

SEED MICROMORPHOLOGICAL STUDY ON ENDEMIC AND SUBENDEMIC SPECIES OF *VERONICA* L. (PLANTAGINACEAE JUSS.) IN IRAN

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Abstract

Seed morphology of 12 Iranian endemic and subendemic species of *Veronica* was studied using scanning electron microscope (SEM). Seven qualitative and quantitative characters were measured using SEM micrographs and stereomicroscopy. The seed shape of most species is ovate and plano-convex. The size of seeds ranges from 1.25 × 0.75 mm in *V. khorassanica* to 2.5 × 1.75 mm in *V. viscosa* Boiss. The ornamentation of seed coat is reticulate-verrucate in *V. khorassanica*, *V. czerniakowskiana*, *V. mazanderanae* and *V. rubrifolia*, reticulate-rugate in *V. acrotheca*, *V. aucheri*, *V. viscosa* and *V. intercedens*, rugose in *V. microcarpa*, *V. chionantha* and *V. rechingeri*, and reticulate-porate in *V. gaubae*. The testa cells are polygonal in ten species and irregular in two species. Micromorphological characters of seeds are useful in specific and subspecific delimitations of Iranian *Veronica*.

Introduction

Veronica L. (Plantaginaceae *sensu* APG, 2003; formerly Scrophulariaceae) is the largest genus within tribe Veroniceae Bartling. with ca. 450 species (Albach *et al.*, 2004a). Endemic species are important on both global and local levels, as they provide unique genetic diversity for further studies and provide local people with priceless services (Newmark, 2002). Fischer (1981) introduced the genus *Veronica* in Flora Iranica with 56 species and arranged these species in five sections. According to the flora of Iran the genus includes 61 species with 18 endemics among them (Saeidi-Mehrvarz, 2003). Alborz and Zagros mountains of Iran are an important center of speciation of *Veronica*. Many endemic species of this genus occur in Alborz range, such as *V. siaretensis* E. Lehm., *V. rechingeri* M.A. Fisch., *V. mazanderanae* Wendelbo and *V. chionantha* Bornm. In foothills of Kopet-Dagh in northeastern Iran, some species such as *V. khorassanica* Czerniak. and *V. czerniakowskiana* Monjuschko are endemic (Saeidi-Mehrvarz, 2005). The distribution range of *V. acrotheca* Bornm. & Gauba and *V. rubrifolia* Boiss. is in western Iran, whereas *V. rechingeri* and *V. gaubae* Bornm. occur in northern Iran. These endemics of *Veronica* and most of the ones restricted to Iran and adjacent regions belong to two subgenera, *Pocilla* (Dumort.) M. Mart. Ort., Albach & M.A. Fisch. and *Pentacepala* (L. B. Moore) Garn.-Jones.

Macro- and micromorphological characters of seed are of essential systematic importance within *Veronica* (Yamazaki, 1957; Juan *et al.*, 1994; Martínez-Ortega and Rico, 2001; Munoz-Centeno, 2006). Several authors such as Elisens and Tomb (1983) and Barthlott (1984) have emphasized the phylogenetic and systematic value of the structural character of the seed coat, due to their low phenetic variation. The shape of seed has been traditionally used in *Veronica* as an important taxonomic character (Martínez-Ortega and Rico, 2001). Güld (2013) studied seed morphology of six Turkish species of *Veronica* viz. *V. bozakmanii* M. A. Fisch, *V. arvensis* L.,

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V. triphyllos L., *V. polita* Fr., *V. hederifolia* L. and *V. cymbalaria* Bodard by SEM. Martínez-Ortega and Rico (2001) studied seed morphology and its systematic significance in some *Veronica* species (Scrophulariaceae), mostly from the Western Mediterranean. Some studies have been carried out on Iranian species, seed and fruit micromorphological study (Saeidi-Mehrvarz *et al.*, 2001a,b). The seed morphology of ten Iranian *Veronica* species have been reported by Saeidi-Mehrvarz *et al.* (2001b). It was the first step of seed micromorphological study on *Veronica* of Iran. The present study aims to record the seed micromorphological characters of 12 Iranian endemic and subendemic species of genus *Veronica* and evaluate their taxonomic significance for the first time.

