

Seed morphology and anatomy of *Hypericum elegans* Steph. ex Willd.

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Abstract: *Hypericum elegans* is a rare perennial distributed primarily in Central and Eastern Europe. Seed morphology and anatomy in *H. elegans* was studied on the basis of a seed sample from its only locality in Poland. Scanning electron microscopy revealed that the seed coat of mature seeds is composed basically of 3 cell layers: epidermal, subepidermal and sclerenchymatic. They are documented graphically here.

Key words: *Hypericum*, carpology, seed sculpture, seed coat

1. Introduction

The genus *Hypericum* L. includes about 420 species representing various life forms: small trees, large shrubs, and herbaceous plants, including perennials and annuals (Stevens 2007). They are distributed mostly in the temperate zone and in mountains in the tropical zone.

In Poland the genus is represented by 8 species. Undoubtedly the rarest among them is *Hypericum elegans*, discovered in Poland in 1984, in the SE part of the country (Brzeg *et al.* 1988). It is classified as a continental-subcontinental element of the Polish flora (Rothmaler *et al.* 2005). It is a rare perennial herb, distributed primarily in Central and Eastern Europe.

Considering that seeds provide many diagnostic features, they are often subject to detailed morphological and anatomical studies.

So far, few studies of this type have been conducted in relation to the genus *Hypericum*, as seed structure has been described in only several species (Netolitzky 1926; Corner 1976; Robson 1981).

Hypericum seeds develop from anatropous, tenuinucellar ovules with a two integuments (Netolitzky 1926; Corner 1976; Szabes & Morozova 1991). The outer integument is composed of 2, while the inner one of 2 or more cell layers (Corner 1976; Szabes & Morozova 1991).

The seed coat develops from both integuments. According to Netolitzky (1926), the outer epidermis of the testa (i.e. of the outer integument) consists of relatively large cells, with brown contents (tannin compounds). *Hypericum quadrangulare* has a flat outer epidermis, while *H. elodes* has horseshoe-shaped cells (Netolitzky 1926). The inner epidermis of the testa, according to the cited author, is composed of cells with thickened wall angles, while according to other authors (Corner 1976; Robson 1981), the inner testa epidermis deteriorates and is invisible in mature seeds.

The outer epidermis of the tegmen (i.e. of the inner integument) is transformed into a mechanical layer. It consists of sclerenchyma cells with strongly thickened walls in the front plane, undulated-stellate in outline (Netolitzky 1926; Corner 1976). According to Corner (1976), this layer is characteristic of the tribe Hypericoideae. Other cell layers of the tegmen are compressed to an unstructured membrane (Netolitzky 1926; Corner 1976). According to Olen Dorf (1907), the mechanical layer is formed by the inner testa epidermis, while the tegmen is compressed and does not participate in development of the seed coat.

Seeds of *Hypericum* form the endosperm, which is reduced to 1-2 layers of thin-walled cells with oil contents (Corner 1976). The embryo is straight, with more

or less developed cotyledons (Netolitzky 1926; Corner 1976).

This study was aimed to investigate and describe details of morphological and anatomical structure of the seed coat of *H. elegans*.

2. Material and methods

One *Hypericum elegans* seed sample was collected from the single Polish local population situated close to the village of Kały II near Zamość (SE Poland). The sample consisted of 50 seeds. For the anatomical analysis, dry seeds were soaked in 70% alcohol for 24 hours, and next transverse sections of the central part of the seed and superficial scraps were made. The sections were mounted on glass slides and observed under a light microscope. Morphological analysis was based on observations of dry seeds under a stereomicroscope and scanning electron microscope (SEM). For SEM observations, 5 seeds from each sample were used: after sputtering with gold. The seeds were measured using NIS-Elements software for digital analysis of microscopic images.

3. Results

Seeds of *Hypericum elegans* are oblong, rounded at both ends, 1200-1300 μm long, 420-460 μm in diameter (Fig. 1). They are slightly curved, with a narrow elongated trace after funiculus on the incurving edge (Figs. 2-3). The seed interior is filled nearly completely with a well-developed embryo (orthotropous, with well-developed cotyledons).

The seed surface is delicately alveolate. Interestingly, the seed sculpture looks slightly different under a light microscope (stereomicroscope) than in a SEM. Under the light microscope, seed sculpture is delicately alveolate, with a regular hexagonal pattern, honeycomb-like, slightly wider transversely than longitudinally (Fig. 2). In the SEM image, seed sculpture is also delicately alveolate, but cells are not so regular, pentagonal or hexagonal, isodiametric or slightly wider longitudinally (Figs. 1 and 4). The difference results from the anatomical structure and the mechanism of image creation in both cases. In the SEM image, the surface of the outer layer of the seed coat is visible. It is very delicate, composed of thin-walled cells. In mature seeds, the layer is collapsed, and the outline of radial walls of the deeper (subepidermal) layer is visible under the stereomicroscope.

