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Scanning electron microscopy of the surface structure of seeds from the genus *Epilobium* in Fennoscandia for determining the species

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The surfaces of seeds from nineteen species of *Epilobium* in Fennoscandia were investigated with scanning electron microscope. The photographs were taken of seeds at magnifications of approximately 45 or 90 times, respectively, depending on the size of the seed. In addition, photographs of four times higher magnification were taken of the base of each seed, as well as detailed pictures of the seed surface at a magnification of about 560 times. In general, the photographs of the seeds were found to provide sufficient evidence to identify the species.

Introduction

In his classical monograph on *Epilobium*, Haussknecht (1884) briefly discussed the seeds from a morphological point of view. However, the description of the structure was approximate only, and did not provide rules on how to classify the species on the basis of this information. In the 1970's, Oredsson and Snogerup treated species of *Epilobium* in a series of publications (e.g. Oredsson & Snogerup 1975, 1976, 1977). Kytövuori (1972) presented a morphological, taxonomical and ecological study of four species of *Epilobium* and the appearance of their seeds. During the same decade when the use of the scanning electron microscope (SEM) became more common, the possibilities to analyze the seed structure were improved. Several investigators, such as Berggren (1974), Denford & Karas (1974), Skvortsov & Rusanovitch (1974), Raven & Raven (1976), and Seavey et al. (1977), have described the appearance of the seeds of different species of *Epilobium* on the basis of SEM photographs. Still, not even these references provide any clear rules on how to distinguish the species from each other. In a recent paper by Akbari & Azizian (2006), some local species of *Epilobium* were grouped into five types on the basis of seed morphology and seed coat sculpturing.

In the present work, the surface structure of species of *Epilobium* seeds in Fennoscandia is investigated with SEM.

Material and Methods

The work studies seeds of the *Epilobium* species treated in the Finnish Field Flora (Hämet-Ahti et al. 1998, 2005). The seeds were obtained from herbarium specimens deposited in the Botanical Museum of the University of Helsinki. First, the seeds were carefully selected and prepared under a stereo microscope to find representative objects. In general, only small differences were observed between seeds from the same species, and the pictures to be presented must therefore be considered

representative for the species in question. The investigation was focused on the convex (”dorsal”) side of the seeds, since the flattened (”ventral”) side has less pronounced surface structures. The coma (Fig. 1a) was removed with a scalpel from each seed and the seed was mounted on conductive carbon tabs and spottened with gold. Micrographs of the specimens were then taken with a Cambridge Instruments SEM (model S 360) using the secondary electron detector. The scanning

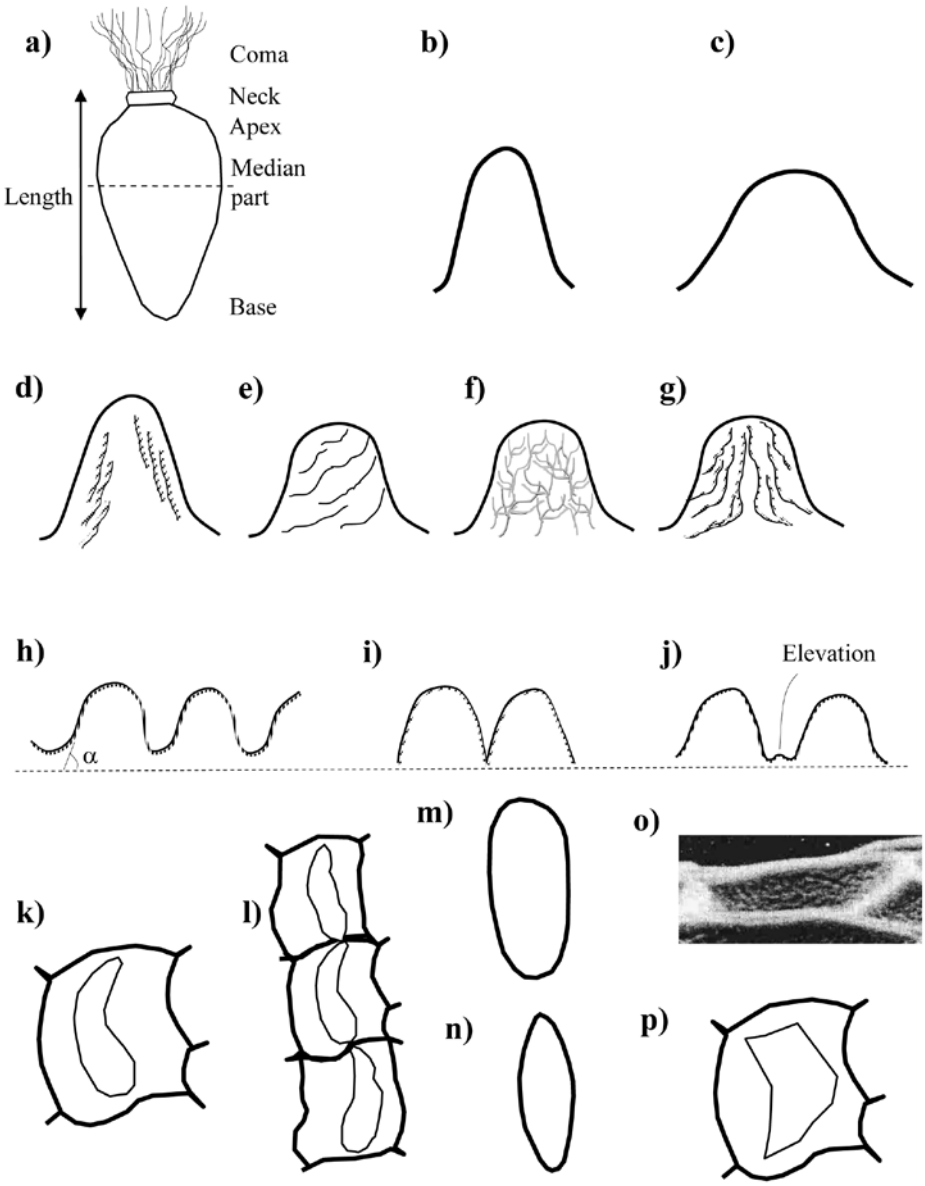

Fig. 1 Illustration of notation: *a*) (*Epilobium*) seed, *b*) parabola-shaped papilla, *c*) hemispheric papilla, *d*) parabola-shaped papilla with parallelly running furrows in the base-point direction of the papilla, e) hemispheric papilla with spiral lines *f*) hemispheric papilla with furrows in random directions, *g*) hemispheric papilla with radially running furrows, *h*) papilla with small base angle (α), *i*) papilla with large base angle, *j*) ridges between the papilla rows in the longitudinal direction of the seed, *k*) cell with crest-like papilla, *l*) ridge (cell with crest-like papillae in a row), *m*) oblong-ovate seed (longitudinal cross-section), *n*) fusiform (spindle shaped) seed, *o*) cell with pit, and *p*) cell with angular papilla.

electron microscope was operated at an accelerating voltage of 15 kV. These stages of the work were carried out at the Laboratory of Inorganic Chemistry at Åbo Akademi University in Turku, Finland.

