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ULTRASTRUCTURAL ASPECTS IN THE RHIZOME OF *DROSERA ROTUNDIFOLIA* L. VITROPLANTULES

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ABSTRACT. The transmission electronic microscopy studies made on the *Drosera rotundifolia* L. vitroplantules – at cell and tissue level – allowed us point out the presence of some tissue elements in the stem, specific for the reserve parenchyma, in the present case in the rhizome, situated in the basal zone of the foils rosettes. In the cortex tissue cells of this organ, we have remarked rich starch deposits, the leucoplasts being transformed in starch cells. At the same time, we observed a significant difference between the sections made through the *D. rotundifolia* green vitroplantules rhizome, of which vacuoles were missing stains (both in the epidermis and the external bark cells), meanwhile in the vitroplantules with foil rosettes, red, the cell vacuom of most cells presented corpuscle like formations, strongly electron dense, or the entire vacuole mass appeared dark coloured. Experiments made previously by us, with different categories of phytoinculs (but also following cell researches made on divers epidermis of coloured petals), to which tissues were contrasted with osmic acid and uranil acetate, in vacuoles with antocians (leuco- or stains), their content was corpusculated, alike the phenomenon produced in the *D. rotundifolia* vitroplantules rhizome cell vacuoles, of red colour. Antocians, in the *D. rotundifolia* vitroplantules, are present not only in the leaf cell vacuom, but in the rhizome cells.

Keywords: *D. rotundifolia* vitrocultures, rhizome ultra-structure, starch corpuscle, antocians

INTRODUCTION

In the ultra structural studies made by us, on *Drosera rotundifolia* L. vitroplantules organs (Turcuş *et al.*, 2008 a-d), we subscribe the studies made by part of the authors mentioned previously, at the level of the plantlet rhizome.

The rhizome is a stem region, interposed between the root basis and the aerial stem. At *D. rotundifolia*, the rhizome was studied for its histo-anatomical characteristics, by Stănescu (2008), in her PhD thesis.

It is interesting to notice that in the scientific literature, in descriptions of the *D. rotundifolia* plants, the rhizome was omitted by many authors (Nitschke, 1861; Darwin, 1875; Warming, 1872; Bruce, 1905; Tarnavschi, 1957; Ţopa, 1961; Ciobanu, 1967; Sălăgeanu and Péterfi, 1972; Ciocârlan, 2000, and others). Most of the authors, in the morphological analysis and the anatomical description of stem components of the *D. rotundifolia* plants, insisted only in describing the flower stalk.

On examining the ultra structural aspects, differentiated in the cells of different organs from the *D. rotundifolia* vitroplantlets, in sections made through the basal region of the roots, we observed the existence of some cells with high content of starch corpuscles. These aspects determined us to thoroughly check the bibliography related to *D. rotundifolia* plants morphology, and we discovered, in the paper of Kawiak and colab. (2003), the use of explants taken from rhizome (with a length of 10mm), when initiating vitrocultures of *D. angelica*, *D. binata* and *D. cuneifolia*.

We mention the fact that the plant vitrocultures grow on a sacharose rich media (20g/l). In consequence, in the heterotrophic metabolism of the *D. rotundifolia* vitrocultures the exceeding glucose molecules get polymerized in starch, which is massively deposited in the plastids stroma, which transforms in starch corpuscles, which accumulate in the reserve parenchyma of the rhizome.

In the present paper, we present electron microscope aspects observed in the rhizome tissue of *D. rotundifolia* vitroplantlets, respectively in the epidermis and stalk cells, the cortex consisting in reserve parenchyma.

MATERIAL AND METHODS

The analyzed biologic material, used for *D. rotundifolia* vitroplantlets rhizome cells analysis, consisted in fragments taken from the basal region of the roots, which – as specified in literature (Kawiak and colab., 2003 and Stănescu, 2008) – corresponds morpho-anatomically to a rhizome (Fig. 1). Above this region we observe the aerial aerial stem, which supports the leaf petioles, disposed in rosettes.

Rhizome fragments, taken from Drosera rotundifolia vitroplantlets were fixed in glutar aldehyde solution 2,7%, for one hour, and afterward tissue samples were post fixed in osmic acid 2% and consequently dehydrated in three successive acetone baths of increasing concentrations; finally, the tissues were included in Epon 812.

