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ROLE OF SEED MICRO-MORPHOLOGY IN THE TAXONOMY OF SAPONARIA (CARYOPHYLLACEAE)

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Saponaria species are morphologically very similar so that their taxonomy has been a challenging subject for a long time. In order to clarify the taxonomy of the genus, materials deposited in the herbaria (TARI, IRAN and FUMH) of Iran were examined. Seed micro-morphology of 10 speceis of the genus *Saponaria* L. (Caryophyllaceae) were studied. Averag Linkage clustering filamentograms based on about 14 quantitative and qualitative traits were identified and coded to be very close to morphological cluster phonograms, and quantitative traits related to the seeds were carefully measured using the software, also PCA analysis was performed and the most variable characteristics were determined, including seed shape, cell shape and, cell margin shape, length to grain width ratio and length to cell width ratio. The micro-morphological characters of the seeds were evaluated as possible taxonomical characteristics of the species. Seed micro-morphology varied among the species and its taxonomic value was significant in distinguishing taxa at the species level.

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Key words: Caryophyllaceae; S*aponaria*; seed micro-morphology; taxonomy; Iran

ریزریخت‌شناسی دانه و نقش آن در تاگزونومیکی گونه‌های جنس Saponaria از تیره میخک

عالمه دشتی: مؤسسه تحقیقات جنگل‌ها و مراتع کشور، سازمان تحقیقات و آموزش و ترویج کشاورزی، تهران، ایران

گونه‌های Saponaria از نظر مورفولوژیکی بسیار مشابه هستند به طوری که تفکیک تاگزونومیکی آنها یک موضوع چالش برانگیز برای مدت زمان طولانی بوده است. به منظور روشن ساختن تاکسونومی این جنس در ایران نمونه‌های هرباریومی (هرباریوم مرکزی ایران (TARI)، مؤسسه تحقیقات گیاهپزشکی (IRAN) و دانشگاه مشهد (FUMH) مورد مطالعه قرار گرفتند. صفات ریزریخت‌شناسی دانه از ۱۰ گونه از جنس .Saponaria L از تیره Caryophyllaceae مورد بررسی قرار گرفتند. صفات کمی مربوط به دانه با استفاده از نرم‌افزار measurement به دقت اندازه‌گیری شدند. فنوگرام خوشه‌ای به روشAverag Linkage بر اساس حدود ۱۴ صفت کمی و کیفی که با کد (۰و۱) مشخص شدند سپس آنالیز PCA انجام گرفت و متغیرترین صفات مشخص شدند که مهترین صفات تفکیک کننده شامل شکل دانه، شکل تزئینات سلول، شکل حاشیه سلول و صفات کمی شامل نسبت طول به عرض دانه، نسبت طول به عرض سلول بودند. این فنوگرام تا حدود زیادی مطابق با فنوگرام خوشه‌ای مرفولوژی رده‌بندی می‌شود و خصوصیات ریزریخت‌شناسی دانه‌ها به عنوان خصوصیات تاکسونومیکی گونه مورد ارزیابی قرار گرفت. صفات ریزریخت‌شناسی دانه در میان گونه‌ها متغیر بود و ارزش تاکسونومی آن در تاکسون‌ها در سطح گونه معنی‌دار بود.

INTRODUCTION

The family Caryophyllaceae includes three subfamilies: Alsinoideae Burnett, Caryophylloideae Arn. and Paronychioideae A. St. Hil. ex Fenzl (Bittrich, 1993). The genus *Saponaria* is a member of Cayophylloideae subfamily with about 40 species,

