Seed Morphology and Seed Coat Sculpturing of *Epilobium* L. Species (Onagraceae Juss.) from Iran

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Abstract: The seeds of 32 specimens belonging to 15 species from 2 sections of the genus *Epilobium* L. (Onagraceae Juss.) were studied by both a light and scanning electron microscope (SEM). Seed morphology of the examined specimens exhibits some variation in shape and size. Seed size ranges between 0.97 mm and 2.2 mm in length. They are ovate, oblong-ovate, and narrowly ovate (ovate-attenuate). The results of SEM investigation of seed coat sculpturing also revealed 5 distinct types of surface structure of the seeds, which are mainly correlated to external morphology of the species.

Key Words: Epilobium, Onagraceae, Seed coat, Seed morphology, SEM

Introduction

The genus *Epilobium* L. is one of the largest genera in the family Onagraceae Juss., with about 185 species throughout the world (Raven, 1976) and 18 species in Iran. The genus contains 2 sections in the Flora Iranica area: Chamaenerion Tausch and Epilobium (Raven, 1964). The species of Epilobium are fairly uniform in external appearance and the genus is a taxonomically difficult group. In addition to vegetative and reproductive characteristics, the features of the seeds have long been employed as an important taxonomic feature within the genus, but most light microscopic examinations are concerned with general shape and size rather than details of surface ornamentation, as was Haussknecht (1884) in his comprehensive monograph of the genus made in an attempt to utilise seed morphology for diagnostic purposes. In recent years, various but limited studies on seed morphology have utilised scanning electron microscopy (SEM) (Berggren, 1974; Skvortsov & Rusanovitch, 1974; Seavey et al., 1977). Since there has been no comprehensive study of seed characters in Iranian species of *Epilobium*, the present work considered seed micro-morphology of 15 species in 2 sections of *Epilobium* from Iran to evaluate seed surface structure as a taxonomic character.

Materials and Methods

Seeds of 15 species from 2 sections of *Epilobium* were provided from the herbaria of TARI, IRAN and SBUH. Specimens investigated are the following:

Section: Chamaenerion

E. dodonaei Vill. (Mazandaran-Ramsar, Mozafarian & Abuhamzeh 42623, TARI).

E. dodonaei Vill. (Mazandaran-Noshahr, Sabeti 7635, TARI).

E. stevenii Boiss. (Azerbaydjan-Sbalan, Asadi 78954, TARI).

E. stevenii Boiss. (Azerbaydjan-Shah bill, Asadi & Mozafarian 29931).

Section: Epilobium

E. hirsutum L. (Mazandaran-Plour, Sheikh Akbari & Moghadam 2006, SBUH).

E. hirsutum L. (Gilan-Asalem, Foroughi 2584, TARI).

E. parviflorum Schreb. (Azerbaydjan-Arasbaran, Moghadam 2024, SBUH).

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E. parviflorum Schreb. (Mazandaran-Ramsar, Sabeti 7635, TARI).