In a detailed anatomical analysis, 3 cell layers of the seed coat of mature seeds can be distinguished (Figs. 5-6). The outer layer, developed from the testa epidermis, consists of thin-walled, isodiametric cells with straight radial walls. The cell outline is visible in SEM images (Fig. 4). The second, subepidermal layer is com-



Fig. 1. *Hypericum elegans* Steph. ex Willd., seed (SEM)



Fig. 2. *Hypericum elegans* Steph. ex Willd., seed (LM)

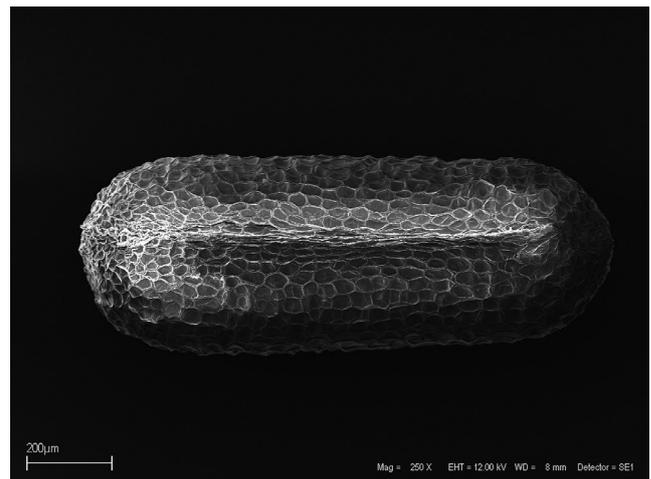


Fig. 3. *Hypericum elegans* Steph. ex Willd., seed (SEM), visible a narrow elongated trace after funiculus

posed of isodiametric cells, regular, hexagonal, slightly wider transversely than longitudinally. Radial walls are straight, somewhat thickened in angles (Fig. 7). Outer periclinal walls are thin and in mature seeds they colla-

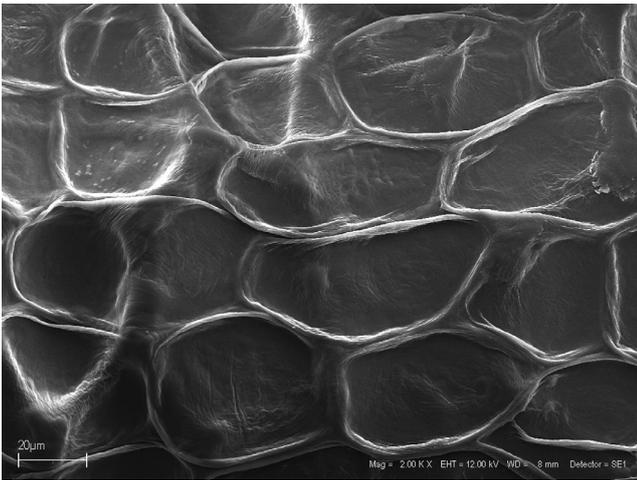


Fig. 4. *Hypericum elegans* Steph. ex Willd., surface of seed coat (SEM)

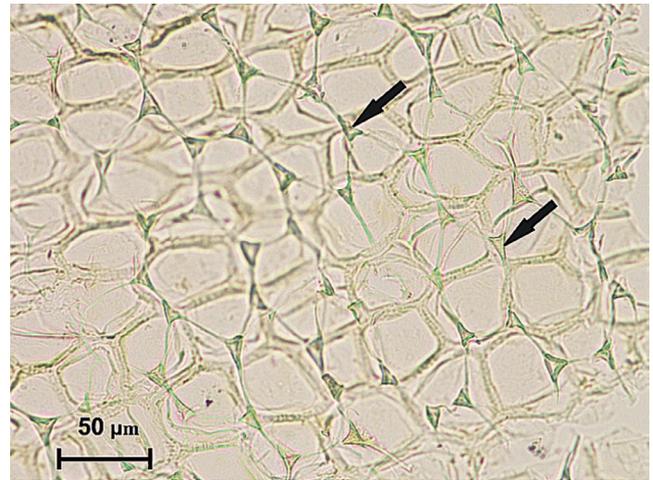


Fig. 7. *Hypericum elegans* Steph. ex Willd., surface view of subepidermal layer (LM), the radial walls thickened in angles (arrow)

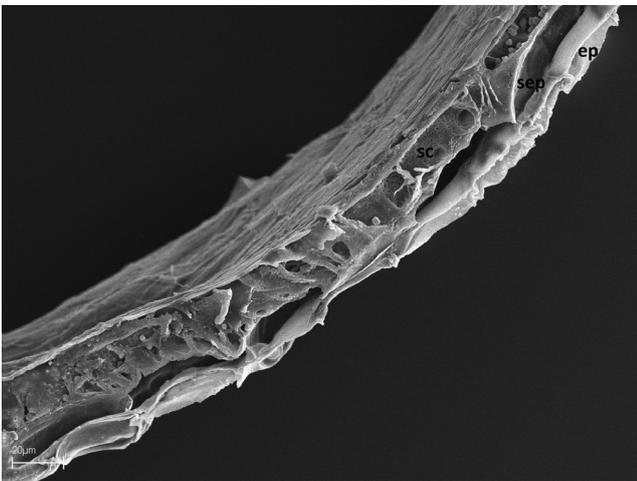


Fig. 5. *Hypericum elegans* Steph. ex Willd., cross section of seed coat (SEM)
Explantations: ep – layer of epiderma, sep – subepidermal layer, sc – sclerenchyma layer