The blocs with tissue samples were sectioned in ultra microtome.

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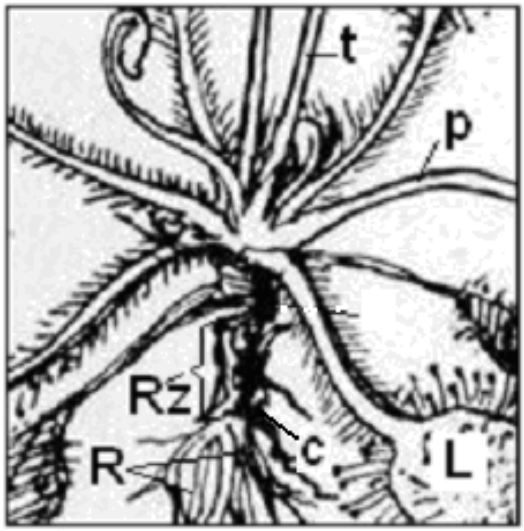

Fig. 1 The connecting region between root, gullet, rhizome and floral stalk, at Drosera rotundifolia L. plants (where: c – gullet, L – leaf lamina, t – floral stalk, p – leaf petiole, R – toots, Rz – rhizome)

The sections were contrasted with a solution of osmic acid and uranil acetate.

The compositions were examined with the transmission electron microscope Jeol JEM 1010. The most representative images can be observed in the 3-5 figures.

The D. rotundifolia vitroplantlets were drawn from cultures realized from propagul, consisting in mini rosettes regenerated from the same type of inoculs, vitrocultivated on mineral and organic Murashige-Skoog (1962) media, modified by us, with no glycine, with added vitamins – HCL thiamine, HCl pyridoxine and nicotinic acid – 0,1mg/l each (instead of 0.5mg/l in the original recipe), with 20ml/l sacharose (instead of 30g/l, quantity prescribed by the mentioned authors), the media missing 2,4-D or other growing regulators; the media solidification was made with 7g/l agar-agar, instead of 10g/l as mentioned in the original recipe; the media pH was adjusted at a value of 5,7, before boiling in autoclave.

After inoculation, the cultures were transferred to the growing chamber and illuminated with fluorescent light, white, with a light intensity of 1700 lux and a photo period of 16h/day. The temperature was 20-24OC.

In the present experiment we operated two variants of D. rotundifolia vitroplantlets: with tissue samples drawn from plants with green leaflets (fig. 2A) and with rhizome tissue, drawn from plantlets with red leaflets (fig. 2B); the last were drawn from vitrocultures realized from a previous subculture – on the described media, but which had in the substrate 2,5mg/l tidiazuron (TDZ). In our opinion, this synthesis cell quinine induces the red colour in the cell vacuole content of the D. rotundifolia vitrocultures. After Crowder and colab. (1990), the red colour is due to antocians.

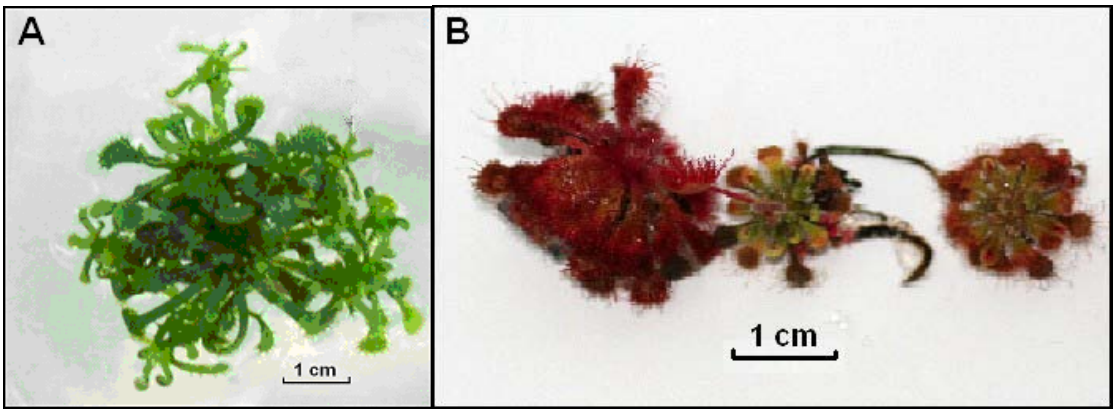

Fig. 2 Mini rosettes of Drosera rotundifolia L.: A – with green color leaflets and B – with red color leaflets, cultivated in similar conditions, but the B vitrocultures originate in a previous culture, made on Murashige-Skoog (1962) media with added 2,5 mg/l tidiazuron (TDZ)