Materials and Methods

This study was mainly based on the specimens deposited in Tehran University Herbarium (TUH) and Guilan University Herbarium (GUH). Approximately 10 seeds from each taxon were analyzed. Macromorphological observations were carried out under a Stereoscopic Microscope (SM). For SEM, seeds were directly mounted on metallic stubs using double-sided adhesive tape and then coated with gold for 6 min in a sputtering chamber before observed under SEM. The SEM examination was carried out under a VEGA/TESCAN SEM, at an accelerating voltage of 15 kV, in Razi Metallurgical Research Center (RMRC) in Tehran. The terminology used to describe seed coat surface sculpturing follows mainly Juan *et al.* (1994), Martínez-Ortega and Rico (2001) and Mounzu-Centeno *et al.* (2006). Circumscription of subgenus follows the system by Albach *et al.* (2004a).

Results and Discussion

The seed size in the studied Iranian *Veronica* ranges from 1 to 3 mm in length and 0.5 to 2 mm in width. Seeds of *Veronica* species are ovate, cymbiform, flattened, plano-convex or cyathiform in shape (Table 1). Color of seeds are dark brown to yellow. The cells are mostly polygonal in studied taxa with exception of *V. chionantha* and *V. rechingeri*, where they are irregular. There is some variation in depth, thickness and ornamentation of anticlinal and periclinal walls. Periclinal wall mostly was flattened and it may have had ornamentation that forms a secondary sculpture. Sculpturing pattern showed four different type: reticulate-verrucate, reticulate-rugose, rugate and reticulate-porate. Among the species examined, only *V. gaubae* showed type of reticulate-porate. The seed morphological characters of the studied taxa are shown in Table 1 and Figs 1-4. A taxonomic key to the species and subspecies of Iranian *Veronica* based on seed characters has been generated for their identification.

A taxonomic key to the species and subspecies of *Veronica* of Iran.

- 1 Seeds yellow to brown..... (2)
 - Seeds dark brown..... (12)
- 2 Seeds cymbiform..... (3)
 - Seeds ovate, flattened..... (4)
- 3 Seed coat rugate, anticlinal wall with medium depth, periclinal wall concave and smooth, terminal funicular attachment.....*V. microcarpa*
 - Seed coat reticulate-rugate, anticlinal wall obscure, periclinal wall convex and corrugate, subterminal funicular attachment.....*V. aucheri*
- 4 Seed coat surface reticulate-verrucate..... (5)

- Seed coat surface not reticulate-verrucate.....(9)
- 5 Seeds plano-convex, brown..... (6)
- Seeds cyathiform or subcyathiform, yellow to brown..... (7)
- 6 Thick anticlinal wall with medium depth, flat periclinal wall with a large conspicuous central wart.....*V. khorassanica*
- Thin and shallow anticlinal wall, flat periclinal wall with a central wart not very conspicuous.....*V. czerniakowskiana*
- 7 Brown, cyathiform, smooth in dorsal face, thin anticlinal wall, funicular attachment.....
.....*V. mazanderanae*
- Yellow to brownish, cyathiform or subcyathiform, cristate, thick anticlinal wall, papillate or micro reticulate.....(8)
- 8 Cyathiform, yellow, with deep ridge on dorsal face.....*V. rubrifolia* subsp. *rubrifolia*
- Subcyathiform, brownish, ridge on dorsal face is not deep.....
.....*V. rubrifolia* subsp. *respectatissima*
- 9 Seed coat rugate, irregular cells, indistinct cell boundaries, subterminal funicular attachment.....*V. rechingeri*
- Seed coat reticulate, polygonal cell, anticlinal wall with medium depth, terminal funicular attachment.....(10)
- 10 Flattened to cyathiform, yellow, seed coat reticulate-rugate, periclinal wall without any pore.....(11)
- Plano-convex, brown, seed coat reticulate-porate, periclinal wall with a central pore.....
.....*V. gaubae*
- 11 Flattened, with a keel at dorsal face.....*V. viscosa*
- Cyathiform, dorsal face rough and without a keel.....*V. intercedens*
- 12 Seed coat rugate, irregular cells, ring structures in dorsal face.....*V. chionantha*
- Seed coat reticulate-rugate, polygonal cells, without any special structures in dorsal face.....
.....*V. acrotheca*

