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یافته‌های نوین در علوم زیستی
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انتشارات دانشگاه خوارزمی

گزارشی از صفات ریزریخت‌شناسی دانه و کپسول در سرده *Scrophularia* (تیره گل‌میمونیان) در ایران

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چکیده. در مطالعه حاضر، تزئینات سطح دانه در ۳۱ گونه (شامل ۳۴ جمعیت) و تزئینات سطح کپسول در پنج گونه از سرده *Scrophularia* پراکنده در ایران، با استفاده از میکروسکوپ الکترونی نگاره (SEM)، مورد بررسی قرار گرفت. سطح دانه در تمام نمونه‌های مورد مطالعه، به صورت مشبّک و دارای تزئینات داخلی نردبانی‌شکل است. با این حال، به واسطه وجود تنوّع در جزئیات مربوط به شکل کلی و الگوی دیواره سلول‌های اپیدرمی، تزئینات موجود در سطح دانه به چهار الگوی متفاوت قابل تقسیم‌بندی است: (۱) سلول‌های اپیدرمی با شکل کاملاً نامنظم؛ (۲) سلول‌های اپیدرمی با شکل نامشخص، به طوری که فاصله سلول‌ها قابل تشخیص نیست؛ (۳) سلول‌های چندوجهی مشبّک دارای دیواره‌های مشخص که به صورت موّاج یا وزیکولی نیست. این الگو خود به دو زیرگروه شامل سلول‌های باریک طویل و سلول‌های پهن تقسیم می‌شود؛ (۴) سلول‌های چندوجهی مشبّک دارای دیواره‌های موّاج و گاهی وزیکولی که به دو گروه دارای سلول‌های باریک طویل و سلول‌های پهن تقسیم می‌شود. همچنین سطح دانه در نمونه‌ها می‌تواند دارا یا فاقد حجره‌ها و دیواره‌های مشخص باشد. به علاوه، تنوّع قابل توجهی در ابعاد، شکل و رنگ دانه‌ها حتّی در یک نمونه مشاهده شد. بررسی‌های انجام گرفته بر روی تزئینات سطح دانه حاکی از آن است که اطلاعات به دست آمده از این صفت، کاربرد قابل توجهی در تقسیم‌بندی گونه‌های *Scrophularia* ندارد.

واژه‌های کلیدی. تزئینات، حجره‌دار، طایفه خرگوشک، مشبّک، میکروسکوپ

Introduction of seed and capsule micromorphological features of the genus *Scrophularia* (Scrophulariaceae) in Iran

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Abstract. In the present study, seed surface ornamentations in 31 species (34 populations) and the capsule surface sculpturing in five species of the genus *Scrophularia* L. distributed in Iran were examined using Scanning Electronic Microscopy. Based on the published seed surface terminologies, all examined seeds show the reticulate pattern with scalariform inner ornamentations. However, there is enough but untrustworthy variations in epidermal cell shapes and the walls' characteristics to divide the seed surface sculpturing patterns into four different groups: (1) cells with completely irregular shape; (2) cells without distinct shape so that their boundaries are not clear; (3) reticulate polygonal cells bordered with distinct walls neither undulate nor vesiculate, divided in two subgroups; forming elongated narrow cells and wide cells; (4) reticulate polygonal cells bordered with undulated or sometimes vesiculate walls divided in two subgroups; forming elongated narrow cells and wide cells. In addition, all the species' seeds can be alveolate, ridged or without distinct alveoli or ridges regarding their surfaces. There have also been variations in size, shape and color of the seeds even in a single individual. There are no applicable features of capsule surface sculpturing analyzed here, based on which species could be classified in certain groups.

Keywords. alveolate, microscope, ornamentation, reticulate, Verbasceae

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INTRODUCTION

Scrophularia L. (commonly known as figwort) with about 250 taxa (Judd et al., 2008) worldwide and about 60 species in Iran (Bayat & Attar, 2016; Ranjbar & Rahchamani, 2018, 2019), is among the largest genera of Scrophulariaceae sensu stricto which belongs to one of the taxonomically most difficult groups in the family (Attar & Hamzehee, 2006; Attar et al., 2011; Ranjbar et al., 2017; Mabberley, 2018).

The genus is mostly found throughout the Northern Hemisphere (Heywood, 1985), but concentrated in Asia with only a few species in Europe and North America. The genus *Scrophularia* grows in different ecological habitats in Iran. Although it occurs throughout mountainous regions (e.g. *S. azerbaijanica* Grau), it can be found in forests (e.g. *S. megalantha* Rech.f.), riversides (e.g. *S. subaphylla* Boiss.), and rarely along the roadsides (e.g. *S. variegata* M.Bieb.). *Scrophularia*, morphologically, is characterized by opposite or alternate leaves, open two-lipped flowers and compound cymes at the end of the stems which can be frondose (having leaves along the peduncles) or non frondose. The most important problem in the taxonomy of the genus is the unclear borders among several species.