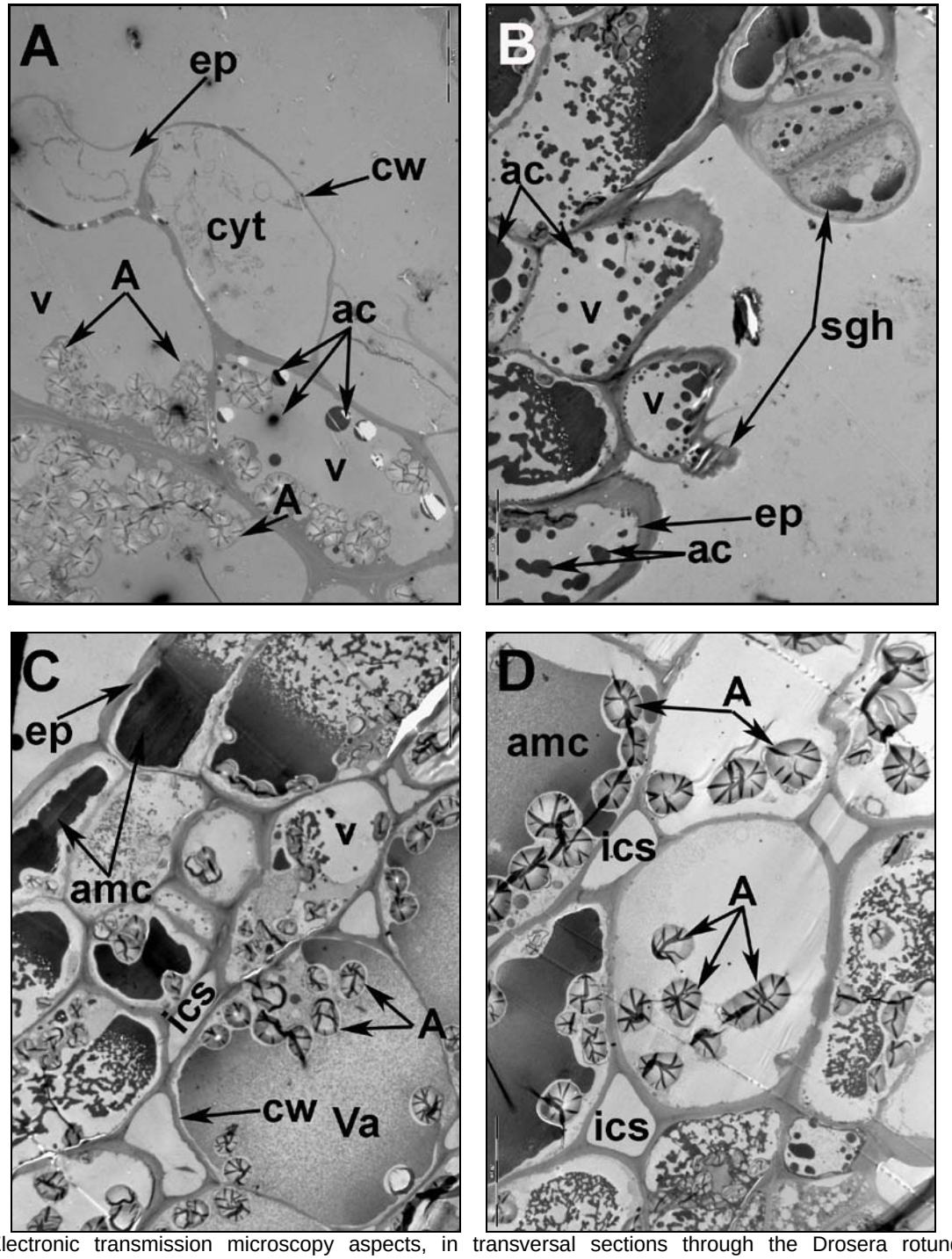

Fig. 4 Electronic transmission microscopy aspects, in transversal sections through the Drosera rotundifolia L. vitroplantules rhizome: A – epidermis and cortex cells aspect, in sections of Drosera rotundifolia L. green vitroplantules, and B-D – sections through rhizomes from red plants, which represent electron dense deposits (corpuscle like or massive), in the epidermal and cortex cells vacuoles of which plastids comprises starch (where: A – starch plastids; amc – antocians massive conglomerates; cyt – cytoplasm; ac – antocians corpuscles; cw – cell wall; ics – inter cell space; V – vacuole; Va – vacuole with antocians).