Seed Types

Gross Classification

With respect to the surface structure of seeds of *Epilobium* a division into four groups can be made: *A*. Seeds with irregular cell walls forming reticulum, *B*. Seeds with papillae, *C*. Seeds with ridges, and *D*. Seeds with pits. The convex part of the tangential wall of the epidermal cells is here referred to as a *papilla*. The special terminology used to describe the surface structure is defined in Fig. 1.

A. Seeds with irregular cell walls forming reticulum
The most prominent characteristic of the seed surface is an irregular polygonal reticulum (network) formed by irregular polygonal epidermal cells with very thin cell walls.

B. Seeds with papillae
The papillae are shaped differently by the strongly arched outer cell walls of the epidermal cells. In most of the species, the papillae are isolated from the adjacent cells by a network of pronounced (rigid) radial walls. The papillae of the specific species may be deformed, forming lines, creases and wrinkles in different directions.

C. Seeds with ridges
The structure of the seed is characterized by rectangular, often almost square cells in parallel rows in the longitudinal direction of the seeds. The surface of the cells is convex and is enclosed by clear cell walls. Along the central axis of the cells there is a longitudinal ridge, formed by strongly squeezed papillae (Fig. 1*k*). The crest in a cell may lean to one or the other side, and such adjacent cells which have merged into longitudinal rows form ridges (Fig. 1*l*). The ridges may be disrupted at intervals. The distances between the ridges at the seeds are larger than the distances between the rows of papillae in seeds without ridges, likely due to the fact that the cells are strongly stretched in the direction of the periphery of the seed.

D. Seeds with pits
By flattening of the central papilla in seeds with papillae, pits have aroused in the surface structure of the seeds. Seeds in this group have generally been described as smooth, but later and at large magnification it has become obvious that the seed surface is uneven due to extremely thickened and somewhat concave outer walls of the cells, which gives rise to shallow pits (Fig. 1*o*). The epidermal cells of the seeds in this group are clearly limited and usually irregularly polygonal, and sometimes square-like. In the literature, the term *foveolate* is sometimes used for this kind of seed structure with small pits with rounded contours (Stearn 1992). However, it seems like *alveolate*, in fact, would be a better characterization of this kind of rough seed surfaces with pits constrained by elevations with angular contours.

Species-Wise Treatment

The following species of the genus *Epilobium* are treated (after the name of the species, the country from where the seeds stem is reported: [F] = Finland, [N] = Norway, [S] = Sweden).

Group A: *E. angustifolium* L. [F]

Group B: *E. parviflorum* Schreb. [S], *E. hirsutum* L. [F], *E. montanum* L. [N], *E. collinum* C.C.Gmel. [N], *E. roseum* Schreb. [S], *E. lamyi* F.W. Schultz [F], *E. tetragonum* L. [S], *E. obscurum* Schreb. [F], *E. davuricum* Fisch. [F], *E. palustre* L. var. *palustre* [F], *E. palustre* L. var. *lapponicum* Wahlenb. [F], *E. laestadii* Kytövuori [F], *E. hornemannii* Rchb. [N].

Group C: *E. adenocaulon* Hausskn. western race [F], *E. adenocaulon* Hausskn. eastern race [F], *E. ciliatum* Raf. [F], *E. glandulosum* Lehm. [N].

Group D: *E. alsinifolium* Vill. [N], *E. lactiflorum* Hausskn. [S], *E. anagillidifolium* Lam. [N].

E. angustifolium (Fig. 2)
The seeds are 1.1–1.4 mm long and asymmetric. In the longitudinal direction of the seed, elevations and valleys, forming a sack-like appearance, can be seen. The fine structure of these consists of irregular polygonal cells with very thin walls that form an irregular polygonal reticulum (network). This is the most distinguished feature of the seed surface. The cells lack papillae and appear ”in disorder”.

E. parviflorum (Fig. 3)
The seeds are small, with a length of 0.8–1.0 mm. Their form is ovate and roundish, i.e. the cross section is plump on the dorsal side of the seed. The apex of the seed is blunt and lacks a neck (cf. Fig. 1a). The size of the seeds is like those of *E. roseum* but the colour is lighter. The papillae of *E. parviflorum* are narrow and randomly distributed while those of *E. roseum* are somewhat larger, denser and appear in many rows. Like *E. roseum*, the papilla point is parabola shaped (Fig. 1*b*) and has more or less (±) parallelly running creases and furrows in the base-point direction of the papilla (Fig. 1*d*). The height of the papillae is roughly the same as that of *E. roseum*, i.e. about 20 µm. They are located in the centre of the cells and are delimited from each other by clearly appearing cell walls.

