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Enigmatic cavity in the ovule of *Polycnemum arvense* L. (lower core Caryophyllales)

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Summary: The interintegumentary airspace typical of many core Caryophyllales and new-revealed presumably schizogenic chalaza cavity in the chalazal bulge parenchyma successively develop and collapse in the ovule of *Polycnemum arvense*. The former one is speculated to promote the epichalazal growth of the nucellus for accelerating the campylotropous to amphitropous shaping of the ovule. The chalaza cavity is presumed to deposit temporarily reserves supplied by the chalazal bulge cells, basal body and integuments.

Keywords: ovule, interintegumentary airspace, chalaza cavity, *Polycnemum arvense*

Core Caryophyllales have characteristically bitegmic ovules (Takhtajan 1966). Their outer and inner integuments fit tightly to each other throughout but in chalazal antiraphe, where they diverge to make an interintegumentary airspace in many representatives. Presumptive exotegmen (= outer epidermis of the inner integument) and presumptive endotesta (= inner epidermis of the outer integument) constitute distinct smooth walls of this air space. The interintegumentary airspaces were described in representatives of Aizoaceae (Ilyina & Poddubnaya-Arnoldi 1983), Amaranthaceae (Savina 1983), Basellaceae (Naumova 1983a), Cactaceae (Naumova 1983b), Chenopodiaceae (Bouman 1984), Didiereaceae (Naumova 1983c), Molluginaceae (Plisko 1991a), Nyctaginaceae (Bouman 1984), Phytolaccaceae (Bouman l. c.), Portulacaceae (Batygina 1983; Plisko 1991b), Stegnospermataceae (Kamelina 1983) and Talinaceae (Veselova et al. 2012).

However, the interintegumentary airspace is accompanied by another cavity in the chalazal bulge of the ovule of *Hablitzia thamnoides* M. Bieb. (Chenopodiaceae) shown by Kamelina (2001: Fig. 3). Kamelina (l. c.) completely ignored this supernumerary cavity as she seemed to have considered it an artifact. A cavity was likewise described in the chalazal antiraphe of the ovule of *Polycnemum arvense* L. at rather advanced developmental stage (Veselova & Dzhalilova 2017). It is strikingly different from the typical interintegumentary airspace in its indistinctive uneven walls. The typical interintegumentary air space with overt smooth walls was also revealed in an earlier developmental stage of the ovule (Veselova et al. 2016). The former cavity was accordingly challenged to be a derivative of the typical interintegumentary air space (Veselova & Dzhalilova 2017).

The cavities in the ovules of core Caryophyllales are certainly worth being scrutinized.

Materials and methods

The inflorescence of *Polycnemum arvense* L. is an open frondose thyrsus. So, stages from early flower buds to ripe fruits are present. Inflorescences were collected from indigenous plants in