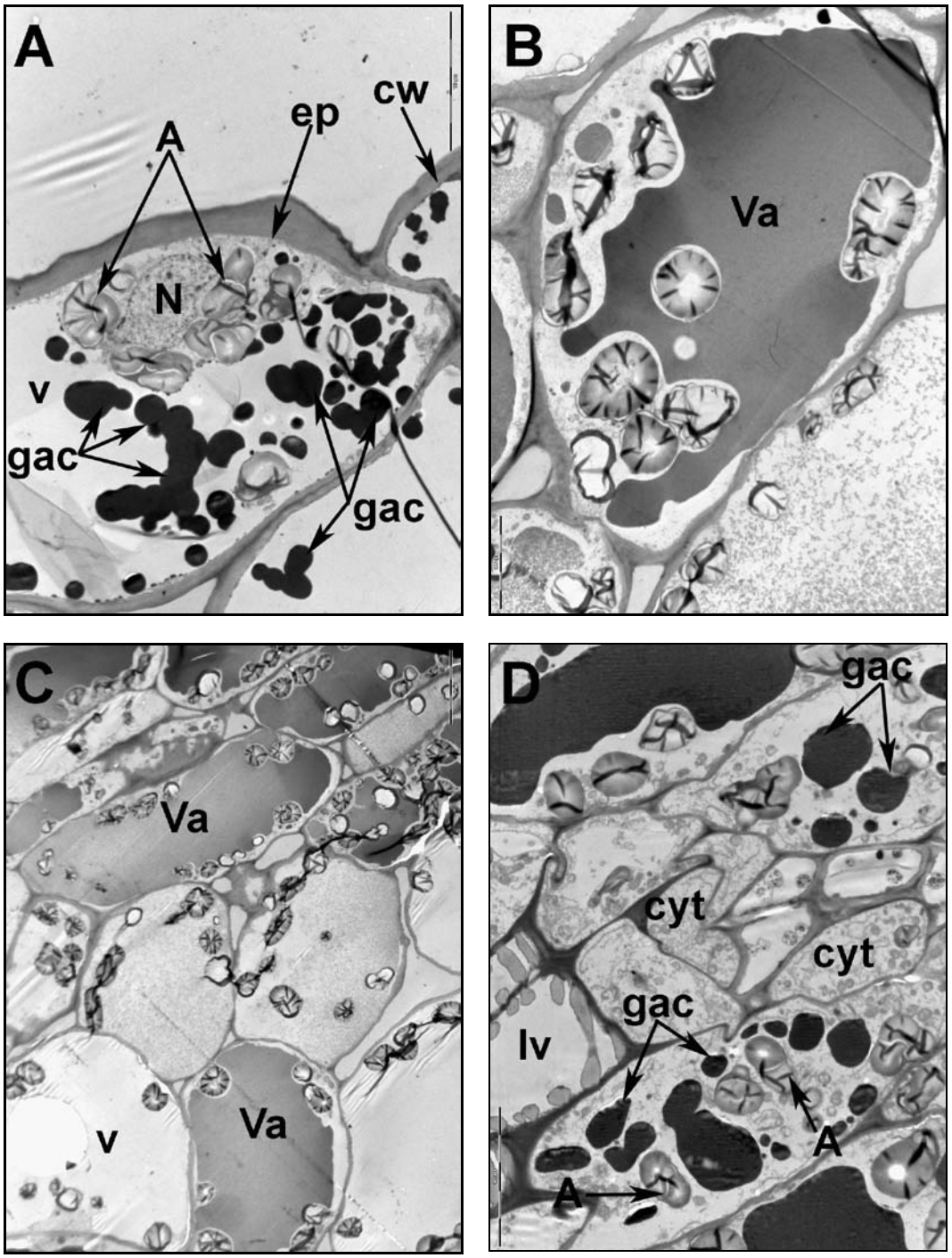

Fig. 5 Comparative aspects among cells from more or less red *Drosera rotundifolia* L. vitroplantules rhizomes; A – rhizome epidermal cells from red vitroplantules and B – from extremely red vitroplantules; C – cortex tissue visualized in sections through rhizomes from vitroplantules of very variable red color (where: A – starch plastids; cyt – cytoplasm; gac –granular antocians corpuscles; ep – epidermis; cw – cell wall; N – nucleus; V – vacuole with no antocians; Va – vacuole with antocians; lv – ligneous vessel).

