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THE SEED COVERING ANATOMY OF SIX HERBACEOUS SPECIES FROM CENTRAL-EASTERN EUROPE

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Abstract: In this study we describe and compare the anatomy of the seed coverings of six herbaceous species from three families native to Central-Eastern Europe: *Dichanthium ischaemum* and *Bromus arvensis* (Poaceae), *Inula ensifolia* and *Cirsium arvense* (Asteraceae), *Trollius europaeus* and *Pulsatilla montana* (Ranunculaceae). Based on the anatomical examination of diaspores, we observed that the seed coat of one-seeded fruits as diaspores was underdeveloped and thin compared to the more complex pericarp. Furthermore, within the same family, smaller diaspores had a simpler seed covering structure than larger ones. We consider that there are just a few publications examining the anatomy of the seed coverings of wild-growing species of non-agricultural interest, and with our study we would like to complete this gap.

Keywords: Asteraceae, diaspore, pericarp, Poaceae, Ranunculaceae, seed anatomy, seed coat, tissue.

Introduction

The science of seed anatomy can be used in multiple ways and can be applied in numerous fields of biology. Understanding the anatomical structure of the seeds is crucial in taxonomy [5, 20]. Generally, seed anatomy is more similar between closely related species, e.g. species from the same family, than between distantly related species. For example, it is known that seeds of species of Fabaceae are hard-seeded, they have outer integuments of densely packed palisade cells and waxy cuticles that make the seed water-impermeable [3]. Seed morphology and anatomy can be unique to certain species or genera and can be used for identification of plants only by their seeds [5, 6]. This can be important in archaeology and archaeobotany due to the long endurance of these reproductive structures in the soil. Understanding the anatomy of the seed in cultivated plant species can be important in agricultural science but also in pharmacy in the case of medicinal plants where the seed is used [25, 28, 30]. Ecology and behaviour of the seeds in their environment is often reflected by their anatomy, for example, dispersal strategy [9, 10, 16, 18, 34] or dormancy type [11, 14] is strongly determined by seed anatomy and morphology.

There are numerous publications about the seed anatomy and morphology of plants of agricultural importance [19, 22], or comparative seed anatomy of closely related species such as members of the Apiaceae [5, 20], which can be easily identified only by their seeds. However,

we have observed a lack of publications in the field of seed-covering anatomy of wild-growing plants, even from the most common plant families. In the same way, comparisons between distantly related species are also scarce.

The objective of our study was to describe and compare the diaspore covering anatomy of six herbaceous plant species native to Central-Eastern Europe.

Material and Methods

Seed collection

We collected diaspores of six common herbaceous plant species from temperate grasslands or ruderal habitats in Transylvania, Romania, which belonged to three common plant families of this region (Table 1.). The diaspores we selected for our study were mostly one-seeded, simple, dry, indehiscent fruits: grains (caryopses), achenes (cypselaes), and nutlets, and in a single case (*Trollius*) seeds from dry dehiscent fruits (follicles). Diaspores were cleaned of appendages before sectioning.

Table 1: Species studied, their family, diaspore type, and literature reviewed for identification of the pericarp and the seed coat. Nomenclature follows Tutin et al. (1964–1980).

Species	Family	Diaspore type	Literature
<i>Dichanthium ischaemum</i>	Poaceae	fruit	Nakamura et al. 2009; Osman et al. 2012; Krishnan & Dayanandan 2003;
<i>Bromus arvensis</i>	Poaceae	fruit	
<i>Inula ensifolia</i>	Asteraceae	fruit	Aguado et al. 2011; Galastri et al. 2010; Marzinek and Oliveira 2010;
<i>Cirsium arvense</i>	Asteraceae	fruit	
<i>Trollius europaeus</i>	Ranunculaceae	seed	Özdemir et al. 2008; Tobe & Keating 1985; Xuhan & Lammeren 1994; Chute 1930
<i>Pulsatilla montana</i>	Ranunculaceae	fruit	

Methods applied for sectioning of the diaspores, staining and fixation of these sections

For each species, three seeds were randomly selected for the anatomical characterization. Seeds were prepared for sectioning with an ultra-microtome. We first fixed the samples in glutaraldehyde (2.7%) for 24 h; they were dehydrated using an increasing range of acetone concentrations (30, 50, 70, 80, 90 and 100 %) for 1 h each. After fixation and dehydration, seeds were embedded stepwise in Epon 812 resin and acetone (100%) solutions with an increasing proportion of Epon 812 and lastly in pure Epon 812 resin for 24 h each. After the polymerization of the embedding agent with seeds in an oven at 60 °C for 48 h, the seeds were sectioned. We used a Leica UC 6 ultra-microtome with glass knife to obtain sections of 0.9-1.3 µm thickness and stained them using Epoxy Tissue Stain. We took pictures of each section with a CCD-digital camera connected to an Olympus BX 51 optical microscope.