E. hirsutum (Fig. 4)
The seeds are small, 0.8–1.1 mm long, and they are slightly larger than those of *E. parviflorum,* and are ovate. The apex of the seed is broad and lacks a neck. The papillae are densely located and high (approx. 35 µm) and appear in irregular, longitudinally interrupted rows, which are not straight. The shape and appearance of the papillae vary due to secondary deformations, which may arise, e.g. when the seed has dried. The outer cell wall, which forms a convex, hemispherical papilla (Fig. 1*c*), has on its surface numerous ± spirally running fine elevated lines (Fig. 1*e*) that are characteristic for *E. hirsutum.*

E.montanum (Fig. 5) and *E. collinum* (Fig. 6)
The seeds of these two species are very similar and show only minor differences. The seeds have a pointed and slightly narrowing base and the apex is neck-less and blunt. The ovate seeds

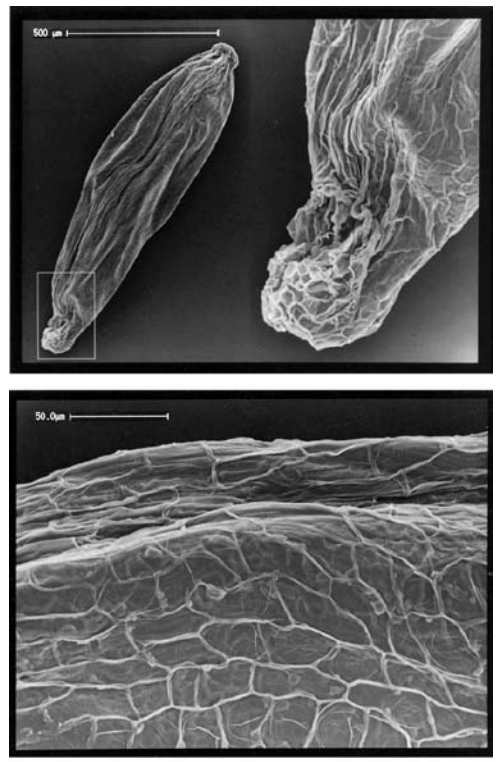

Fig. 2. *E. angustifolium*

of *E. collinum* are smaller (0.9–1.1 mm) than the oblong-ovate seeds of *E. montanum* (1.0–1.3 mm) and have smaller and denser papillae. The height of the papillae of *E. collinum* is approx. 20 µm and for *E. montanum* approx. 30 µm and the shapes of the papillae are different: The basal parts of the papillae are less steep in *E. collinum*, i.e. the base angle formed against the seed surface is smaller (α in Fig. 1*h*). This makes the furrows between the papillae more shallow. In *E. montanum* the papillae seem more free-standing and the basal parts of them are steeper than for *E. collinum*, i.e. the base angle α is larger (Fig. 1*i*). In *E. montanum* the cell walls between the rows of papillae appear as ridge-like formations in the longitudinal direction of the seed (Fig. 1*j*), while in *E. collinum* such ridges do not appear. The papillae of *E. collinum* and *E. montanum* both have small furrows on the convex cell surface that run in different directions (Fig. 1*f*). It should be stressed that *E. lamyi* has papillae of the same type as *E. montanum,* but they are somewhat larger and more sparsely distributed.