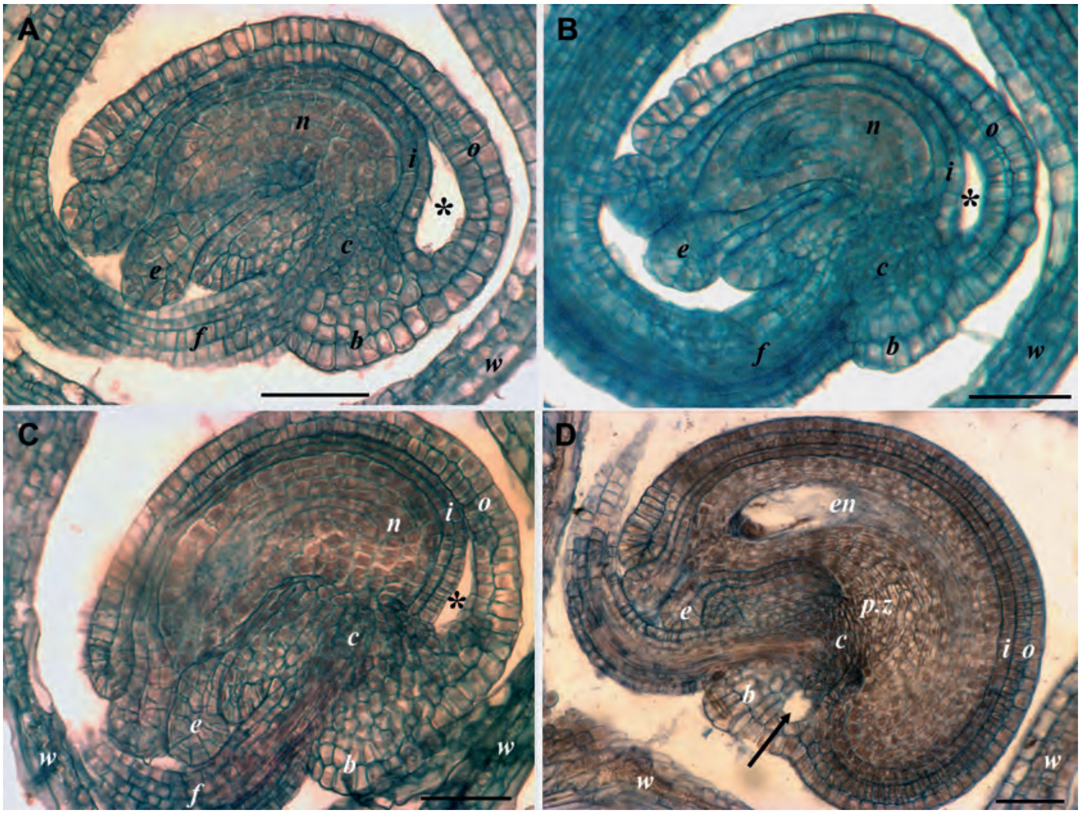

Figure 1. Interintegumentary airspace dynamics. A – ovule at megaspore tetrad developmental stage; B – ovule at mature megaspore developmental stage; C – ovule at mature embryo sac developmental stage; D – ovule at zygote developmental stage. *b* – chalazal bulge; *c* – chalaza; *e* – endostome; *en* – endosperm; *f* – funicle; *i* – inner integument; *n* – nucellus; *o* – outer integument; *p.z* – cell proliferating zone of the nucellus; *w* – ovary wall; *arrow* – chalaza cavity; *asterisk* – interintegumentary air space. Scale bars = 50 μm.

Rostov Region, Russia, and fixed with FAA fixative (1 : 18 : 1; 70% ethyl alcohol). The fixed material was rinsed with 70% ethyl alcohol, dehydrated in ascending ethyl alcohol series, ethyl alcohol / xylol mixtures and embedded in paraffin wax. Ca. 10 μm thick microtome sections of the ovules were mounted on glasses, deparaffinized, rehydrated and successively stained with Alcian Blue and Rawitz's Haematoxylin according to Barykina at al. (2004). The preparations thus prepared were dehydrated and embedded in Canada Balm. Micrographs were taken under light microscope Univar (Reichert) equipped with digital camera DCM-510.

Results

During megaspore tetrad stage, the ovule is campylotropous, crassinucellate and bitegmic (Fig. 1A). The inner integument adjoins the nucellus. The integument is mostly 2-layered except for the multi-layered endostome. The outer integument of larger cells is also 2-layered. Both integuments adjoin each other nearly throughout. There is only the chalazal antiraphe, where the outer integument is far away from the inner counterpart to make a large interintegumentary airspace. The walls of this space are distinctive and smooth, as they are epidermises of the integuments. The cells of both cell layers of the detached part of the outer integument are clearly narrower than its other cells (Fig. 1B). Small-celled parenchyma occupies the chalazal body. A

