ANATOMICAL STRUCTURE OF AFRICAN VIOLET (*Saintpaulia ionantha* L.) VITRO- AND EXVITROPLANTLETS

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Abstract. In this article we study the histo-anatomical structure of vegetative organs of African violet (Saintpaulia ionantha L.) vitro- and exvitroplantlets in comparison with similar aspects at the same organs of the greenhouse plants (control lot). The phytoinoculs vitroculture period was 120 days, the *ex vitro* acclimatization for the exvitroplantlets needed 30 days, and the greenhouse cultivar was 2 years old. Finally, we found that only rootlets of the vitroplantlets had a primary structure because at stemlets level has been identified the cambium presence still the vitroculture period. The cortical parenchyma cells at vitro- and exvitroplantlets was larger and less compact in comparison with those of control lot. Also, in the vitroplantlet rootles and stemlets the report cortex:central cylinder was much higher and vascular bundle was very poorly represented that at exvitroplantlets, but especially in comparison with these aspects in the plants grown in natural conditions. The spongy parenchyma at leaflets from *in vitro* culture was composed of fewer cell layers which was larger and less compact in comparison with those of exvitroplantlet leaf homologous layers and with the same layers from the leaf of greenhouse plants. At *in vitro* leaflets the peryphloemic protective mechanical tissue was at an early forming stage. However, we consider these differences as being due to the plants normal ontogenetic development.

Keywords: vitroculture, acclimatization, *Saintpaulia*, anatomical structure

INTRODUCTION

The *in vitro* regenerated plantlets have some histological changes, together with morphological, physiological and biochemical ones in comparison with plants grown in the natural life. These changes may be a direct consequence of the major and specific vitroculture conditions (especially high humidity, the culture medium rich in inorganic ions, vitamins and sugars, with the increased osmotic lever, constant atmosphere, without air currents and O_2 or CO_2, but rich in ethylene, with additional lighting, the heterotrophic nutrition, possible mixotrophic) [6]. Since these plantlets transferring in the natural conditions, they must pass through a functional adaptation stage were the histo-anatomical structure and ultrastructure of exvitroplantlets suffer gradual changes for successful support the septic regime.

At the moment of transferring into the septic medium an important role in exvitroplantlet survivals is vitroleaflet histo-anatomy, especially the epidermal cells and that mesophyll. Histological studies have shown that the plum and apple [2, 3] or birch [12] leaflets regenerated *in vitro* had a poorly developed palisade tissue in comparison with those grown in natural conditions. Moreover, there is a positive correlation between the mesophyll cells number decrease and changing the stomata structure and function [11, 15].

In present research we aimed to identify the hysto-anatomical changes of vegetative organs of *Saintpaulia* plants grown *in vitro* and their structural organizations occurring during the acclimatization of septic medium, compared with those grown in natural conditions of life, then to find new procedures to improve rate of survival in this final stage of micropropagations.

MATERIALS AND METHODS

The plant material consisted of vegetative organs (roots, stems and leafs) of some African violets **vitroplantlets**, which are at a vitroculture stage of 120 days on average mineral base (MB) Murashige - Skoog (MS) [5] modified by us, namely without glycine, with the addition of 1 mg/l vitamins (thiamine hydrochloride, pyridoxine HCl and nicotinic acid) instead of 0.1 mg/l and 0,5 mg/l according to original recipes, 100 mg/l meso-inositol, 20 g/l sucrose (instead of 30 g/l sucrose in the recommendations of the authors) and only 7 g/l agar, instead of 10 g/l in the original recipe, the culture medium was deprived of regulators growth, environmental pH was adjusted, prior autoclaving at value of 5.7, culture was performed in containers of 2/7 cm, placed on shelves lit by fluorescent light, white with a luminance of 1700 lux and a 16-h day length response light/24 h, in a heat treatment between $23^oC \pm 2^oC$, during light period and $20^oC \pm 2^oC$, in the dark period. Cross sections made to highlight the anatomy of vitroplantlets organs were made at the moment of transferring to soil; they had a beam waist of propagul 2.5-3.0 cm.

Exvitroplantlets were grown for 30 days in *Top soil* substrate, which was produced by frame cultures, in a biobase [14], distributed in specially incubators arranged by us for this activities [13]. Lighting and temperature regime in the growth medium exvitroculture was identical to that provided during vitroculture.