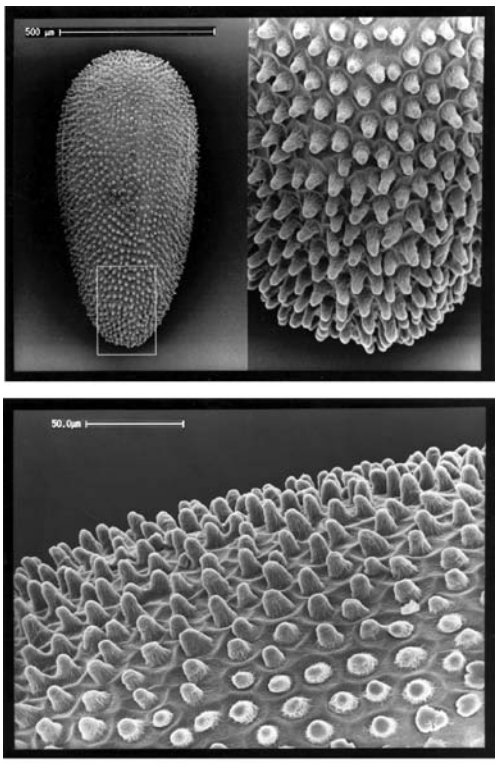

Fig. 3. *E. parviflorum*

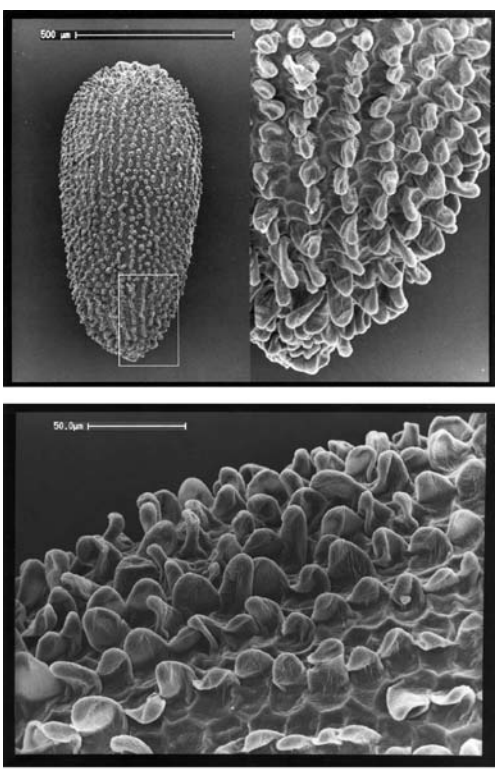

Fig. 4. *E. hirsutum*

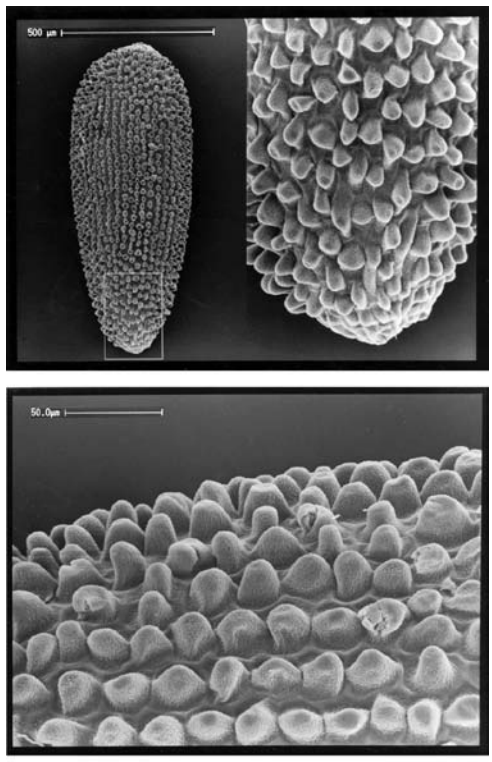

Fig. 5. *E. montanum*

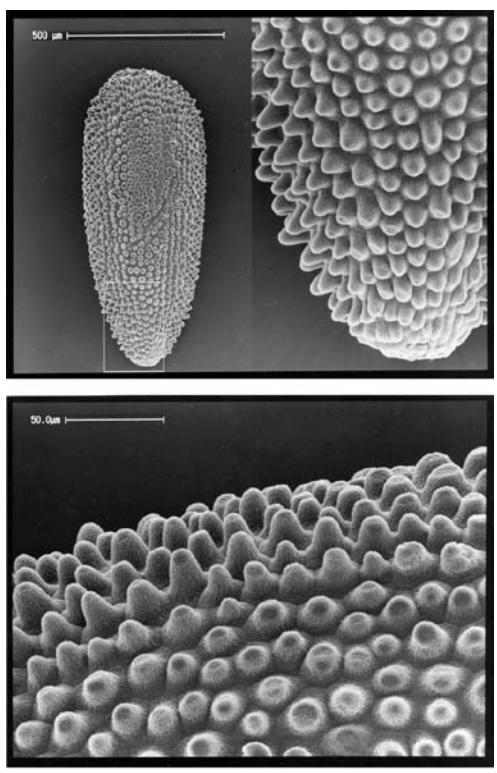

Fig. 6. *E. collinum*

E. roseum (Fig. 7)
The seeds are 0.9–1.2 mm long and of ovate shape, neck-less with a blunt apex. The size of the seed is similar as in *E. parviflorum* and the colour is dark brown. The cells of *E. roseum* are small and polygonal with prominent cell walls; the papillae are very centrally located in the cells. They are small, pointed, dense and appear in many small rows. The papilla point has, as in *E. parviflorum,* a parabola shape (Fig. 1*b*) and both species have ± parallelly running furrows in the base-point direction of the papilla (Fig. 1*d*). As in *E. collinum*, the papilla height is approx. 20 µm but the overall size is smaller and they appear denser. Compared to the papillae of *E. parviflorum,* they are of approximately the same height, but are thicker and more densely distributed.

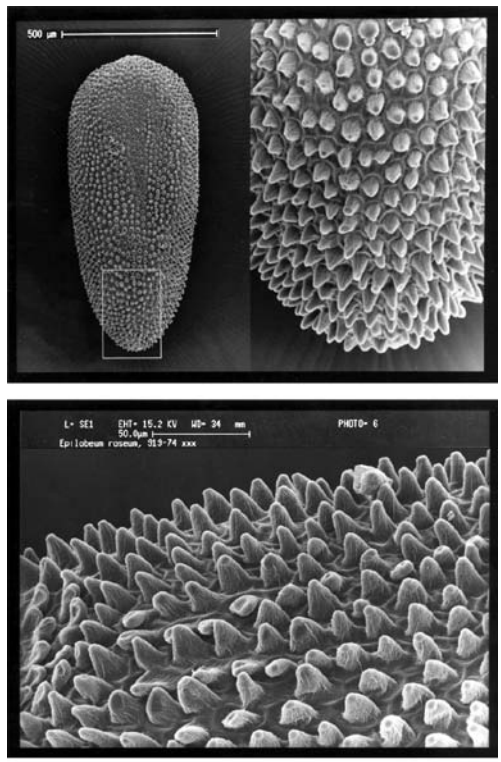

Fig. 7. *E. roseum*

E. lamyi (Fig. 8) and *E. tetragonum* (Fig. 9)
The seeds of these two species are strikingly similar, and only minor differences exist. Their lengths are 0.8–1.0 mm and they are dark brown. The shape of the seeds is ovate and slightly roundish. The apices of the seed for both species are rounded, but blunter in *E. lamyi*. The base of the seed of *E. lamyi* is somewhat pointed while it is rounded in *E. tetragonum*. Both species have papillae that are large and high (ca 20–30 µm). The papillae of *E. lamyi* resemble those of *E. montanum* (Fig. 1*i*), i.e. the papillae are steep and the base angle is large in the peripheral direction of the seed. The papillae of *E. tetragonum* (Fig. 1*j*) are of the same type as for *E. collinum* (Fig. 1*h*), i.e. less steep (lower base angle α) in the peripheral direction. In turn, in the longitudinal direction of the seed, *E. tetragonum* (Fig. 1*j*) shows ridge-like elevations formed by the cell walls between the rows of papillae, and the papillae are somewhat thicker and more sparsely distributed. The most characteristic difference between *E. tetragonum* and *E. lamyi*, however, is the width of the seed (approx. 0.38 mm vs. 0.43 mm).

E. obscurum (Fig. 10)
The seeds are small, 0.7–0.9 mm, and their shape is ovate and neck-less. The base of the seed is somewhat pointed, and the apex of the seed is slightly rounded but not as abruptly as in *E. lamyi*. The papillae are deformed and, seen from above, differently angular (Fig. 1*p*). They appear in longitudinal characteristic rows where the bases of the papillae partly overlap, which, depending of the shape and irregularity of the papillae, makes the rows appear curved and disrupted.

E. davuricum (Fig. 11)
The dark brown seeds are 1.3–2.0 mm long and narrowly ovate. The seed is broadest close to its median part, which is characteristic of *E. davuricum* compared to *E. palustre* var. *palustre*, the seeds of which are broadest above (cf. Fig. 1a) the median part (Kytövuori 1969). The base of the seeds of *E. davuricum* is, furthermore, slightly more pointed and the width is larger. The neck of the seed is relatively tall (approx. 0.3 mm) and is as long as broad, which is characteristic of *E. davuricum*. The papillae are somewhat lower than those of *E. palustre* var. *palustre* and *E. laestadii* and appear in irregular rows. On their surfaces the papillae show numerous radial furrows like those of *E. palustre* var. *palustre* (Fig. 1*g*).