RESULTS AND DISCUSSIONS

We know presently that to Drosera plants, growing in nature or in vitro, the red or reddish colour (even cyclamen), of the leaf and stem cells is due to the presence in the vacuole juice of antocians, organic plant stain, relatively common in plants world (Cachiţă şi Crăciun, 1990). But, in the scientific literature we found no reference regarding the dependence of the presence or absence of this plant pigment, in the vacuole juice of different Drosera cells (in our case D. rotundifolia), of different conditions in the environment, no matter if natural or in vitro.

The electron microscope images, made for transversal sections through D. rotundifolia vitroplantlets rhizome cells, red or green colour, are

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relevant for similarities or differences between the two types of plants.

From the 3 (A-D) figure and the 4 A figure we observe that the epidermis cells of the D. rotundifolia vitroplantlets rhizome miss the starch corpuscles; at the same time, these cells are very poor in figurative elements. On the other hand, the cortex cells of the D. rotundifolia vitroplantlets rhizome have in the cell plasma large deposits of starch, transforming the plastids in large starch corpuscles, which present a central hillum, prolonged and ramified. The cell plasma is pellicle like and the vacuoles are large, with no antocians; rarely, in some vacuoles the existence of sphere phosphor-lipid corpuscles was mentioned, with leuco-antocians absorbed in their structure, and which became electron dense in contact with the osmic acid (Cachiţă şi Crăciun, 1990).

In the figure 4 we can observe, in comparison, aspects surprised in sections through the D. rotundifolia vitroplantlets rhizome, green (fig. 4A) and red (fig. 4B-D). As results from figures 4B-C, both the vacuom of the red vitroplantlets epidermis and the vacuom of the bark parenchyma cells, present rich corpuscle formations (fig. 4B), either highly electron dense masses, mostly in the vacuole juice of the epidermis (4C), or corpuscle formations, very fine. On the other hand, the proof that the sections belong to the rhizome is made by the sessile secretive hairs, present at epidermis level (fig. 4B), and the existence of high starch deposits, transformed in starch corpuscles (fig. 4C-D), emphasized both in the cells with high content of antocians, and in cells in which the antocians have a ramified constitution, which tend to conglomerate and transform in a compact mass, strongly electron dense. Unlike the rhizomes without antocians, to which the epidermis has a senescent aspect (fig. 3A and B and fig. 4A), the rhizomes with antocians have cells with a more vital aspect, with vacuole integrity (proof that the bio-membranes – especially tonoplast – are integer). In these cells – in section sides – we easily observe the presence of starch corpuscles. In these cells (fig. 5A and B), the plastid starch deposition process is very advanced.

We remark also the fact that, as can be seen in figure 5 (C and D), in the deposition parenchyma present in the rhizome cortex a very heterogeneous population of parenchyma cells is present. Generally, at the bark periphery (in epidermis vicinity), the cells are longer and have a vacuole content very rich in antocians, which appear either corpuscle like or conglomerated, or as a uniform mass, more or less electron dense. No matte how deep we search in the bark (getting closer to the central cylinder), as much the vacuom is fragmenting (fig. 5D), but the starch corpuscles persist, and in some cells the formations which contain antocians are no more present in all the deposition parenchyma cells (fig. 5C).

CONCLUSIONS

Through our studies, for the first time in the scientific literature, we proved the presence of starch corpuscles in the *D. rotundifolia* vitroplantules rhizomes. At the same time, we pointed out the presence of antocians in the epidermis cells vacuom and in the cells of the external bark of rhizome, at red colour vitroplantlets.

At *D. rotundifolia* vitroplantules, we emphasized the presence of glandular sessile hairs, on the rhizome epidermis, situation which proves the fact that the organ belongs to the stem system.

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