found in temperate Eurasia, mainly in the Mediterranean region. This genus was formerly represented in Iran by eight species, distributed in north (Gilan, Mazandaran and Golestan), north east (Khorasan), northwest (Azerbaijan, Zanjan) and west (Hamedan and Kurdestan), south (Kerman, Hormozgan and Sistan-Baluchestan) and in center (Tehran, Markazi, Yazd and Qazvin Provinces). According to the study of herbarium species, the species of this genus are distributed in these areas. More than half of the species occurring in Iran are reported from the north and north-east of the country (Rechinger, 1988). Most of the Iranian *Saponaria* species are morphologically similar, therefore their taxonomy has been controversial for long time. The importance of seed micromorphology in classification of taxa in Caryophyllaceae has long been recognized by different authors (Barkoudah, 1962; Amini & al. 2011; Mostafavi & al. 2012; Moore & Webb. 1978; Punt & Hoen. 1995; Pax & Hoffman. 1934; Yildiz. 2002. Bittrich (1993) belived that seed micro-mophology, especially the cell surface shape, is a valuble diagnostic character for distinguishing taxa at species level in the family. In Caryophyllaceae, seeds are small in size (0.4-3 mm long) and they are black, brown, or nearly white in color (Bittrich, 1993). Their shapes are different (i.e. reniform, pyriform, or orbicular) and they are rarely winged (Bittrich, 1993). The testa is variously sculptured by papilliform cells, but they are rarely completely smooth (Bittrich 1993). A few seed micromorphological studies have been performed on the subfamily Alsinoideae (including the genera *Sagina* L. (Crow, 1979), Arenaria L. (Wofford, 1981) and *Velezia* L. (Poyraz & Ataslar 2010).

According to Simmler (1910), *Saponaria* is divided to two subgenera including *Saponariella* Simmler and *Saporhizaea* Simmler.

According to the latest morphological studies, the genus *Saponaria* in Iran comprises 10 species (Dashti & al., 2014). In this study, seed micromorpholpgy of 10 *Saponaria* species belonging to two subgenera are investigated to evaluate its taxonomic value.

MATERIALS AND METHODS

Seeds were taken from the herbarium material deposited in TARI, IRAN, FUMH,. The specimens were studied using Flora Iranica (Rechinger 1988), Flora de I Iran (Parsa 1951), Folra of Turkey (Hedge 1967), Flora of the USSR (Gorshkova 1936), Flora Europaea (Chater, 1964) and Monographie der Gattung *Saponaria* (Simmler 1910).

The list of the specimens and their localities are shown in table 1. Seeds were stabilized on aluminum stubs and coated with a thin layer of gold. Then, the specimens were studied using scanning electron microscope (SEM), model EM 3200. Micro-morphological measurement were calculated using Carnoy, a digital measurements software (Scholes & al., 2002). Some characters including seed length and width, cell length and width, seed shape, cell shape, cell ornamentation, and cell margin were measured. Averag Linkage clustering filamentograms based on about 14 quantitative and qualitative traits were identified and coded. Also PCA analysis was performed and the most variable characteristics were determined.

Table1. The list of the *Saponara* species used for seed micro-morphological study and their localities.

Taxa	Localities
Saponaria officinalis L.	Tehran: Azadshahr, National botanical Garden, 1320 m, Dashti 98960 (TARI)
S. Bodeana Boiss.	Khorasan: Between Bojnurd and Golestan Forest, Slope N. Kuhe Kurkhod, 2000-2700 m, Assadi & Hamdi 85595 (TARI)
S. cerastioides Fisch. ex C. A. Mey.	Mazandaran: Savad kuh, 1983 m, Zabihi 43505 (TARI)
S. esfandiarii Assadi	Hormozga: Bandar Abbas, Ghotbadad, Baghestan, Damtang village, Baz Mnt. 500-2000 m, Mozaffarian 49988 (TARI)
S. viscosa C. A. Mey.	Azerbaijan: Uromie lake, Jazire Ashak, Zehzad 83311 (TARI)
S. orientalis L.	Azerbaijan: 2 km N. E. Khalkhal, 1800-2000 m, Mozaffarian & Nowrouzi 34060 (TARI)
S. iranica Dashti, Assadi & Sharifnia	Kerman: Baft, Hararan, 2900 m, Yazdani 4956 (IRAN)
*S. floribunda (*Kir. & Kar.) Boiss.	Yazd: 50 km E. Bafgh, Mt. Hamsuk village, 2300-2600 m, Assadi & Bazgosha 56090 (TARI)
S. makranica Rech. f.	Esfahan: 6 km from Naein to Anarak, 1440 m, Mozaffarian 79240 (TARI)
S. kermanensis Bornm.	Kerman: Lalehzar, Baghabad, 2600 m, Foroughi & Assadi 17893 (TARI)