Plants from greenhouse - which served as control - came from a culture which was realised in septic medium, in the second year of life.

Anatomical structure of organs was studied on sections made by hand practiced trough fresh plant material with anatomical razor, in the transverse plane

and colored with 'Congo Red' and green iodine [1]. The location of section was at middle of roots or stem leght and the leafs was collected from plant middle. For each experimental variant were made and examined microscopically every 30 sections per sample. The representative images were photographed. I used the manual drawing of the anatomy observed under the microscope for their presentation as explicit. Because we haven't identified major changes between the vegetative organs structures of *ex vitro* cultivated plants and those of *in vivo*, for this we have made a single schematic representation of each one.

RESULTS

A. Issues relating to anatomical structure of control plant organs, harvested from greenhouse

a) *The anatomical structure of adventitious roots from greenhouse plants*

African violet under natural life conditions - taken as control in these experiments - were also generated *in vitro*, and then acclimatized to the septic medium. At the moment of the use they were in an advanced growth stage inside the greenhouse (2 years), reason why the rootlets of these plants were adventitious, not embryonic origin.

Transverse sections through the rough adventitious roots of African violets have had a circular shape, this rhizodermis presented some flaking phenomens and were identified rare piliferous sinks (Fig. 1A).

In cross section through the roots of the control plant could be seen the beginning of the secondary structure, marked by the presence of both lateral meristems, namely phelogen and cambium. The phelogen generated, to outwards, 4-5 cell layers with suberified cell walls, and to interior of this secondary meristem, the pheloderma was generated, consisting of a single layer with large cells, round and with cellulosic cellular walls.

The following cortex layers were represented by polygonal cells, compact, and the last layer - endodermis - was well differentiated.

Pericycle, as the first layer of the central cylinder, delimits the secondary phloem ring generated by cambium. This secondary meristem gave more secondary wood inwards, rather than outwards secondary phloem, secondary wood being composed of ordered strings of xylem vessels, separated by libryform (intensely lignified fibres, with thick walls). Liberian fascicle alternated with wooden fascicle, marking a poliarhe root type (Fig. 1).

