THE ASPECTS REGARDING *CHRYSANTHEMUM* VITRO- AND EXVITROPLANTLETS ANATOMICAL STRUCTURE

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Summary: This study was consecrate to observe the histoanatomical structure of roots stems and leafs of *Chrysanthemum* vitroplantlets, in their vitroculture period and of exvitroplantlets, at 30 days from their septic medium transfer. The registered observations were compared with those realized at similar organs level of greenhouse plants (control lot). The noted differences between vitroplantlets anatomical structure and that greenhouse plants had, in special, ontogenetic bases.

Keywords: histoanatomy, vitroplantlets, acclimatization, *Chrysanthemum*

INTRODUCTION

The „in vitro" newly formed vegetative organs are histoanatomically and physically affected by special culture conditions (Cachiţă and co. 2004). During the acclimatization process of „in vitro" generated plantlets to natural conditions of life, they must undergo a period of functional adapting, in which the entire histoanatomical structure and ultrastructure of vitroplantlets suffer a gradual change of their morphophysiology in order to adapt easier to a septic medium.

Romano and Martins - Loução (2003) have developed a morphoanatomical and physiological study over the cork tree leaflets (*Quercus suber*), during the vitroculture and also during the exvitroplantlets acclimatization period. As a result of the researches made by them, it was concluded that the vitroleaflets had opened stomata and destroyed annex cells, while at the acclimatized plantlets the leaflets had stomata with a functional system for closing and opening them, their anatomic structure having large intercellular spaces and a lower density of mesophyllum cells, but having only one layer of palisade cells. The acclimatized plantlets leaflets had a mesophyllum with small intercellular spaces and a high density of cells, the palisade parenchyma being made up of two or three layers of cells. During the acclimatization, the thickness of the leaf had increased and also its compactness and cellular differentiation degree.

MATERIAL AND METHOD

The vitroplantlets provided by Chrysanthemum uninodal, apical minicuttings which was 120 days „in vitro" maintained on basal medium (BM) Murashige – Skoog (MS) (1962), changed by us (Vancea & Cachiţă 2001), without grown regulators; pH was adjusted to 5,7, prior autoclaving; the vitroculture was made in recipients with 2/7 cm dimensions, and after inoculation, they were incubated at irradiance with white fluorescent light with 1700 lx intensity and 16/24 h light photoperiod, at 23 ˚C ± 2˚C in the light period and 20˚C ± 2˚C in the darkness period. The transversal sections realized for regarding the vitroplantlets organs anatomical structure was made in the moment of their soil transfer, after Andrei and Paraschivoiu (2003) method.

The exvitroplantlets was growth in incubators for 30 days, in "Top soil" substratum, made in a biobase (from Stei City, Bihor County), soil resulted from a vital activity of worm cultures growth on vermicompost (Vancea and co., 2000), which was ready in incubator trays, with 5/22/35 cm dimensions (Vancea and Cachiţă 2001).

The illuminations and the temperature regime in growth medium of exvitrocultures were identical as the vitroplantlets had in the vitroculture period.

The greenhouse plants, which were considered the control lot, were provided by a culture made in septic medium.

For vegetative organ structure imagines presentation we made the manual picture of anatomical structure shown to microscope, for the best explications.

RESULTS AND DISCUSSIONS

a) The anatomic structure of adventitious roots (Fig. 1 A).

The radicular system of greenhouse chrysanthemum plants, also resulted from „in vitro" cultures and then transferred „ex vitro" – environment in which they have developed for two years – is derived not from embryonic rootlets, but through an adventitious way, the same as the ones of vitro- and exvitroplantlets.