Some authors have determined the genus circumscription of *Veronica* using seed characters (Yamazaki, 1957; Juan *et al.*, 1994; Mortinez-Ortega and Rico, 2001; Munoz-Centeno *et al.*, 2006). In all cases these characters were useful at infrageneric rank. Seed coat in most species was reported as reticulate-verrucate. This pattern was found as common in *Veronica* and in most species of subg. *Pentasepala* (Munoz-Centeno *et al.*, 2006). In our study it appears in subg. *Pentasepala* twice, in *V. khorassanica* and *V. czerniakowskiana*. It seems that seed coat in the ancestor of subg. *Pellidosperma* (E.B.J. Lehm.) Assejeva, *Stenocarpon* (Boriss.) M.M. Mart. Ort., Albach & M.A. Fisch., *Chamaedryis* (W.D.J. Koch) M. Mart., *Pocilla* and *Pentasepala* was reticulate-verrucate (Munoz-Centeno *et al.*, 2006). According to Albach *et al.* (2004b) *V. czerniakowskiana* is a sister clad to a group of *Veronica* species, such as *V. jacquinii*, *V. turrillana* and *V. bombycine*. These species also show reticulate-verrucate pattern (Munoz-Centeno *et al.*, 2006). Among the species of subg. *Pentasepala*, perennials exhibit reticulate-verrucate in *V. aucheri* Boiss. and *V. acrotheca*, and rugate in *V. chionantha* and *V. rechingeri*. These patterns have been mentioned as rare cases in earlier studies (Mortinez-ortego and Rico 2001, Munoz-Centeno *et al.*, 2006). *V. acrotheca* and *V. aucheri*, despite similar seed coat and shape, are separable in some characters, such as thickness of anticlinal cell wall.