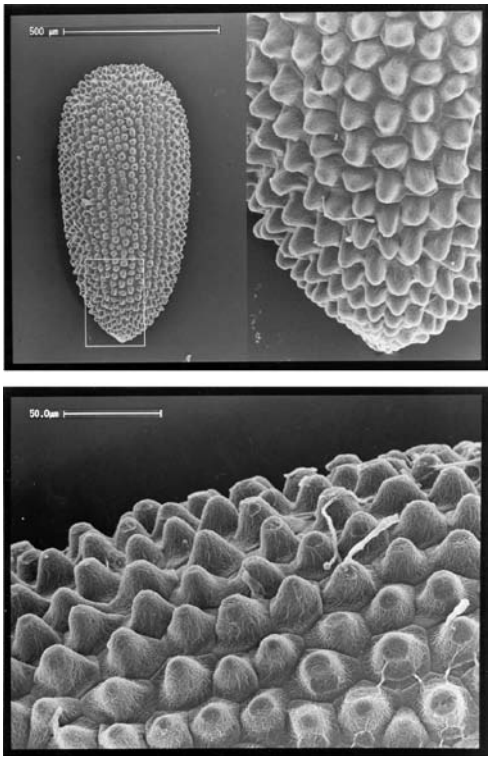

Fig. 8. *E. lamyi*

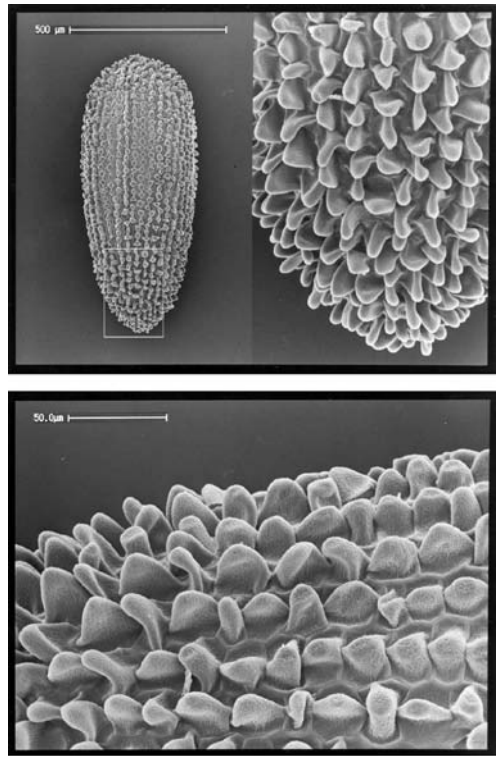

Fig. 10. *E. obscurum*

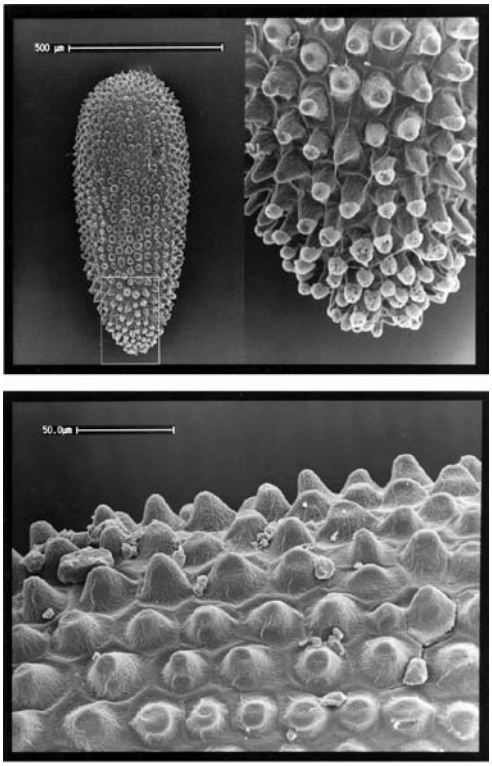

Fig. 9. *E. tetragonum*

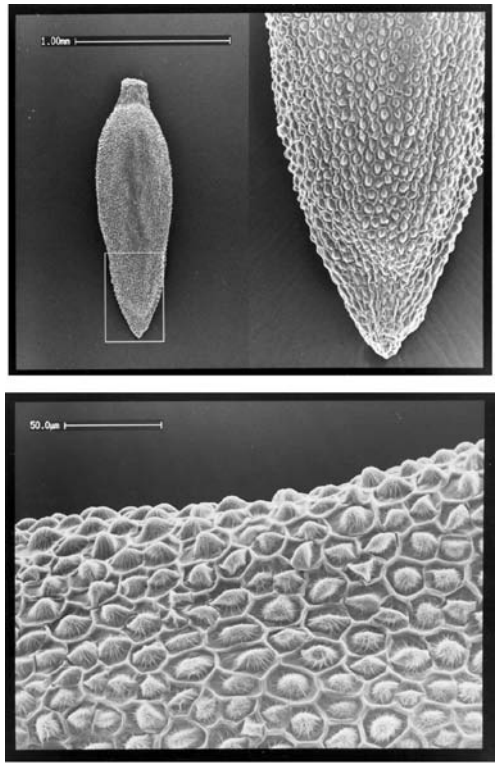

Fig. 11. *E. davuricum*

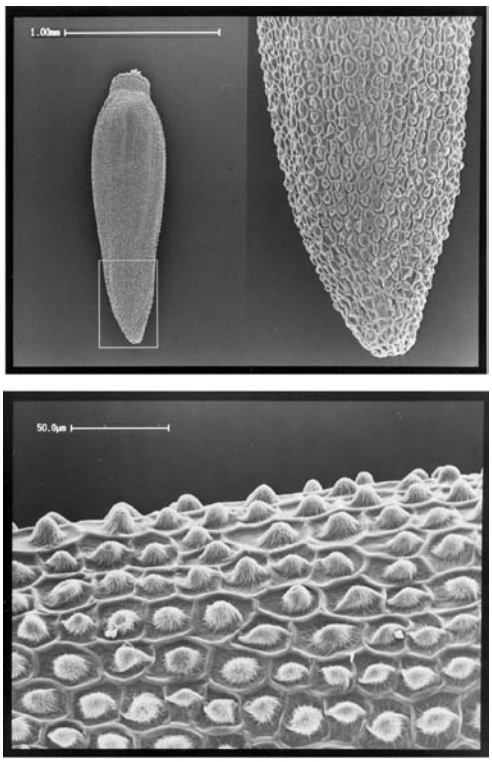

Fig. 12. *E. palustre* var. *palustre*

E. palustre var. *palustre* (Fig. 12)
The brown and relatively large seeds are 1.3–2.1 mm long, and much lighter that the seeds of *E. davuricum.* The narrowly ovate seed is broadest above its mediate part, while it is broadest closer to the median part in *E. davuricum*. Compared to *E. davuricum*, the base of the seed is less pointed. The length of the neck of the seed (approx. 0.1 mm) is about half of the width. The papillae are as high as they are broad (approx. 10 µm) and appear in longitudinal rows. On their surfaces the papillae show numerous radial furrows like those of *E. davuricum* (Fig. 1*g*).

