# ROOTS AND LEAFS HYSTO-ANATOMY OF *Cymbidium hybridum* CULTIVATED „IN VITRO” AND „EX VITRO”

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**Abstract.** In this experiment we tried to found structural differences of Cymbidium hybridum roots and leaflets depending on culture conditions (“in vivo”, “in vitro” or “ex vitro”). At the end of the experiment we concluded that there are no structural differences between plants, except the normal ontogenetic changes. So, at the level of “in vivo” roots were observed many conductive fascicle, comparatively with “in vitro” or “ex vitro” roots, and at the “in vivo” and the “ex vitro” roots structure was identified the methaxylem, while, at the “in vivo” roots, this elements does not appear. The vitro- and exvitroleaflets mark a low chloroplast density, comparatively with control lots (“in vivo” cultivated leafs).

**Keywords:** *Cymbidium*, hysto-anatomy, vitroculture, exvitroculture

## INTRODUCTION

”In vitro” neoformat vegetative organs are physiologically and hysto-anatomically affected by the special conditions of culture [2, 3, 7, 8]. During the acclimatization process to natural conditions, the “in vitro” generated plants must have a functional adaptation process, the entire structure and ultrastructure must suffer a “maturation” change in order to “ex vitro” life regime support with successful [18].

Smith [18] analyzed “in vitro” neoformated roots comparatively with “ex vitro” roots (generated in sterile sand) at red maple (*Acer rubrum* Red Sunset) plantlets. “In vitro” rhysogenesis consisted in a more primary roots formation and less of the secondary roots, side of the “ex vitro” lots of plantlets. “In vitro” neoformated roots contained excessive cortical tissue, less xylem, fewer and very poorly developed absorbent hairs, with a poor resistance, against the “ex vitro” roots. After 8 - 10 weeks of exvitroculture, 50% of vitroroots - which had damaged the cortical and the vascular tissue - died. However, most anatomical differences between “ex vitro” roots and “in vitro” roots - who survived - had disappeared after 16 - 20 weeks of exvitroculture.

Fuernkranz and collaborators [11] have performed anatomical studies on *Prunus* roots formation initial stages, from the $4^{th}$ day after vitroplantlets were transferred to aseptical conditions, have shown that initial cell division take place in cambium and in phloem, causing a proliferation of tissue around the stem circumference. In this proliferating mass, many cells containing colors sealed inclusions, identified as fat, staining with Sudan II technique. Gradually, the fat inclusions disappear and were replaced with clear spaces, presumed to be vacuole. All sprout which was examined had visible functional links between the stem vascular system and the initial roots.

Vitroleaflets anatomy - especially the epidermal and mesophyllian cells structure - has most important implications when the vitroplantlets is transferring into the septic medium. A physiological and morfo-anatomical study on cork tree (*Quercus suber*) leaflets during vitroculture and “ex vitro” acclimatization period was made [18]. The authors reported that vitroleaflets stoma was open and guard cells destroyed, while those acclimatized plants have the closed stomata; their anatomical structure is characterized by intercellular large spaces and low quantity of foliar mesophyll, but with a differentiated palisade cells layer. Leafs of acclimatized plantlets shows a sunny leafs structure, with small intercellular spaces, high cells density and two or three palisade cells layers. During acclimatization process increased leaf thickness, and also its compaction and cell differentiation.

Epidermal and mesophyllian cells structure of strawberry vitroleaflets was improved by increasing the intensity of light at 250 μmol $m^{-2}.s^{-1}$ PPF and $CO_2$ concentration was increased to 3000 ppm, in the absence of sucrose in the medium, during “in vitro” rooting period [12], with a successful in their acclimatization process.

Also, by adding 2% polyethyleneglicol (PEG), in a culture medium to vine (*Vitis vinifera*) [9], shortened the required time for “ex vitro” acclimatization, because those which were treated with PEG vitroleaflets had a less mesophyll, a palisade layer better defined (with lower intercellular spaces) and a greater chloroplasts number in cells, compared with the vitroleaflets structure grown on medium without PEG. Therefore, the vine anatomy and physiology growth on the vitroculture medium with the PEG addition was similar to those grown in the greenhouse.

## MATERIALS AND METHODS

Culture medium was Murashige – Skoog (1962) based medium [13] adapted by Blidar and their collaborators for *Cymbidium* [4], without growth regulators and the conditions in growing room, were the following: 23 – 25 °C temperature, culture lighting has been assured with fluorescent tubes, issuing white light with 1700 lux intensity and 16/24h light/day photoperiod.

The exvitroculture was made in substratum consist in perlite, being maintained in same conditions like the vitroculture, anterior described.

After 12 months of vitroculture (when the stems had 2.0 - 2.5 cm waist), parts of the vitroplantlets were

sacrificed by carrying out cross-sections at the level of vegetative organs, and some were transferred to the septic medium, in special exvitroculture conditions (high humidity, supplementary illumination, without air currents) to be studied from the anatomical point of view, to 30 days after "ex vitro" transfer (the end of acclimatization period when they survive the greenhouse conditions without our support). As control lot were chosen plants from greenhouse, obtained by "in vitro" culture and adapted to septic medium, but at two years in „in vivo" regime (Table 1).