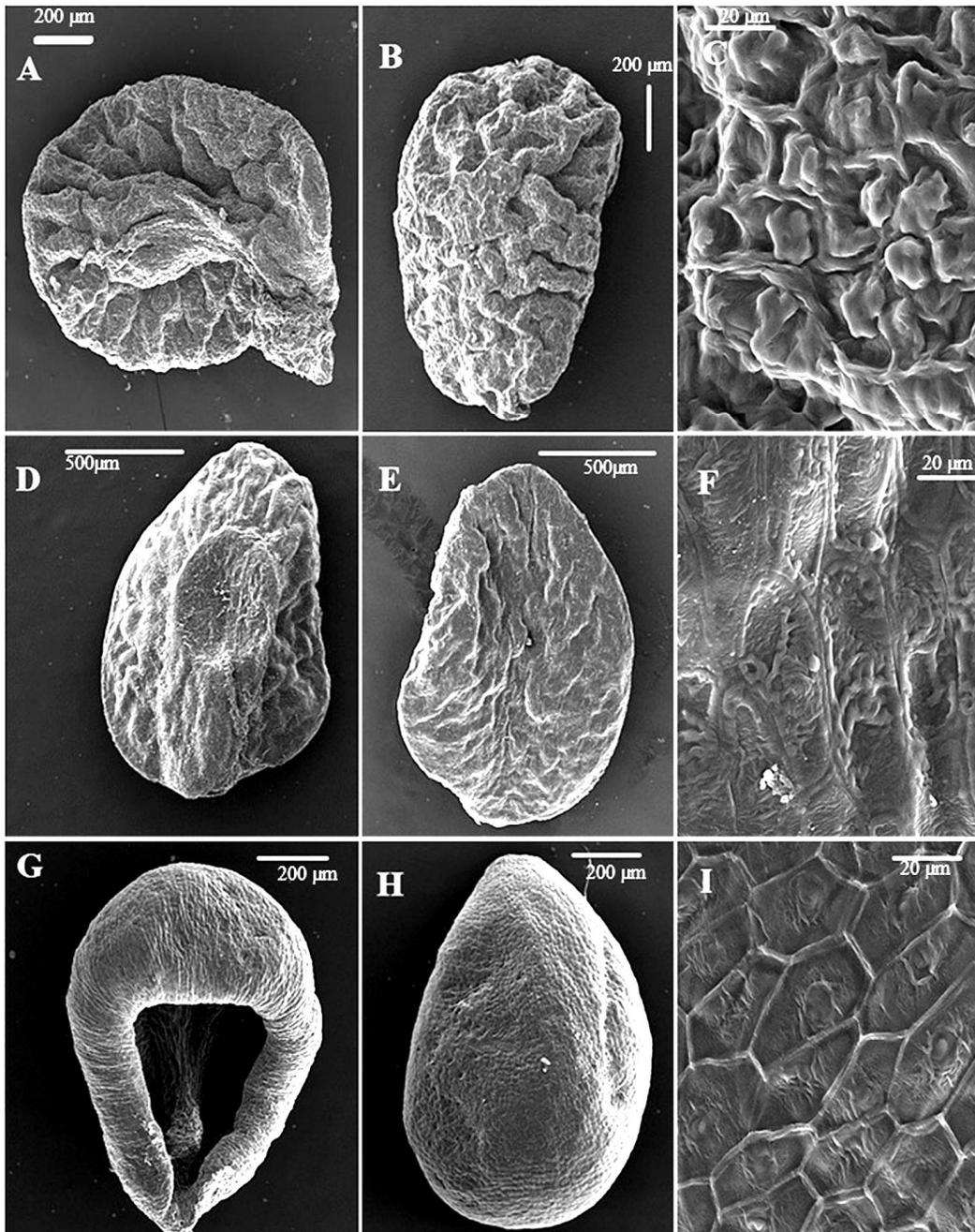


Fig. 1. SEM Micrographs of *Veronica* seeds. A, D, G Ventral face, B, E, H Dorsal face, C, F, I seed coat. A-C: *V. khorassanica*, D-F: *V. czerniakowskiana*, G-I: *V. mazandranae*.

