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Dynamics of reserve substance allocation in the ovule and developing seed of *Polycnemum arvense* L. (Polycnemaceae, lower core Caryophyllales)

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Summary: Seeds of *Polycnemum arvense* are similar with typical centrosperms in accumulating reserve starch in the perisperm and reserve proteins in the embryo. The developing seed is supplied with precursors of the reserve substances from distant source. Some carbohydrates are deposited as transitory starch in all ovule structures except the core nucellus and in the early embryo. The transitory starch of the exotesta, tegmen, abfunicular epidermis of the nucellus, nucellar cap, abfunicular strand of the nucellus cells and embryo seems to be completely utilized *in situ*. The transitory starch of the chalaza protrusion, basal body and endotesta is likely to be partly consumed by the developing endosperm and embryo and partly re-deposited in the perisperm as the reserve starch. The perisperm parenchyma dies off thereafter. Starch deposition in the ovule and seed is accordingly two-stepped. The proteins are deposited one-stepped in the embryo to substitute the starch, in the endospermic cap covering radicula and in the exotesta and endotegmen where they are co-precipitated with tannins. The proteins of the embryo are reserve substances. The endospermic cap is functionally similar with the aleurone layer of grass grains. It hydrolyses the reserve starch of perisperm during germination. The proteins deposited in the endospermic cap are likely to be a reserve of necessary hydrolytic enzymes. The proteins co-precipitated with the tannins in the exotesta and endotegmen are evidently neither transitory nor reserve; their function is to be explored. The endosperm transmits nutrients to the developing embryo but it accumulates neither transient nor reserve substances.

Keywords: ovule, seed, endosperm, embryo, reserve starch, transient starch, reserve proteins, perisperm, nutrient allocation dynamics, *Polycnemum arvense*

Nutrients enter the ovule through funicular vascular bundle to be dispersed in various developing sporogenous and gametophytic structures, in the embryo and extra-embryonic tissues which transiently or finally accumulate reserve substances. Patterns of their dispersals are taxon-specific (Bewley et al. 2013). There is accordingly a system of specialized structures in the ovules and developing seeds that directionally conduct metabolites (Billings 1901; Maheshwari 1950; Batygina & Shamrov 1994a, b; Shamrov 1994, 2008) and transiently deposit reserve substances (Zinger 1958; Plisko 1982).

The ovules of Caryophyllales plants characteristically have no specialized structures such as podium, postament, integumentary vascular bundles and tapetum whereas their embryo sacs have ephemeral antipodals (Yakovlev 1983; Kamelina 2009). Some unique patterns of transport routes and deposition sites of reserve substances in the ovules and seeds of these plants are therefore anticipated. However, embryologists and carpologists have mainly been scrutinizing the localization of reserve substances in various constituents of ripe seeds (Netolitzky 1926; Irving et al. 1981; Yakovlev 1983; Lawrence et al. 1990; Elamrani et al. 1992; Coimbra & Salema 1994; Prego et al. 1998). The dynamics of transporting and accumulating reserve substances has only been described in *Dianthus chinensis* L. (Buell 1952), *Spinacia oleracea* L. (Wilms

1980) and *Amaranthus hypochondriacus* L. (Coimbra & Salema 1994) and sketchy described in *Phytolacca americana* (Zheng et al. 2010) and *Chenopodium quinoa* L. (Prego et al. 1998; López-Fernández & Maldonado 2013).

We investigated the dynamics of allocation of reserve substances in the ovule and developing seed in *Polycnemum arvense* L. This is a distant member of the basal clade AAA of core Caryophyllales (Masson & Kadereit 2013; Veselova et al. 2016). It has presumably retained some ancestral characteristics of reserve substance allocation in its ovules and seeds under development.

Materials and methods

Shoots bearing flower buds, flowers and fruits of varying degree of maturation were taken from indigenous plants of *P. arvense* in the Rostov Region, Russian Federation. The shoots were fixed in FAA fixative, rinsed in 70% ethyl alcohol and embedded in paraffin wax. Microtome sections 8 to 12 µm thick were deparaffinized, rehydrated and stained with Alcianic Blue and tinted with Ravitz's Hematoxylin for general picture, with Procyonic Blue for proteins or with Sudan IV for cutin or processed either with Periodic acid-Schiff Reaction (PAS) for polysaccharides or with Ferric Chloride Reaction for tannic substances. The preparations thus processed were dehydrated and embedded into Canada Balm. All above-mentioned treatments correspond with Barykina et al. (2004).

Micrographs were taken under light microscope Univar (Reichert) equipped with digital camera DCM-510.

Results

The mature ovule (Fig. 1A) is campylotropous, crassinucellate, bitegmic (Veselova et al. 2016). The outer 2(3)-layered integument mostly adjoins the inner one, but it is separated from the latter in its basal antiraphe part where interintegumentary air space thus results. Mostly 2-layered inner integument tightly adjoins the nucellus throughout and is terminated by multilayered endostome. There is a hypostase under the nucellus as a 2–3-layered plate of alive, seriate, narrow, thin-walled cells. The chalaza consists of small-celled parenchyma. Larger parenchyma cells constitute raphe region and epifunicular antirapheal protrusion of the chalaza. The vascular bundle runs through massive funicle up to the hypostase to ramify thereunder and to end blindly in small-celled chalaza parenchyma.

Transformations of ovule structures

The fertilization of the embryo sac triggers the campylotropous ovule to grow and to change into amphitropous seed by the time of development stage of early globular embryo (Fig. 1A–C). The antirapheal part of the nucellus proliferates while the epifunicular protrusion of the chalaza becomes leveled.