E. palustre var. *lapponicum* (Fig. 13)
The seeds are large, 1.8–2.4 mm, and narrowly ovate. Compared with *E. palustre* var. *palustre* the seed is clearly larger and its neck section is broader (approx. 0.3 mm vs. 0.2 mm), with a neck length to width ratio of about 1:2. In a light microscope the seed surface looks smooth, but the SEM reveals clear papillae. These are arranged in longitudinal rows and the papillae are smaller than those of *E. palustre* var. *palustre* (smaller than 10 µm). A characteristic feature is that the walls between the cells are mostly straight and square-shaped, while they are clearly curved in *E. palustre* var. *palustre*.

E. laestadii (Fig. 14)
The seeds are 1.3–1.9 mm long, light brown and narrowly ovate. Their neck is relatively narrow and about as broad as long (approx. 0.2 mm). The papillae appear in longitudinal rows and are about 7 µm tall. The epidermal cell contours are irregularly polygonal. The walls between adjacent cells are curved in some cells.

E. hornemannii (Fig. 15)
The seeds are 1.0–1.4 mm long and clearly smaller than those of *E. alsinifolium*, and are slimmer than the seeds of *E. anagallidifolium*. They are also somewhat smaller than the seeds of *E. lactiflorum*. The seeds are narrowly ovate and their necks are relatively small. The papillae are prominent and can after drying be folded.

E. adenocaulon (Figs. 16, 17)
The Finnish Field Flora (Hämet-Ahti et al. 1998) treats two races of *E. adenocaulon,* a western (Fig. 16) and an eastern one (Fig. 17). For the western race, the seeds are 1.1–1.4 mm long, dark brown and with higher, irregular and ± clearly disrupted ridges. The seeds wind in different directions and their shape is not overall as symmetrical as the other seeds in the group. The seeds have a characteristic base, which is long, crooked and slim. The sides of the seed are almost parallel and narrow as the base and the apex is approached. The narrowing is not, however, as smooth and gradual as the apex is approached, but is abrupt at the transition to the appendix-like neck. The seeds in the capsule overlap so the base of the seed above lies far below the upper part of the lower seed. This crowding and the resulting pressure give rise to the formation of a hollow in the base of the upper seed. The height of the ridges is approx. 20 µm and the distance between them is considerable (about 30 µm). For the eastern race the seeds are 0.8–1.2 mm long and light brown, with straighter and less clearly disrupted ridges. The seeds are