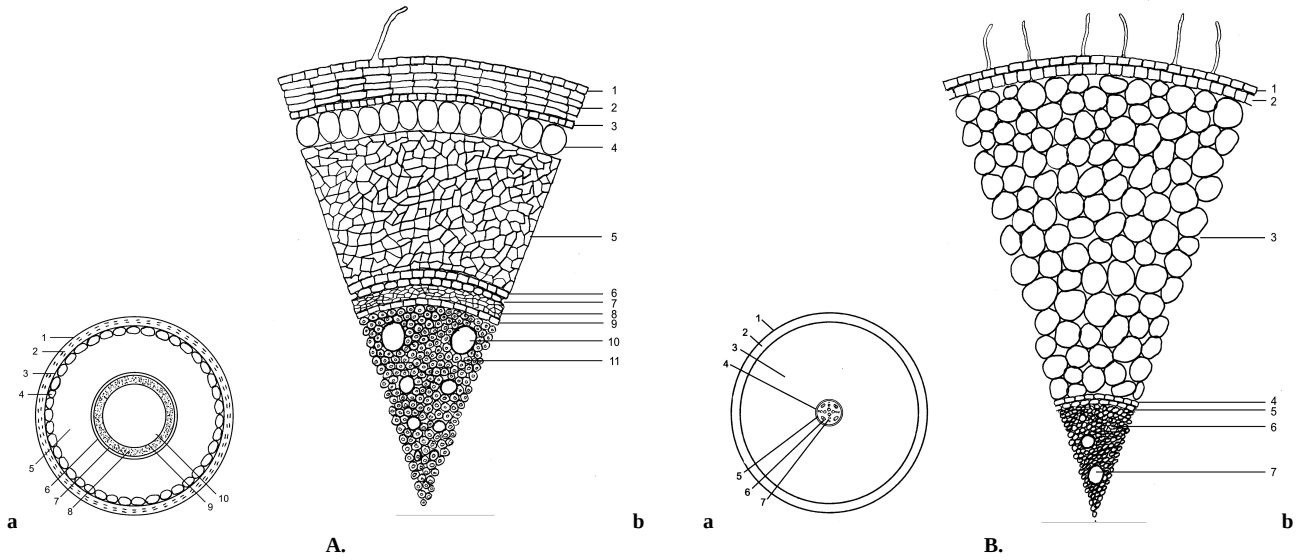

Figure 1. Schematically representation of African violets (*Saintpaulia ionantha* L.) plant root anatomical structures, which was cultivated in greenhouse (control) and "ex vitro", at *30 days* from their transfer in septic medium (A) and "in vitro" (B): a – scheme, b – detail (**A**: 1 – rhizodermis; 2 – suber; 3 – phelogen; 4 – pheloderma; 5 – cortical parenchyma; 6 – endodermis; 7 – pericycle; 8 –secondary phloem; 9 – cambium; 10 –secondary xylem; 11 – libryform; **B**: 1 – rhizodermis with piliferous sinks; 2 – exodermis; 3 –cortical parenchyma; 4 – endodermis; 5 – pericycle; 6 –primary phloem; 7 – primary xylem) (bars mean 100 μm).

b) *The stem anatomical structure of greenhouse plants*

In cross sections through African violet stem has been observed that it had a wavy shape (Fig. 2A).

The first cortex cell layers made a primary phellem, formed by simple impregnation of the cell walls with suberin. The following cortex layers were composed of round cells, which were intercellular space, representing a storage parenchyma of starch granules. The last cortex layer, namely endodermis, was well differentiated, as the first cell layers of central cylinder, pericycle (Fig. 2A).

In the central cylinder, vascular bundle – by collateral-open type - resulted from cambium function, which generated external secondary phloem and secondary xylem inwards. At the top of each liberian vessel were protective scllerenchyma arcs and between vascular bundles were reported scllerefied pithy rays.

Secondary xylem was composed from timber vessels ordered in parallel strings, surrounded by libryform. In the sections made by us, they could see the pericycle origins of adventitious roots (Fig. 2A). Medullar parenchyma has been well represented, the round cells of this being a veritable store of starch granules, substantially larger dimensions as comparatively with the amyloplasts from cortical parenchyma level. The ratio of cortex and central cylinder was 1:1.

c) *The foliar limb anatomical structures of greenhouse plants* (control)

In cross section made through of hypostomatice leafs of control African violet plants was observed that the median nervure is very much proeminent at the lower face (abaxiale) of foliar limb, but the adaxiale nervure part, between the two ”wings” of foliar limb, presented a concavity. Upper epidermis cells were large and rectangular; they are many tectory hairs, pluricellular, unbranched and longer, described by us [7], and secretor hairs, with a constitution identical to those present and *Chrysanthemums*. The leaves were pubescent.

Otherwise, be carried out sections through leafs from bottom propagul regions were observed three vascular bundles, while in the median leaf nervure harvested from the apical propagul area was revealed only a single vascular bundle, which signifies that the two beams was ulterior differentiated in plant ontogenetically development, the fact no identified at *Chrysanthemums*, where the leaf structures was not affected by leaf locations on the stem. After Deliu [4], the vascular bundle numbers can be different to the same species or even to the same individual.

Procambium generated primary xylem to the superior face of foliar limb and primary phloem to the inferior of this. Vascular bundles were protected by a collenchymas tissue following additional cellulose deposition at the cellular wall levels (Fig. 3A).