E. montanum L. (Tehran-Shemshak, Sheikh Akbari & Niknam 2012, SBUH).

E. montanum L. (Gilan-Asalem, Asadi & Wendelbo 18411, TARI).

E. lanceolatum Seb. & Mauri (Azerbaydjan-Arasbaran, Gobham & Wendelbo 37487)

E. lanceolatum Seb. & Mauri (Gilan-Astara, Mousavi & Runemark 21618, TARI).

E. minutiflorum Haussk. (Mazandaran-Kandovan, Yousefi 1439, TARI).

E. minutiflorum Haussk. (Tehran-Darake, Sheikh Akbari & Moghadam 2018, SBUH).

E. minutiflorum Haussk. (Markazi-Arak, Sheikh Akbari & Niknam 2017, SBUH).

E. confusum Haussk. (Mazandaran-Ramsar, Asadi & Masoumi 21465, TARI).

E. confusum Haussk. (Lorestan-Shool abad, Runemark & Lazari 26433, TARI).

E. palustre L. (Mazandaran-Ramsar, Runemark & Mousavi 21661, TARI).

E. palustre L. (Isfahan-Natanz, Asadi & Foroughi 18073, TARI).

E. roseum Schreb. subsp. *subsessile* (Boiss.) P.H.Raven (Mazandaran-Gachsar, Sheikh Akbari & Niknam 2013, SBUH).

E. roseum Schreb. subsp. *subsessile* (Boiss.) P.H.Raven (Gilan-Ramesh, Ahmadi 35126, IRAN).

E. anatolicum Haussk. subsp. *anatolicum* (Tehran-Taleghan, Terme, Mousavi & Tehrani 28845, IRAN).

E. rechingeri P.H.Raven (Mazandaran-Lar valley, Asadi & Wendelbo 13398, TARI).

E. rechingeri P.H.Raven (Mazandaran-Kandovan road, Mousavi & Karavar 33858, IRAN).

E. rechingeri P.H.Raven (Tehran-Karaj, Asadi 83119, TARI).

E. frigidum Haussk. (Mazandaran-Ramsar, Runemark & Masoumi 36754, TARI).

E. frigidum Haussk. (Hamedan-Alvand mountain, Asadi & Mozafarian 36818, TARI)

E. frigidum Haussk. (Kordestan-Ghorveh, Asadi 75346, TARI).

E. ponticum Haussk. (Mazandaran-Noshahr, Izadyar 30030, IRAN).

E. ponticum Haussk. (Gilan-Asalem, Gauba 31118, IRAN).

E. gemmascens C.A.Mey. (Mazandaran-Talesh, Mousavi, Delghandi & Tehrani 33658, IRAN).

E. gemmascens C.A.Mey. (Azerbaydjan-Marand, Asadi & Olfat 68530, TARI).

In order to determine the average seed sizes, 10 seeds from each species were measured (Table 1). For SEM studies, seeds were directly mounted on stubs and covered with gold. Photographs were taken with a JEOL JAX-840 SEM. All the specimens were examined, but only the clearest photographs representing each seed coat sculpturing type were selected and illustrated.

Results

The characteristics of seed morphology are listed in Table 1. It appears that the size and shape of seeds are variable within the species of the 2 sections recognised by Raven (1964). Seed size ranges between 0.97 and 2.2 mm in length. Two species from the Chamaenerion section, E. dodonaei Vill. and E. stevenii Boiss., and E. palustre L. from the section Epilobium have large seeds (Table 1). The other seeds are of average size (1.03-1.4 mm long), except E. minutiflorum Haussk. seeds, which are small (0.97 mm long). The shapes of the seeds observed are mainly oblong-ovate to ovate (Figure 1), except E. dodonaei, E. stevenii (Chamaenerion section), E. confusum Haussk., and E. palustre (section Epilobium), which have narrowly ovoid seeds (Figure 1). Most species of Epilobium have papillose seeds and SEM investigation of the seed surfaces shows some variation between the species. Five types of seed surface ornamentation are recognised in this group.

Type I: Type I is characterised by surface cells with prominent and regularly rectangular radial walls. Their tangential walls are compressed into irregularly shaped prominences, which are often collapsed. This type is recognised on the seeds of 2 studied species from the *Chamaenerion (E. steveii & E. dodonaei)* section (Figure 1. K and L).

Type II: Seed surface cells are polygonal in outline,

Seed characters/Species		Shape	Length (mm)	Breadth (mm)	Surface type
Section Chamaenerion	E. dodonaei	Narrowly ovate	1.99	0.5	I
	E. stevenii	Narrowly ovate	2.2	0.5	Ι
	E. hirsutum	Ovate	1.4	0.48	II
	E. parviflorum	Ovate	1.09	0.5	II
	E. montanum	Oblong-ovate	1.09	0.36	III
	E. lanceolatum	Oblong-ovate	1.12	0.4	III
	E. minutiflorum	Oblong-ovate	0.97	0.34	II
Section <i>Epilobium</i>	E. confusum	Narrowly ovate	1.4	0.4	II
	E. palustre	Narrowly ovate	1.93	0.58	II
	E. roseum subsp. subsessile	Oblong-ovate	1.02	0.41	II
	E. anatolicum subsp. anatolicum	Oblong- ovate	1.05	0.41	II
	E. gemmascens	Oblong-ovate	1.03	0.39	II
	E. frigidum	Oblong-ovate	1.13	0.35	IV
	E. ponticum	Ovate	1.12	0.42	IV
	E. rechingeri	Oblong ovate	1.35	0.44	V

Table 1. Comparison between seed size, shape, and seed coat sculpturing in Epilobium species. Sizes are given as averages.

with a convex portion having clearly distinct spiral lines that are centrally located at the centre of tangential walls. Each cell is isolated from its adjacent cells by prominent and rigid radial walls (Figure 2. A). Type II is observed in most of the species of the section *Epilobium* (*E. minutiflorum*, *E. confusum*, *E. anatolicum* Haussk. subsp. *anatolicum*, *E. gemmascens* C.A.Mey., and *E. roseum* Schreb. subsp. *subsessile* (Boiss.) P.H.Raven) (Figure 2. B-G).

Type III: There are no prominent radial walls in this type. The surface cells are arranged in an irregular pattern; the tangential walls of cells are collapsed in the centre (Figure 2. H and I). This type is indicated by only 2 species of the section *Epilobium (E. montanum* L. and *E. lanceolatum* Seb. & Mouri).