Moreover, the importance of several characters such as shape of corolla and staminods in the systematics of the genus is still ambiguous. Based on a comprehensive phylogenetic study, the family Scrophulariaceae s.s. is delimited into seven tribes (Stevens, 2017), according to which *Scrophularia* and *Verbascum* L. form one well-supported clade called the tribe *Scrophularieae*. Very closely related to *Scrophularia* are *Verbascum* and *Oreosolen* Hook. F. (Oxelman et al., 2005). *Scrophularia* and *Verbascum* were placed in the tribe *Verbasceae* (Freeman & Scogin, 1999). However, they were all transferred into *Scrophularieae* (Olmstead et al., 2001; Oxelman et al., 2005; Stevens, 2017).

The close relationship between *Scrophularia* and *Verbascum* has been suggested by previous molecular studies (Ghahremaninejad et al., 2014; Riahi & Ghahremaninejad, 2019), as well as similarities in seed and embryo characters (Thieret, 1967) and leaf anatomy (Lersten & Curtis, 1997).

Seed micromorphology of some Scrophulariaceae genera such as *Striga* Lour. (Jones & Safa, 1982), *Paulownia* Siebold & Zucc. (Vujičić et al., 1993), *Cordylanthus* Nutt. & Benth. (Chuang & Heckard, 1972), *Orthocarpus* Nutt. (Chuang & Heckard, 1983), *Kickxia* Dumort. (Juan et al., 1998) and *Verbascum* (Juan et al., 1997; Attar et al., 2007) has been the subject of study. In general, and according to Chuang and Heckard (1983), the seed shape is directly related to the insertion point in the fruit. It is proved that there are some striking similarities between the species of the genus *Orobanche* L. and some species of *Scrophularia* in some morphological characters especially some of the seed features, such as the terminal insertion and the reticulate ornamentation (Juan et al., 2000) which shows the closeness of the two families. Based on the features of the tangential wall in the seeds epidermal cells of the genera of the family Scrophulariaceae studied by Juan et al. (2000), two groups were established from which *Verbascum* and *Scrophularia* are in the first group with membranous walls.

The aim of the present study is to screen the seed and capsule surface ornamentation in order to see if they are appropriate for a reliable classification of the genus at the infrageneric level.

MATERIALS AND METHODS

All the analyzed information on the species, collection data and the vouchers used are given in Table 1. Seeds were collected from living plants whenever possible, otherwise from herbarium specimens. The species of the genus *Scrophularia* examined here (including 31 species with some populations of the six species), are deposited at HKS, IRAN and TUH (acronyms according to Holmgren et al., 1990). The species which are not included in this analysis are closely similar to species examined, some of them are rare and some others are collected from the samples that possess no seeds. For the SEM studies, clean seeds were fixed on aluminum stubs using double-sided adhesive and were coated with a thin layer (ca. 25 nm) of gold-palladium. The SEM micrographs were taken by a Zeiss SEM-960A (Germany) from the middle of the seeds, mostly at magnification of 600x. At least, four seeds from each specimen were scanned to ensure the consistency of seed coat ornamentations. 10 seeds at least were measured to record the accurate information about morphology and size parameters. The terminology of Sutton (1988) and Juan et al. (1997) have been used to describe the seed surface patterns. Regarding capsule surface ornamentation, all the species were taken from the same samples as mentioned.

Table 1. Voucher information and results of seed micromorphological characters for the studied specimens.