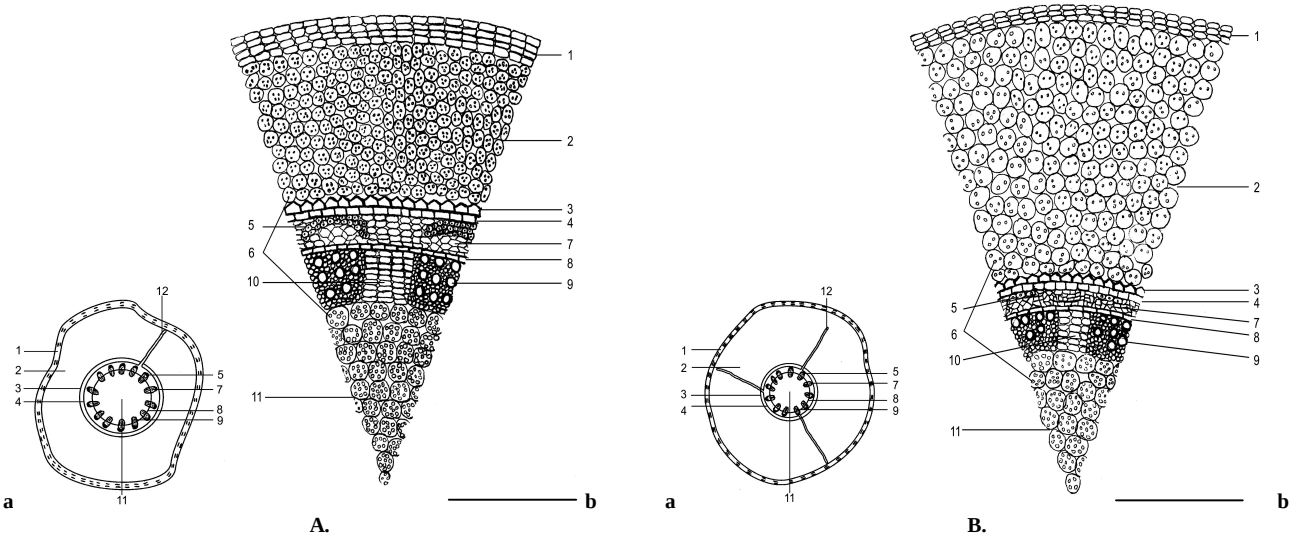

Figure 2. Schematically representation of African violets (*Saintpaulia ionantha* L.) plant stem anatomical structures, which was cultivated in greenhouse (control) and ”ex vitro”, at *30 days* from their transfer in septic medium (A) and ”in vitro” (B): a – scheme, b – detail (1 – suber; 2 – cortical parenchyma; 3 – endodermis; 4 – pericycle; 5 – scllerenchyma arcs; 6 – starch granules; 7 – secondary phloem; 8 – cambium; 9 – secondary xylem; 10 – scllerefied pithy rays; 11 – medullar parenchyma; 12 – adventitious roots) (bars mean 100 µm).

B. Issues regarding anatomical structure of vitroplantlet vegetative organs

a) The adventitious rootlet anatomical structures of vitroplantlets

At vitroplantlets, anatomical structure has been highlighted as being in a primary form. The cross-section contour was circular, rhizodermis with piliferous sinks, exodermis unistratified, the cortical parenchyma poorly organized, consisting of oval cells with thin and cellulosic walls, with intercellular spaces more larger than that seen in greenhouse plants, presented endodermis in interior, which has cells whose walls had no thickening (Fig. 1B).

Immediately below endodermis was present pericycle, which delimit a small central cylinder (the report cortex : central cylinder was 6:1), with vascular bundle poorly represented, and in central the extremely low medullar parenchyma (Fig. 1B).

b) The stemlet anatomical structures of vitro-plantlets

African violet vitroplantlet stemlet cross sections contours were circular, slightly wavy (Fig. 2B).

The primary phellem from exterior was thinner and the cortical parenchyma cells, oval and thin, being larger and less compact, with lower starch deposit, compared to that seen in plants grown in the greenhouse.

Under cortex was present endodermis unistratified, followed by pericycle (Fig. 2B), then the central cylinder composed of vascular bundles, with an intrafacicular cambium ring, between phloem and xylem, less represented that control group. Secondary meristem which was dedifferentiate in the central cylinder, namely cambium, appeared to *in vitro* plantlets too, in which case, the mechanic tissue was poorly represented, and the ratio between the cortex and central cylinder was 2:1. In place instead, to the central cylinder level, distinguish rays directed outwards, which marked the adventitious root neoforming to stemlet level. Certainly, adventitious roots neoforming was present in greenhouse plants, but the process was decreased as intensity, probability performance of sections which capture the process, in this case, being lower.

c) The foliar limb anatomical structures of vitro-plantlets

The foliar limb of vitroplantlet leaflets had the same bifacial dorsiventrale structure, as those of control plants. Instead, the foliar limb adaxiale face - at vitroleaflets - was almost flat, and at the abaxiale, the median nervure was least prominent, compared with

the control, whose protuberance were very evident. Epidermis, upper and lower, were - as in greenhouse plants - unistratified, with isodiametrice cells (Fig. 3B), but the walls of these cells had no cuticle, only many tectory and secretor hairs.

