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Stalactite development in the exotesta cell walls of *Amaranthus retroflexus* L. (Amaranthaceae): an unusual way of cell wall lysis

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Summary: The stalactites, unique structures that are specific for Amaranthaceae s. lat., are numerous cross-walled, smooth-contoured masses of precipitated tannic substances in the thickened outer cell wall of the exotesta cells. The stalactites strikingly differ from typical depositions of tannic and other non-crystalline substances in plant cell walls. The latter, unlike stalactites, are deposited in layers parallel to the cell wall or they evenly or gradually impregnate the cell wall. The stalactites are initially detected as isolated, closed, smooth-walled, globular cavities in the inner papillae of the cell wall at the heart embryo developmental stage. These cavities elongate inwards during cell wall differentiation and thickening. They are filled with insoluble tannic substances at the immature seed developmental stage. The stalactites are solid masses of tannic (and phytomelanin) substances, which fill pre-developed cross-walled cavities in the outer cell wall of the exotesta cells. These cavities are completely isolated by the cell wall layers from the plasma membrane at each stage of their development. The cavities arise and elongate lysigenously. The cavity-generating lysis of the cell wall is a unique process that most likely takes place in the depth of the cell wall separated from the formatively active plasma membrane. Moreover, it affects only specific areas of the cell wall and results in smooth-walled cavities. The mechanism of such lysis needs to be further investigated.

Keywords: *Amaranthus*, seed coat, exotesta, cell wall lysis, stalactite, tannic substance, anatomy

Stalactites, designated by Meunier (1890), are typical formations of exotesta (outer layer of seed coat) of many representatives of Amaranthaceae s.l. (Meunier 1890; Netolitzky 1926; Butnik 1991; Butnik & Zhapakova 1991; Kühn et al. 1993). They look like darker stripes in the thickened outer cell wall of exotesta cells. These cell walls have been accordingly described as striated by some botanists (Kühn et al. 1993; Kuegle & Gabel 2004). However, the stalactites are cross-walled (Meunier 1890; Netolitzky 1926) to oblique (Meunier 1890; Sukhorukov & Zhang 2013) in sharp contrast to typical cell-wall striation which is always in the plane of the cell wall being a manifestation of the arrangement of cellulose fibrils (Frey-Wyssling 1976).

The cross-walled arrangement of stalactites sometimes provokes the latter to be even considered as local thickenings of the cell wall which 'hang down' into the cell lumen (Butnik 1991; Butnik & Zhapakova 1991; Salakhova et al. 1992). Nevertheless, these formations were confidently assigned to be internal constituents of rather flat outer cell walls of exotesta cells (Meunier 1890; Netolitzky 1926; Kowal 1954; Suárez Ramos & Engelman 1980; Tikhomirov & Fedorova 1997; Sukhorukov & Zhang 2013).

The cross-walled stalactites resemble to some extent cross-walled ectodesmata inherent in outer cell walls of certain surface cells. The latter always contact the protoplast's surface with their inner termini (Frey-Wyssling 1976). Unlike the ectodesmata, the stalactites never contact the protoplast's surface. Therefore, no similarity between ectodesmata and stalactites was affirmed by Netolitzky (1926) almost 90 years ago.

The stalactites were interpreted as pit canals (Plisko 1991; Veselova et al. 2009, 2012a). This interpretation is irrelevant for the same reason.

Hanausek (1915) considered the stalactites as cutin intrusions into the cell wall, but neither cutin nor suberin was histochemically detected in stalactites (Netolitzky 1926; Kowal 1954; Suárez Ramos & Engelman 1980; Tikhomirov & Fedorova 1997; Veselova et al. 2014). The latter are no way parts of the cuticle (Meunier 1890; Netolitzky 1926). These formations are mostly interpreted now as local depositions of colored tannic substances alias phlobaphene (Netolitzky 1926; Kowal 1954; Tikhomirov & Fedorova 1997; Sukhorukov & Zhang 2013). Tannic substances are typical components of the cell wall in seed coats (Pal et al. 1990; Zaprometov 1993; Bakhtenko & Kurapov 2008; Bewly et al. 2013), where they evenly or gradually impregnate the cell wall (Zaprometov 1993; Bakhtenko & Kurapov 2008) and do not constitute the cross-walled stripes. Moreover, hardly discernible stalactites have also been revealed in colorless exotesta cell walls of white seeds of *Amaranthus caudatus* L. and *A. hypochondriacus* L., respectively (Suárez Ramos & Engelman 1980; Veselova et al. 2014), which have no colored tannic substance at all in their seeds.