Integuments. The endostome cells that line the micropyle canal become destroyed when the pollen tube grows through the canal. Other cells of both integuments proceed. The inner integument grows faster than the outer one in its basal antirapheal part. Thereof, the inner integument presses against its outer counterpart to close the interintegumentary chamber at the development stage of zygote (Fig. 1B). Both integuments contribute to the seed coat which also incorporates the outer cells of disappearing chalaza protrusion. The integument cells accumulate

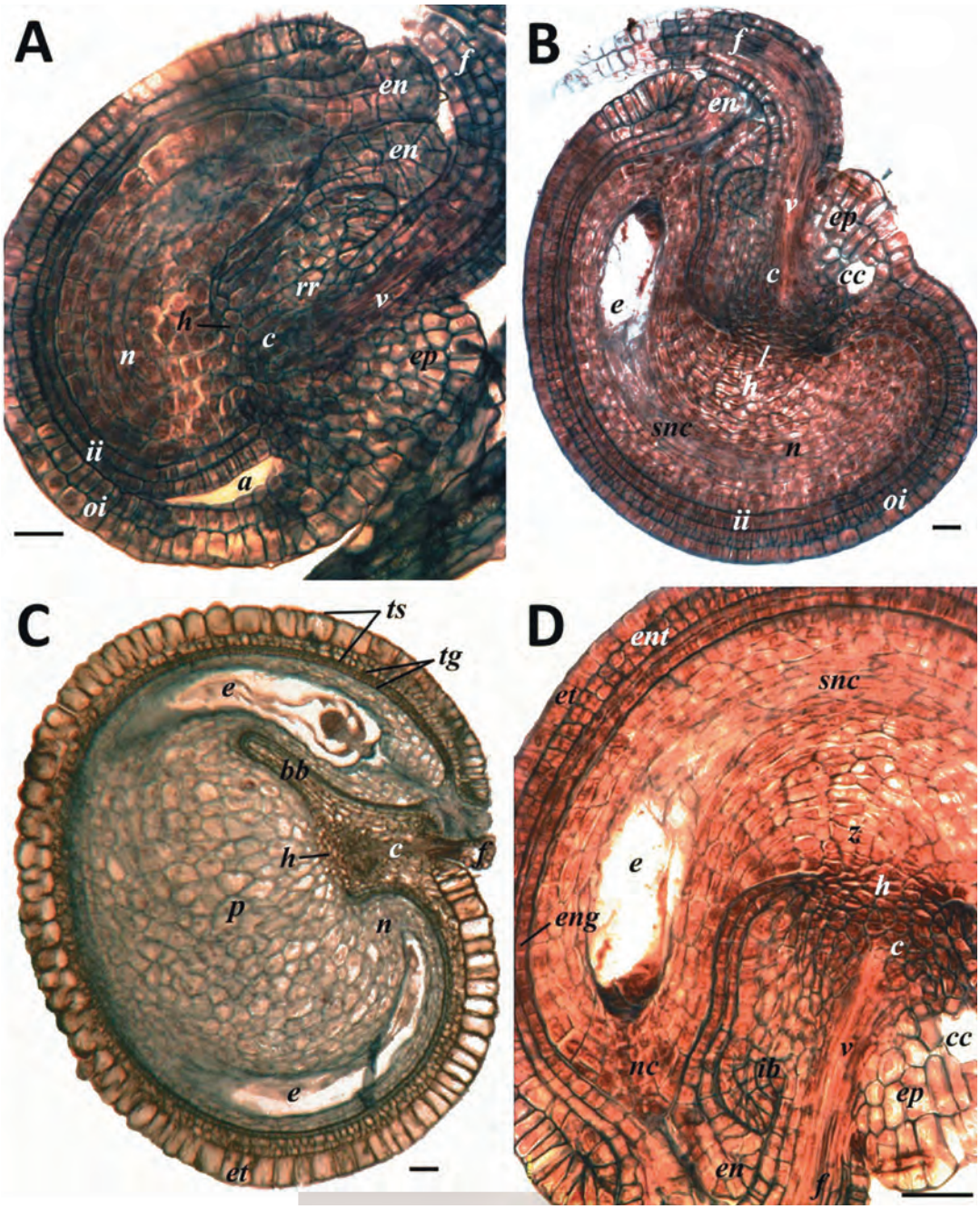

Figure 1. Campylotropous ovule to amphitropous seed transition. A – campylotropous ovule at the development stage of embryo sac; B – ovule at the development stage of zygote; C – amphitropous seed at the development stage of globular embryo; D – initial perisperm at the development stage of zygote. *a* – interintegumentary air space; *bb* – basal body; *c* – chalaza; *cc* – chalaza cavity; *e* – endosperm; *en* – endostome; *eng* – endotegmen; *ent* – endotesta; *ep* – epichalazal protrusion; *et* – exotesta; *f* – funicle; *h* – hypostase; *ib* – initial basal body; *ii* – inner integument; *n* – nucellus; *nc* – nucellar cape; *rr* – raphe region of the chalaza; *snc* – abfunicular strand of the nucellar cells; *oi* – outer integument; *p* – perisperm; *tg* – tegmen; *ts* – testa; *v* – vascular bundle; *z* – cell multiplication zone (precursor of the perisperm). Scale bars = 20 µm.

tannins by the development stage of early globular embryo. Histological differentiation of the seed coat becomes clear at the same time (Fig. 1C).

The outer integument differentiates into 1-layered exotesta and 1(2)-layered endotesta (Fig. 1C, D). The exotesta cells develop thick outer cell wall (Fig. 2B) whereas those of endotesta remain thin-walled. The cells nearby the micropyle die off and lose their contents at the development stage of early globular embryo (Fig. 2A). Other testa cells remain alive up to the development stage of late torpedo embryo.

The inner integument gives rise to the ephemeral exotegmen and permanent endotegmen (Fig. 2A). The former one is already destroyed up to a membrane of indistinguishable separate cells at the development stage of early globular embryo. The endotegmen remains alive up to the development stage of late torpedo embryo. Its cells develop fibrous thickenings on their inner cell walls and accumulate aureate tannic bodies.

Nucellus. Zone of proliferation of nucellus cells arises distally of the hypostase at the development stage of zygote (Fig. 1D). Series of cells generated there fan out of this zone to differentiate gradually into the storage cells of the perisperm being a characteristic tissue of the seed (Fig. 1C). The growing perisperm takes the central part of developing seed and pushes the arcuate endosperm away from the hypostase (Fig. 1C, D). Abfunicular epidermal and subepidermal cells of the nucellus divide tangentially (Fig. 1D). However, the abfunicular side of the nucellus remains thin because it is concomitantly consumed from inside by the growing endosperm.

The antirapheal part of the nucellus expands at the development stage of proembryo and thus enhances amphitropy of developing seed (Fig. 1C). The nucellar cap lengthens by means of elongation of its constituent cells to result in a deep groove between itself and the growing perisperm at the development stage of zygote (Figs 1D; 2A).