RESULTS

Three different seed shapes including renifom-elliptic, reniform-circular and reniform-pyriform were observed in the *Saponaria* species examined. Most of the species, including *S. cerastioides* Fisch. ex C. A. Mey., *S. viscosa* C. A. Mey., *S. orientalis* L. and *S. kermanensis* Bornm., have reniform- circular seeds. Also the species of *S. officinalis* L., *S. bodeana, S. iranica* Dashti, Assadi & Sharifnia and *esfandarii* Assadi have reniform-elliptic seeds. Seed shapes in only two species including *S. floribunda* (Kir. & Kar.) Boiss. and *S. makranica* Rech. f. are reniform-pyriform (tables 2 and 3; figs. 3-4).

Seed ornamentations were verrucate, verrucate-reticulate and rugose., Most of the investigated species had verrucate ornaments (*S. orientalis, S. kermanensis, S. makranica, S. floribunda,* S. *iranica, S. esfandiarii, S. ceratioides and S. viscosa*), S. *officinalis* (with verrucate-reticulate ornamentation) and *S. bodeana* (with rugose ornamentation) Table 3; figs. 3-4

Among the studied species, *S. viscosa* (0.94×0.96 µm) and *S. makranica (*44.68×33.37 µm) have the smallest and largest seed length and width respectively (figs 3 and 4; table 2). The largest cell length (216.65 µm) was observed in *S. kermanensis* and the smallest cell length (0.73) in *S. bodeana.* The largest cell width (133.40 µm) was observed in *S. kermanensis* and the smallest cell width in *S. bodeana* (figs. 3 and 4; table 2).

Seed color in most cases were dark brown, while in *S. bodeana* was light brown and blackish brown in *S. officinalis* and *S. cerastioides* (table 3).

Five different seed cell shapes including oblong, elongated oblong, linear oblong, polygonal and elongated polygonal were found in the *Saponaria* species examined. *Saponaria officinalis*, *S. orientalis* and *S. kermanensis* were oblang and *S. viscosa* was elongated oblang. While in *S. floribunda* was linear. *S. bodeana, S. cersatioides* were polygonal, *S. esfadiarii* and *S. makranica* elongated polygonal (tabel 3, figs 3-4).

Two different seed cell margine including dentate and sinuate were found in the *Saponaria* species examined, most of the species, including *S. officinalis, S. viscosa, S. orientalis, S. iranica, S. floribunda* and *S. kermanensis* have dentate seed cell margin, while in *S. bodeana*, *S. cerastioides*, *S. esfandiarii* and *S. makranica* have sinuate seed cell margin (table 3, figs 3-4)

Selected seed micro-morphological characters of possible taxonomic importance in the examined taxa are summarized in the tables 2 and 3, selected SEM micrographs of the seeds and their surfaces are shown in figs. 3-4. Some characters of the seeds were variable among diferent species of *Saponaria*, like for example: shape, color and margin of the testa cells. A set of characters were found as distinctive for the species (tables 2, 3) as described in detail below. The result show that, with draw of the results of the analysis seed micro-morphology have significant role in identification of species.

Table 2. Comparison of seed micro-morphological quantitive characters in *Saponaria* species. Abbreviations: L, seed length; W, seed width; CL, cell length; CW, cell width; CD, cell distance; N, number of ornamentation in 0/5 micron.