All representatives of core Caryophyllales have colorless proanthocyanidins in their underdeveloped seed coats (Bate-Smith & Ribéreau-Gayon 1959; Suárez Ramos & Engelman 1980; Bittrich & do Carno Amaral 1991). These substances are also polyphenols but less polymerized than the tannic ones. The proanthocyanidins are believed to precipitate in stalactites of white seeds (Suárez Ramos & Engelman 1980), but they are unlikely to make these formations discernible. Besides, the cause of local cross-walled precipitation of proanthocyanidins is still to be discovered.

Therefore, the stalactites seem to be special inner formations in the cell walls which either accumulate (colored) tannic substance or not.

Stalactite development data could elucidate the nature of these formations, but such data are still to be obtained. Even the most extensive investigation of Suárez Ramos & Engelman (1980) only shows dynamics of tannic substance accumulation in the stalactites and not the stalactite development per se. Meunier's (1890) data are also rather incomplete. In his opinion, the stalactites are rod-like masses of some substance somehow associated with provisional inner papillae of the cell wall. They abut upon the cuticle and intrude inwards into the cell wall matrix with the thickening of the wall. However, these formations really never contact the cuticle (Netolitzky 1926; Kowal 1954; etc.).

The aim of this study is to describe the formation of the stalactites to fill this gap. We have investigated stalactite development in exotesta cell walls of black-seeded *Amaranthus retroflexus* as a model, because this species has long been known to have conspicuous large stalactites in its exotesta (Netolitzky 1926; Kowal 1954; Tikhomirov & Fedorova 1997).

Materials and methods

Inflorescence fragments at different developmental stages from flower buds to dehiscing fruits with ripened seeds were sampled from wild plants in western Moscow Region and immediately fixed in a FAA fixative. Fixed material was dehydrated in a series of ethyl alcohols of increasing strength and embedded into paraffin wax through ethyl alcohol-xylene mixtures according to Prozina (1960). Microtomic 10 μm thick sections were mounted on slides and dewaxed by

xylene. Some preparations were directly embedded in Canada Balm to retain natural color of the structures. Other ones were rehydrated according to Prozina (1960) and then they were histochemically processed according to Barykina et al. (2004) as follows:

Rawitz Alum Hematoxylin staining;
PAS reaction for polysaccharides;
Alcian Blue staining for unlignified cell walls;
Ruthenium Red staining for pectin;
Phloroglucinol-hydrochloric acid reaction for lignin;
Sudan IV or Sudan Black staining for lipoid substances;
Procion Blue staining for proteins;
Light Green staining for proteins;
Ferric chloride reaction for tannic substances.

The preparations thus processed were dehydrated and embedded into Canada Balm according to Prozina (1960).

Micrographs were taken under light microscopes Axioplan 2 imaging (Leica) and Univar (Reichert) equipped with digital cameras AxioCam MR and DCM-510, respectively.

Results

There is certain synchronicity between embryo sac/embryo development and seed coat formation in the investigated plants. Accordingly, stalactite development is described in accordance with traditional stages of embryogenesis.

Because the initial stages of stalactites development were seemingly not revealed in previous investigations, we traced the exotesta formation from the mature embryo sac stage, when there is still no sign of transformation of the outer epidermis of outer integument into the exotesta.

Mature embryo sac stage. The campylotropous ovule has 2 integuments (Fig. 1A), which start changing into the seed coat. The outer epidermis of the outer integument consists of small, evenly thin-walled cells with a relatively large nucleus and small vacuole/s (Fig. 1B). The cuticle on this cell layer is indiscernible.

Post-fertilization stage. The outer cell layer of the outer integument shows discernible indication of initiating exotesta. Its cells are enlarged; some are flattened paradermally (Fig. 1C). The cells contain abundant amyloplasts with compound starch grains which mask the cell nucleus and vacuoles. Their outer cell walls are slightly thicker than the other counterparts. Polysaccharides, including pectin, and proteins are detected in all cell walls of exotesta cells. The cuticle on the exotesta surface is indiscernible.

Globular proembryo stage. The exotesta cells are mostly anticlinally elongated (Fig. 1D). They have large vacuole/s, few amyloplasts with compound starch grains and a few yellow inclusions of tannic substance. The outer cell wall of exotesta cells is a little bit thicker than the other cell walls. It appears homogeneous due to abundance of polysaccharides and proteins. Thin cuticle is visible on the exotesta surface.