The outline of the transversal section through the chrysanthemum plant roots, no matter the culture type ("in vitro", ex vitro" or greenhouse) was circular. At greenhouse plants and exvitrorootlets, the rhizodermis presented exfoliation phenomena, one could observe short and scarce absorbent hairs (Fig. 1 A), and at the vitrorootlets, the rhizodermis had a single layer, made up of parenchyma cells, tightly united between them and also presented numerously short unicellular absorbent hairs (Fig. 1 B). The cortical parenchyma, at greenhouse plants was made up of more spherical or oval cells, with their cellulose cellular walls, among which one could distinguish intercellular spaces, and at vitroplantlets this was better represented, compared with the same tissue from control root, but also of exvitroplantlets rootlets. The stem cortex presented, inside, endodermis, well distinguished, at the level of the three previously mentioned sections (Fig. 1 A). In all three radicular sections, made at greenhouse plants,

at exvitroplantlets, or at vitroplantlets, it was distinguished the presence of a exodermis made up of a single layer of cells, among which there was no intercellular space, with cellular walls infiltrated with suberin.

The central cylinder is placed in the median area of the transversal section and is limited, outside, by the pericycle, with thin cellulose walls cells; at greenhouse plants and at exvitroplantlets the central cylinder had characteristic elements for a secondary structure, the conductive tissue, in its greatest part, is of cambial origin. The central cylinder at vitroplantlets was not well organized, the primary conductive tissue of opened collateral type was placed in free phloem which alternated with xylem and represented the most characteristic part of the central cylinder.

The pith is placed centrally in the rootlets section and best represented at vitroplantlets (Fig. 1 B), comparatively to the greenhouse ones (Fig. 1 A).

The vascular cambium, present only at greenhouse plants and exvitroplantlets roots, was circular (Fig. 1 A) and generated two concentric rings, the phloem one thinner, and the xylem one, thicker, strongly lignified, with different diameter vessels, speeded in the fundamental mass of libryform, the root center being represented by a lignified pith parenchyma, compressed by the conductive tissue.

b) The anatomic structures of stem

At greenhouse plants, the median area of the main stalk had a mix structure (with elements of transition from primary structure to the secondary one), while the apical area presented a primary anatomic structure.