Taxa	**Characters**							
	L (µM)	w (µm)	L/w	CL (µm)	CW (µm)	CL/CW	CD (µm)	N
S. officinalis	1.74	1.60	1.08	108.84	42.50	2.56	17.51	5
S. bodeana	1.20	1.18	1.01	0.73	0.51	1.43	8.13	3
S. cerastioides	1.12	1.06	1.05	164.01	64.07	2.55	4.48	8
S. esfandiarii	34.02	31.29	10.08	17.54	8.76	2.002	8.21	14
S. viscosa	0.94	0.96	1.02	152.86	42.72	3.57	6.34	6
S. orientalis	1.09	1.03	1.05	101.12	87	1.16	28.43	5
S. iranica	1.15	0.88	1.30	150.03	66.02	2.27	2.47	5
S. floribunda	1.10	0.71	1.54	173.63	30.32	5.72	4.95	14
S. makranica	46.68	33.37	1.39	12.11	34.11	3.52	4.17	14
S. kermanensis	1.16	1.18	0.98	216.65	133.40	1.62	5.8	4

Table 3: Comparision of seed micropmorphological data in *Saponaria* species (qualitative characters). Abbreviations: S, shape; O, ornamentation; SC, seed color; CS, cell shape; CM, cell margine.

Taxa	**Characters**				
	S	**O**	**SC**	**CS**	**CM**
S. officinalis	reniform-elliptica	vrerrucate-reticulate	blackis-brown	oblang	dentate
S. bodeana	reniform-elliptica	rugose	light-brown	polygonal	sinuate
S. cerastioides	reniform-circular	verrucate	blackish-brown	polygonal	sinuate
S. esfandiarii	reniform-elliptica	verrucate	dark-brown	elongated-polygonal	sinuate
S. viscosa	reniform-circular	verrucate	dark-brown	elongated-polygonal	dentate
S. orientalis	reniform-circular	verrucate	dark-brown	oblang	dentate
S. iranica	reniform-elliptica	verrucate	dark-brown	elongated-polygonal	dentate
S. floribunda	reniform-pyriform	verrucate	dark-brown	linear oblang	dentate
S. makranica	reniform-pyriform	verrucate	dark-brown	elongated-polygonal	sinuate
S. kermanensis	reniform-Circular	verrucate	dark-brown	oblang	dentate