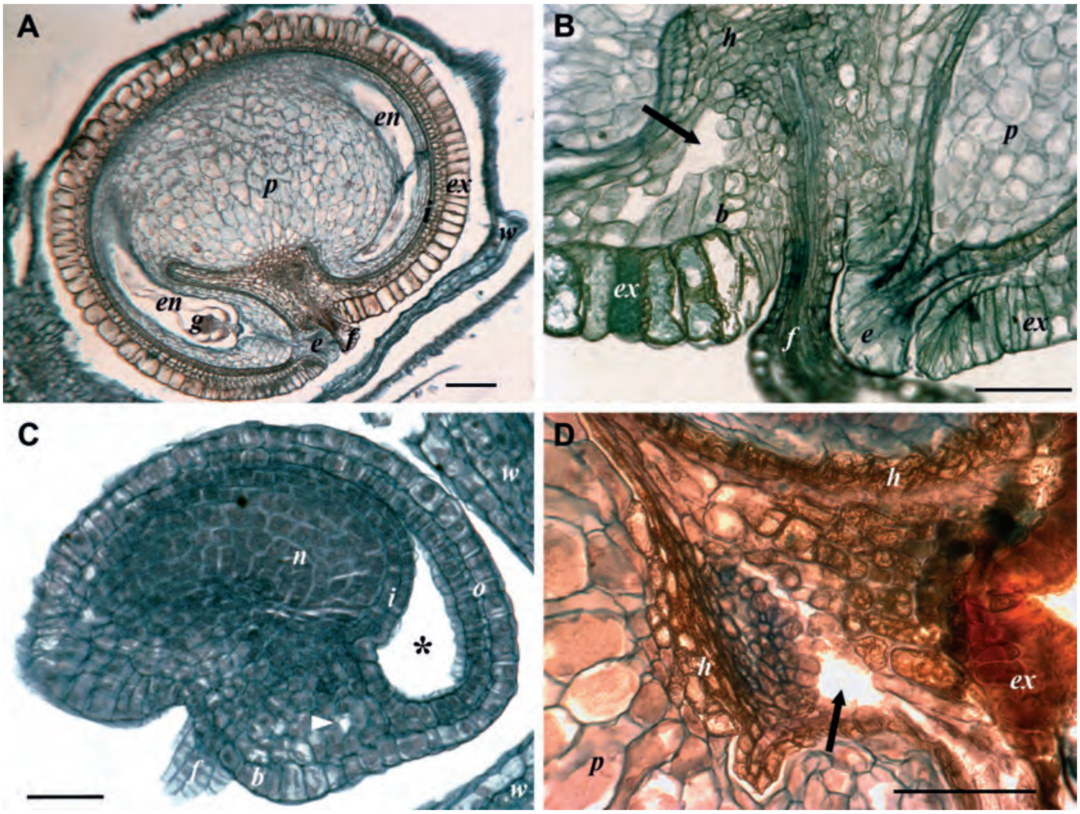

Figure 2. Chalaza cavity dynamics. A – ovule at early globular embryo developmental stage; B – chalaza cavity at early globular embryo developmental stage; C – ovule at binucleate embryo sac developmental stage; D – chalaza cavity at mature embryo stage. *b* – chalazal bulge; *e* – endostome; *en* – endosperm; *ex* – exotesta; *f* – funicle; *g* – globular embryo; *h* – hypostase; *n* – nucellus; *p* – perisperm; *w* – ovary wall; *arrowhead* – initiating chalaza cavity; *arrow* – chalaza cavity; *asterisk* – interintegumentary airspace. Scale bars = 50 μm.

small chalaza bulge protrudes over the funicle opposite the raphe (Fig. 1A–C). This bulge mostly consists of larger-celled parenchyma.

The campylotropous ovule changes into an amphitropous one after fertilization of its embryo sac (Fig 1D). The outer integument becomes partly 3-layered due to periclinal cell divisions in its inner epidermis. The nucellus changes drastically. A cell proliferating zone develops in it above the hypostase. The epidermal and subepidermal cells of the nucellus also frequently divide periclinally to result in nucellus thickening and its epichalazal growing towards the chalazal bulge. The interintegumentary airspace narrows as the nucellus grows. It completely disappears by the endosperm initiation developmental stage (Fig. 1D). The epichalazal growing of the nucellus and conjoined integuments progresses coherently further on, till the micropylar and chalazal parts of the ovule become one-leveled by the early globular embryo developmental stage (Fig. 2A). The chalazal bulge aligns and merges with the epichalazal outgrowth of the ovule, its outer cells differentiate into the exotesta indistinguishable from that of integumentary origin (Fig. 2B).

Another cavity starts developing in the chalazal bulge parenchyma a little bit earlier than the embryo sac fertilization developmental stage (Fig. 2C). This cavity is quite distinct at the endosperm initiation developmental stage (Fig. 1D). It enlarges towards the hypostase and endotesta, the latter one being thereof splitted (Fig. 2B). Thus, developing cavity sharply differs from the former interintegumentary airspace in its 'blurry' uneven walls. There is no sign of destroyed cells in these walls up to the very end of cavity existence (Fig. 2D).

The chalaza cavity under consideration remains nearly until the mature embryo developmental stage. It collapses and disappears as the seed progresses.

Discussion

Two cavities successively form and collapse in the chalazal antiraphe of the ovule of *P. arvense*. They are located very close but slightly spaced. Besides, they develop differently.

The interintegumentary airspace typical of many core Caryophyllales (Kapil & Vasil 1963; Bouman 1984) forms first. The detached part of the outer integument is constituted by narrower cells in all of its layers (Fig. 1B). These narrower cells indicate their more frequent anticlinal cell divisions. These more frequent cell divisions seem to cause local overgrowth of the outer integument discordant with the inner integument growth. Local discordant overgrowth of the outer integument results in its protruding and detaching from the inner one.

The interintegumentary airspace is exogenous. It is traditionally considered air-containing, but its content has likely never been explored. It invariably looks empty in our preparations. However, this 'airspace' could really be permanently or temporarily filled with a kind of secret like the ovary cavity in some angiosperms (Rudall et al. 1998; Igersheim et al. 2001; Ronse De Craene & Wanntorp 2008).

The interintegumentary airspace collapses soon after the nucellus has started its epichalazal growth. This airspace possibly promotes epichalazal growth of the nucellus and thus accelerates the change of the ovule from campylotropous to amphitropous.

The chalaza cavity develops endogenously in the parenchyma of the chalazal bulge after the interintegumentary airspace. The cavity walls are highly uneven and indistinct, but the absence of any sign of cell destruction within makes us believe this cavity to be of schizogenic origin.

The chalaza cavity looks empty in all preparations examined. This might be an artifact of the technique used. This cavity appears in early seed development. It exists, when the nucellus is differentiating into the perisperm and accumulating reserves. The cavity collapses by the time the perisperm and embryo have completed. Thereof, the chalaza cavity seems to participate in reserves deposition. The developing seeds are repeatedly reported to have specific formations for temporary storing reserves supplied by the funicular vascular bundle (Buell 1952; Plisko 1982; Zinger 1958). Being near the funicular vascular bundle end, the cavity under consideration could be a constituent of such formations. It could accumulate starch hydrolyzate and some other substances which are supplied by the chalazal bulge cells, the basal body and integuments mostly during early embryo development (Veselova & Dzhalilova 2017).

Figure 3 in Kamelina (2001) shows that the chalaza cavity is not confined in *Polycnemum*. Other members of core Caryophyllales are worth being screened for the presence of such a cavity.

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