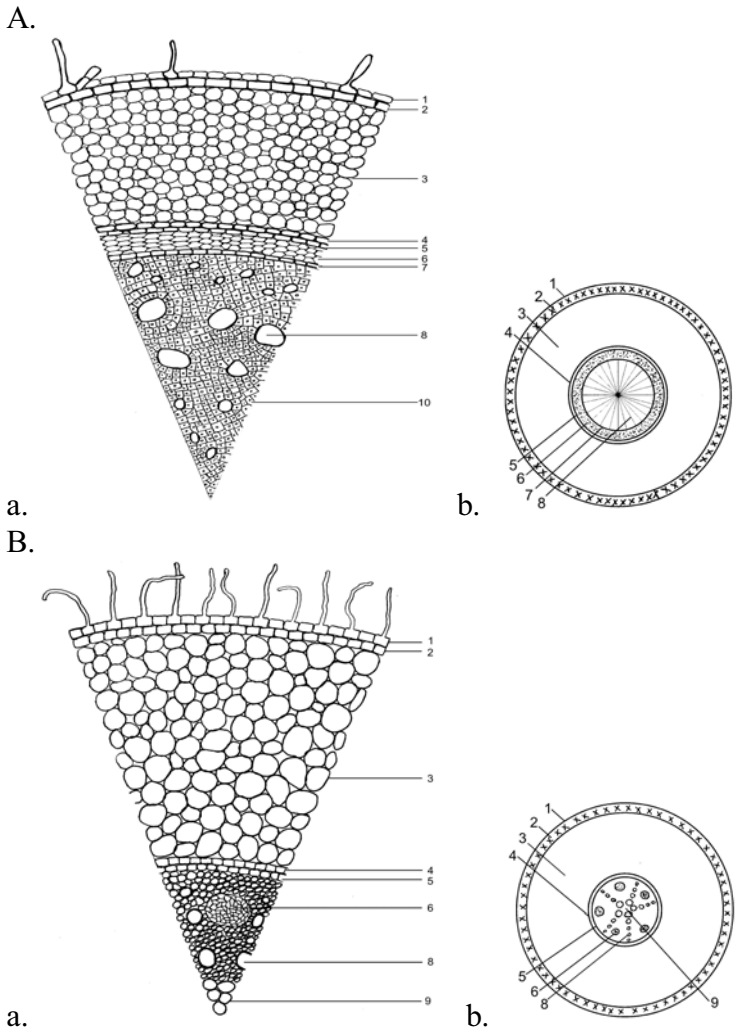

Figure 1. The schematically representations of chrysanthemum (*Chrysanthemum morifolium* Ramat var. *Lamet*) roots anatomical structure, cultivated in greenhouse (control lot) and "ex vitro" (after 30 days from their transfer in septic medium) (A), as well as those "in vitro" cultivated (B): a – detail, b - scheme (1 – rhizodermis, with absorbent hairs; 2 – exodermis; 3 – cortical parenchyma; 4 – endodermis; 5 – pericycle; 6 – phloem (primary in B and secondary in A); 7 – cambium; 8 – xylem (primary in B and secondary in A); 9 – pith; 10 - libryform).

The transversal sections made through the main stem of the control, vitro- and exvitroplantlets presented a circular outline, slightly corrugated.

The epidermis was with only one layer, with small cells, izodiametrical, tightly united together, with cutinized external cellular walls at greenhouse plants, with a beginning of cuticle at exvitroplantlets and without this layer at vitroplantlets, and without thectory hairs, at control plants (Fig. 2 A), but numerously at vitroplantlets (Fig. 2 B).

At greenhouse plants, but also at exvitroplantlets, the stem cortex was made up of oval cells, with the first 3 – 4 cell layers from the close neighborhood of collenchymased epidermis, being followed by many layers of parenchyma cells, and the last layer of the stem cortex, endodermis, not different from the anterior

one. As a result, the pericycle did not distinguish, as the first layer of cells from the central cylinder. The stem cortex of vitroplantlets had cellular walls, of collenchyma cells, thickened by cellulose, and the parenchyma cells are bigger, comparatively to ones of control.