Heart embryo stage. The exotesta cell lumens are filled with a homogeneous (Fig. 1E) or coarse-grained (Fig. 1F), yellow-brown mass of tannic substance. No organelles are detectable in most

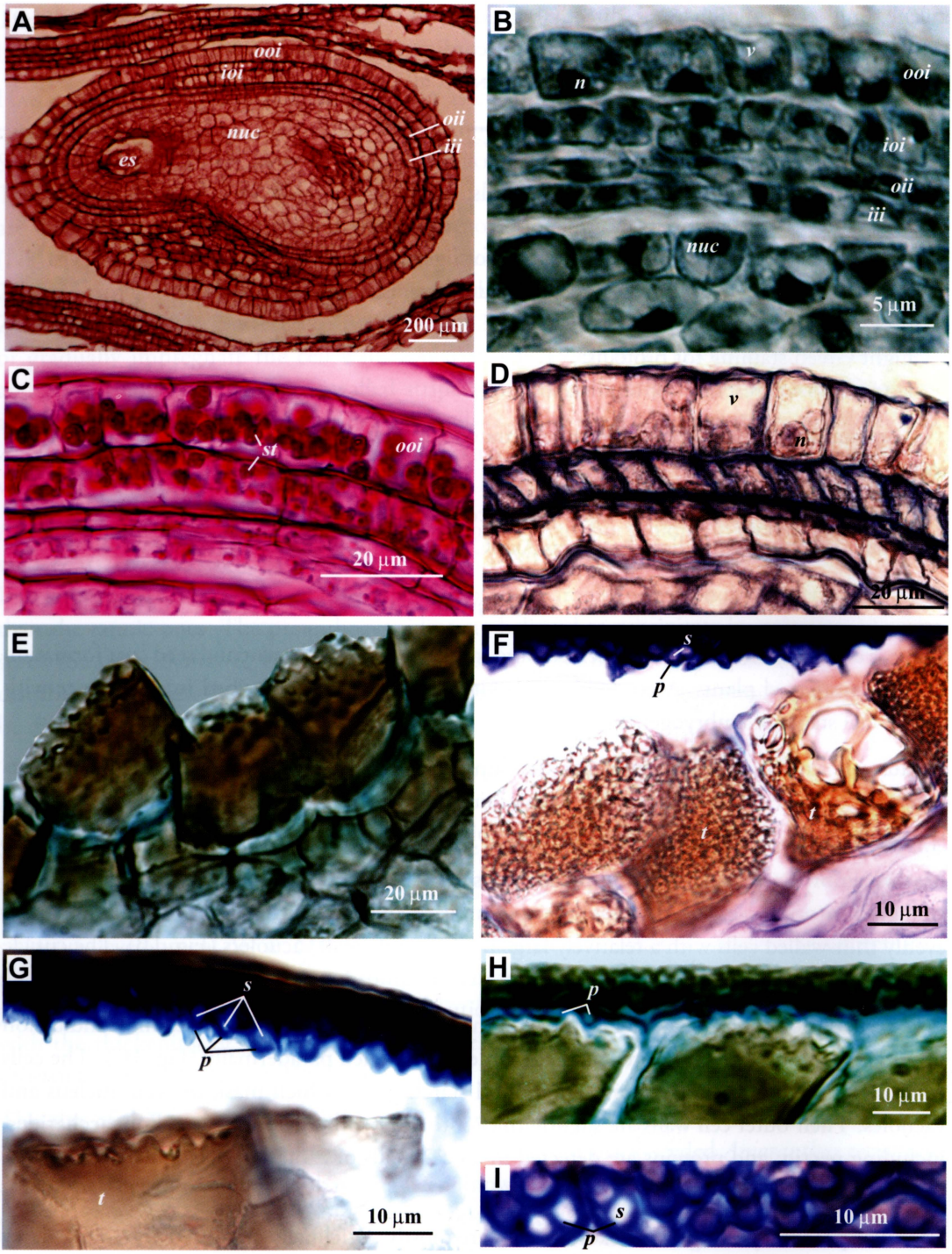

Figure 1. Early stages of seed coat development, transverse (A–H) and oblique paradermal (I) sections. A – ovule, mature embryo sac stage (Rawitz Alum Haematoxylin); B – integuments, mature embryo sac stage (Rawitz Alum Haematoxylin + Light Green); C – initiated seed coat, post-fertilization stage (PAS reaction); D – developing seed coat, globular proembryo stage (Alcian Blue); E – outer surface of the protoplasts of developing exotesta cells, heart embryo stage (Procion Blue); F – protoplast contents of the exotesta cells near micropyle, heart embryo stage (Alcian Blue); G – protoplast relief, cell wall counter-relief and initial stalactite cavities, heart embryo stage (Alcian Blue); H – outer cell walls of the exotesta cells, heart embryo stage (Procion Blue); I – outer cell walls of the exotesta cells, heart embryo stage (Alcian Blue). ▶

exotesta cells under light microscope (Fig. 1E). Only those located near remnant micropyle still contain large amyloplasts embedded in tannic mass. Diffuse proteins and presumable dextrins are also detected in all exotesta cells.