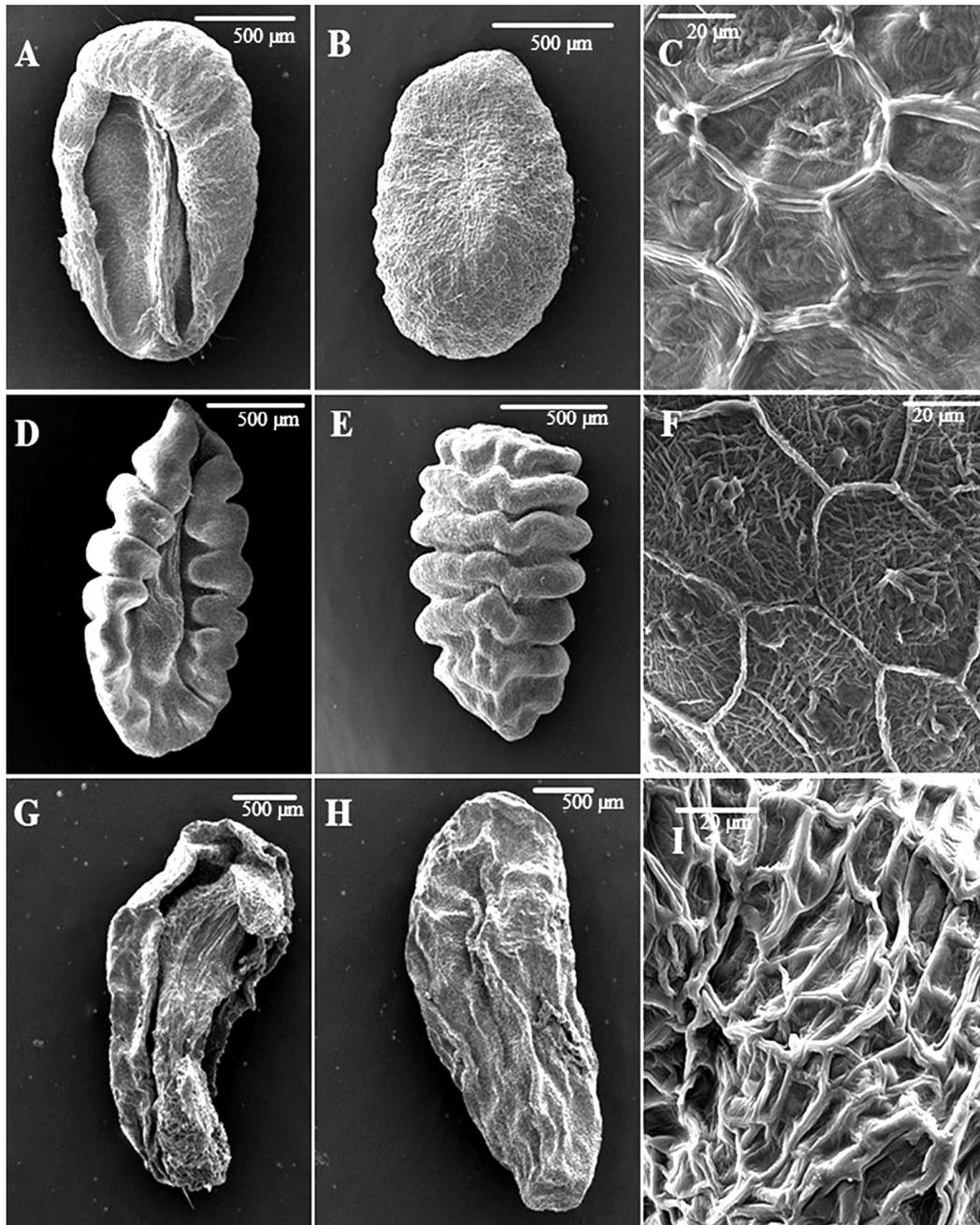


Fig. 2. SEM Micrographs of *Veronica* seeds. A, D, G Ventral face, B, E, H Dorsal face, C, F, I, seed coat. A-C: *V. rubrifolia* subsp. *respectatissima*, D-F: *V. rubrifolia* subsp. *rubrifolia*, G-I: *V. acrotheca*.