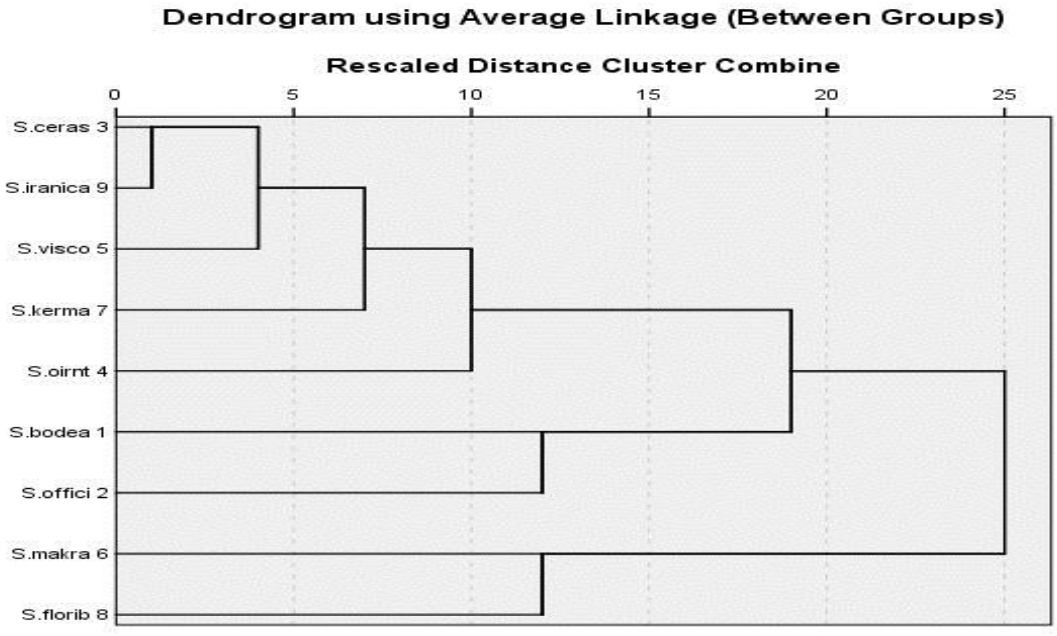

Fig. 1: Clustering phenogram on the basis of quantitative and qualitative characters of seeds of *Saponaria* species (abbreviations: S. ceras= *S. cerastioides*; S. new= *S. iranica*; S. visco= *S. viscosa*; S. kerma= *S. kermanensis*; S. orient= *S. orientalis*; S.bodea= *S. bodeana*;, S. offici= *S.officinalis*; S. makra= *S. makranica*; S. florib= *S. floribunda*.

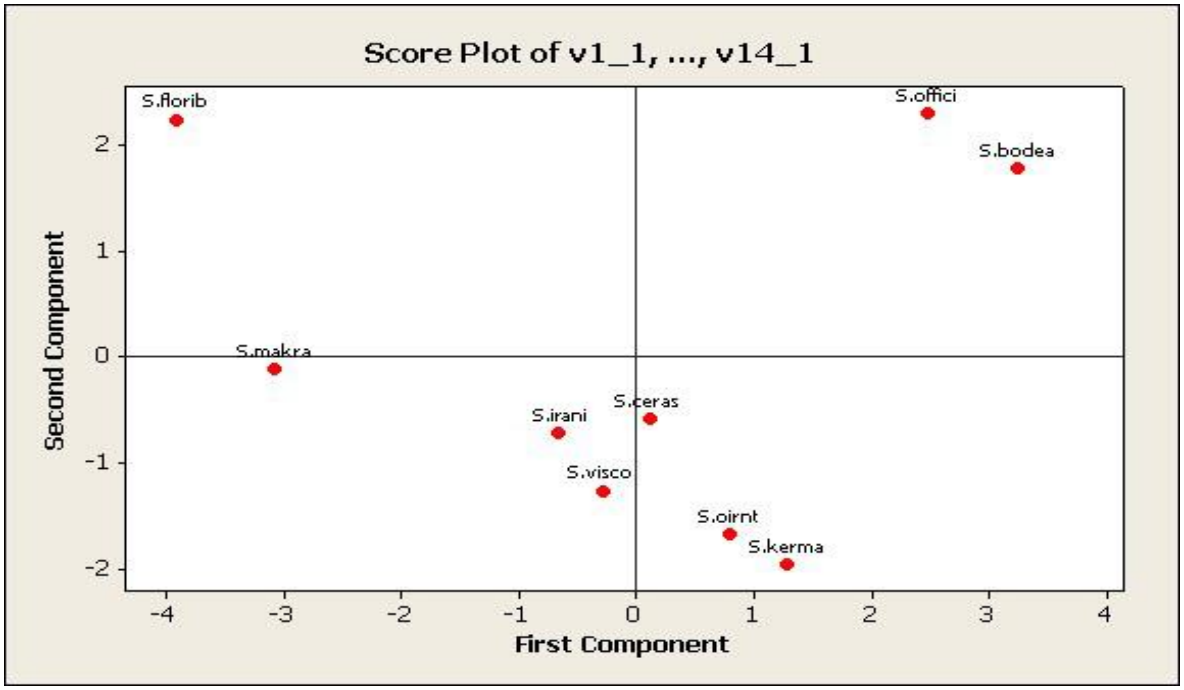

Fig. 2: Parameters of main component 1 and 2 (PCA) based on quantitative and qualitative seeds data of *Saponaria* species.