The outer cell wall is rather thick and uniquely shaped. Its outer surface is smooth, whereas its inner surface is papillose (Fig. 1F, G). This relief of the inner surface of cell wall conforms to the counter-relief of the outer surface of protoplasts (Fig. 1G). The cell wall is differentiated into 2 layers. The outer subcuticular one is the thinnest, homogeneous, yellow of tannic substance and unstainable by Alcian Blue (Fig. 1G). The much thicker inner layer is also homogeneous, but it has proteins and no tannic substance. The proteins are more copious in the innermost part of the layer and in papillae (Fig. 1H). Each papilla contains a very regular smooth-walled cavity (Fig. 1G, I), which is completely closed and isolated from the cuticle as well as from the plasma membrane by the cell wall matter. No contents are indicated by applied histochemical processing in these cavities.

Late torpedo embryo stage. The outer cell walls make up to 40% thickness of the exotesta (Fig. 2A). Decreased cell lumens are filled with a homogeneous fawn mass; the organelles are indiscernible (Fig. 2A–C). Smoother inner relief of cell wall and outer counterpart of protoplast are still visible in some exotesta cells (Fig. 2B), but both are lost in the others (Fig. 2C). The outer cell wall is distinctively 2-layered; the thinner outer layer is impregnated by tannic substance which makes it naturally beige-yellow (Fig. 2A); the thicker inner layer is tannic-free and naturally colorless. The boundary between the two layers is undulate. Both layers show very similar staining by Alcian Blue or PAS reaction, and only an extremely thin subcuticular zone remains unstained. The same zone becomes visible after Sudan IV or Sudan Black staining due to its contrast to the stained cuticle (Fig. 2B).

Procion Blue staining reveals various contents of proteins in the cell wall: the highest in its outer layer, the lowest in the inner cell wall layer (Fig. 2C).

The former globular intra-papilla cavities are changed into rather long cross- and smooth-walled formations without any detectable contents (Fig. 2D). They extend through both layers of the cell wall except of its outermost and innermost zones (Fig. 2A–C). These cavities are thus completely separated from the cuticle and the plasma membrane.

Immature (light-colored) seed stage. The cell lumens are highly flattened and filled with homogeneous yellow-brown contents (Fig. 2E). The outer cell wall makes up to 80% thickness of the exotesta. It is light brown throughout and unstainable by most procedures applied (Fig. 2E). However, PAS reaction makes visible 2 zones of the cell wall. The thicker inner zone is red-stained whereas the thinner outer zone remains unstained (Fig. 2F). The latter one might correspond to the outermost unstainable zone of the cell wall at preceding developmental stages, but it is much thicker. The proteins are mostly in the inner zone of the cell wall and in cross-walled cavities (Fig. 3A). The protein-accumulating zone is thinner than the PAS-stained one (comp. Figs 2F and 3A). The cross-walled cavities are very distinctive (Fig. 3A, B) and filled by a yellowish tannic substance accumulated mostly in their outer parts (Fig. 3B).

iii – inner epidermis of inner integument; oii – outer epidermis of inner integument; es – embryo sac; nuc – nucellus; ooi – outer epidermis of outer integument; ioi – inner epidermis of outer integument; n – nucleus; v – vacuole; st – starch in amyloplasts; p – cell-wall papilla; s – stalactite cavity; t – tannic substance.