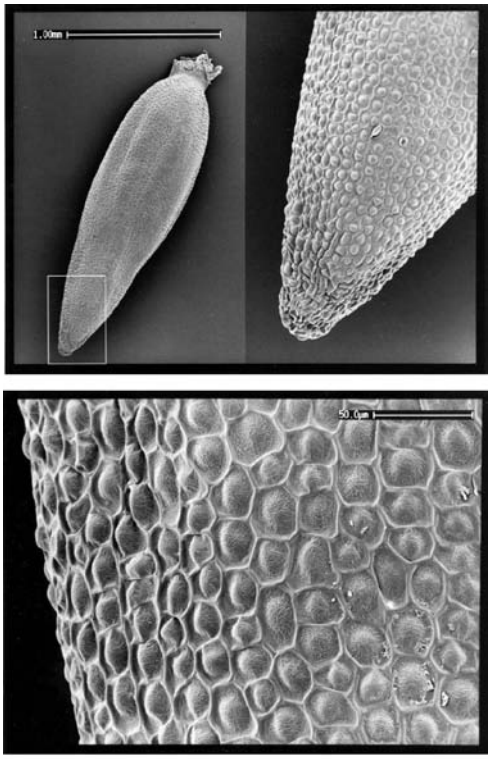

Fig. 13. *E. palustre* var. *lapponicum*

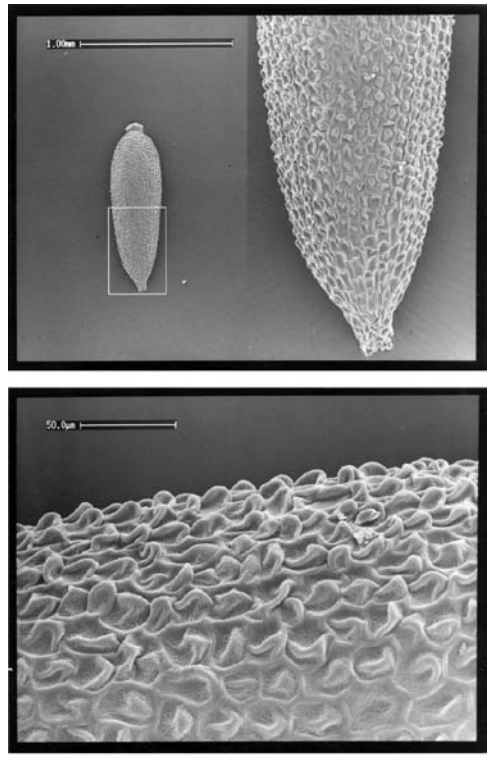

Fig. 15. *E. hornemannii*

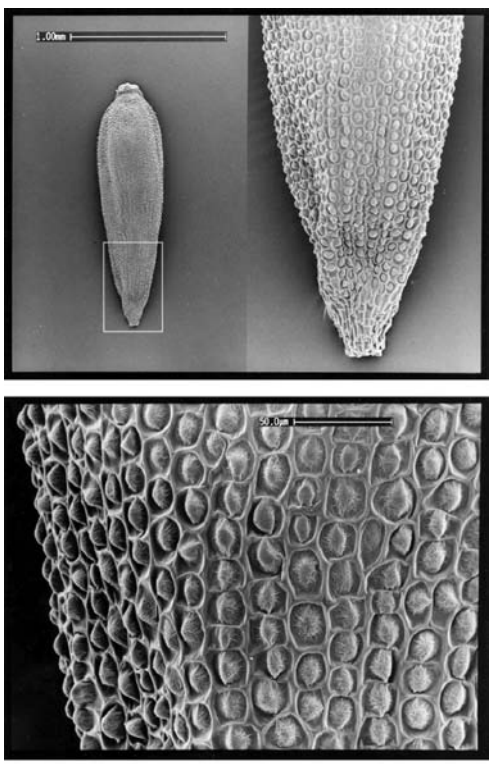

Fig. 14. *E. laestadii*

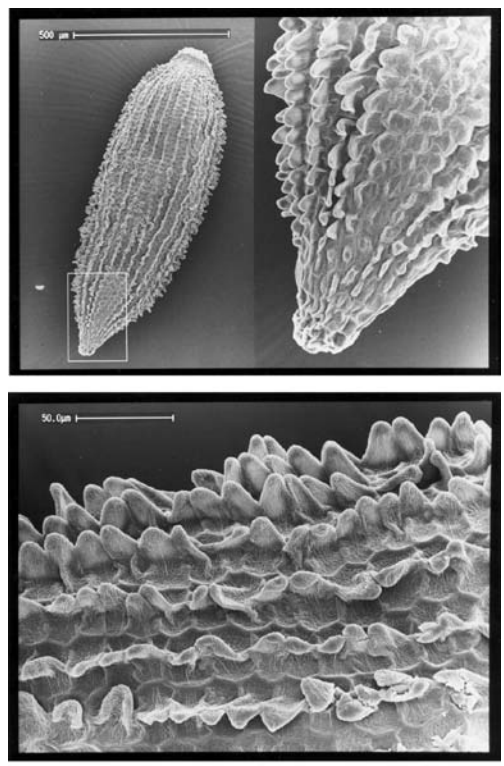

Fig. 16. *E. adenocaulon* (western race)

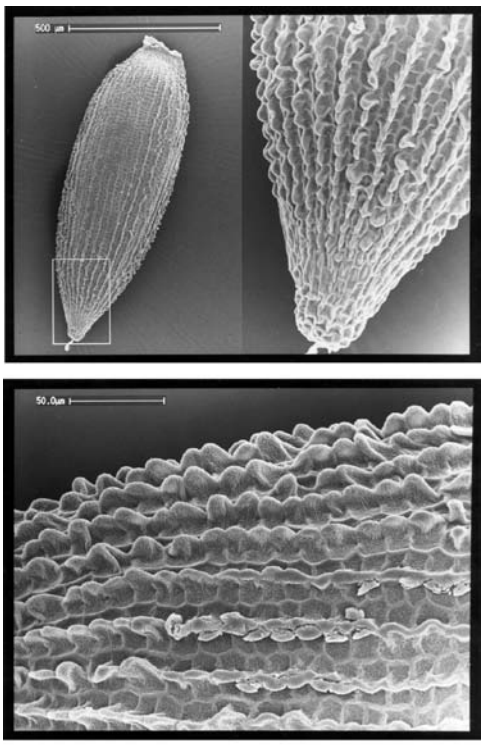

Fig. 17. *E. adenocaulon* (eastern race)

straighter and the shape is more regular and symmetric. The height of the ridges is approx. 15 µm.

E. ciliatum (Fig. 18)
The seeds are 1.1–1.3 mm long and resemble the seeds of *E. adenocaulon* with a long and pointed base, but their width is not as uniform but fusiform (spindle shaped, Fig. 1*n*). Both the base and apex of the seed of *E. ciliatum* are straighter than in the western race of *E. adenocaulon*. The ridges of *E. ciliatum* resemble more those of the eastern race of *E. adenocaulon* and are usually regular, straight and less disrupted than for the western race. The height of the ridges is approx. 30 µm and increases as the base of the seed is approached.

E. glandulosum (Fig. 19)
The seeds are 0.9–1.2 mm long and their sides are almost parallel. The blunt base and apex abruptly narrow and are almost round. The length and width of the seed are thus characteristic of *E. glandulosum* and can be called oblong-ovate (Fig. 1*m*). The neck section of the seed is negligible. The ridges are regular, run longitudinally at about 25 µm from each other, slightly disrupted and approx. 25–30 µm high.

E. alsinifolium (Fig. 20)
The seeds are 1.3–1.8 mm long and almost of the same size as those of *E. palustre*, but are dark as the seeds of *E. davuricum*. The shape is narrowly ovate. The seed neck width is double that of the length and is somewhat broader than in *E. anagallidifolium*. Earlier, the seeds were considered smooth and thus referred to be foveolate. However, the term alveolate could also be used for them, since the seed surface is irregular with pits and elevations that are not rounded (Fig. 1*o*). The cells are irregularly polygonal and their centres are concave. The cell walls are prominent, and extremely thickened. The walls between cells are clearly curved, while they are more angular in *E. anagallidifolium*. Adjacent cells with parallelly running cell walls in the longitudinal direction of the seed give rise to apparent rows. This is characteristic for *E. alsinifolium* by contrast to *E. anagallidifolium*.

E. lactiflorum (Fig. 21)
The seeds are 1.1–1.4 mm long, light brown and somewhat larger than in *E. hornemannii* but smaller than the seeds of *E. alsinifolium*. The shape is narrowly ovate. The neck of the seed is narrow and as long as broad. The narrow neck is characteristic, just as in *E. hornemannii*. Like *E. alsinifolium*, the cells appear in longitudinal rows. In general, the seeds have been described as being smooth.

E. anagallidifolium (Fig. 22)
The seeds are small, 0.8–1.1 mm, relatively dark, and their shape is slim and narrowly ovate, but broader than those of *E. hornemannii*. The seed neck is very short and broad. The seeds have usually been described as smooth and thus belonging to the foveolate type. The cells are irregularly polygonal, the cell centre is concave, with small pits. The cell walls are more angular than those of *E. alsinifolium*. Adjacent cells in the longitudinal direction of the seed do not appear in longitudinal rows like the cells of *E. alsinifolium* and *E. lactiflorum*.