Identification of tissue types of the pericarp and seed coat

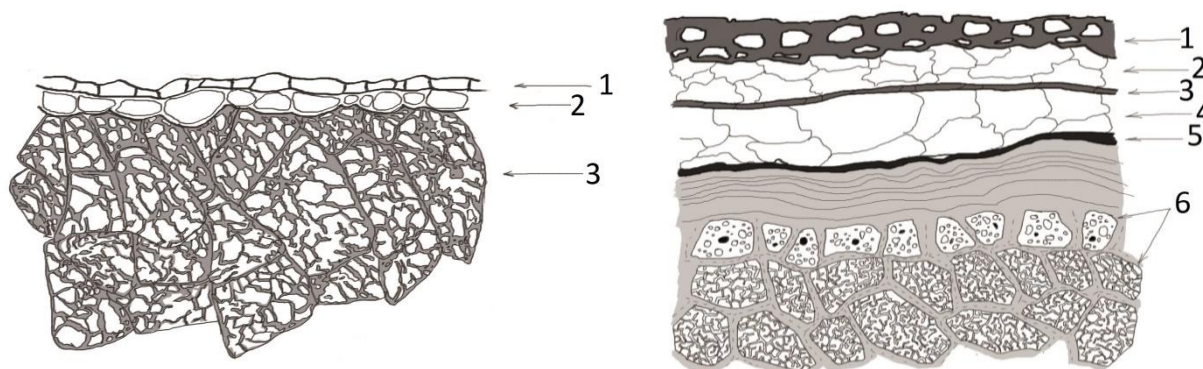
We identified and described different tissue types based on simple cell morphology and colour. Furthermore, we differentiated between pericarp and seed coat, reviewing publications examining the seed anatomy of other, closely related species (Table 1).

Results

A. Poaceae family

Dichanthium ischaemum (L.) Roberty

The diaspore of this species is a relatively small caryopsis, which has a very simple seed covering anatomy consisting of two cell layers over the endosperm (Fig. 1a). The outer layer is represented by the pericarp and is formed by flat epidermal cells. Below the pericarp lies the seed coat, which is also represented by a single cell layer of very small, crushed epidermal cells, not always visible. In contrast to the seed coat, the aleurone layer is clearly visible and consists of usually isodiametric cells with thick cell walls. These cells sometimes have different sizes and their shape varies from moderately flattened to round-shaped (Fig. 1a). The endosperm is clearly distinguishable from the aleurone layer by its large irregularly shaped and ruminate cells. This is characterised by uneven and enlarged inner surface of the cells formed by ingrowths of the cell wall (Fig. 1a).



a. small caryopsis of *Dichanthium ischaemum*

b. large caryopsis of *Bromus arvensis*

Fig. 1: Diaspore covering structures of Poaceae:

- a. 1 – pericarp with epidermal cells, 2 – aleurone layer with larger isodiametric cells, 3 – endosperm with large parenchyma cells;
- b. pericarp with 1 – sclerenchyma cells, 2 – flattened smaller parenchymatous cells, 3 – thin elongated parenchymatous cells, 4 – flattened larger parenchymatous cells, 5 – seed coat with crushed cells appearing as black thin layer, 6 – endosperm with large isodiametric cells.

Bromus arvensis L.

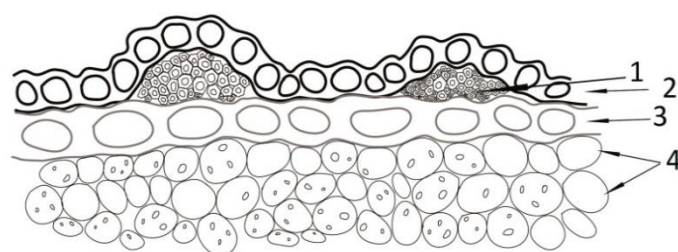
The caryopsis of this species is relatively large and has a more complex covering structure than that of the previous species (Fig. 1a and b). The outer part of the pericarp is formed by two layers of more or less isodiametric sclerenchyma cells. Underneath this lignified tissue are flattened, irregularly shaped parenchyma cells in two rows, which are followed by a compressed layer of elongated cells. This is followed by two rows of large, thin-walled, sometimes flattened parenchyma cells (Fig. 1b). The seed coat is very thin and appears as a dark layer of compressed cells forming a single layer over the aleurone cells (Fig. 1b). The outer cell wall of the aleurone cells is very thick, sometimes thicker than the protoplast of the cells. The endosperm is formed by irregularly shaped cells usually larger than the aleurone cells. They have

ruminate cell walls, which increase the inner surface of the cells, similar to the previous species (Fig. 1a and b).