Basal body. The groove mentioned above is immediately filled by both integuments. The inner one remains 2-layered, but it folds to enter the groove. The outer integument locally expands in raphe region to fill this folding (Fig. 1B–D). This intruding outgrowth of the outer integument alias basal body (Bouman 1984, 1992) remains up to the development stage of early torpedo embryo. Thereafter, the cells of the basal body become empty and collapse (Fig. 2B) whereas the tegmen areas covering the basal body become merged into a single layer. Growth of the central cells of the perisperm results in remnants of the basal body to be completely destroyed. The basal body is thus absent in the mature seed.

Hypostase initiates simultaneously with the inner integument. The hypostase cells are initially hardly distinguishable from surrounding counterparts (Fig. 1A, B). The hypostase is a 2–3-layered plate of juxtaposed narrow flattened cells at the development stage of mature embryo sac. It is 3–4-layered at the development stage of zygote (Fig. 1D) and its cells are already enlarged and isodiametric at the development stage of globular embryo. Abfunicular cells of the hypostase are further enlarged and adfunicular ones are flattened at the development stage of late globular embryo or early torpedo embryo. The hypostase resultantly becomes convex (Fig. 1C). The cell walls of hypostase cells are tannin-impregnated at the development stage of early globular embryo (Fig. 1C), whereas aureate tannin-like bodies are deposited in the protoplasts.

Chalaza. Small-celled chalazal parenchyma under the hypostase remains alive up to the development stage of immature seed (Fig. 2B). Its cells have large nuclei and intensely stainable

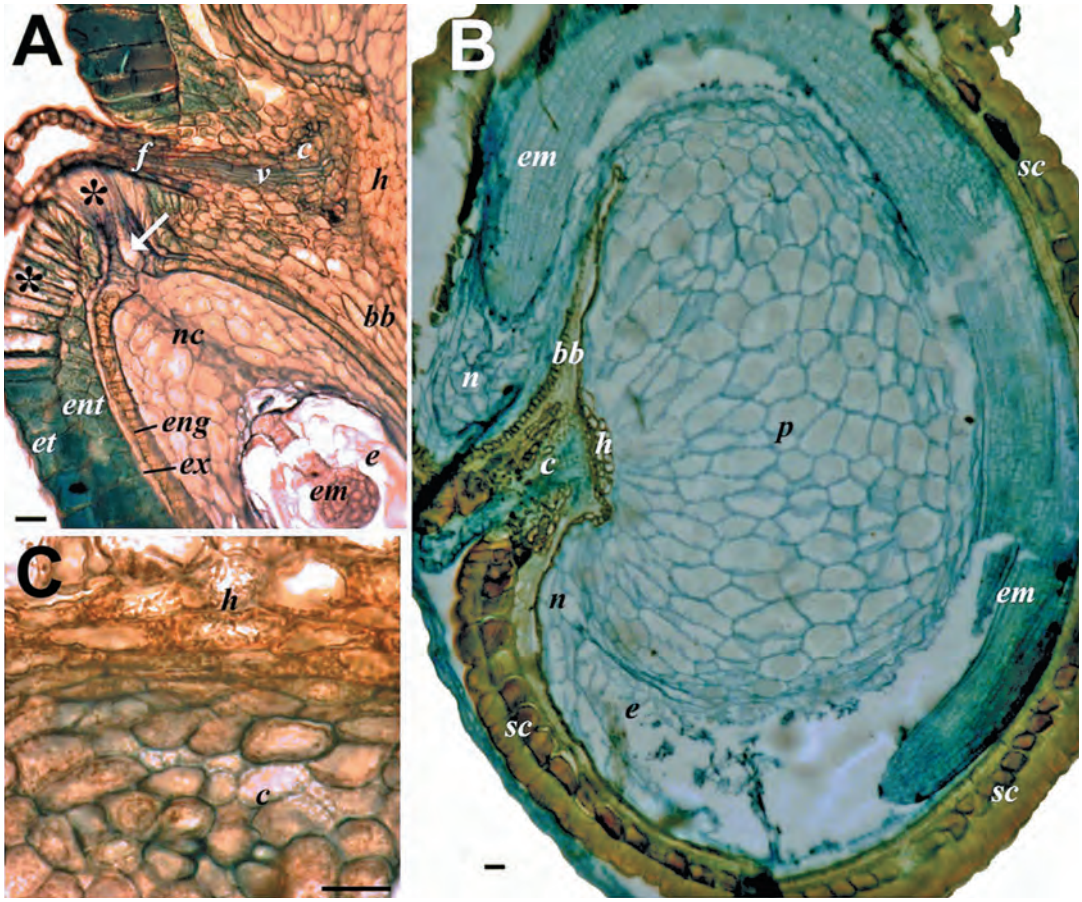

Figure 2. Seed structures at mid and late development stages. A – micropylar part of seed at the development stage of late globular embryo; B – seed with underdeveloped embryo; C – hypostase-chalaza boundary in the immature seed. *arrow* – micropyle; *asterisks* – empty cells of the exotesta and endostome; *bb* – basal body; *c* – chalaza; *e* – endosperm; *em* – embryo; *eng* – endotegmen; *ent* – endotesta; *et* – exotesta; *ex* – exotegmen; *f* – funicle; *h* – hypostase; *n* – nucellus; *nc* – nucellar cap; *p* – perisperm; *sc* – seed coat; *v* – vascular bundle. Scale bars = 20 µm (A, B), 10 µm (C).

cytoplasm, the cell walls are neither lignified nor tannin-impregnated, but they are stainable with Alcianic Blue (Fig. 2C). The vascular bundle ramifies and terminates in this parenchyma.

Chalaza cavity. Schizogenous chalaza cavity originates in the chalaza protrusion at the development stage of zygote (Fig. 1B). Its origin coincides with closing of the interintegumentary air space (Veselova et al. 2017). The chalazal cavity is enlarged at the development stage of early globular embryo. It resultantly stretches from the small-celled chalazal parenchyma under the hypostase to the endotesta base (Fig. 3), the latter one being partly split by the cavity. The chalazal cavity remains nearly up to the development stage of developed embryo. This cavity is then filled by loose parenchyma cells containing a tannin-like substance (Fig. 5 A). The chalaza cavity is undetectable in the mature seed.