Foliar mesophyll had no changes compared to those described to greenhouse plants, except that spongy parenchyma was composed of fewer cell layers, and these were larger and less compact, the intercellular spaces was more evident, compared with the homologous layer of greenhouse plant leafs. Mechanical protective tissue from around the vascular bundles, from median nervure level was at an early formation stage and vascular bundles were three - generated by the cambium activity – in the foliar limb of leaflets harvested from bottom area of vitroplantlets and only one in those taken from the apical part of its.

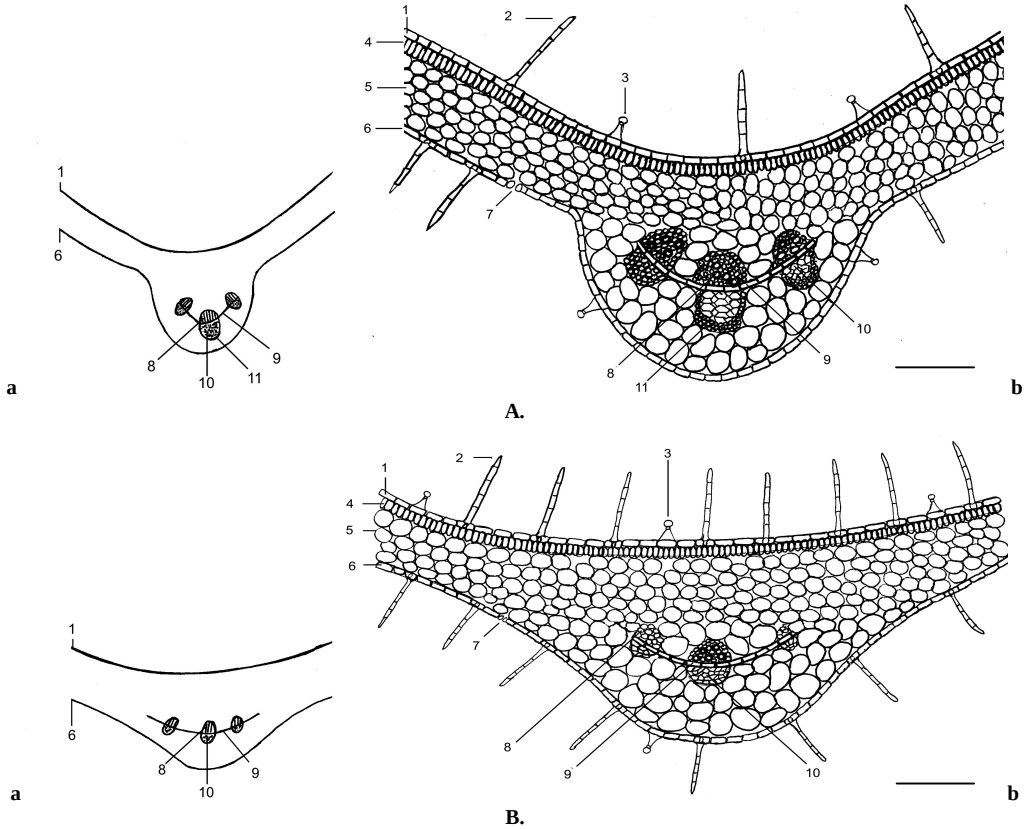

Figure 3. Schematically representation of African violets (*Saintpaulia ionantha* L.) plant foliar limb anatomical structures, which was cultivated in greenhouse (control) and "ex vitro", at *30 days* from their transfer in septic medium (A) and "in vitro" (B): a – scheme, b – detail (1 – upper epidermis; 2 – tectory hair; 3 – secretor hair; 4 – palisade tissue; 5 – spongy tissue; 6 – bottom epidermis; 7 – stomata; 8 – primary xylem; 9 – intrafascicle cambium; 10 – primary phloem; 11 – mechanic colenchymised tissue arcs) (bars mean 100 µm).

