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Ultrastructural changes in *in vitro* callus plant cells producing secondary metabolites

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The objective of our research was to analyze the ultrastructure of the callus cells of *Leontopodium alpinum* (Edelweiss), *Fragaria x ananassa* (strawberry) and *Cotinus coggygria* (smokebush) producing large amounts of secondary metabolites.

Unlike plants, the *in vitro* callus has the peculiarity of proliferating (meristem-like) and at the same time synthesizing tissue-specific compounds with physiological significance in different stages of development. The cell population of the callus consists of two types of cells: those which divide and those which are producing the secondary metabolites, in different ratios, depending on the signals from the culture environment.

Our TEM observations showed that in the metabolic active callus cells a complex network of membrane-bound organelles are involved in the synthesis, sorting, transport, degradation, storing or exocytosis of secondary metabolites such as anthocyanin pigments, flavonoids, polyphenols, tannins, oils, polysaccharides, alkaloids, many of them with antioxidant activity and with potential biotechnological value.

Our results indicate that in the experimental conditions of the *in vitro* culture, callus cells have developed a complex endomembrane system that includes endoplasmic reticulum, Golgi apparatus, trans-Golgi network, multivesicular bodies, multilamellar bodies, membranous vesicles and vacuoles (vacuom) as an end point of the catabolic pathway.

In the biosynthetic active callus cells, the entire cellular machinery is subordinated to the production of secondary metabolites, evidencing phenomena of autophagy, through which different useful cellular components are recycled. Finally, the secondary metabolites are irreversibly stored in large amounts in the vacuoles, at the tonoplast level, some of them into the cytoplasm, into the modified plastids or on the external face of the cell wall.

In the intercellular space we noticed excreted vesicles, or most often, a reticulated extracellular matrix, probably involved in cell protection, recognition and integration.

Keywords: *callus plant cells, TEM, secondary metabolites.*

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Not applicable.

Conflicts of Interest

The authors declare no conflict of interest.

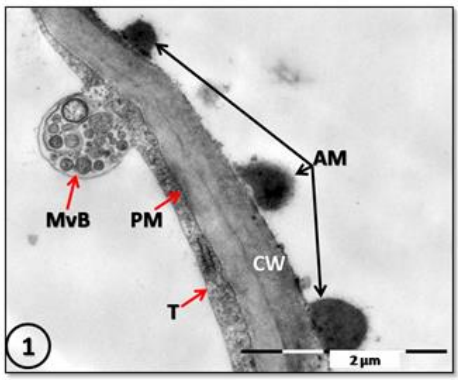

Figure 1. *Leontopodium alpinum* non-pigmented callus cells: multivesicular body (MvB) involved in the transport of secondary metabolites. Amorphous material (AM) excreted and stored at the external side of the cell wall (CW). PM = plasma membrane; T = tonoplast.

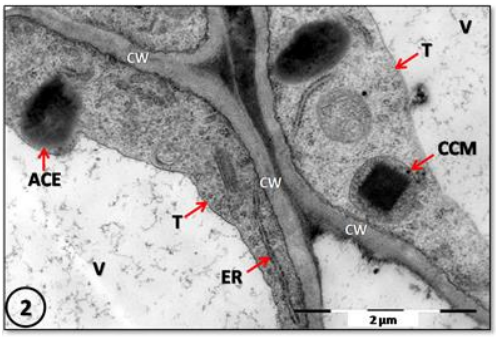

Figure 2. *Leontopodium alpinum* non-pigmented callus cells: secondary metabolites stored as fibrillar deposits in the vacuole, amorphous material in cytoplasmic endosome (ACE) and crystal containing microsome (CCM). Endoplasmic reticulum (ER) involved in biosynthesis process. CW = cell wall; V = vacuole; T = tonoplast.

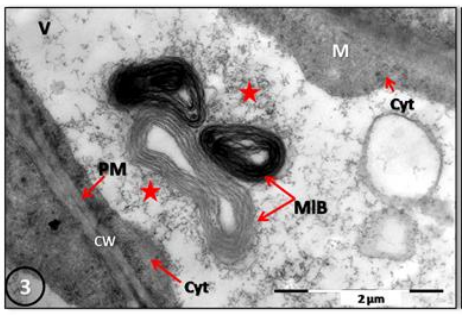

Figure 3. *Leontopodium alpinum* red pigmented callus cells: multilamellar bodies (MlB) into the vacuole (V) as a possible result of the autophagy process. Filamentous secondary metabolites (red stars) stored into the vacuole. CW = cell wall; PM = plasma membrane; M = mitochondria; Cyt = cytoplasm.

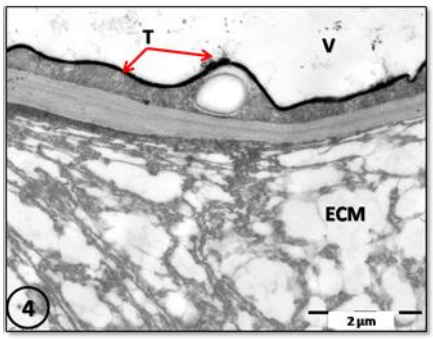

Figure 4. *Fragaria* × *ananassa* (strawberry) red pigmented callus cells: the deposition of the anthocyanin pigment at the tonoplast (T) level and in the vacuole (V); the surface secretion of the extracellular matrix (ECM) with fibrillar and spherical components, probably involved in cell protection, recognition and integration.