Dynamics of starch allocation

Ergastic substances were detected in all constituents of developing seed except for the endosperm (Figs 3; 5A). Only clusters of small roundish intensively Alcianic Blue-stained bodies were discernible on the place of destroyed endosperm cells near tips of growing cotyledons and radicula (Fig. 5B).

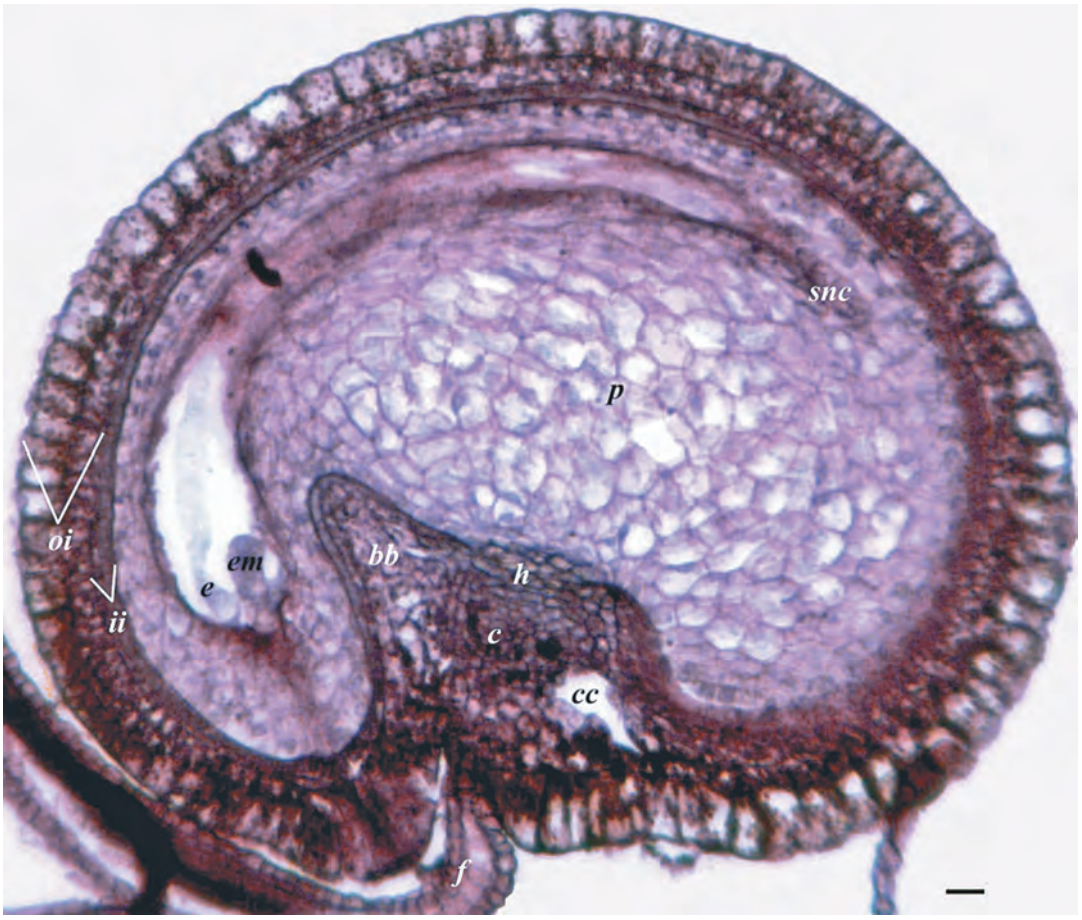

Figure 3. Starch localization in the seed at the development stage of proembryo (PAS reaction). *bb* – basal body; *c* – chalaza; *cc* – chalaza cavity; *e* – endosperm; *em* – proembryo; *h* – hypostase; *ii* – inner integument; *oi* – outer integument; *p* – perisperm; *snc* – strand of the nucellar cells. Scale bar = 20 μm.

Transient starch as simple starch grains is accumulated in the ovule by the time of fertilization. Its amount is further increased at the development stage of proembryo (Fig. 3). All cells of both integuments contain abundant transient starch (Figs 3; 4A), the amyloplasts of the exotesta cells being the largest. Similar large amyloplasts are also in the cells of the basal body (Fig. 4B) and chalaza protrusion around the schizogenous chalaza cavity (Fig. 4A). Small amyloplasts are in the cells of the nucellar cap (Fig. 4C) and in nucellar epidermal cells around their nuclei (Fig. 4B). Arcuate strand of elongate, thin-walled, starch-containing cells is at the abfunicular side of the nucellus (Figs 1B, D; 3). This cell strand is ephemeral and soon substituted by the growing nuclear endosperm (Fig. 3). The central part of the nucellus predetermined to give rise to the perisperm is completely starch-free (Figs 3; 4C). The small cells of the chalaza under the hypostase are also starch-free (Figs 3; 4C).

The integument layers successively lose the transient starch as the seed progresses. The differentiating exotegmen is the first to become starch-free by the development stage of early globular embryo. The exotegmen is obliterated soon thereafter. The endotegmen loses its transient starch after the exotegmen, though it remains alive for a rather long period of time, viz. up to the development stage of late embryo (Fig. 5A). Most exotesta cells contain the transient starch up