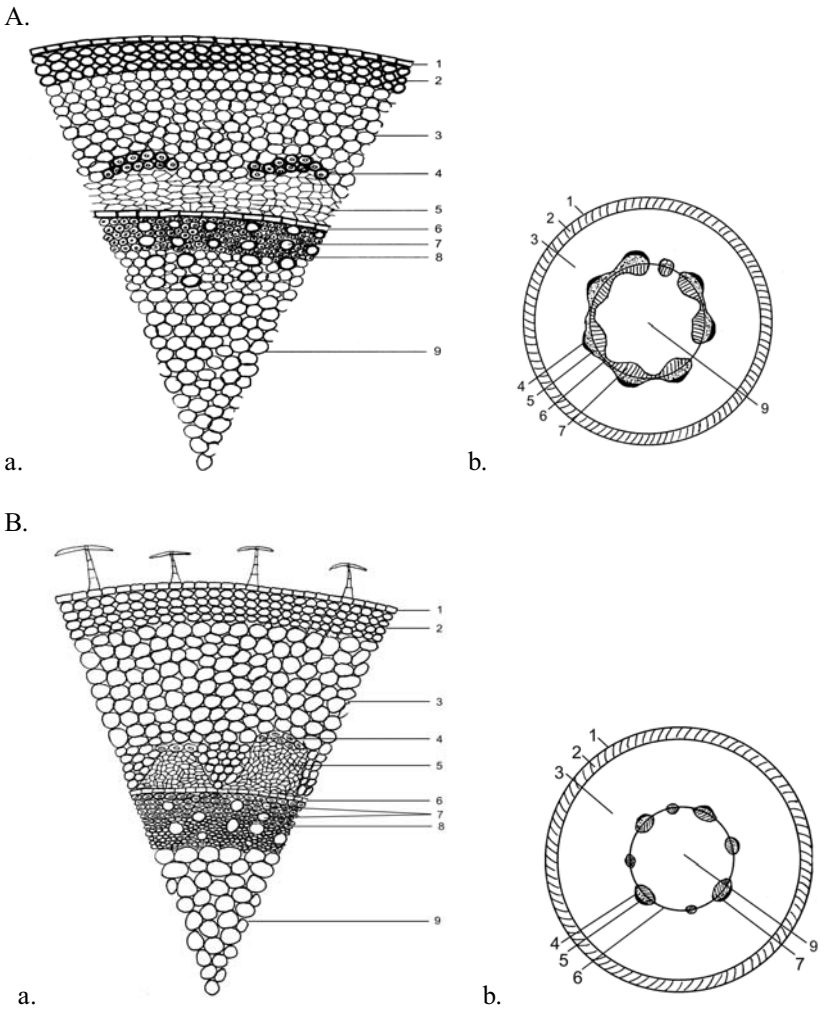

Figure 2. The schematically representations of chrysanthemum (*Chrysanthemum morifolium* Ramat var. *Lamet*) stems anatomical structure, cultivated in greenhouse (control lot) and "ex vitro" (after 30 days from their transfer in septic medium) (A), as well as those "in vitro" cultivated (B): a – detail, b - scheme (1 – epidermis, with or without thectory hairs); 2 – collenchymas; 3 –cortical parenchyma; 4 – scllerenchyma arcs; 5 –secondary phloem; 6 – cambium; 7 –secondary xylem; 8 – libryform; 9 – pith).

No matter the type of culture, at a radicular level, without endodermis and pericycle, the *central cylinder*, of ring type, started at the external limit of the vascular bundle, of opened collateral type, arranged on a single circle (of eustel type), as a consequence of vascular cambium functioning, that generated the secondary phloem towards the exterior and the secondary xylem towards the interior. At the exterior, the phloem was protected by scllerenchyma arcs. This vascular *cambium* ring was very well developed at greenhouse plants. The report among stem cortex and the central cylinder was of 1:2 (Fig. 2 A); the conductive tissue of vitroplantlets stem was weakly developed, compared with the one met at greenhouse plants, the report among stem cortex and central cylinder– at vitrostems - being of 1:1.

At greenhouse plants, the center of the stem was filled up by pith, made up of oval and round cells with thin cellulose cellular walls, met at vitroplantlets, but also quantitatively smaller at exvitroplantlets, and the component cells were bigger than the ones of the control.

c) The anatomic structure of leaf lamina

If in the transversal section through the control leaf lamina one can see that the median vein was very *prominent*, on the inferior side, abaxial surface, and on the superior side, adaxial surface – between the „wings" of the lamina – this one being slightly convex, at vitroplantlets leafs- gathered and structurally analyzed after *120 days* from inoculation of the mini seedlings – it presented a small delicate vein, without prominences.

The lamina, amfystomata (Petruş – Vancea and Cachiţă, 2004) have superior and inferior *epidermis*, in one layer, with izodiametrical cells, without cuticles in the case of vitroplantlets leafs. At the level of the two epidermis thectory hairs were present, in a „T" shape, but also secretor hayrs, approximately 10 times smaller than the thectory ones, also having a basal cell, a „leg" of the hair and a secretor cell in the superior side, the two components being tied through a link cell, like a "neck".