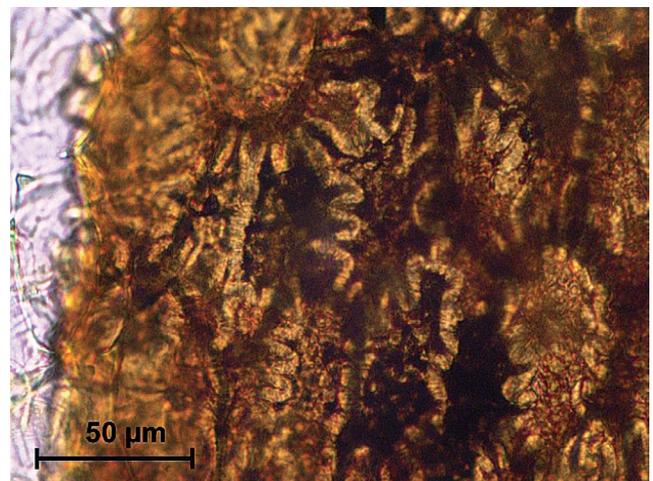


Fig. 8. *Hypericum elegans* Steph. ex Willd., surface view of sclerenchyma layer (LM)

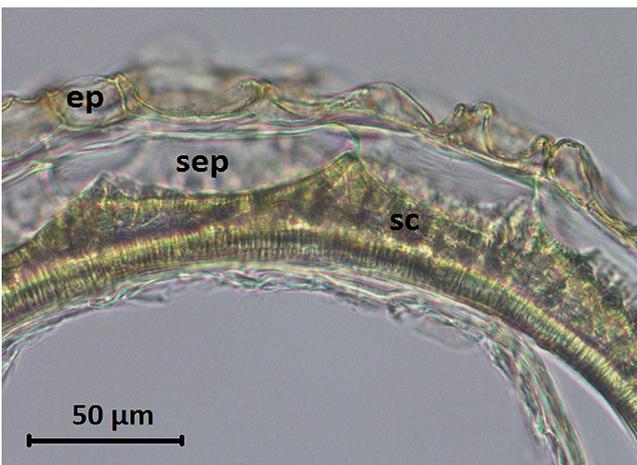


Fig. 6. *Hypericum elegans* Steph. ex Willd., cross section of seed coat (LM)
Explantations: ep – layer of epiderma, sep – subepidermal layer, sc – sclerenchyma layer

pse together with the walls of epidermal cells. Inner periclinal walls are thickened, fused with cells of the

underlying sclerenchyma. The layer of subepidermal cells gives the image of seed sculpture observed under a stereomicroscope (Fig. 2). The third layer of cells of the seed coat of mature seeds forms sclerenchyma. It is composed of isodiametric cells with strongly thickened and porous walls (Fig. 5). Radial walls are strongly undulated (Fig. 8). The surface of these cells is visible after removal of the outer layers. The other cell layers of integuments are compressed and do not participate in seed coat formation.

The embryo of *Hypericum elegans* nearly completely fills the seed interior. It is orthotropous, with well-developed cotyledons, which account for 1/3 of embryo length. The whole embryo is surrounded by 2 layers of endosperm cells.

4. Discussion

The observed seed structure in *Hypericum elegans* is consistent to a large extent with earlier descriptions

of *Hypericum* seeds (Mađalski 1982; Bojnanský & Fargašová 2007). However, on the basis of the analysis of the seed coat of mature seeds it is not possible to determine which elements of the seed coat develop from the outer integument, and which from the inner integument. In the seed coat of *H. elegans*, 3 cell layers can be distinguished. The outermost layer is the epidermis of the testa, as reported consistently by many authors (Netolitzky 1926; Corner 1976; Robson 1981). Results of this study indicate that this layer consists of thin-walled cells, but they are not large and do not accumulate any brown substances (tannin compounds), which contrasts with the report of Netolitzky (1926). The subepidermal layer is composed of large, regular cells with slightly thickened radial walls (thickened only in their angles). This is consistent with Netolitzky's (1926) observations. It is probably the inner epidermis of the testa, which does not disappear completely, as suggested by some authors (Corner 1976; Robson 1981), but remains there and shapes the seed sculpture visible

under a light microscope. This layer is fused with the underlying cell layer. The third layer of the seed coat is composed of cells that are undulated-stellate in surface view. All walls of these cells are strongly thickened and porous. They form the sclerenchyma layer mentioned also by other authors (Netolitzky 1926; Corner 1976; Robson 1981). The other cell layers of the integument are compressed to an unstructured layer, as reported previously (Netolitzky 1926; Corner 1976).

In conclusion, results of this study partly confirm the earlier findings about the outermost epidermal and innermost sclerenchymatic layers of the seed coat. However, they are separated by a subepidermal layer, composed of large cells with slightly thickened radial walls, which shapes the seed sculpture visible under a light microscope.

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