Taxon	Voucher data	Seed length (mm)	Seed width (mm)	Seed pattern
S. amplexicaulis	Lorestan: Khorramabad, 24147-TUH	1±0.2	0.5±0.1	- / 4a
S. atropatana	Azarbayejan: Khoy, Foruragh, Shurik, 69952-TUH	0.75±1.5	0.5±0.1	Alveolate/4b
S. azerbaijanica	Azarbayejan: Arasbaran, Khodafarin, 17356-TUH	1±0.1	0.5±0.1	Alveolate/4a
S. chlorantha	Kordestan: Maryvan to Saghez, Agieh village, 387-HKS	0.5±0.1	0.3±0.1	- /1
S. crassicaulis	Tehran: Shemshak to Dizin, 14536- TUH	1.4±0.1	0.6±0.1	- / 4a
S. crassiuscula	Bakhtiari: Borujen, to Dorahan, Doudelu Mountain, 57239-TUH	0.8±0.2	0.5±0.1	Alveolate/2
S. crenophila	Kordestan: Divandareh to Saghez, Irankhah pass, 7604- HKS	0.7±0.3	0.4±0.2	Alveolate/4a
S. deserti	Baluchestan: 50 km to Bazman from Bampour, 21540-TUH	1.4±0.5	0.5±0.3	Alveolate/4b
S. farinosa	Kohkilouyeh-Boyer Ahmad: Dehdasht, Toyeh-She, 46914-IRAN	1.15±0.15	0.7±0.2	-
S. frigida subsp. *frigida*[1]	Khorassan: Neyshabur, Soumeeh, Binaloud, 39129-IRAN	1.25±0.25	0.7±0.2	Alveolate/4b
S. frigida subsp. *frigida*[2]	Kerman: Baft, Gugher, Bondar, 31979-TUH	1.4±0.1	0.5±0.1	Alveolate/4b
S. frigida subsp. *haussknechtii*[1]	Kerman: Hezar Mountain, 39138- IRAN	1.4±0.3	0.5±0.2	Alveolate/4b
S. frigida subsp. *haussknechtii*[2]	Markazi: Arak, Sefidkhani elevation, 10633-TUH	1±0.1	0.5±0.1	Alveolate/4b
S. gaubae [1]	Mazandaran: Sang-Deh, 30 km S.E. Pole-Sefid, 39381-IRAN	1±0.7	0.6±0.1	Alveolate/3b
S. gaubae [2]	Gilan: Amarlou, Barreh-Sar to Damash, 39224-IRAN	1.25±0.15	0.75±0.15	Alveolate/3b
S. glauca	Fars: Shiraz, Sabzpushan Mountain, 39145-IRAN	0.8±0.1	0.5±0.1	- / 2
S. ilwensis	Azarbaijan: Kalibar, Nabijan, Kalan Mountain, 39146- IRAN	0.85±0.15	0.6±0.1	Ridged/4b
S. kurdica	Azarbayejan: Urumieh, Band, 70083- TUH	0.9±0.1	0.4±0.1	- / 4a
S. leucoclada	Kerman: Between Doulatabad and Siruieh, 70177-TUH	2±0.1	1.1±0.1	Ridged/4b
S. libanotica	Esfahan: Akhore mountain, 39168-IRAN	1.1±0.2	0.5±0.1	Alveolate/3a
S. megalantha	Mazandaran: Ramsar, Neydasht, 8409-TUH	0.5±0.1	0.4±0.1	Alveolate/1
S. mesopotamica	Kordestan: Saghez to Mrivan, Heshlagh Pol village, 4237-HKS	0.6±0.1	0.4±0.1	Alveolate/3b
S. nervosa subsp. *boisseriana*	Kordestan: Bidjar, Hamzeh-Arab Mountain, 39178-IRAN	1.5±0.2	0.7±0.1	Alveolate/4b
S. oxysepala	Ardebil: Ghotur_Sou, Sabalan Mountains, 39198-IRAN	0.9±0.2	0.5±0.1	Ridged/4b
S. pruinosa [1]	Kordestan: 55 km to Baneh from Marivan, 36346-TUH	1.8±0.2	0.75±0.15	Alveolate/3b
S. pruinosa [2]	Kermanshah: 7 km after Paveh to Ravansar, 17837-TUH	1±0.2	0.65±0.15	Alveolate/4a
S. pruinosa [3]	Mazandaran: Rudehen elevations, 10623-TUH	1.3±0.1	0.7±0.1	Alveolate/4a
S. sanguinea	Lorestan: Oshtoran- Kooh, Saravand to Gahar lake, 39234-IRAN	1.3±0.2	0.6±0.2	Alveolate/4a
S. scoparia	Khorassan: Sabzehvar, Soltan Abad to Ghochan, 39230-IRAN	1.1±0.2	0.55±0.25	Alveolate/1
S. scopolii [1]	Gilan: Ispili, Larekhani, 18473-TUH	0.8±0.1	0.4±0.1	- /1
S. scopolii [2]	Gilan: Asalem to Khalkhal (forest), 39232-IRAN	0.85±0.15	0.4±0.1	- /1
S. striata [1]	Yazd: Tarzjan, 28175-TUH	1.3±0.1	0.7±0.1	Ridged/4b
S. striata [2]	Markazi: Arak, Emarat elevation, 10645-TUH	1.45±0.15	0.8±0.1	Ridged/4a

Table 1. continued.