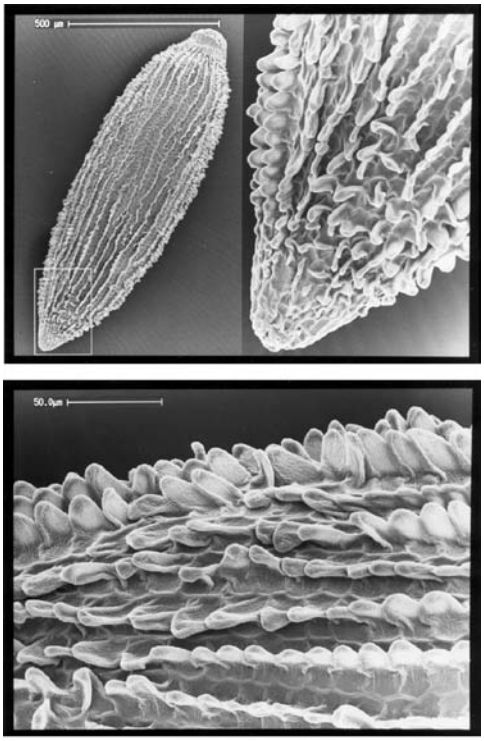

Fig. 18. *E. ciliatum*

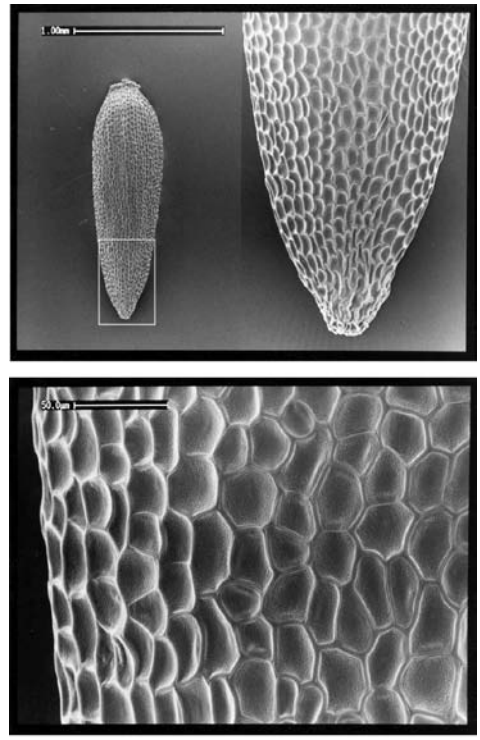

Fig. 20. *E. alsinifolium*

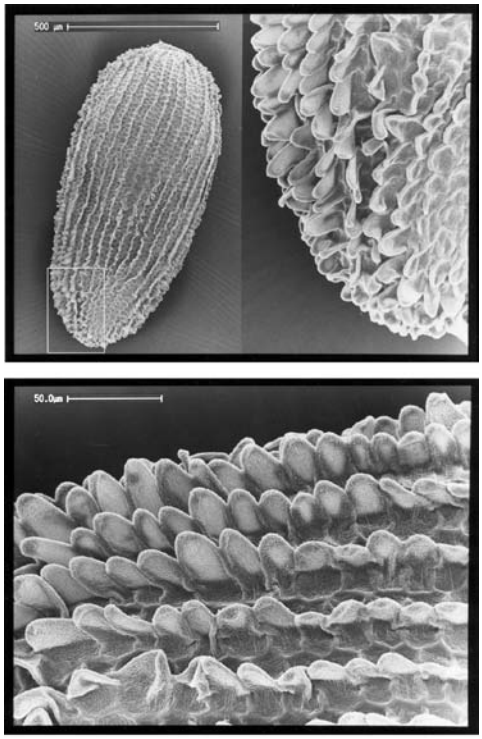

Fig. 19. *E. glandulosum*

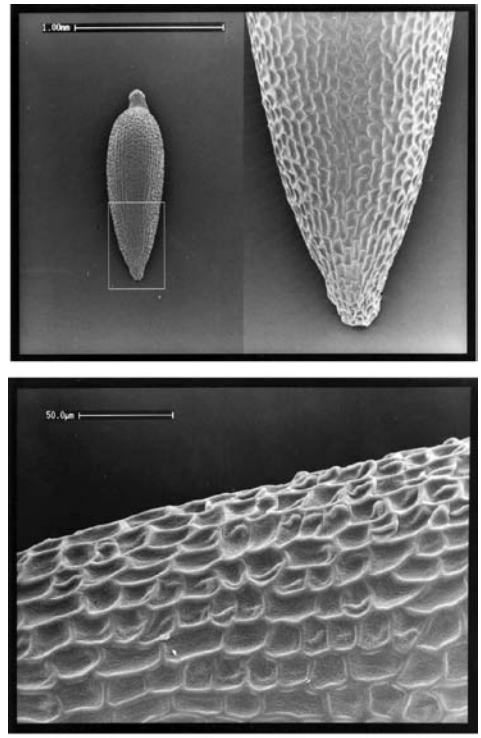

Fig. 21. *E. lactiflorum*

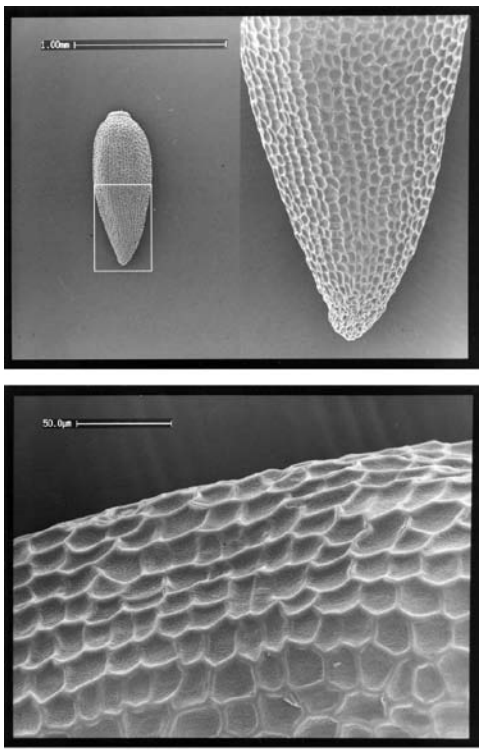

Fig. 22. *E. anagallidifolium*

Discussion

In analyzing seeds of the genus *Epilobium* it may be advantageous to make use of SEM images. This is particularly true for seeds with similar morphology belonging to the same group. The method is not, however, suitable for developing a unique key for classification of the different species of *Epilobium*, due to its inherent limitations: The seeds should be carefully selected and be of good quality. Secondary deformations may be relatively common and they may make the determination difficult. These may be due to different reasons, e.g. the seeds have dried on the plant, at the pressing or at the vacuum treatment in the electron microscope. The work that has been presented in this paper is based on SEM photography, but the results may also facilitate the determination using an optical stereo microscope.

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