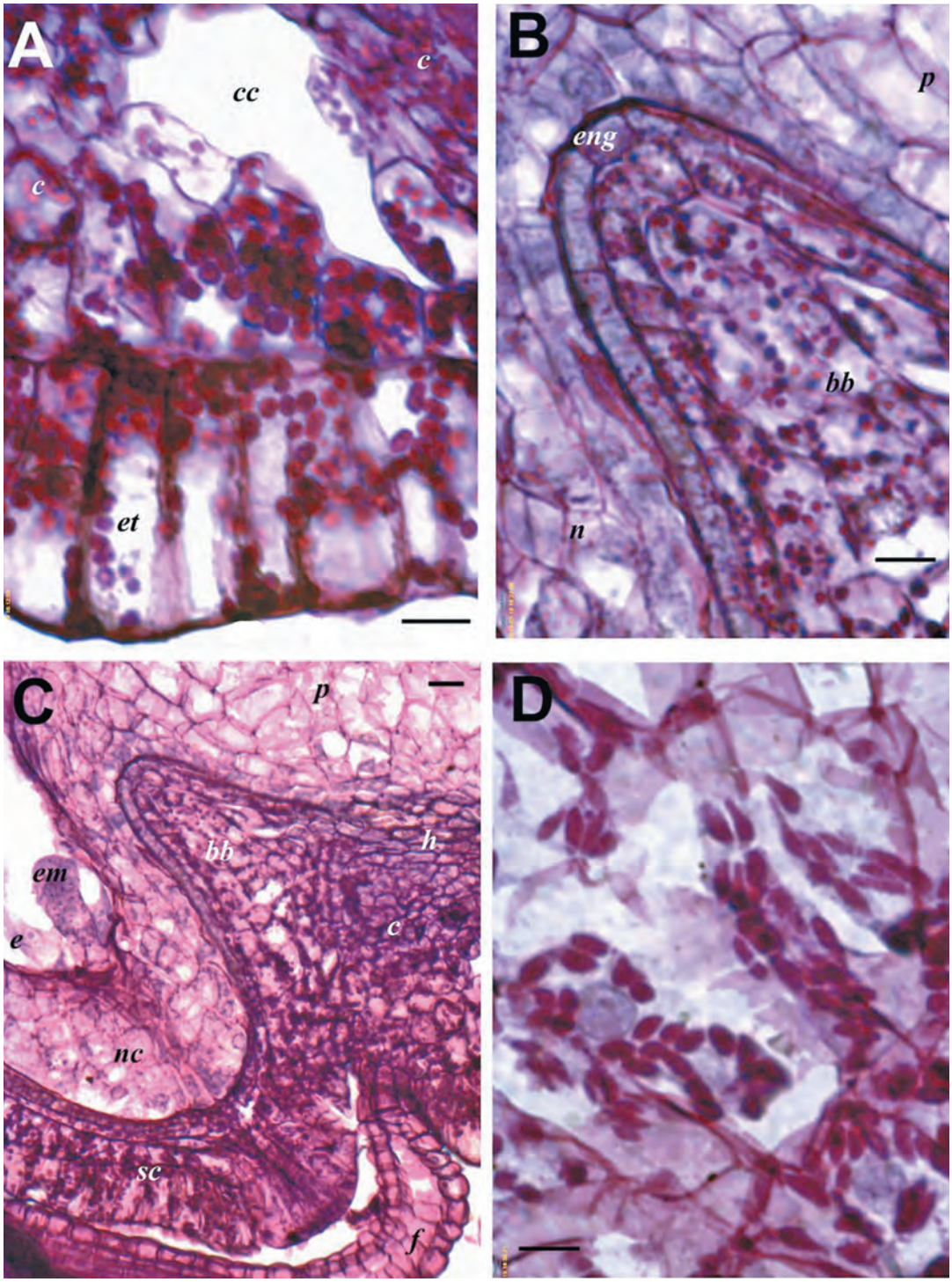

Figure 4. Starch localization in the seed structures. A – chalaza cavity region at the development stage of proembryo; B – developing basal body at the development stage of proembryo; C – proembryo and nucellar cap; D – perisperm at the development stage of early torpedo embryo. *bb* – basal body; *c* – chalaza parenchyma; *cc* – chalaza cavity; *e* – endosperm; *em* – embryo; *eng* – endotegmen; *f* – funicle; *h* – hypostase; *n* – nucellus; *nc* – nucellar cap; *p* – perisperm; *sc* – seed coat. Scale bars = 10 µm.