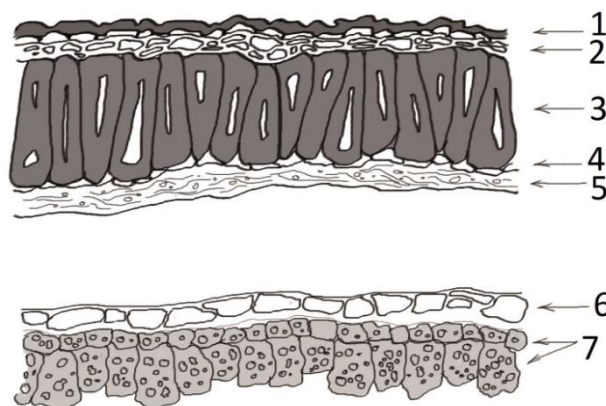
B. Asteraceae family

Inula ensifolia L.

The relatively small achene (cypsela) of this species has a simple pericarp and seed coat anatomy consisting of only three layers (Fig. 2a). The outer part of the pericarp (exocarp) is formed by cubic epidermal cells with thick cell walls. Underneath are sclerenchyma bundles, but these appear only locally in the region of the ribs of the achene, and between the ribs there is no sclerenchyma or other tissue to be found (Fig. 2a). The mesocarp is therefore underdeveloped and is represented poorly and only locally. The endocarp is not visible and may be absent. The seed coat is clearly distinguishable and is formed by large isodiametric epidermal cells arranged in a single row. The endosperm consists of isodiametric cells with thin walls (Fig. 2a).



a. small achene of *Inula ensifolia*



b. large achene of *Cirsium arvense*

Fig. 2: Diaspore covering structures of Asteraceae:

- a. pericarp: 1 – epidermis with cubic cells and with thick cell walls, 2 – sclerenchymatous bundle in rib; seed coat: 3 – isodiametric living epidermal cells with thick cell walls; endosperm: 4 – parenchymatous, round cells;
- b. pericarp: 1 – epidermis with thick-walled cells, 2 - parenchymatous flat cells, 3 - palisade sclerenchyma; 4 - parenchymatous small and flat cells; seed coat: 5 - parenchymatous small and crushed cells; endosperm: 6 - flattened parenchymatous cells in one layer; cotyledon: 7 – large parenchymatous cells.

Cirsium arvense (L.) Scop.

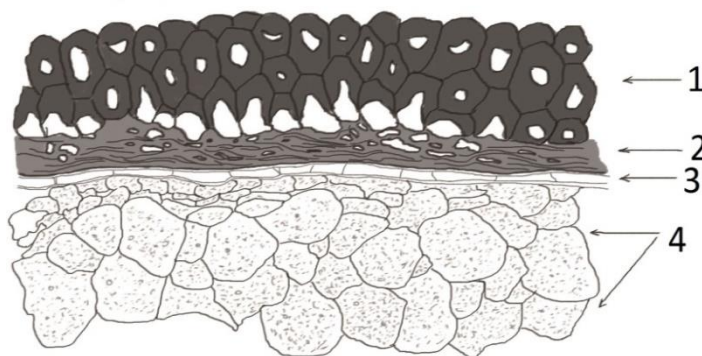
The relatively large achene of this species has a more complex covering anatomy than the previous species from the same family, but we observed also some similarities between them

(Fig. 2a and b). The outer epidermis of the pericarp (exocarp) is formed by small cells with thick exterior and thinner interior walls. The mesocarp begins with a few layers of parenchymatous flat cells with thin walls followed by a thick layer of palisade sclerenchyma forming a single layer (Fig. 2b). The endocarp is poorly visible and is represented by one layer of thin-walled flattened cells. The seed coat is fused with the pericarp and consists of a few layers of compressed small cells. The inner part of the seed, containing the embryo and the endosperm, is separated from the seed coverings (Fig. 2b).

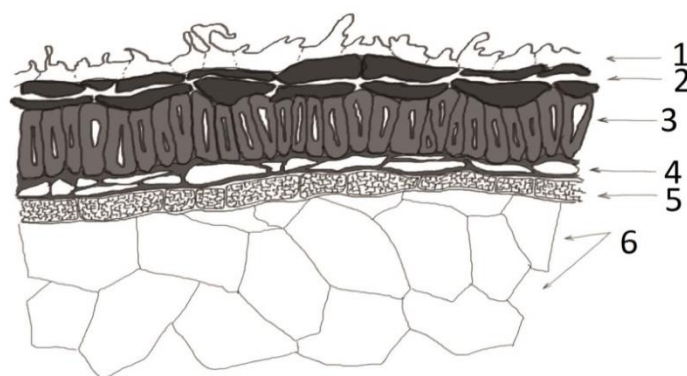
C. Ranunculaceae family

Trollius europaeus L.