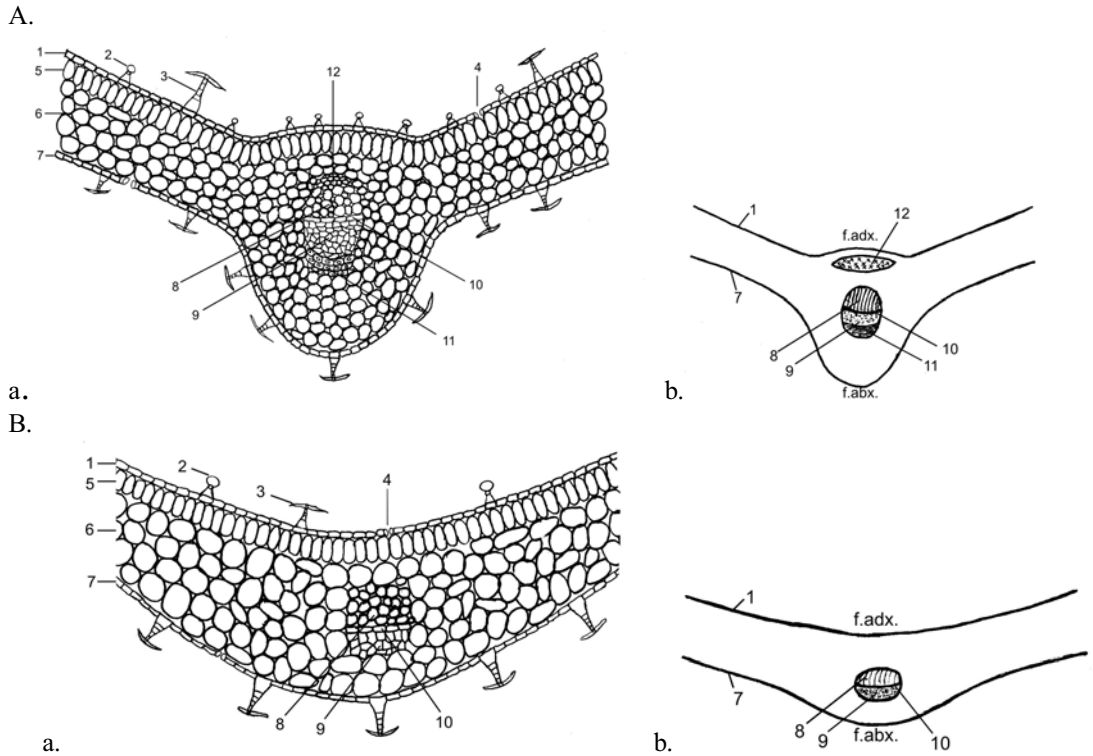

Figure 3. The schematically representations of chrysanthemum (*Chrysanthemum morifolium* Ramat var. *Lamet*) leafs anatomical structure, cultivated in greenhouse (control lot) and "ex vitro" (after 30 days from their transfer in septic medium) (A), as well as those "in vitro" cultivated (B): a-detail, b-scheme (1-superior epidermis; 2-secretor hair ; 3-thectory hair; 4-stomata; 5-palisade tissue; 6-spongy tissue; 7-inferior epidermis; 8-xylem; 9-phloem; 10-cambium; 11-scllerenchyma arcs; 12-collenchymas; f.adx.-adaxial surface; f.abx.-abaxial surface).

The mesophyllum, with a higher density of the greenhouse plants, was represented by a palisade tissue, set immediately under the superior epidermis, with one layer, rich in chloroplasts, but also of a spongy tissue, set from the inferior side of the lamina (bifacial structure), pluristratified, with irregular form cells, with less chloroplasts (Fig. 3 A). After Toma and Rugină (1998) the palisade cells dimension may vary, and the number of layers can be different in accordance with the degree of illumination of the organ, that is the reason why, at vitroplantlets, the mesophyllum was represented by a palisade tissue set in a single layer, maintained also at the level of the median vein, also by a spongy tissue, made up of a smaller number of layers, comparatively with the one from control leafs (taken from greenhouse plants). The intercellular spaces at spongy mesophyllum cells were bigger than the control ones and their density lower. As a result, during acclimatization, the thickness of the leaf increased, its compactness and cellular differentiation degree grew, fact also mentioned by Romano and Martins - Loução (2003) at the cork tree.

In all three lamina sections it was observed that, at the level of the main vein, the conductive tissue, made up of one simple collaterally vascular bundle opened – between xylem and free space is the cambium – with the primary xylem oriented towards the adaxial surface and with the primary phloem towards the abaxial surface. Above the xylem – because of the supplementary thickened with cellulose cellular walls – there is a mechanical tissue, a collenchymas, and over the phloem one could observe a scllerenchyma protective arch. The mechanical tissues, the collenchymas and scllerenchyma type were seen only at the greenhouse plants level and of exvitroplantlets, in a smaller proportion, on the superior side of the main vein, respectively on the inferior area of the vascular bundle from the main vein level (Fig. 3).

CONCLUSION

Histoanatomically, the vitroplantlets – in generally – presented a primary structure of rootlets, steamlets and leaflets, on the other hand, to exvitroplantlets and greenhouse plants was remarket the initiation of some secondary structure elements, those being into a more advanced ontogenetic faze.

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