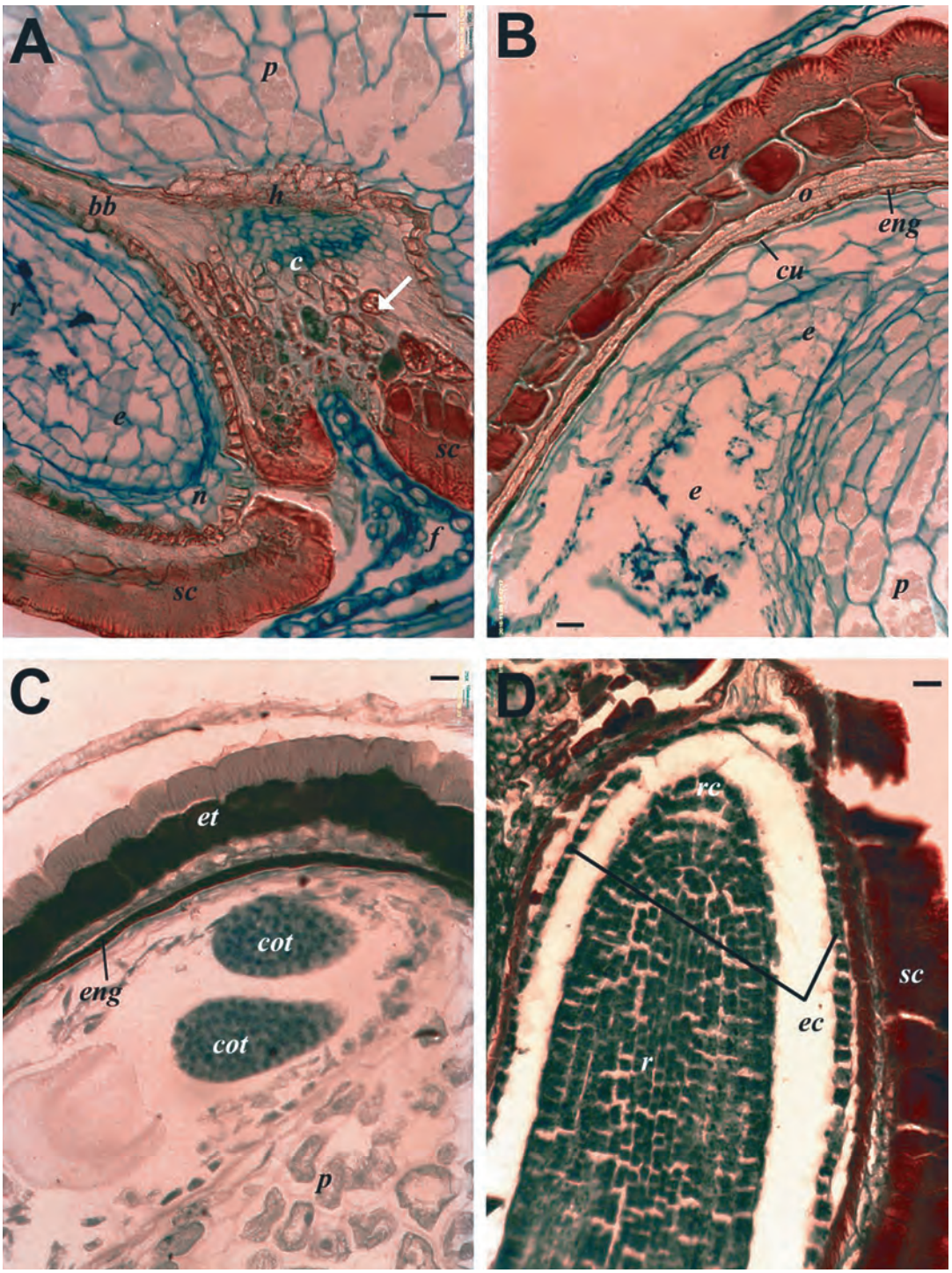

Figure 5. Endosperm and protein-accumulating structures of immature (A–C) and mature (D) seed. A – proximal part of the endosperm around the radicula tip; B – highly obliterate distal part of the endosperm; C – deposited protein in the embryo and seed coat (Procionic Blue Reaction); D – endospermal cap of the radicula tip (Procionic Blue Reaction). *arrow* – parenchyma cells occupying the chalaza cavity; *bb* – obliterated basal body; *c* – chalaza; *cot* – cotyledon; *cu* – cuticle; *e* – endosperm; *ec* – endospermal cap; *eng* – endotegmen; *et* – exotesta; *f* – funicle; *h* – hypostase; *n* – nucellus; *o* – obliterated layers of the seed coat; *p* – perisperm; *r* – radicula; *rc* – root cap; *sc* – seed coat. Scale bars = 20 µm (A–C), 50 µm (D).

to the development stage of globular embryo. They gradually transform their starch into dextrin which coalesces with tannin to fill the cell completely (Fig. 5B). The developing endotesta is the most prolonged to retain the transient starch. Its cells start hydrolysing their transient starch only at the development stage of early torpedo embryo. The endotesta completely loses the transient starch by the time the embryo is grown half of its final length.

The basal body cells become starch-free at the development stage of globular embryo. The cells of chalaza protrusion start lysing their starch at the development stage of 4–5-celled proembryo, especially those lining the chalaza cavity. Their starch grains resultantly become very small (Fig. 4A). They are absent at the development stage of globular embryo.

Disappearing of the transient starch is synchronized with deposition of reserve starch in the growing perisperm whose cells accumulate numerous compound starch grains (Fig. 4D). Scanty transient starch is detectable in the embryo tissues at every development stage except in the mature embryo (Fig. 4C) (Veselova et al. 2016).

There are no deposited carbohydrates in the endosperm of *P. arvense* (Fig. 5A) except for clustered small roundish bodies intensely stained with Alcianic Blue which are discernible on the place of destroyed endospermal cells near tips of growing cotyledons and radicula (Fig. 5B).

Dynamics of protein allocation

Having lost the transient starch at the development stage of globular embryo, the cells of exotesta and tegmen are intensely stained with Procyonic Blue and Ferric Chloride Reaction to evidence accumulation of protein and tannic substance. Both substances are combined into insoluble homogeneous mass which fills the cell (Fig. 5C).

Reserve proteins start being accumulated in the tissues of developing embryo since the development stage of late torpedo embryo. These proteins substitute the transient starch there (Fig. 5C, D).

The endosperm stores no reserve proteins except for its very tip that covers the radicula (Fig. 5D) as evidenced by Veselova et al. (2016).

Discussion

Specialization of the seed constituents of centrosperms for storing substances is usually characterized as follows.

The perisperm of centrosperms is invariably described as the starch storage tissue (Gibbs 1907; Netolitzky 1926; Devine 1950; Buell 1952; Irving et al. 1981; Lawrence et al. 1990; Elamrani et al. 1992; Coimbra & Salema 1994; Zheng et al. 2010). The residual endosperm capping the radicula is reported to store proteins and lipids (Coimbra & Salema 1994; Prego et al. 1998). The embryo is mostly characterized to be specialized to store proteins (Lawrence et al. 1990; Reguera & Haros 2017) or proteins and lipids (Netolitzky 1926; Irving et al. 1981; Elamrani et al. 1992; Coimbra & Salema 1994; Prego et al. 1998). However, Buell (1952) failed to detect reserve proteins in embryos of *Dianthus chinensis* L. and Divine (1950) described embryos of *Lychnis alba* Mill. as storing starch. The investigated seeds of *Polycnemum arvense* are quite typical of centrospems' seeds in storing proteins in the embryo tissues and residual endosperm and in storing starch in the perisperm. However, dynamic allocation of these reserve substances in developing seed seems to be specific.

Every reserve substance enters the ovule and the developing seed as water-dissolved low molecular weight precursors. The latter are specifically transported to the storing sites to be polymerized and deposited there (Bewley et al. 2013). The soluble precursors of reserve substances cannot be detected with the technique we applied. The trajectories the metabolites are running through the seed are therefore conjectural.

The soluble precursors of the reserve substances are evidently transported into the ovule through its funicle. Buell (1952) identified 2 sources of such precursors in *D. chinensis*. All precursors are eventually supplied from distant autotrophic parts of the plant body. Some quantity of the precursors is directly delivered from the distant source to the ovule through the vasculature. However, a bulk of carbohydrates is deposited in the funicular tissue as a transient starch at the pre-megasporogenetic development stage. This starch is completely hydrolysed as the embryo sac develops in the nucellus and carbohydrates released are transferred to the ovule. The funicular tissue which has accumulated the starch is thus a nearby source of soluble assimilates to be supplied to the ovule.

The ovule of *P. arvense* seems to have no nearby source of soluble precursors of nutrient substances, because its funicle accumulates neither transient starch nor transient proteins up to the development stage of fertilization. Instead, abundant transient starch is deposited in two integuments, basal body, chalaza protrusion and nucellar cap at the pre-fertilization development stages; some starch is also deposited in the abfunicular cell strand of the nucellus.