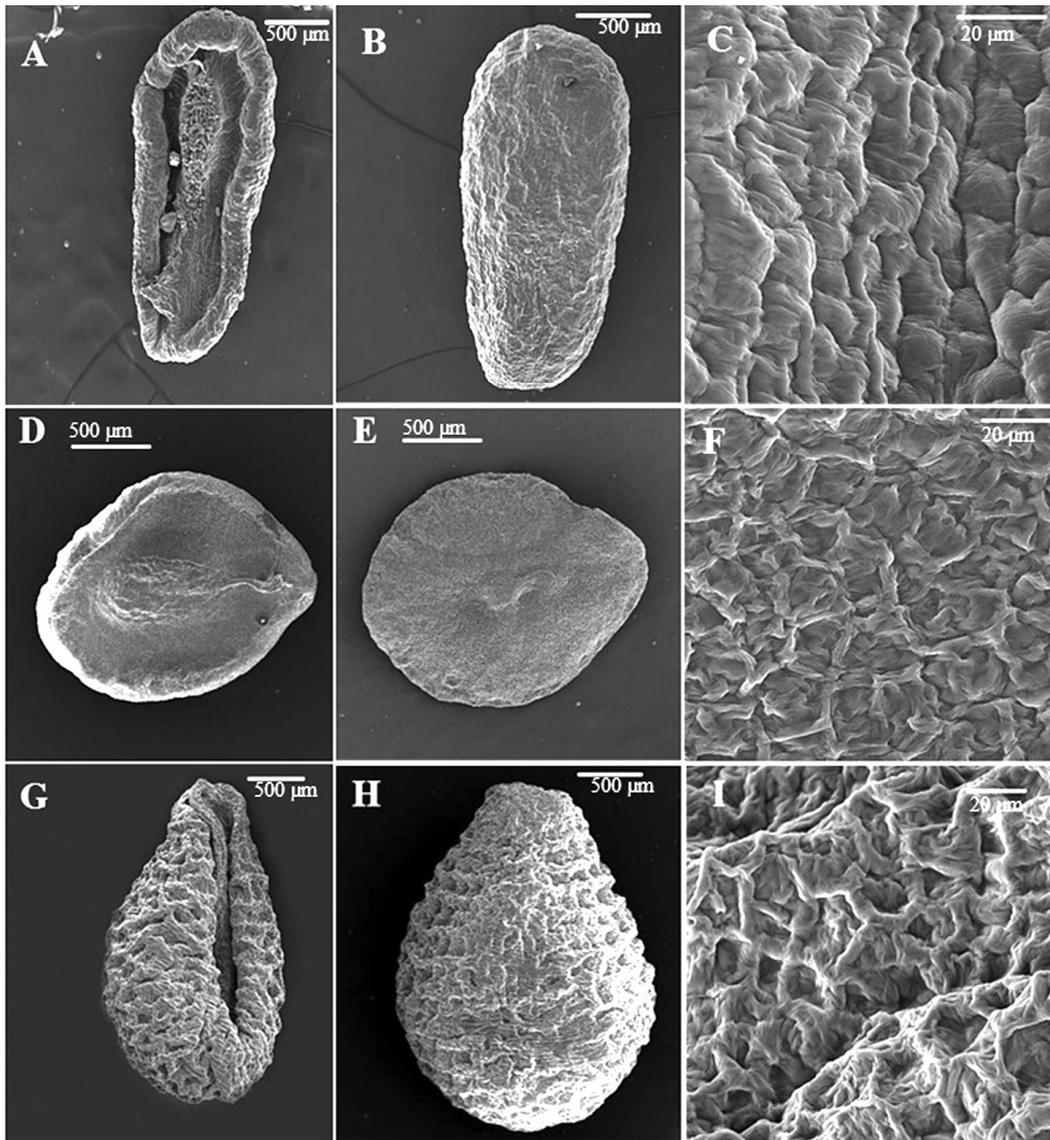


Fig. 3. SEM Micrographs of *Veronica* seeds. A, D, G Ventral face, B, E, H Dorsal face, C, F, I, seed coat. A-C: *V. aucheri*, D-F: *V. viscosa*, G-I: *V. intersedens*.

Dorsal face of seed coat in *V. chionantha* has a particular structure that is introduced here for the first time. Four to five raised rings with a central cavity that form a pillar. Among the species from subg. *Pentasepala* only *V. rechingeri* shows flattened seeds. *V. gaubae* of subg. *Pentasepala* exhibit a particular pattern in seed coat. It is reticulate-porat because of a periclinal wall with a central pore. This pattern has not been reported so far. *V. viscosa* and *V. intercedens* Bornm. from subg. *Pocilla* show reticulate-rugate. Earlier this pattern was reported in *V. stylophora* Popov and