C. Issues regarding anatomical structure of exvitroplantlet vegetative organs

a) The adventitious root anatomical structures of exvitroplantlets

At exvitroplantlets, 30 days after transfer to soil, was observed a secondary structure in the early stage.

Cross-section contour was circular and rhizodermis has rare piliferous sinks (Fig 1A), being flaking.

The cortex tends to a compact structure like this identified in greenhouse plants and presented phelogen, which generated outwards, four cell layers, their cellular walls being bold, and inwards this secondary meristem forming pheloderma, consisting of a large and round cell layer, with primary cellulosic cellular walls. The following cortex layers were represented by polygonal cells, without intercellular spaces, and the last cortex layer, endodermis - well differentiated - protect central cylinder. Under endodermis was pericycle, which delimits secondary phloem generated from cambium. Central cylinder was less organized compared with those present in roots of greenhouse plants, vascular bundle representing the largest part of it. Medullar parenchyma of rootlets from *ex vitro* culture was poorly represented, being compressed by vascular bundle, like in greenhouse.

b) The stemlet anatomical structures of exvitroplantlets

At exvitroplantlets, after 30 days of acclimatization, the stem had the same structure as those plants cultivated in greenhouse, but in a different ontogenetically phase.

Cross-section contour of exvitroplantlet stemlets was circular and slightly wavy (Fig. 2A). The first two layers of cortex cells have a primary phellem, formed by simple impregnation of the cell walls with suberine; this was followed by cortex layers composed from round cells, representing a storage parenchyma of starch granules, less than the greenhouse plants. Endodermis, unistratified, continues with pericycle, under which was the central cylinder, composed of vascular bundles, arranged on a single circle. Unlike plants from the greenhouse, the intrafacicular cambium ring, between liberiane beams and wood beams was poorly developed, but better than the vitroplantlets.

Like the plants from greenhouse and vitroplantlets, in the exvitroplantlets internal structure could highlight neoforming adventitious roots, started from the layers of the central cylinder, more precisely from the pericycle. Central cylinder center was occupied by medular parenchyma, well represented and very rich in amyloplasts. Report cortex:central cylinder maintained 1:1, as greenhouse plants, by medullar parenchyma contributing (Fig. 2A).

c) Foliar limb anatomical structure of exvitro-plantlets

In cross section through leaflets from *ex vitro* culture was observed that the median nervure was proeminent on the bottom (abaxiale) face, but less intense than in the greenhouse plant leafs, and concave in the adaxiale face between the two "wings" of foliar limb. Both epidermis of foliar limb were composed of a single cell layer, showing both tectory and secretor hairs (Fig. 3A).

Foliar mesophyll was represented by a palisade tissue, unistratificat, with cell rich in chloroplasts and a spongy tissue which, during the acclimatization period, had increased number of layers and not presented differences significant compared to that observed in the leafs of plants grown in greenhouse (control).

At the median nervure level of leaflets provided from basal region of exvitroplantlets, and at leafs of vitroplantlets group were reported three vascular bundles, collaterally-open type, in a parenchyma well represented, unlike the one present in foliar limb of leaflets from *ex vitro*, located at the top of plant.

Similar situations were described after the some studies on the anatomical structure of vegetative organs of vitro- and exvitroplantlets, compared with those grown in greenhouse, made by us [9], at *Chrysanthemum*. The authors mentioned have concluded that at rootlets, stemlets and leaflets of *Chrysanthemum* grown *in vitro* were no revealed the structural abnormalities, but some ontogenetic differences, the vitroplantlets showing a primary structure of vegetative organs, while at exvitroplantlets level was distinguish some elements characteristic of secondary structure, a structure already in advanced at greenhouse plants.