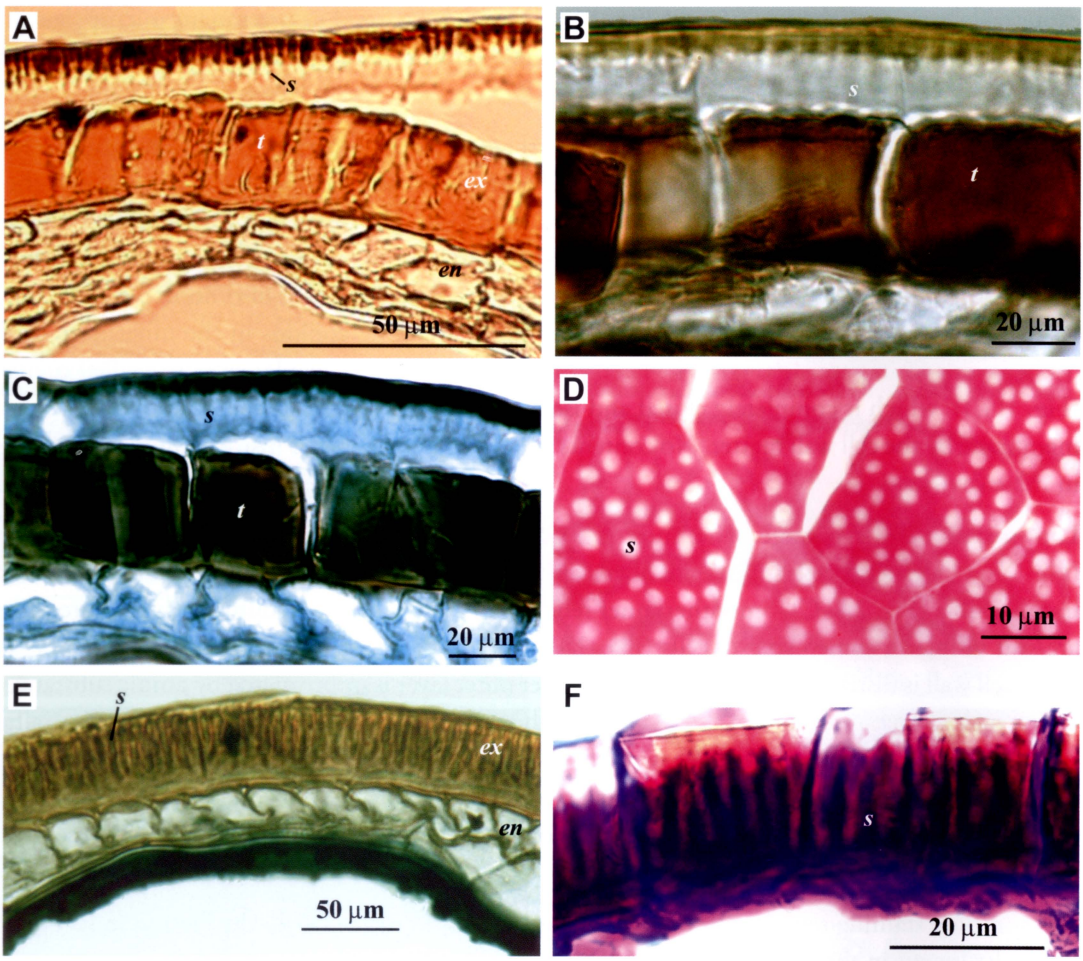

Figure 2. Late stages of seed coat development, transverse (A–C, E, F) and paradermal (D) sections. A – exotesta, torpedo embryo stage (unstained); B – exotesta, torpedo embryo stage (Sudan IV); C – exotesta, torpedo embryo stage (Procion Blue); D – outer cell walls of the exotesta cells, torpedo embryo stage (Ruthenium Red); E – seed coat, immature seed stage (Alcian Blue); F – exotesta, immature seed stage (PAS reaction).
s – stalactite cavity; t – tannic substance in the exotesta cell; ex – exotesta; en – endotesta.

Mature seed stage. The lumens of exotesta cells are even more flattened (Fig. 3C), some are empty, but others filled with a homogeneous yellow-brown mass which reacts with Sudan IV or Sudan Black indicating the presence of lipids. The exceedingly thick outer cell wall is colored throughout by the tannic substance. Its natural brown color intensifies outwards thus indicating outward increasing of content or polymerization grade of this substance (Fig. 3C). Sudan IV tinges the whole cell wall with pinkish. The outer half of the cell wall is affected neither by reagents for polysaccharides nor by reagents for proteins, but the inner one is slightly stained by Light Green as well as by Procion Blue (Fig. 3C). The latter one also slightly stains contents of the cross-walled cavities.

Every cross-walled cavity contains a central mass of insoluble tannic substance of the same color as the cell wall per se (Fig. 3C, D). This mass is partly to completely surrounded by a darker layer in very many cavities, but it is partly to completely replaced by the dark substance in a few ones (Fig. 3D). The darker substance makes the cavities contrasting under transmission light microscopy,