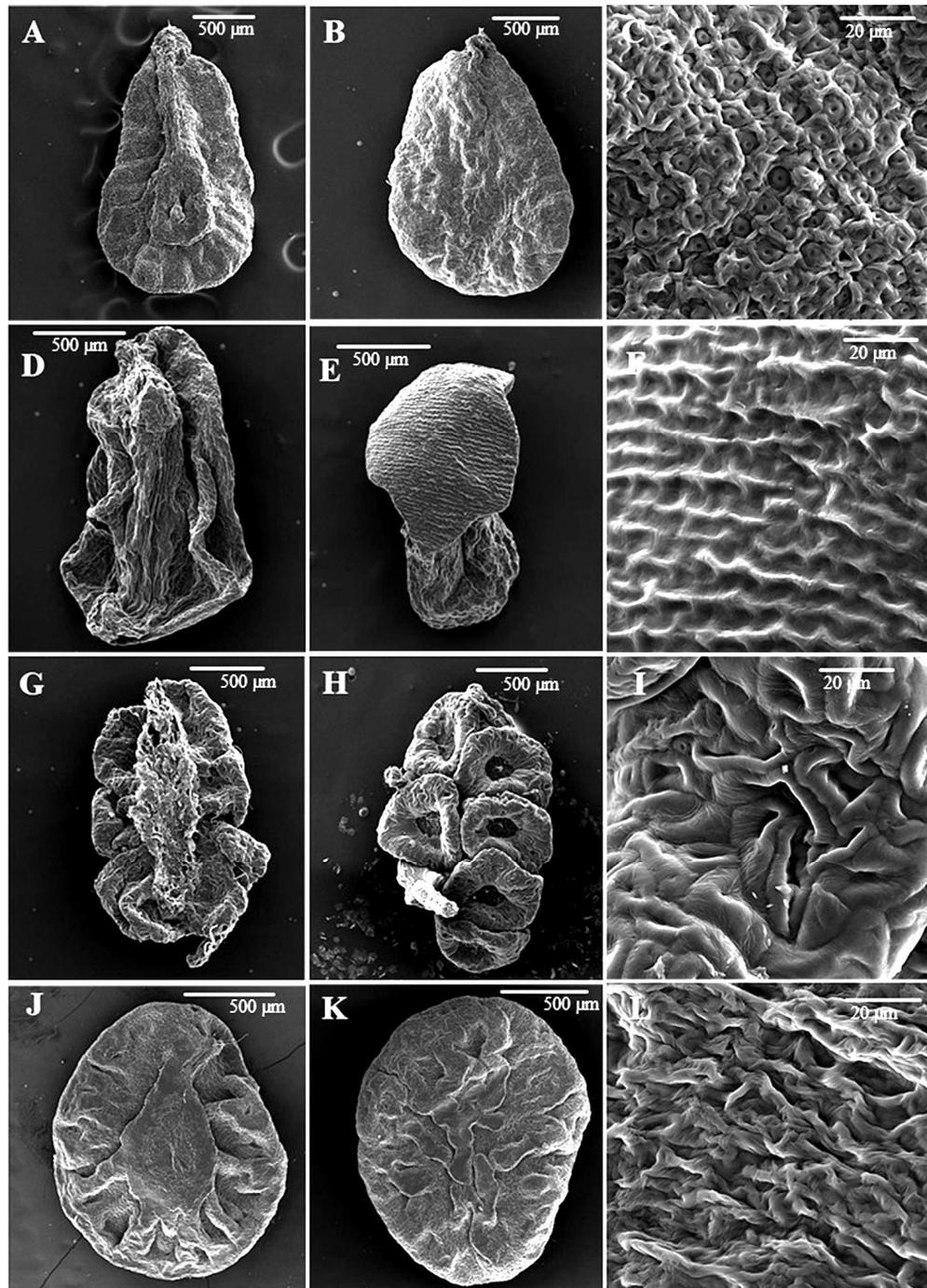


Fig. 4. SEM Micrographs of *Veronica* seeds. A, D, G, J Ventral face, B, E, H, K Dorsal face, C, F, I, L seed coat. A-C: *V. gaubae*, D-F: *V. microcarpa* Boiss., G-I: *V. chionantha*, J-L: *V. rechingeri*.

Table 1. Characteristic features of the seeds of investigated *Veronica* species in Iran.