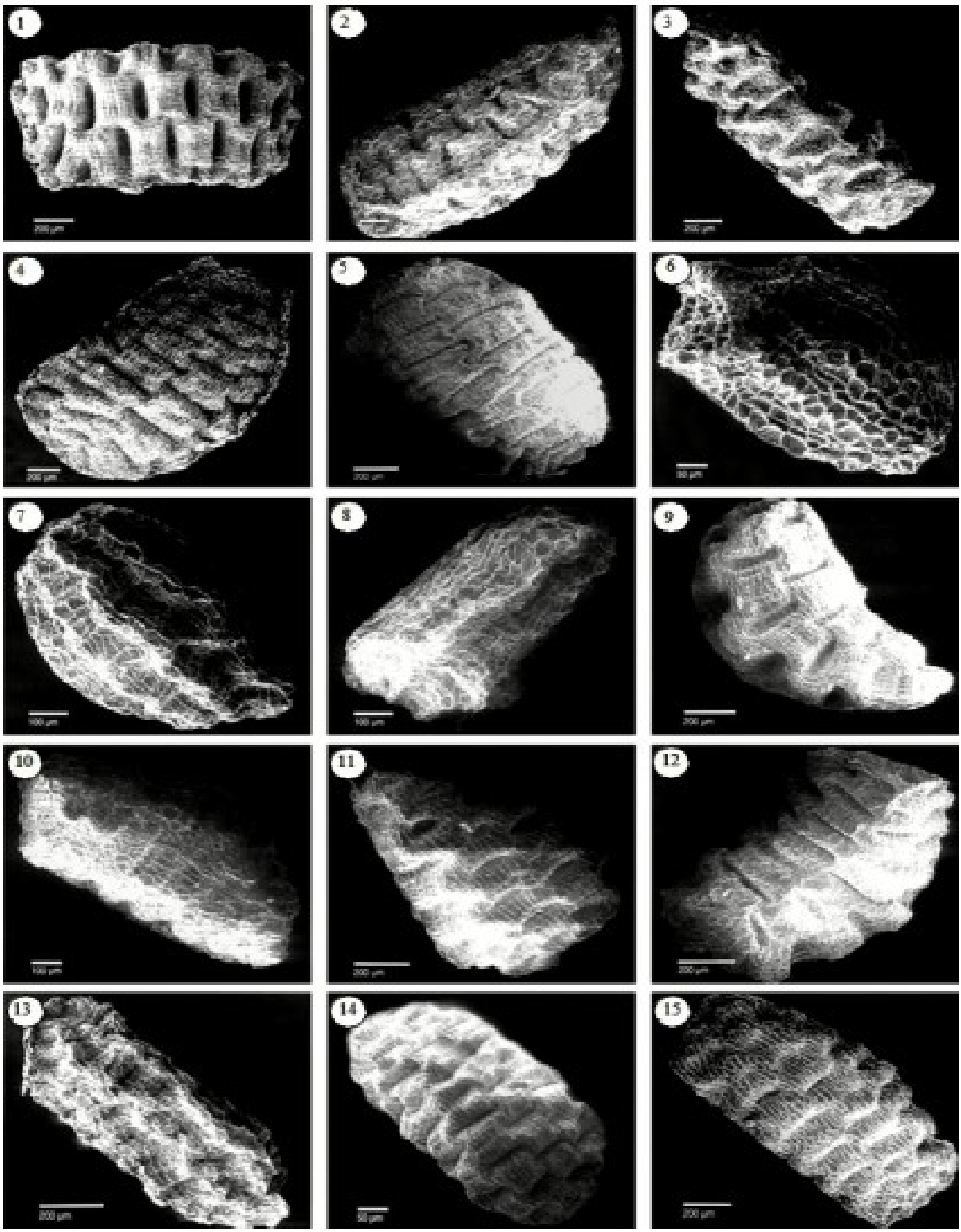
S. striata [3]	Khorassan: Quchan, Chenaran, Akhlamad fall, 27512-TUH	1.2±0.1	0.5±0.1	Ridged/3a
S. subaphylla	Esfahan: Khansar, Ghale-Bala-Mohammad Mt., 39318-IRAN	1.5±0.1	0.6±0.1	Alveolate/4a
S. svartiana	Azarbayejan:15 km after Salmas to Urumieh, 39302-IRAN	0.9±0.1	0.6±0.1	Alveolate/4b
S. syriaca	Semnan: 8 km N.E.of Momen-Abad, 39300-IRAN	1.5±0.1	0.8±0.1	Alveolate/3b
S. umbrosa [1]	Mazandaran: Karaj, Chalus road, Pole Zanguleh, Kamarbon, 33296-TUH	1±0.1	0.5±0.1	- /4b
S. umbrosa [2]	Golestan: Golestan park, after Mirza Baylu to Sulgerd, 25445-TUH	0.8±0.1	0.5±0.1	- /3b
S.variegata subsp.*rupestris*	Mazandaran: Kelardasht to Pit-Sara, 39324-IRAN	1.5±0.1	0.6±0.1	Alveolate/3b
S.variegata subsp.*cinerascens*	Mazandaran: Ilka, Varvasht mountain to Kamarbon, 39316-IRAN	1.25±0.25	0.5±0.1	Alveolate/3b
S.variegata subsp.*variegata*	Gilan: Talesh, Aghvelar village, 28978-TUH	1.5±0.1	0.6±0.1	Alveolate/3b
S. vernalis [1]	Golestan: Gorgan, Shamushak forest, 47677-IRAN	0.9±0.1	0.4±0.1	- / 4b
S. vernalis [2]	Mazandaran: Tunekabon, Jannat-Rudbar, 21238-TUH	1.2±0.1	0.7±0.1	- / 3b
S. xylobasis	Fars: Estahbanat, Chute mountain, 39397-IRAN	1.4±0.1	0.6±0.1	- / 2