Tabel 4. The comparison of the variance between 14 quantittive and qualitative characters by use of principal component Analysis extraction method (In factor analysis, the main component of the first with the highest of variance was 39.496 0/0 and the main component of the second with the variance of 20.136 0/0 had the highest share in the estimation of similarities and differences between species).

Component Matrix[a]		
	Component	
	1	2
v1=length seed (L)	-.304	.820
v2=wight seed (w)	.184	.930
v3=L/W	.934	.085
v4=cell length (CL)l	.204	-.089
v5=cell wight (CW)	-.271	-.112
v6=CL/CW	.894	.186
v7=N (number of ornamentation in 0/5 mic)	.956	-.149
v8=D (distance between cells)	-.320	.157
v9=seed shape (Ss)	.825	-.399
v10=color seeds (C)	.464	-.595
v11=seed cells (S)	.732	.073
v12=center cell (CC)	.159	-.669
v13=margin of cell (CM)	.079	.386
v14=ornamentation seed (O)	.930	-.072

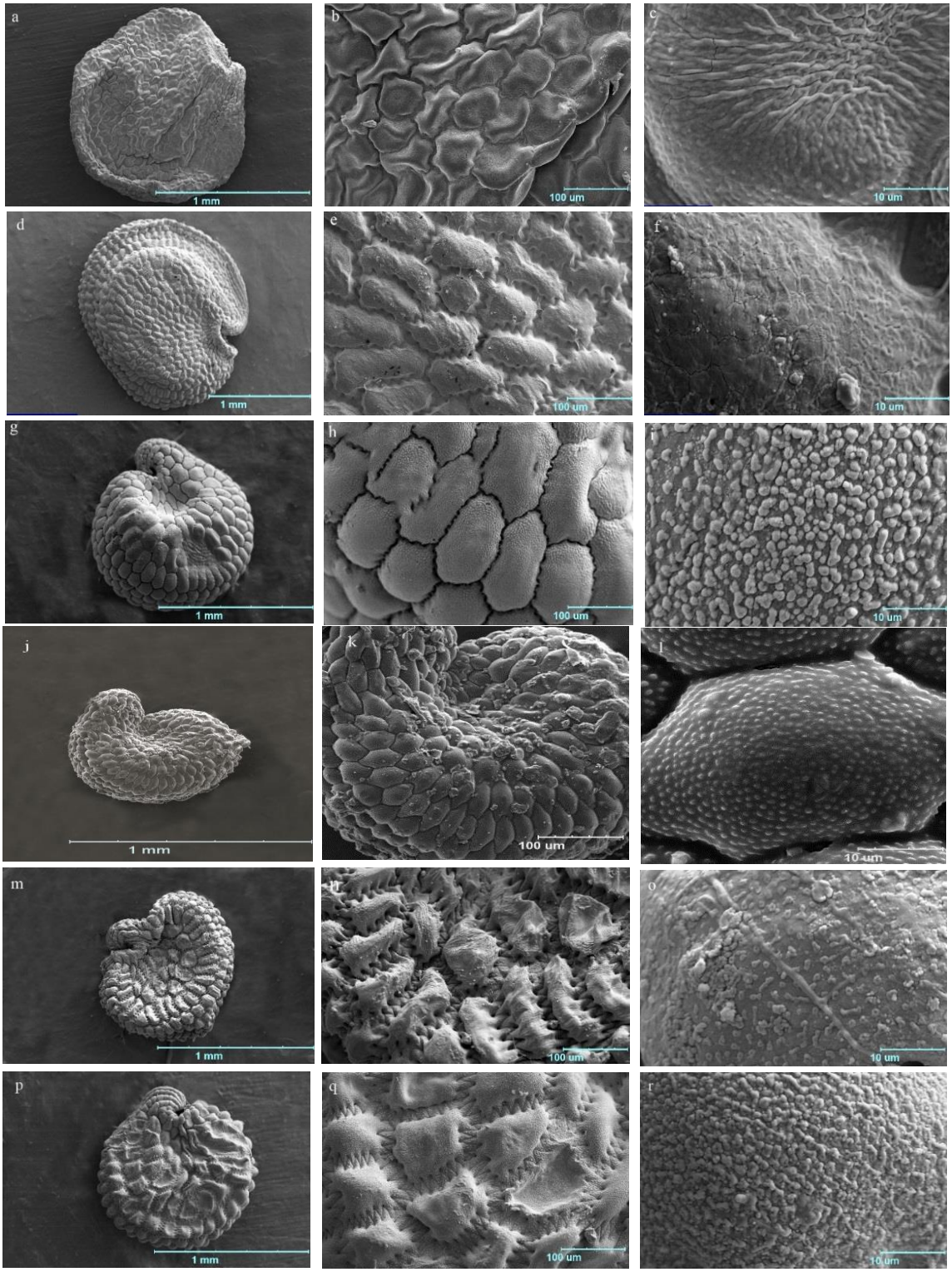

Fig. 3: SEM micrographs of seed micro-morphological characteristics of *Saponaria* species: a, d, g general appeance; b, e, h-testa cells; c, f, I cell shape. a, b, c (*S. bodeana*); d, e, f (*S. officinalis*) and g, h, I (*S. cerastioides*); j, m, p-gereral appearance; k, n, q, testa cells; I, o, r shape cells; j, k, l (*S. esfandarii*); m, n o (*S. viscosa*) and p, q, r (*S. orientalis*).