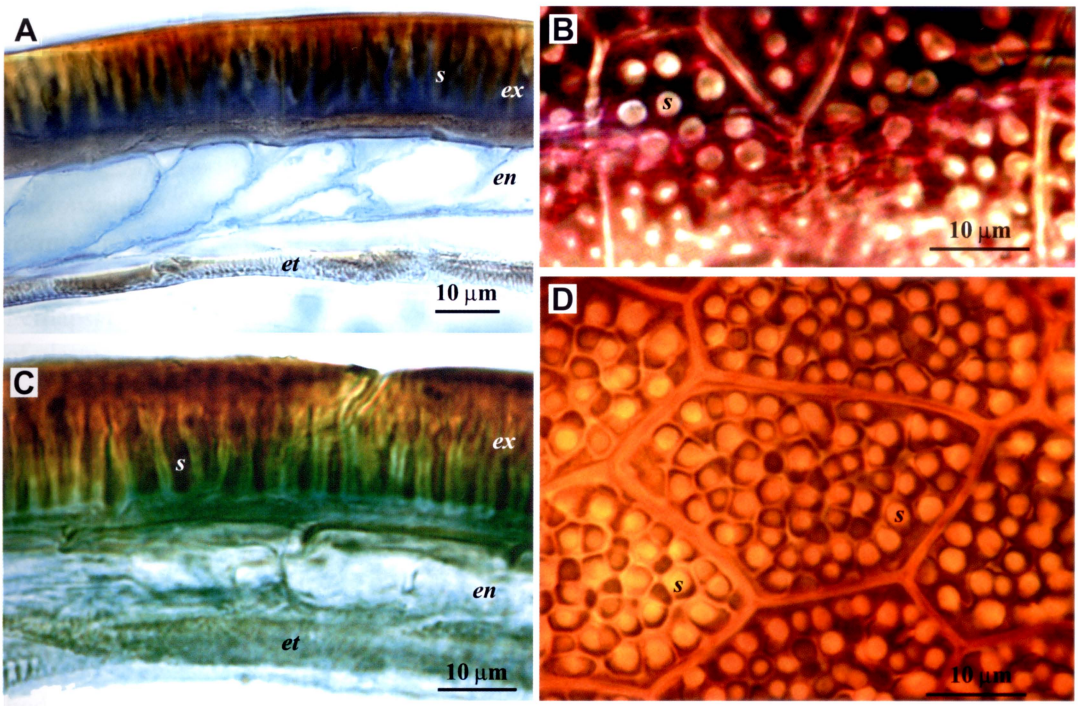

Figure 3. Stalactites in the exotesta-cell walls of immature (A, B) and mature (C, D) seed, transverse (A, C) and paradermal (B, D) sections. A – Procion Blue; B – PAS reaction; C – Procion Blue; D – unstained. s – stalactite; ex – exotesta; en – endotesta; et – endotegmen.

but the cavities are hardly discernible under SEM (Fig. 4) because of the consistency of cavity contents as well as of the cell wall is likely to be the same. When filled with insoluble tannic substance, the cavities under consideration fit well the stalactites as conceptualized by Meunier (1890), Kowal (1954), Tikhomirov & Fedorova (1997) and Sukhorukov & Zhang (2013).

Discussion

The data obtained show that the stalactites coincide with cross-walled masses of a colored insoluble tannic substance in the cell wall. However, it is unlikely that they came into existence by means of a simple local deposition of this substance in the cell wall matter. These masses are preceded by the cell-wall interior cavities. The tannic substance fills the pre-developed cavities. These cavities are worth beeing considered as stalactites and not the tannic masses per se. In other words, the stalactite development is mostly a development of inner cavities in the cell wall.

These cavities appear to be empty at all stages preceding the immature seed developmental stage. This appearance could be an artifact, however. The stalactite cavities could accumulate tannic substance from the very beginning, but this substance may be highly water and ethyl alcohol soluble when it is not compound with proteins, polysaccharides or phospholipids etc. (Schofield et al. 2001; Hagerman & Butler 1991; Mueller-Harvey 2001; Zaprometov 1993; Bakhtenko & Kurapov 2008). Therefore, the stalactite cavity contents may have been washed out under the process of dehydration-rehydration, whereas the tannic substance is firmly fastened in the cell wall at the same time due to interaction with the cell wall matter. Condensed tannic substance becomes detectable in stalactite cavities at immature seed developmental stage.

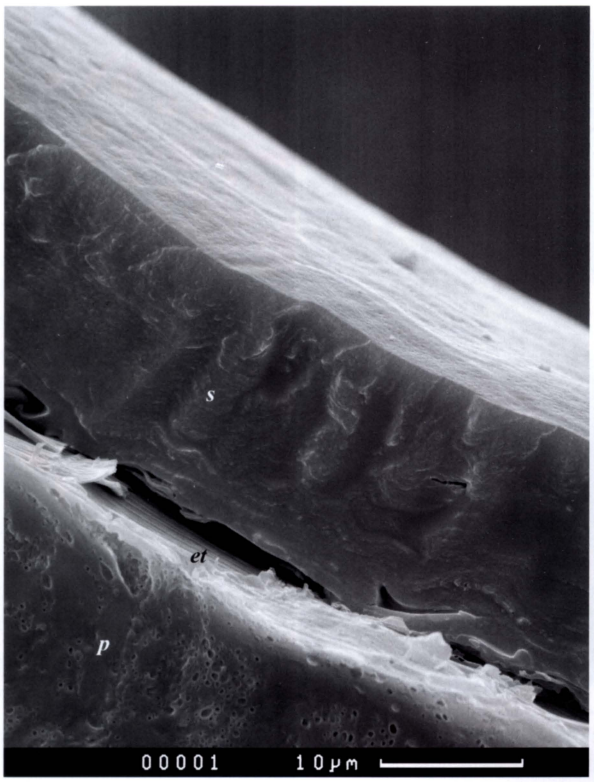

Figure 4. Cross-sectioned outer cell wall of exotesta cell of mature seed, SEM. s – presumable stalactite; p – perisperm; et – endotegmen.