RESULTS

Seed shape and surface

As it is observed in Figures, *S. pruinosa*[1] Boiss. (Fig. 1), *S. azerbaijanica* Grau (Fig. 2) and *S. deserti* Del. (Fig. 3) show the alveolate type, whereas *S. leucoclada* Bunge (Fig. 4) and *S. striata* Boiss. (Fig. 5) show the ridged type and *S. chlorantha* Kotschy & Boiss. (Fig. 6), *S. umbrosa*[1] Dumort. (Fig. 7) and *S. umbrosa*[2] (Fig. 8) do not have a clear alveoli or ridge. *S. pruinosa*[3] (Fig. 9), *S. frigida* Boiss. subsp. *haussknechtii*[2] Bornm. ex Grau (Fig. 10), *S. frigida* subsp. *frigida*[2] (Fig. 11), *S. frigida* subsp. *frigida*[1] (Fig. 12), *S. variegata* M.Bieb. subsp. *cinerascens* (Boiss. in Tchihat.) Grau (Fig. 13), *S. variegata* subsp. *variegata* (Fig. 14), *S. variegata* subsp. *rupestris* (M.Bieb.) Grau (Fig. 15) are some more examples of alveolate type. Seed surface ornamentations have been also examined. As it is shown in Figures, *S. megalantha* (Fig. 16) possesses irregular cells, *S. glauca* Decne. ex Benth. (Fig. 17) has shapeless cells and *S. kurdica* Eig (Fig. 18), *S. syriaca* Benth. (Fig. 19), *S. pruinosa*[3] (Fig. 20), *S. leucoclada* (Fig. 21) and *S. pruinosa*[1] (Fig. 22) all contain reticulate cells. The seeds vary from oblong (e.g. *S. pruinosa*) to elliptic (e.g. *S. umbrosa*[1]) and prismatic (e.g. *S. frigida* subsp. *frigida*[2]) in shape (see Figs. 1, 7, 11). It is important to mention that all the types could be found in all the species' capsules. Among the studied species, the size of seed ranges from 0.3 (e.g. *S. megalantha*) to 0.9 mm (e.g. *S. umbrosa*) in width and from 0.7 (e.g. *S. atropatana* Grossh.) to 1.9 mm (e.g. *S. leucoclada*) in length (Table 1). Some seeds have an attenuate (e.g. *S. azerbaijanica*), truncate (e.g. *S. leucoclada*), apiculate (e.g. *S. chlorantha*), obtuse (e.g. *S. umbrosa*[2]), or rounded (e.g. *S. variegata* subsp. *variegata*) beak (see Figs. 2, 4, 6, 8, 14 respectively), whether in all dimensions or two, although it can be in one as well. The spectrum of mature seed color ranges from dark brown (e.g. *S. megalantha*) to black (e.g. *S. leucoclada* – data not shown).

However, orange to light brown colors are recognized in immature seeds. Given the fact that three different types of seed surface patterns are identified for species in the present study, we can make a relatively thorough artificial classification, based on which seed coat can be alveolate (Figs. 1-3), ridged (Figs. 4, 5) - which are arranged longitudinally in numerous rows, or without distinct alveoli or ridges sometimes forming clear longitudinal undulate walls (Figs. 6-8). As the alveoli show significant differences in depth and slight differences in length and width among the species, the ridges demonstrate some differences which can help classify the species into different types as discussed later. If the ratio of length to width calculated in each species is 1.8 ± 0.4 mm, it is considered as ridge and if the ratio is 4.5 ± 0.5 mm, it represents alveolus (measuring at the same magnification). The seed coat is far deeply alveolate in *S. pruinosa*, *S. scoparia* Pennell, *S. svartiana* Gabrielian, *S. syriaca*, *S. mesopotamica* Boiss. and *S. crassiuscula* Grau. whereas *S. azerbaijanica*, *S. gaubae* Bornm., *S. libanotica* Boiss., *S. nervosa* Benth., *S. sanguine* Grau., *S. subaphylla* Boiss., *S. variegate* subsp. *variegata* are deeply alveolate and *S. atropatana* Grossh., *S. crenophila* Boiss., *S. deserti*, *S. frigida*, *S. megalantha*, *S. variegata* subsp. *rupestris*, *S. variegata* subsp. *cinerascens* are shallowly alveolate (see Figs. 1-3 and also 9-15).



Figures 1-15. Alveolate types. 1. *S. pruinosa*[1] (far deeply). 2. *S. azerbaijanica* (deeply). 3. *S. deserti* (shallowly). 4,5. ridged type: 4. *S. leucoclada* (far deeply) and as the largest seed, 5. *S. striata*[2] (deeply). 6-8. without alveoli or ridges type and sometimes longitudinally undulate: 6. *S. chlorantha* (as the smallest seed). 7,8. *S. umbrosa*[1,2]. 9. *S. pruinosa*[3], 10. *S. frigida subsp. haussknechtii*[2]. 11. *S. frigida subsp. frigida*[2]. 12. *S. frigida subsp. frigida*[1]. 13. *S. variegata subsp. cinerascens*. 14. *S. variegata subsp. variegata*. 15. *S. variegata subsp. Rupestris*