Pre-fertilization accumulation of the transient starch in the outer cell layer of the outer integument was revealed in *Spinacia oleracea* L. (Wilms 1980) and sporadically in other dicotyledons (Savchenko 1973). Both integuments accumulate the transient starch in *Dianthus chinensis* but at the development stage of globular embryo (Buell 1952). The integument-accumulated starch is hydrolysed after fertilization in *S. oleracea* (Wilms 1980) and at the development stage of cotyledon initiation in *D. chinensis* (Buell 1952).

The transient starch is accumulated at pre-fertilization development stage in both integuments and in the basal body in *P. arvense*. This starch is successively hydrolysed in exotegmen, endotegmen, basal body, exotesta and endotesta, respectively.

Postponed hydrolysis of the starch in the endotesta could result from deficiency of lytic enzymes in its cells. The deficient lytic enzymes could be supplied from outside. There is the integumental tapetum of tenuinucellate ovules that is known to be able to hydrolyse contents of neighboring cells (Zinger 1958). By analogy, the forming tegmen could be a source of necessary enzymes for the endotesta in *P. arvense*. The tegmen tightly adjoins the testa and the cuticular layer in between seems enough permeable as it is too thin and usually undetectable under the light microscopy. However, the tegmen also adjoins the basal body which is comparable with the endotesta as a derivative of the inner cell layer of the outer integument. Contrary to the endotesta, starch lysis in the basal body is not postponed. Therefore, the causes of late starch lysis in the endotesta and the source of lytic enzymes there remain to be explored

Forming seed coat certainly consumes some transient starch accumulated in the integuments.

The exotesta cells probably need a large amount of deposited transient starch to develop very thick outer cell walls and to produce abundant tannins. A rather large amount of the starch seems

to change additionally into dextrin, the latter combining with the tannin into homogeneous masses. These tannin-dextrin masses fill the cells and remain there to seed maturity (Fig. 5). These masses should not be considered reserve substances. There are only exotesta cells around the micropyle that completely lyse their contents. Their lysis products could be used by the developing proembryo or re-deposited in the forming perisperm. Therefore, the transient starch deposited in the presumptive exotesta is likely to be nearly completely consumed *in situ*.

The endotegmen cells also need carbohydrates to make fibrous thickenings on their cell walls. They probably extract the necessary carbohydrates from the scanty transient starch they have accumulated. They probably completely consume this scanty transient starch to make their fibrous cell walls.

The starch-storing cells of exotegmen, endotesta and basal body obliterate. Therefore, the transient starch they have deposited could be consumed by the developing endosperm/embryo or stored in the perisperm if only transmitted into the nucellus.

The integuments and basal body tightly adjoin the nucellus in the developing seed. However, the cuticle of endotegmen and the cuticle of nucellus combined constitute a rather thick cuticular barrier (Fig. 5B) that seems impermeable to solutes. The cuticular barrier is broken only at the site where the pollen tube has grown into the nucellus and destroyed the cuticle of the latter one (Zinger 1958). The cells of endostome and exotesta around the micropyle autolyse their contents after the pollen tube has grown through the micropyle. Resultant hydrolysate could directly be absorbed into the nucellar cap to nourish developing proembryo. The strand of elongate cells traversing the nucellar cap (Fig. 4C) seems to corroborate such absorption.

However, mass of dead integumentary cells around the micropyle seems to be an inefficient conductor of solutes from the seed coat into micropylar canal. Most carbohydrates released from hydrolysed transient starch of the endotesta, exotegmen and basal body are therefore likely to be transferred into the small-celled parenchyma of chalaza under the hypostase. This parenchyma also gains nutrients from the distant source *via* the vascular bundle. The hypostase is thus located on the routes of metabolite transportation in the developing seed.

The hypostase is considered a specialized structure to attract soluble nutrients from the funicle to the chalaza and to direct these substances to archesporium, megaspore, embryo sac and endosperm, respectively (Zinger 1958; Bhatnagar & Johri 1972; Boesewinkel & Bouman 1984; Batygina & Shamrov 1994b; Shamrov 2008). It is also thought to supply the embryo sac with enzymes and physiologically active substances (Zinger 1958; Boesewinkel & Bouman 1984), to stop excessive growing of the embryo sac (Bhatnagar & Johri 1972; Boesewinkel & Bouman 1984), to maintain increasing of the perisperm tissue as well as to regulate water exchange through the hilum in the ripe seed (Bhatnagar & Johri 1972; Boesewinkel & Bouman 1984).

The perisperm cells of *P. arvense* are centrifugally prolate (Figs 1C; 2B) as if they were specialized to transfer substances from the hypostase to the megaspore, embryo sac and endosperm, respectively. If so, these cells indirectly evidence that the hypostase *per se* can conduct solutes from the small-celled parenchyma of chalaza at least to the development stage of globular embryo, though the hypostase cells are filled with tannic inclusions and accumulate tannin in their cell walls already at the development stage of early globular embryo (Veselova et al. 2016). Accumulation

of tannin could block the apoplastic transport but it is unlikely to affect the symplastic one (Boesewinkel & Bouman 1984).

The adfunicular cells of hypostase become flattened at the development stage of torpedo embryo. Such cells are believed less efficient for conducting solutes (Veselova & Dzhalilova 2017). The schizogenous chalazal cavity was speculated to participate in transportation of the solutes past the hypostase (Veselova & Dzhalilova l. c.). This speculation seems unconvincing now.

The perisperm tissue has no stored reserve substances up to the development stage of heart embryo. It consists of prolate cells oriented to the endosperm. These cells have large pits in their cell walls. Therefore, the perisperm is mostly a conducting tissue at the earlier development stages that transfers solutes from the hypostase to the endosperm/embryo. Some transient starch accumulated in the cells of integuments, basal body and chalaza protrusion is likely to be re-deposited in the perisperm as a stored starch. However, the perisperm starts accumulating the reserve starch before the transient starch is exhausted. Consequently, the perisperm mostly gains the carbohydrates to be deposited from the distant source.