The maximum content of proteins is also detectable there at this stage. Accumulation of proteins in the stalactite cavities seems to be a precondition of condensation of soluble tannic substance into solid masses. The nature of the proteins and mechanism of their accumulation in stalactite cavities are still to be investigated.

The stalactite tannic substance is partly substituted by a darker substance in stalactites of definitive exotesta cells. We believe this substance to be a phytomelanin, another common constituent of the seed coat (Zaprometov 1993; Paredes et al. 2009).

The earliest indication of stalactite cavities appears at heart embryo developmental stage, when the exotesta cells highly accelerate thickening of their outer cell wall. The outward surface of the protoplast becomes micropapillose and the inward surface of the outer cell wall acquires its micropapillose counter-relief in *A. retroflexus*, just as in *A. hypochondriacus* L. (Suárez Ramos & Engelman 1980) and in *Chenopodium* species (Meunier 1890). An ultrapapillose surface is characteristic of the protoplast at initial stage of primary cell wall development (Frey-Wyssling 1976), when the cells produce numerous cytoplasmic vesicles containing the matter of cell wall matrix. These vesicles move to the plasma membrane and merge with it to release their contents into the forming cell wall via exocytosis. Intense incorporation of vesicle membranes into the plasma membrane unbalances membrane recycling. The protoplast surface becomes papillose to enlarge plasma membrane. Thickening of the cell wall slows down in a short period of time. The vesicle production concomitantly slows down, membrane recycling re-balances and protoplast surface becomes smooth.

The micropapillae under consideration highly exceed the ordinary ultrapapillae. They are certainly too large to be results of exocytosis. Besides, these micropapillae appear at heart embryo developmental stage, long after the primary cell wall started forming. Sudan IV staining reveals a very thin subcuticular layer of the cell wall (Fig. 1G). This layer is likely to be attributed as the primary cell wall. If so, the papillae are formed on the secondary cell wall. Therefore, the micropapillae of the secondary cell wall in exotesta cells essentially differ from ultrapapillae of the forming primary cell wall. However, these formations are similar in their dynamics: both appear at the period of intense thickening of cell wall and disappear when the thickening slows down. Perhaps, the micropapillae also enhance thickening of the cell wall by means of enlarged surface of plasma membrane which bears more sites for releasing substances of cell wall matrix and for more cellulose-synthesizing complexes.

Initial stalactites appear as solitary globular cavities in micropapillae of the cell wall. They grow longer inwards with the thickening of the cell wall. They are completely isolated from the plasma membrane by the innermost part of cell wall from the very beginning to the very end. Then, stalactite initiation and development strikingly differ from development of plasmodesm canals and pit canals, the latter being the results of discontinuous formation of the cell wall (Esau 1977; Frey-Wyssling 1976). The stalactite cavity could evolve from either a growing crystal or local lysis of the cell wall. However, the shape of these cavities does not fit the shape of any crystalline body, therefore the stalactite cavity more likely results from the local lysis of the cell wall.

The stalactite cavity has unexpectedly a smooth wall which is similar to other lysigenous smooth-walled hollow formations, sieve pores and vessel perforations (Esau 1969; Frey-Wyssling 1976). However, there are definite differences between the stalactite formation and the local lysis of the cell wall that gives rise to sieve pores as the latter takes place in contact with the formatively active smooth plasma membrane (Esau 1969). Such a lysis is certainly caused and controlled by the latter one. The stalactite cavities, in contrast, are initiated and develop more distantly from the formatively active plasma membrane on a molecular scale. Their smooth wall cannot be shaped by the smooth plasma membrane.

Local lysis of the cell wall that gives rise to vessel perforation occurs just after the total autolysis of the protoplast has completed, thus releasing certain cell wall-lytic enzymes. These enzymes are believed to affect only unlignified cell walls and median plates without plasma membrane control (O'Brien 1970; Roberts 1976; Benayoun et al. 1981; Fukuda 1996; Nakashima et al. 2000; Ye 2002; Endo et al. 2009; Myburg & Sederoff 2014). The smooth wall of the perforation accordingly results from preceding patterned polymerization of lignin in the cell wall. However, neither lignin nor some other special protecting matters were detected around the presumptive stalactite cavities in *A. retroflexus*. Besides, the vessel perforations do not elongate with thickening of the cell wall because they form after the latter one has attained its definitive thickness. Therefore, the mechanism of stalactite cavity formation differs from the mechanism of perforation formation.