The diaspore of this species is represented by a seed, which, until mature, is enclosed within a follicle. The outer part of the seed coat is formed by lignified U-shaped cells arranged in two or three rows (Fig. 3a). This is followed by crushed irregular shaped parenchyma cells and finally by flat, rectangular epidermal thin-walled cells arranged in one row. The endosperm consists of round and large parenchyma cells (Fig. 3a).



a. seed of *Trollius europaeus*



b. nutlet of *Pulsatilla montana*

Fig. 3: Diaspore covering structures of Ranunculaceae:

- a.** seed coat: 1 – lignified U-shaped cells, 2 – crushed irregularly shaped parenchymatous cells, 3 – flat, epidermal-like cells; endosperm: 4 – large parenchymatous rounded cells;
- b.** pericarp: 1 – irregularly shaped epidermal cells with thick cell walls; 2 – flat subepidermal parenchymatous cells; 3 – sclerenchymatous palisade; seed coat: 4 – flattened parenchymatous cells of different sizes with irregular side walls; 5 – flattened parenchymatous cells with a more regular shape and similar sizes; endosperm: 6 – irregularly shaped, large parenchyma cells.

Pulsatilla montana (Hoppe) Rchb.

The elongated, relatively small, anemochorous nutlet of this species is densely covered by trichomes, which are part of the outer epidermis of the pericarp. The outer epidermis forms the exocarp, constructed of irregularly shaped cells with thick cell walls (Fig. 3b). This is followed by the mesocarp consisting of two rows of flat subepidermal cells and underneath with a palisade sclerenchyma (Fig. 3b). The endocarp is reduced or not visible. The seed coat is fused with the pericarp and is formed by two distinct tissue layers: the upper part of one or two layers of flat irregularly shaped parenchyma cells and the lower part by larger, elongated rectangular cells (Fig. 3b).

Discussion

We observed certain similarities between species of the same family but also between species from more distantly related families. There are very few studies describing in detail the seed coat and pericarp anatomy of members of Ranunculaceae and we found no publications concerning the genera *Pulsatilla* and *Trollius*. In general, the pericarp of the diaspores studied was formed by an epidermal exocarp, followed by a mesocarp often containing sclerenchyma and parenchyma, and finally an endocarp also epidermal but often crushed or not visible. An exception was the small caryopsis of *Dichanthium ischaemum* where the pericarp and the seed coat was formed only by a single cell layer.

The seed coat in the case of one-seeded fruits was usually underdeveloped, consisting of a single or a few layers of living cells with thin walls. In the case of seed as diaspore, the seed coat was much more developed, containing sclerenchyma with protective role and also parenchyma formed by living cells. One explanation for this is that in one-seeded fruits as diaspores, the pericarp takes over the protective role of the seed coat and therefore the seed coat is less developed (Tsou and Mori 2002).

Smaller one-seeded fruits as diaspores had a very simple covering anatomy formed by two or three layers and tissue types. The larger diaspores, by contrast, had much thicker and histologically more complex coverings formed by numerous tissue types with cells of various morphology and composition. We assume that since larger diaspores are more likely to remain on the soil surface (Bekker et al. 1998), they need additional protection against seed predators (Hulme 1998, Alcántara et al. 2000, Honek & Martinkova 2003, Lundgren & Rosentrater 2007) and extreme and potentially damaging environmental conditions such as UV radiation, frost or heat (Gashaw & Michelsen 2002, Daws et al. 2006).

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ANATOMIA ÎNVELIȘULUI SEMINAL LA ȘASE SPECII ERBACEE DIN EUROPA CENTRALĂ

(Rezumat)

În acest studiu descriem și comparăm anatomia învelișului seminal (tegumentului seminal și pericarpului) la șase specii erbacee din trei familii, autohtone în Europa Centrală: *Dichanthium ischaemum*, *Bromus arvensis*, *Inula ensifolia*, *Cirsium arvense*, *Trollius europaeus*, *Pulsatilla montana*. Examinând anatomia acestor diaspore am observat că tegumentul seminal la speciile cu semințe închise în fruct uniseminal a fost slab dezvoltat și subțire față de pericarp. Pe lângă acestea, comparând diasporele de diferite dimensiuni între speciile aparținând aceleași familii am constatat că diasporele mici au un înveliș seminal mai simplu și mai subțire ca diasporele mai mari. Considerăm că există puține publicații examinând anatomia învelișului seminal la speciile spontane și cu acest studiu am dori să contribuim la acest subiect.

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