The transient starch in the abfunicular epidermis of the nucellus seems to be completely consumed *in situ* to maintain repeating cell divisions of this tissue.

The abfunicular cell strand in the nucellus is completely digested by growing endosperm. Therefore, the transient starch in these cells could hardly be added to the starch stored in the perisperm, but it is evidently consumed by the endosperm.

The antipodals of *P. arvense* are similar with their counterparts of other centrosperms (Yakovlev 1983) in being ephemeral. The chalaza-facing end of the endosperm is quickly specialized into the haustorium (Veselova et al. 2016) to compensate destroyed antipodals. There is this haustorial end of the endosperm that destroys and consumes the abfunicular strand of starch-containing cells of the nucellus.

The endosperm is reported to accumulate starch grains in *Chenopodium album* L., *Phytolacca* spp., *Trianthema monogyna* L. (Dahlgren 1939) and *Dianthus chinensis* (Buell 1952) and protein globules in *Talinum* spp. (Veselova et al. 2012) at earlier development stages. The endosperm of *Polycnemum arvense* contrasts with them in that it does not accumulate insoluble ergastic substances up to the completion of the embryogenesis. Clustered small roundish bodies intensely stained with Alcianic Blue are only detectable on the place of endosperm cells which have been destroyed by tips of growing cotyledons and radicula. Similar bodies were earlier revealed in empty dead cells of the nucellus which adjoin the developing embryo sac of the species under consideration (Veselova et al. 2016). These bodies seem to be insoluble carbohydrates similar to the mucilage (Luppa 1977). Thus, the endosperm of *P. arvense* principally absorbs dissolved nutrients to nourish the developing embryo. It becomes nearly completely destroyed by the latter one except for the small endospermic cap covering the radicle. This cap consists of homogeneous flattened cells with reserve proteins therein (Fig. 5D). It resembles the aleurone layer in the cereal grains. Such an endospermic cap is reported to hydrolyse the reserve starch in cells of dead perisperm to nourish the seedling (López-Fernández & Maldonado 2013; Burrieza et al. 2014). The endospermic cap of *P. arvense* appears to function similarly.

Developing embryo accumulates the starch which is substituted with the proteins as aleurone granules at embryo maturing.

Conclusion

Polycnemum arvense is fundamentally similar to other centrosperms both in the localization pattern of reserve substances in mature seed and in allocation dynamics of deposited nutrients in the ovule and developing seed.

The proteins to be deposited are precipitated in different structures *in situ* in one-step. The proteins deposited in the embryo tissues and endospermic cap could certainly be used as a source of (hydrolytic) enzymes and amino acids in germinating seed. These proteins should therefore be considered reserve ones. The proteins and tannin are co-precipitated in the cells of exotesta and tegmen. These combined precipitates seem to be insoluble and unavailable to be consumed by the inner structures of the seed. Whatever be the function of the seed coat-deposited proteins, these substances should consequently be interpreted as neither transient nor reserve ones.

Starch deposition in the ovule and seed is two-stepped. The transient starch is the first to be precipitated and the reserve starch is the second one.

There is a set of depots of the transient starch in the ovule and seed, viz. two integuments, basal body, chalaza projection, nucellar cap, nucellar epidermis, abfunicular strand of the nucellus cells and even the embryo *per se*. The transient starch deposited is hydrolysed thereafter to be differently consumed by the structures of the developing seed. The transient starch of the nucellar cap seems to be totally consumed by growing pollen tube and proembryo. The transient starch of the integuments is certainly used *in situ* for developing characteristic cell wall thickenings of endotegminal and exotestal cells and for producing tannin (and proteins) deposited in the seed coat. The transient starch in the exotegmen is likely to be consumed by the developing endotegmen. The transient starch deposited in the endotesta, chalaza projection and basal body seems to be (partly) used for supplying the perisperm. The transient starch of the endotesta is probably nearly completely transported to the perisperm, though its parenchyma remains alive for a long period of time. There is the transient starch in the abfunicular strand of nucellus cells that is undoubtedly completely consumed by the endosperm, because the latter one annihilates these cells to absorb their lysates for nourishing the embryo.

The perisperm cells die off after the reserve starch has been accumulated therein, their nuclei become deformed. The perisperm parenchyma also dies off after accumulating reserve starch in *Chenopodium quinoa* Willd. (Prego et al. 1998; López-Fernández & Maldonado 2013), but it seems to remain alive and retain large intact nuclei in easy germinating seeds of heterospermous *Atriplex nitens* Schkuhr (Veselova & Kondorskaya 1990). Whether the dying perisperm parenchyma is typical of most centrosperms is to be revealed.

Polycnemum arvense differs from most centrosperms in basal body and schizogenous chalaza cavity. The former one accumulates the transient starch to supply the perisperm. The function of the latter one is to be revealed.

Polycnemum arvense matches *S. oleracea* (Wilms 1980), *C. quinoa* (Prego et al. 1998; López-Fernández & Maldonado 2013), *Amaranthus hypochondriacus* (Coimbra & Salema 1994) and *Talinum* spp. (Veselova et al. 2012) in allocation dynamics and location of nutrients in its ovule and seed. The ovule of *P. arvense* accumulates much more transient starch to the development stage of fertilization than the ovule of *D. chinensis* (Buell 1952). The transient starch is mostly deposited in the integuments and chalaza in *P. arvense*. The transient starch is accumulated in

such structures of *D. chinensis* only at the development stage of early embryogenesis. Instead, abundant transient starch is deposited in the funicle tissue in *D. chinensis*.

The transient starch deposited in the integuments of *P. arvense* seems to be sufficient to maintain seed coat development and also to partly supply the perisperm. The transient starch in the integuments of *D. chinensis* seems to be too late-deposited, too scanty and too soon-vanishing to be sufficient for maintaining seed coat development.

The transient starch accumulated by the endotesta becomes completely exhausted at the development stage of late torpedo embryo in *P. arvense*. Remnants of the transient starch in the endotesta are still detectable in the seed coat dumped by the germinating seed of *Talinum* spp. (Veselova & Dzhalilova, unpubl.).

The centrosperms are evidently polymorphic in dynamics of allocation, storing and consuming of nutrients in their ovules and developing seeds. Possible ecological causes, physiological regulation and taxonomic significance of the revealed polymorphism are still to be explored.

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