The smooth wall of stalactite cavity at any stage of its development clearly shows that cavity elongation is a continuous process. There are two conceivable mechanisms of such a continuous elongation. The cell wall around the initial stalactite cavity could locally be stretched inwards. Certain inward force is necessary for this stretching. Ordinary turgor pressure is unable to generate such a force because it is general and directed outward. Thus, there is only local contraction of

the protoplast that could generate the necessary force. Such a contraction is absolutely unusual for cells of higher plants. Besides, presumed contraction must be strong enough to overcome the cell wall resistance. The cytoplasm would have accordingly been rather massive to contain a powerful contracting apparatus. However, the protoplast looks like a homogeneous mass of tannic substance, proteins and polysaccharides, when the stalactite cavity grows longer in investigated species. We do not think that the cytoplasm is completely destroyed at these developmental stages. Otherwise, neither thickening of the cell wall nor special chemical transformations of the latter one would be possible.

In two *Talinum* species, the exotesta cells generate a large homogeneous mass of tannic substance during the time when the outer cell wall thickens (Veselova et al. 2012b). Only a very thin layer of cytoplasm and a nucleus was detected around this mass. Thus, it is clear that the mass of tannic substance is the content of a very large vacuole. Likewise, the tannic-protein-polysaccharide mass in exotesta cells of *A. retroflexus* is the content of an enormous vacuole, and the surrounding cytoplasm is too thin to be detected by light microscopy. It is difficult to envision how the remaining cytoplasm would create such powerful contractile forces capable of stretching and invagination of the thickening cell wall. Therefore, only internal local lysis of the cell wall can be considered as the mechanism of elongation of stalactite cavities in *A. retroflexus*.

The cells of higher plants are thought to be able to synthesize lytic enzymes for any organic constituent of their cell walls (Frey-Wyssling 1976). But the lysis that is assumed to form the stalactite cavity is quite unusual for cells of higher plants, as it affects strictly defined parts of cell wall that are distant from the active plasma membrane. Certain cell wall-lytic enzyme(s) must accordingly be released through the plasma membrane in inactive form (otherwise, the enzyme(s) would destroy the cell wall adjacent to the plasma membrane and the stalactite cavity would contact the plasma membrane). Inactive enzyme must concentrate in strictly definite sites in the cell wall and be activated later to destroy precisely defined part of the cell wall and produce the isolated stalactite cavity. The activating agents could later be released into the cell wall and diffuse throughout the cell wall to reach the enzyme-occupied site. Agent diffusion evidently needs no special mechanism. However, the defined and precise localization of the inactive enzymes would require special mechanisms.

Two mechanisms are plausible. In the first scenario, the plasma membrane could locally release insoluble inactive enzyme(s) into the cell wall and then produce new layer(s) of the cell wall without enzyme(s). The enzyme-occupied site would thus become isolated from the plasma membrane. By means of activation, an enzyme could produce an isolated stalactite cavity. This process, however, would be unable to maintain cavity elongation. Also, in that case, the exotesta cell wall would have contained small globular stalactite cavities formed at an early developmental stage, or long cross-walled ones that start developing after ceasing thickening of the cell wall. This is not the case in *A. retroflexus*. Another observation contradicts this scenario: if insoluble enzyme masses were repeatedly released into the thickening cell wall and were overlaid by enzyme-free layers of the cell wall, the septae remnants would be seen in the stalactite cavity. This is not the case either. Therefore, this model seems to be unlikely.

In the second scenario, the inactive soluble enzymes that were released by the plasma membrane could be transported along a strictly defined path across the cell wall towards the site of the stalactite cavity formation, where it would eventually initiate and maintain the growth and

elongation of the cavity. However, the mechanisms that control such a directional movement of soluble substances through the cell wall remain to be elucidated. Local activity of cell wall-lytic enzyme(s) resulting in the production of cavities with very smooth walls also remain enigmatic.

Conclusion

The stalactites are solid masses of tannic (and phytomelanin) substance, which fill predeveloped, cross-walled cavities in the outer cell wall of the exotesta cells. Development of the stalactite cavities in the exotesta cell wall of *A. retroflexus* drastically differs from development of any cell wall inner formation known so far. These cavities are completely isolated by the cell wall layers from the plasma membrane at each stage of development. The cavities arise and elongate lysigenously. The cavity-generating lysis of the cell wall is a unique process that, most likely, takes place in the depth of the cell wall that is separated from the formatively active plasma membrane. Moreover, it affects only specific areas of the cell wall and results in smooth-walled cavities. Among the most interesting and challenging biological processes are the processes of plant development and morphogenesis. They often cannot be explained by simple physico-chemical regulation (Frey-Wyssling 1976). Stalactite development is certainly among such intriguing processes, perhaps the most intriguing one and it is worth of further investigations.

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