Type IV: Cells are regularly polygonal and reticulate. The radial walls are the most prominent feature of the seed coat surface. The tangential walls have no convex portion at their centre. This type is observed on the seeds of *E. ponticum* Haussk. and *E. frigidum* Haussk. (Figure 2. J and K). This type has also been called foveolate by Seavey et al. (1977).

Type V: The central prominences of the surface cells are irregularly compressed into a multisided prominence.

There are no prominent radial walls for the cells (Figure 2. L). This type is represented by Iranian endemic species of *Epilobium (E. rechingeri* P.H.Raven).

Discussion

Morphological characters of seeds provide valuable information on the evolutionary classification of flowering plants (Corner, 1976; Takhtajan, 1991). Skvortsov and Rosanovitch (1974) and Seavey et al. (1977) presented and discussed some results from macro- and micromorphological studies of *Epilobium*. In our observations, a number of seed features occur in *Epilobium* that vary between different species, such as seed shape, size, and seed coat sculpturing (Table 1). These features can be used in genus taxonomy.

Seed coat sculpturing consists of repetitive units called surface cells. Special forms of these surface cells are the result of differential thickening in their walls (Denford & Karas, 1974). Many micro-structural details of the seed coat surface are strikingly constant from one sample to another in each species (Figure 2. B and C), while it varies between different species. Although most seeds of the species are type II, some species are separated from others on the basis of this feature.



Figure 1. A: Ovate seed of *E. ponticum*. B: Ovate seed of *E. parviflorum*. C: Oblong-ovate seed of *E. minutiflorum*. D: Oblong-ovate seed of *E. roseum* subsp. *subsessile*. E: Oblong-ovate seed of *E. rechingeri*. F: Oblong-ovate seed of *E. frigidum*. G: Narrowly ovate (ovate-attenuate) seed of *E. confusum*. H: Narrowly ovate seed of *E. palustre*. I: Narrowly ovate seed of *E. stevenii*. J: Narrowly ovate seed of *E. dodonaei*. K: Seed coat surface ornamentation of *E. stevenii* representing type I. L: Seed coat surface ornamentation of *E. dodonaei* representing type I. Scale bars for whole seed images: 200 µm Scale bars for images K and L: 10 µm

Most species of *Epilobium* (section *Epilobium*) have seed coat type II, such as *E. minutiflorum*, *E. confusum*, *E. anatolicum* Haussk. subsp. *anatolicum*, *E. gemmascens*, *E. roseum* subsp. *subsessile*, *E. hirsutum* L., *E. parviflorum* Schreb., and *E. palustre*, but they differ from each other in seed size and shape (Table 1). *E. stevenii* and *E. dodonaei* (section *Chamaenerion*) differ from the section *Epilobium* on the basis of seed size, shape, and seed coat ornamentations (Table 1).

E. montanum and *E. lanceolatum* are morphologically similar and have type III seed coat sculpturing.



Figure 2. A: A surface cell with convex portion of tangential wall and rigid radial walls (arrows points) on seed of *E. gemmascens*. B: Seed coat surface ornamentation of *E. minutiflorum*, representing type II (Tehran region). C: Seed coat surface ornamentation of *E. gemmascens*, representing type II. E: Seed coat surface ornamentation of *E. anatolicum*, subsp. *anatolicum*, representing type II. F: Seed coat surface ornamentation of *E. hirsutum*, representing type II. G: Seed coat surface ornamentation of *E. parviflorum*, representing type II. H: Seed coat surface ornamentation of *E. montanum*, representing type II. B: Seed coat surface ornamentation of *E. montanum*, representing type II. I: Seed coat surface ornamentation of *E. montanum*, representing type III. I: Seed coat surface ornamentation of *E. montanum*, representing type III. I: Seed coat surface ornamentation of *E. montanum*, representing type III. I: Seed coat surface ornamentation of *E. montanum*, representing type III. I: Seed coat surface ornamentation of *E. lanceolatum*, representing type III. J: Seed coat surface ornamentation of *E. ponticum*, representing type IV. K: Seed coat surface ornamentation of *E. frigidum*, representing type IV. L: Seed coat surface ornamentation of *E. rechingeri*, representing type V. Scale bars for images: 10 μm Scale bar for image A: 2 μm

E. ponticum and *E. frigidum* are type IV, but *E. ponticum* has ovate seeds, whereas *E. frigidum* has oblong-ovate seeds (Figure 1. A and F). *E. rechingeri* (only endemic species from Iran) has type V seed coat sculpturing, and this type is very distinct from the others.

Results of this study are correlated primarily with the morphological classification in Flora Iranica. Consequently, seeds in *Epilobium* have unique features that facilitate species recognition and description.

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