DISCUSSIONS

Adventitious rootlets histo-anatomy of vitroplantlets revealed a primary structure with rhizodermis containing piliferous sinks, exodermis unistratified, the cortical parenchyma with round cells with intercellular spaces and very small central cylinder (report cortex : central cylinder was 6:1), vascular bundle very poorly represented. At exvitroplantlets, 30 days after transfer to soil, were the beginning of secondary structure (marking the transition from primary to the secondary anatomical structure) in the early stage, marked by the presence of both lateral meristems, namely phelogen and cambium. After similar researches [8] carried out on *Cymbidium hybridum* vitroplantlets, but also on exvitroplantlets (at 30 days after transferring into the septic medium) or on greenhouse plants (which are in an advanced stage of *ex vitro* growth), we reported that at roots of plants cultivated in greenhouse conditions have indicated a number of wooden beams 14 and all that liberians, while at rootlets provided from *in vitro* or *ex vitro*, the number was halved. This situation is explained by Toma and Rugină [16], but also Deliu [4], at plants grown under natural conditions, in that the vascular bundles number may vary in the same species or even the same individual, as influenced by mineral nutrition of the those plant organisms.

As regards stem structures of vitroplantlets, was signalled the presence of secondary meristem, dedifferentiate in the central cylinder, namely cambium, like at the greenhouse plants, the mechanical tissue was poorly represented and the report cortex and central cylinder was 2:1 (compared with the that of control which was 1:1), the primary phellem from exterior was thinner, compared to that present in the greenhouse plants or exvitroplantlets and cortical parenchyma cells have been more larger and more least compact, compared to the control. At exvitroplantlets, as at the greenhouse plants, the stem marked the beginning of dicotyledonous plants secondary structure. The most starck in parenchimatic cell of stem provided from *ex vitro* culture is normal. At *Saintpaulia ionantha* cv. 'Raving Red' shoot leafs regenerated from callus, stereological analysis of the histological events indicated that there was a progressive decrease in the starch content (percentage volume composition, number and volume of starch grains) of leaf segments which callused and later differentiated into shoots from the first day of culture to day 24 [10].

Foliar limb structures at the median nervure level, at vitroplantlet was revealed by several features, compared to that seen in plants grown under natural conditions, namely: the adaxiale face of foliar limb was almost flat, and at the abaxiale, median nervure was just little prominent, compared with the control, whose protuberance was well outlined; the spongy parenchyma was composed of fewer cell layers, this being more larger and more less compact, compared with those of homologous layer of greenhouse plant leafs; mechanical protector tissue, around the vascular bundles was an early stage of forming. At exvitroplantlets, the anatomical structure presents a borderland stage between the two aspects caught on vitroplantlets and to the greenhouse plants, in terms of spongy and mechanical tissue layer size. Observing the changes which are - during the *Aralia elata* and *Phellodendron amurense* exvitroplantlet acclimatizations - in the leaflet foliar limb structures, Yokota and his collaborators [17] concluded that the plantlet post-acclimatization survival percentages depends of the foliar mesophyll layer development, particularly the palisade cells and vascular connections established at this level, which are dependent on the *in vitro* rooting stage, reason for they recommended to try, since the vitroculture period, any solutions to determine the occurrence of some leaflet anatomy aspects, as close to the normal, and the selecting for micropropagation those most corresponding plants, in that regard. At *Calathea orbifolia* (Linden) Kennedy, the cultivation of plantlets

in temporary immersion system produced thicker leaf chlorenchyma and aquiferous parenchyma, lower stomatal frequency and more epicuticular wax than did those in semi-solid medium [18]. In the case of micropropagated highbush blueberry (*Vaccinium corymbosum* cv. 'Bluetta'), *in vitro*-developed leaf cells were circular and small, the spongy parenchyma was discontinuous and disorganized and formed by 1–2 layers of cells with large intercellular spaces and the palisade to spongy mesophyll thickness ratio was 1:1.5. After rooting *ex vitro*, the first leaves formed under natural conditions showed substantial changes in the anatomical characteristics. After 6 months, the plants produced leaves similar to those in field-grown plants [19]. At *Cymbidium* 'Joy Polis', the acclimatized plants presented morphological and anatomical structure similar to the mother plant (from greenhouse). The anatomical structure of *in vitro* plants did not affect plant survival during the acclimatization process, as this cultivar has great phenotypic plasticity [20]. On the wide side, at black mulberry (*Morus nigra* L.), at 7 day after *ex vitro* transfer, the highest was observed a proportion of woody area occupied by vessels. An important feature of developing woody tissue is the difference in patterns of vessel distribution from the characteristic differentiation patterns of earlywood and latewood vessels in mature wood of ring-porous trees [21].

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