodiaceae prilagođena je slanim, hipersalinastim, kserofitnim i kserohalofitnim zajednicama ili ruderalnim staništima. Detaljno je proučavana morfologija polena šest rodova Chenopodiaceae (Seidlitzia, Atriplek, Bassia, Salsola, Krascheninnikovia i Spinacia). Ove biljne vrste su sakupljene iz različitih fitogeografskih regiona Irana. Palinološko ispitivanje je obavljeno tehnikama skenirajuće elektronske mikroskopije (SEM). Karakteristike polena proučavane u ovoj studiji uključuju prečnik polena i pora, broj i gustinu otvora, interporalno rastojanje (tetivu), odnos tetive/prečnika polena, odnos prečnika pora/prečnika polena, kao i gustinu spinule na tektumu i operkulumu. Koristili smo različite multivarijantne statističke metode da bismo otkrili odnose vrsta. Urađena je i klaster analiza po Ward-u da bi se proverio odnos između vrsta. Oblici polenovih zrna bili su radijalno simetrični, izopolarni, pantopoliporatni i sferoidni. Kod Chenopodiaceae su definisana tri tipa polena, uglavnom na osnovu veličine zrna polena, broja pora, broja mikroehina na membrani pora, gustine spinula i oblika ivice pora.

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