Species	Subgenus	Life Form	Length (mm)	Width (mm)	Shape	Color	Seed Coat	Cell	Funicular Attachment Terminal
<i>V. khorassanica</i>	<i>Pentasepala</i>	Perennial	1 (1.25 ±0.3)	0.5 (0.75 ±0.3)	Ovate Plano-Convex	Brown	Reticulate Verrucate	Polygonal	Terminal
<i>V. microcarpa</i>	<i>Pentasepala</i>	Perennial	1.5 (1.25 ±0.3)	1 (0.85 ±0.4)	Cymbiform Plano-Convex	Brown	Rugate	Polygonal	Terminal
<i>V. czerniakowskiana</i>	<i>Pentasepala</i>	Perennial	1.5 (1.25 ±0.3)	1 (1.15 ±0.2)	Ovate Plano-Convex	Brown	Reticulate Verrucate	Polygonal	Ventral
<i>V. chionantha</i> *	<i>Pentasepala</i>	Perennial	1.5 (1.1 ±0.1)	1.3 (0.8 ±0.1)	Cymbiform Plano-Convex	Dark Brown	Rugate	Irregular	Terminal
<i>V. acrotheca</i> *	<i>Pentasepala</i>	Perennial	1.2 (1.5 ±0.3)	0.9 (1.25 ±0.3)	Cymbiform Plano-Convex	Dark Brown	Reticulate Rugate	Polygonal	Terminal
<i>V. aucheri</i> *	<i>Pentasepala</i>	Perennial	2.5 (1.25 ±0.3)	1.5 (0.7 ±0.2)	Cymbiform Plano-Convex	Brown	Reticulate Rugate	Polygonal	Subterminal
<i>V. rechingeri</i> *	<i>Pentasepala</i>	Perennial	1.5 (1.75 ±0.3)	0.9 (1.4 ±0.1)	Ovate Flattened	Light Brown	Rugate	Irregular	Subterminal
<i>V. gaubae</i> *	<i>Pentasepala</i>	Annals	2 (1.4 ±0.5)	1.5 (1.25 ±0.3)	Ovate Plano-Convex	Brown	Reticulate Perate	Polygonal	Terminal
<i>V. viscosa</i>	<i>Pocilla</i>	Annals	1.8 (2.5 ±0.7)	1.5 (1.75 ±0.3)	Ovate Flattened	Yellow	Reticulate Rugate	Polygonal	Terminal
<i>V. intercedens</i>	<i>Pocilla</i>	Annals	3 (1.75 ±0.3)	2 (1.1 ±0.1)	Ovate Cyatiform	Yellow	Reticulate Rugate	Polygonal	Terminal
<i>V. rubrifolia</i> subsp. <i>respectatissima</i>	<i>Pocilla</i>	Annals	1.5 (1.65 ±0.2)	1 (1.1 ±0.1)	Ovate sub Cyatiform	Yellow to Brown	Reticulate Verrucate	Polygonal	Terminal
<i>V. rubrifolia</i> subsp. <i>rubrifolia</i> *	<i>Pocilla</i>	Annals	1.8 (1.75 ±0.3)	1.2 (1.25 ±0.3)	Ovate Cyatiform	Yellow	Reticulate Verrucate	Polygonal	Terminal
<i>V. mazandaranae</i> *	<i>Pellidosperma</i>	Annals	2 (1.25 ±0.3)	1.5 (1.15 ±0.2)	Ovate Cyatiform	Brown	Reticulate Verrucate	Polygonal	Subterminal

* Endemic species of Iran.

V. capillipes Nevski of this subgenus (Munoz-Centeno *et al.*, 2006). *V. intercedens* is similar to *V. capillipes* with a cyathiform seed shape, unlike *V. viscosa* where it is flattened. The seed shape and seed coat of *V. rubrifolia* are reported to be cyathiform and cristate at dorsal face (Munoz-Centeno *et al.*, 2006; Hassan and Khalik, 2014). In this study, seed shape of *V. rubrifolia* subsp. *respectatissima* M.A. Fischer is found as sub-cyathiform, though it is cyathiform in *V. rubrifolia* subsp. *rubrifolia* Boiss. Two subspecies of *V. rubrifolia* differ in ornamentation of periclinal wall. It is papillate in *V. rubrifolia* subsp. *rubrifolia* and microreticulate in *V. rubrifolia* subsp. *respectatissima*. The finding of reticulate-verrucate seed coat in *V. rubrifolia* in this study is supported by Munoz-Centeno *et al.* (2006) and Albach *et al.* (2008). In *V. mazandranae*, the only representative of subg. *Pellidosperma*, seeds are sub-cyathiform and smooth in dorsal face. This is accordant with the result of Munoz-Centeno *et al.* (2006) who show that the seeds in this subgenus are sub-cyathiform or cymbiform. The finding of seed coat in *V. mazandranae* as reticulate-verrucate is concordant with the observation of Munoz-Centeno *et al.* (2006) in subg. *Pellidosperma*. This study indicates that seed characters, such as shape, size, dorsal surface, testa cells, and surface ornamentation etc. are useful in identification and classification of the *Veronica* species studied. Micromorphological characters of seeds seem reliable for taxonomic delimitation at both specific and subspecific levels in Iranian *Veronica*. Further studies on seed morphology of *Veronica* species based on broader species sampling are needed for more comprehensive conclusion.

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