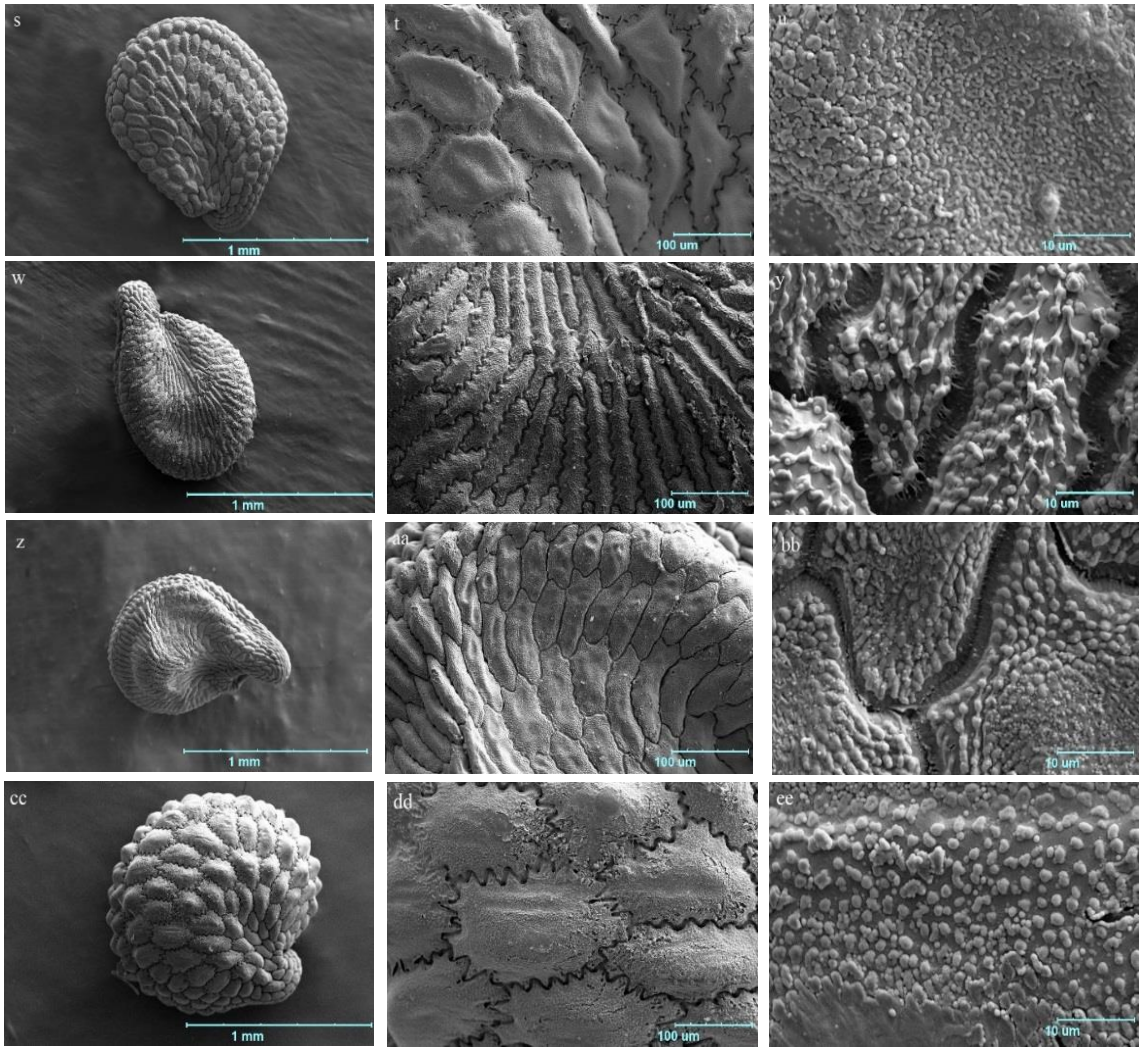

Fig. 4: SEM micrographs of seed micro- morphological charateristics of *Saponaria* species: s, w, z-general appearance; t, x, aa-testa cells and u, y, bb Shape cells; s,t,u (*S. iranica*); w, x, y (*S. floribunda*); and z, aa, bb (*S. makranica*); cc, general appearance; dd, testa cell; ee, shape cell *(S. kermanenesis*).

DISCUSSION

According to the current study, the seeds of *Saponaria* species bear several micro-morphological characters that can be used in the taxonomic treatments.

Considering some basic differences in morphological and micro-morphological characters, *Saponaria bodeana* and *S. officinalis* differ from the rest of species by having petal lamina with two coronal scales at the base (Hedge, 1967; Dashti & al; 2014). These two species differ from the other species in seed color and seed size (fig. 3; table 3). *Saponaria makranica* and *S. floribunda* are similar in seed shape but they differ in seed cell shape and cell margin. Three annual species including *S. viscosa*, *S. iranica* and *S. orientalis* have many similarities in morphological characters (Dashti & al. 2014) which make their recognition difficult, but they show different seed micromorphology. The genera *Gypsophila* and *Ankyropetalum* are most closely related to *Saponaria*, (Simler 1910, Barkouda). The species of *Saponaria* studied here show different patterns of seed surface sculpturing but do not show any distinct protuberances on the cells composing the seed surface. Some of *Gypsophila* specie are morphologically very similar to the species of *Saponaria*, but based on the key to the genera provided by Barkuda, the surface of the seed of *Ankyropetalus gypsophiloides* are composed of elongated polygonal cells with deeply undulate anticlinal walls and distinct spherical protuberance. This is common type of seed micro-scupturing found in

Gypsophila too, (Amini & al 2011) and *Saponaria esfandiarii*, *S. makranica* and *S. iranica*, but they differ in the seed shape and cell margin shape. The results of this study revealed that reniform- elliptical, circular and pyriform seed shapes with elongated polygonal, oblang, linear oblang and polygonal cells as well as cell ornamentation types separate *Saponaria* from *Gypsophila*.

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