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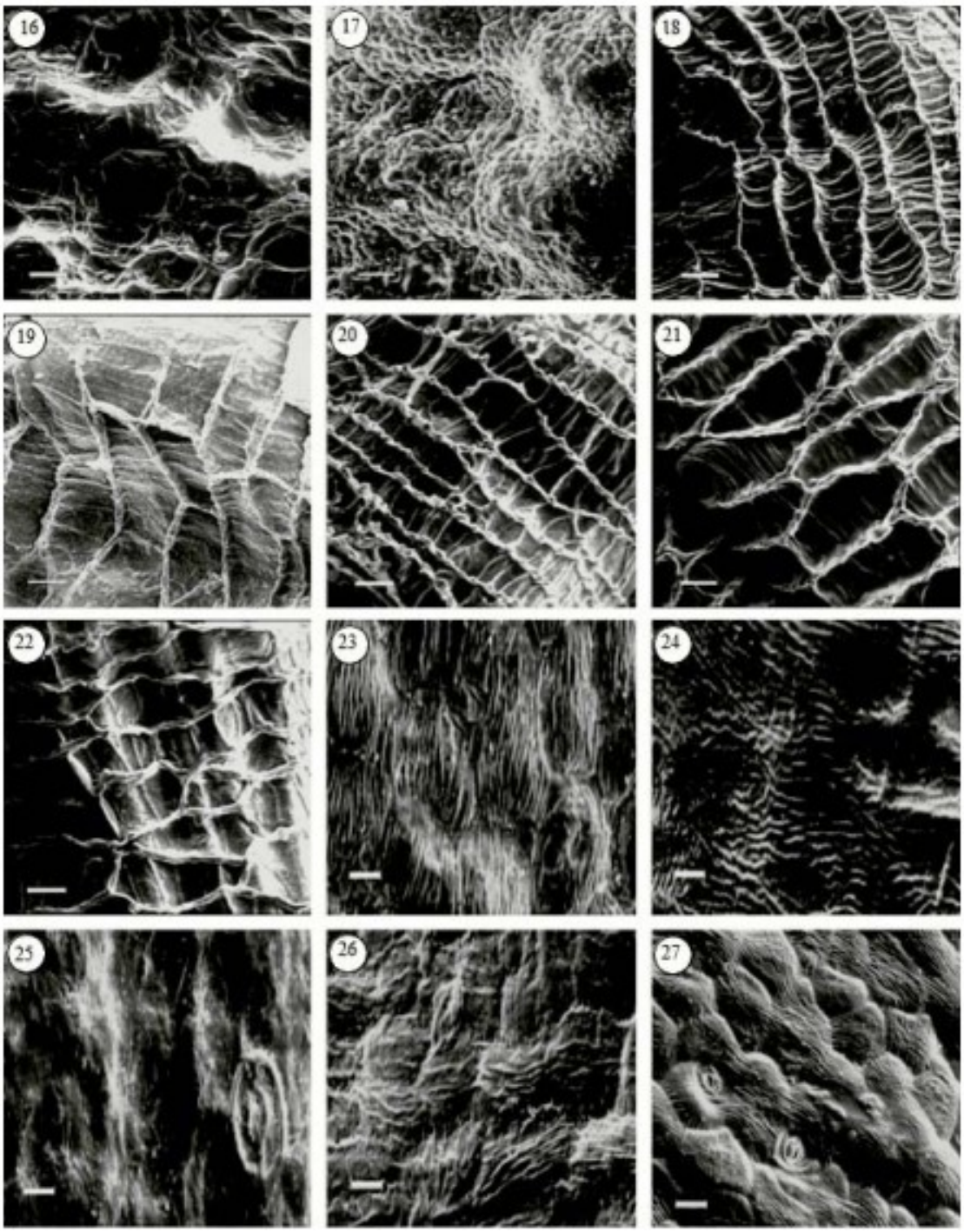

Figures 16-27. Seed surface ornamentation and capsule surface. 16-22. Seed surface ornamentation, scale bar = 20 µm. Irregular cells. 16. *S. megalantha*; shapeless cells. 17. *S. glauca*; reticulate cells. 18. *S. kurdica*. 19. *S. syriaca*. 20. *S.pruinosa*[3]. 21. *S. leucoclada*. 22. *S. pruinosa*[1]. 23-27. Capsule surface, scale bar = 10 µm. 23. *S. amplexicaulis*. 24. *S. atropatana*. 25. *S. azerbaijanica*. 26. *S. crassicaulis*. 27. *S. striata*[2].

Some species such as *S. leucoclada* can be far deeply ridged while *S. ilwensis* C.Koch, *S. oxysepala* Boiss. and *S. striata* are deeply ridged (see Figs. 4, 5). In some species such as *S. amplexicaulis* Benth., *S. chlorantha*, *S. crassicaulis* Boiss., *S. glauca*, *S. kurdica*, *S. scopolii* Hoppe, *S. umbrosa* and *S. vernalis* L., no distinct alveoli or ridges are observed, which form the third group sometimes including longitudinal undulate walls (see Figs. 6-8). Sometimes significant differences

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are found in subspecies or populations of the same species. For example, the seed surface patterns are the same in two far distant populations of *S. umbrosa* and *S. pruinosa*. On the other hand, three subspecies of *S. variegata* show some differences in their shapes and patterns.

Based on the seed surface ornamentation, another classification is also considered which divide all the species into four groups;

(1) cells with completely irregular shape (e.g. *S. megalantha*),

(2) cells without distinct shape so that their boundaries are not clear (e.g. *S. glauca*),

(3) reticulate polygonal cells bordered with distinct walls neither undulate nor vesiculate, consisting two subgroups a-forming elongated narrow cells (e.g. *S. kurdica*), b- forming wide cells (e.g. *S. syriaca*),

(4) reticulate polygonal cells bordered with undulate or sometimes vesiculate walls, consisting two subgroups a- forming elongate narrow cells (e.g. *S. pruinosa*), b- forming wide cells (e.g. *S. leucoclada*) (see Figs. 16-22).

However, the first classification is artificially applicable for classifying the species. It is noticeable that neither of two classifications suggested here, verify each other.

Capsule surface

Since there are no helpful features in capsule surface ornamentations, the examined species could not be classified into some artificial certain groups. Although, some different ornamentations on capsule surfaces could be recognized. Some of the micrographs are illustrated in Figures 23-27. As it is shown, capsule surfaces can be longitudinally striate forming almost orbicular chambers (e.g. *S. amplexicaulis*, Fig. 23), horizontally striate forming various grooves (e.g. *S. atropatana*, Fig. 24), smooth with no special sculpturing (e.g. *S. azerbaijanica*, Fig. 25), irregularly striate forming more clear chambers (e.g. *S. crassicaulis*, Fig. 26), unclearly striate forming irregularly small chambers (e.g. *S. striata*, Fig. 27).

DISCUSSION

According to Juan et al. (2000), the indumentum, capsule dehiscence, structure of the endocarp, seed coat ornamentation, and inner structure of the seeds were the most useful features to determine relationships among the different genera included in the family Scrophulariaceae. Despite the utility of fruit and seed characters for distinguishing the genera in Scrophulariaceae by Juan et al. (2000), seed features are unlikely to classify the species of *Scrophularia* in an accurate way based on the present study. In addition, phenetic analysis of 58 characters revealed that several groups of genera in Scrophulariaceae are closely related on the basis of their fruit and seed features (Juan et al., 2000) which is not true regarding the species of *Scrophularia* using micromorphological features of seed and pollen grains (Daemi, 2009; Rahchamani, 2018). Similarly, seed micromorphological studies on *Verbascum* as the closest genus to *Scrophularia* could not present a complete classification for the genus (Attar et al., 2007). Also, in the family Orobanchaceae as a close family to Scrophulariaceae, characters such as size, shape and ornamentation of the seeds were not found to be very useful in differentiation of taxa; however, other characters of the epidermal seed coat cells confirm the usefulness of seed characters to identify most of the studied species of *Orobanche* (Plaza et al., 2004). Moreover, *Verbascum* and *Celsia* L. also belong to the *Scrophularia* type of the alveolate seeds, characterized by endothelium arranged in ribs and penetrate in endosperm (based on the classification presented by Hartl, 1959). Contrary to most other genera in the family Scrophulariaceae, *Scrophularia*, *Verbascum* and *Oreosolen* are all mainly distributed in Northern Hemisphere -confirming their close relationship biogeographically. Although, they are not surprisingly from the same morphological point of view, their seed surface ornamentations are more or less similar to each other which makes it difficult to make a classification.

As a result, having a short look at Figures 1-15 shows the difficulties in differentiating the species having the alveolate type. It is also shown obviously that *S. pruinosa* (Figs. 1, 9) with two populations from two different locations have almost the same alveoli but different seed surface ornamentations. In *S. frigida* two populations of the same subspecies and the subspecies show variations in their size, shape and particularly in surface (see Figs. 10-12). Likely, three subspecies of *S. variegata* have alveolate seeds, but there are clear differences in their surfaces (see Figs. 13-15). Given the fact that reticulate seed coats have been observed in several genera belonging to different tribes, such as *Digitaleae* (Juan et al., 2000), some of the species examined here show reticulate sculpturing. However, this is not a reliable character for identifying the species of *Scrophularia*. Only *Verbascum* and *Scrophularia* have seeds with longitudinal rows of alveoli or ridges (Juan et al., 2000). According to study on *Verbascum* seeds (Attar et al., 2007) on *Verbascum* and our current data on *Scrophularia*, it seems that seed and capsule surface ornamentations show homoplasy and are not suitable to be relied on. Therefore, the information given here on both seed surface patterns and ornamentations is not helpful. For example,

although seed surface patterns in *S. pruinosa* (Figs. 1, 9) are the same, their ornamentations (Figs. 20, 22) put them more or less in different groups. Although capsule dehiscence in *Scrophularia* is septicidal, which has been proved to have a high systematic value, they do not allow us to put the species even in artificial groups. The results of this study indicate that the characters of seed surface and capsule sculpturing do not provide any diagnostic features useful in separation of the species of *Scrophularia* and presenting natural grouping in the genus. Therefore, these characteristics are not reliable to indicate the homogeneity of the group and probably its monophyly.

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REFERENCES

Attar, F. & Hamzehee, B. 2006. Two new species of *Scrophularia* L. (Scrophulariaceae) from Iran. Feddes Repertorium 117: 508-511.

Attar, F., Keshvari, A., Ghahreman, A., Zarre, Sh. & Aghabeigi, F. 2007. Micromorphological studies on *Verbascum* (Scrophulariaceae) in Iran with emphasis on seed surface, capsule ornamentation and trichomes. Flora 202: 169-175.

Attar, F., Riahi, M., Daemi, F. & Aghabeigi, F. 2011. Preliminary molecular phylogeny of Eurasian *Scrophularia* (Scrophulariaceae) based on DNA sequence data from *trnS-trnG* and ITS regions. Plant Biosystems 145: 857-865.

Bayat, N. & Attar, F. 2016 Leaf anatomical studies on selected species of *Scrophularia* L. (Scrophulariaceae) in Iran. Nova Biologica Reperta 2: 286-297.

Chuang, T. & Heckard, L.R. 1972. Seed coat morphology in *Cordylanthus* (Scrophulariaceae) and its taxonomic significance. American Journal of Botany 59: 258-265.

Chuang, T. & Heckard, L.R. 1983. Systematic significance of seed-surface features in *Orthocarpus* (Scrophulariaceae subtribe Castillejinae). American Journal of Botany 70: 877-890.

Daemi, F. 2009. Micromorpholoy in *Scrophularia* (Scrophulariaceae) in Iran. M.Sc. thesis. University of Tehran.

Freeman, C.E. & Scogin, R. 1999. Potential utility of chloroplast *trnL* (UAA) gene intron sequences for inferring phylogeny in Scrophulariaceae. Aliso 18: 141-159.

Ghahremaninejad, F., Riahi, M., Babaei, M., Attar, F., Behçet, L. & Sonboli, A. 2014 Monophyly of *Verbascum* (Scrophularieae: Scrophulariaceae): evidence from nuclear and plastid phylogenetic analyses. Australian Journal of Botany 62: 638-646.

Hartl, D. 1959. Das alveolierte Endosperm bei Scrophulariaceen, seine Entstehung, Anatomie und taxonomische. Beiträge zur Biologie der Pflanzen 35: 95-110.

Heywood, V. 1985. Flowering Plants of the World. USA: Oxford University Press.

Holmgren, P.K., Holmgren, N.H. & Barnett, L.C. 1990. Index Herbarioum I: The Herbaria of the World, 8th edition, Regnum Veg. 20.

Jones, B.M.G. & Safa, B.S. 1982. Variation of seed-coat Ornamentation in *Striga hermonthica* (Scrophulariaceae). Annals of Botany 50: 629-634.

Juan, R., Fernandez, I. & Pastor, J. 1997. Systematic consideration of fruits and seeds in the genus *Verbascum* (Scrophulariaceae). Annals of Botany 80: 591-598.

Juan, R., Pastor, J. & Fernandez, I. 1998. Morfología y anatomía de frutos y semillas en el género *Kickxia* Dumort (Morphology and anatomy of fruit and seeds of the genus *Kickxia* Dumort). Lagascalia 20: 207-218.

Juan, R., Pastor, J. & Fernandez, I. 2000. SEM and Light Microscope observations on fruit and seeds in Scrophulariaceae from southwest Spain and their systematic significance. Annals of Botany 86: 323-338.

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

Lersten, N. & Curtis, J. 1997. Anatomy and distribution of foliar idioblasts in *Scrophularia* and *Verbascum* (Scrophulariaceae). American Journal of Botany 84: 1638-1645.

Mabberley, D.J. 2018. Mabberley's plant-book: a portable dictionary of plants, their classification and uses (4th edition). New Delhi. Cambridge University Press.

Olmstead, R.G. Depamphilis, C.W., Wolfe, A.D., Young, N.D., Elisons, W.J. & Reeves, P.A. 2001. Disintegration of the Scrophulariaceae. American Journal of Botany 88: 348-361.

Oxelman, B., Kornhall, P., Olmstead, R.G. & Bremer, B. 2005. Further disintegration of Scrophulariaceae. Taxon 54: 411-425.

Plaza, L., Fernandez, I., Juan, R., Pastor, J. & Pujadas, A. 2004. Micromorphological studies on seeds of *Orobanche* species from the Iberian Peninsula and the Balearic Islands, and their systematic significance. Annals of Botany 94: 167-178.

Rahchamani, N. 2018. Systematic study of the genus *Scrophularia* (Scrophulariaceae) in Iran. Ph. D. thesis. Bu-Ali Sina University.

Ranjbar, M., Rahchamani, N., Ghahremaninejad, F. & Zeraatkar, A. 2017. *Scrophularia attariae* and *S. maharluica* spp. nov. (Scrophulariaceae) from south Iran. Nordic Journal of Botany 35: 147-156.

Ranjbar, M. & Rahchamani, N. 2018. The species of *Scrophularia* L. (Scrophulariaceae) with white margined leaves in Flora Iranica. Adansonia 40: 67-87.

Ranjbar, M. & Rahchamani, N. 2019. New Findings on *Scrophularia* sect. *Mimulopsis* (Scrophulariaceae) from Iran. Novon 27: 23 8-247.

Riahi, M. & Ghahremaninejad, F. 2019. The tribe Scrophularieae (Scrophulariaceae): A review of phylogenetic studies. Hacquetia 18: 337-347.

Stevens, P. 2017. Angiosperm Phylogeny Website., http://www.mobot.org/MOBOT/research/APweb./

Sutton, D.A. 1988. A revision of the tribe Antirrhineae. *In*: Thieret, J. 1967. Supraspecific classification in the Scrophulariaceae: A review. Oxford University Press, London. Sida, Contributions to Botany 3: 87-106.

Thieret, J. 1967. Supraspecific classification in the Scrophulariaceae: A review. Sida, Contributions to Botany 3: 87-106.

Vujičić, R., Grubišić, D. & Konjević, R. 1993. Scanning electron microscopy of the seed coat in the genus *Paulownia* (Scrophulariaceae). Botanical Journal of the Linnean Society 111: 505-511.

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