**Table 1.** Origin of studied plant material.

| Culture types | Sectioned vegetative organ types |
|---|---|
| „in vivo" – *control* (from greenhouse)<br>„in vitro" – in the moment of „ex vitro" transfer<br>„ex vitro" – in a 30$^{th}$ days from „ex vitro" transfer | Roots<br>Leaflets - apical<br>- basal |

Sections of fresh plant material were made manually, with shaving blades, in transversal plan [1]. Throughout the section technique, the plant material was wetted and sections were placed - by putting the blade - in tap water at laboratory temperature. The coloration was simple, made in Petri boxes, with one coloring namely ′Congo Red′, used for colouring cellular walls unlignified. Color was freshly prepared, as follows: 3 g of ′Congo Red′ were dissolved in 100 ml distilled water, to which was added 2 ml ammonia. The sections (kept in tap water) were maintained for 1 minute in colour, then washed and placed on the mount, in a drop of water and covered with a slide. Handling sections was done with great finesse, with a spatulate needle. Were made 30 sections per sample. Of these, after coloring was chosen the best 10 sections per sample, which were analyzed immediately using a microscope and the most representative images were photographed.

The section were examined in optical microscope Leitz brand, Webster M. Photographs were taken with a digital camera with a resolution of 640/480/300, with 10X and 40X objective and 10X ocular. Micrometric index was calculated using the Andrei and Paraschivoiu [1] method.

## RESULTS

Regarding roots hysto-anatomy, were not observed differences were observed depending on the type of culture ("in vivo", "in vitro" or "ex vitro").

At vitroplantlets, but also at exvitroplantlets, because of high humidity in the culture substrate, the roots colour was dark green, those that were submersion in the culture and in soil. Being a epiphyte species of orchids, roots anatomical structure of cymbidium present, on the outside, velamenum radicum, a multistratified rhyzodermis, consisting in 4 - 6 isodiametric cell layers, closely united among themselves (Fig. 1 B and C), with water absorb and storage role. At air roots and at greenhouse plant roots, the velamenum have a white – silver colour. The velamenum cell walls of greenhouse plants roots were thicker, compared to those of vitrorootlets and exvitrorootlets velamenum, in the absorbent hairs area (Fig. 1 B).

Regardless of culture type, perpendicular to velamenum radicum is exodermis cells (Fig. 1 B and C), arranged in a single layer of rectangular cells. At plants grown in greenhouse, exodermis was composed of cells with suberified cellular walls, clear highlighting cells of passage at this level, the situation unencountered to vitro- and exvitroplantlets. The cortical parenchyma presented round cells, smaller in exodermis and endodermis neighboring layers (witch delimited this) and higher in the middle of layer. The first cortical parenchyma layer contained chloroplasts. The endodermis and the inter cortical parenchyma layer was unistratified, with polygonal, rectangular, parallel with the central cylinder cells, closely attached to each other and without intercellular spaces, giving a round form to central cylinder.

Central cylinder - reduced in volume over cortical parenchyma - has (at external) a unistratified pericycle, whose cells were lower than those of endodermis, placed on a single circle, being arranged in alternate (xylem with phloem fascicule). At vitroplantlets are not differentiated methaxylem or methaphloem elements, but to the exvitroplantlets and greenhouse plants the methaxylem was well inside the central cylinder (Fig. 1 A). Methaphloem has not been observed.

Depending of nutrition conditions or ontogenetic phase, at "in vivo" roots we observed three times higher number of conductive vessel, comparatively with "in vitro" or "ex vitro" roots.

Medullar parenchyma was present, the methaxylem not replace it entirely, sending medullar ray among vessel (xylem and phloem), defining them.

*Cymbidium* leaf hysto-anatomy - relieved by this method - was not significantly affected by the plants culture conditions (Fig. 2).

Thus, the only differences between the structures of plant foliar limbs from different regime of life consist in: the presence of lower chloroplasts at foliar mesophyll level of vitro- and exvitroleaflets and, compared with the control chloroplasts content which have more intense green colour.

The superior and inferior epidermises presented small, rectangular cells, orientated parallel to foliar mesophyll (Fig. 2).

Foliar mesophyll has a homogeneous structure, being composed by isodiametrical cells, round, uneven size, with intercellular spaces. The cells from the intern part of foliar mesophyll were larger than those from the immediate vicinity of the two epidermises. However, under the two epidermises exists a layer which, from point to point, regularly, present islands of sclerenchyma.

Conductive fascicle, collateral-closed type, were arranged in a row, number of 8-9, forming nerves,

median nerves being the most prominent, the phloem was oriented to inferior epidermis, and the xylem to the superior epidermis, clearly defined by protective arcs of sclerenchyma.

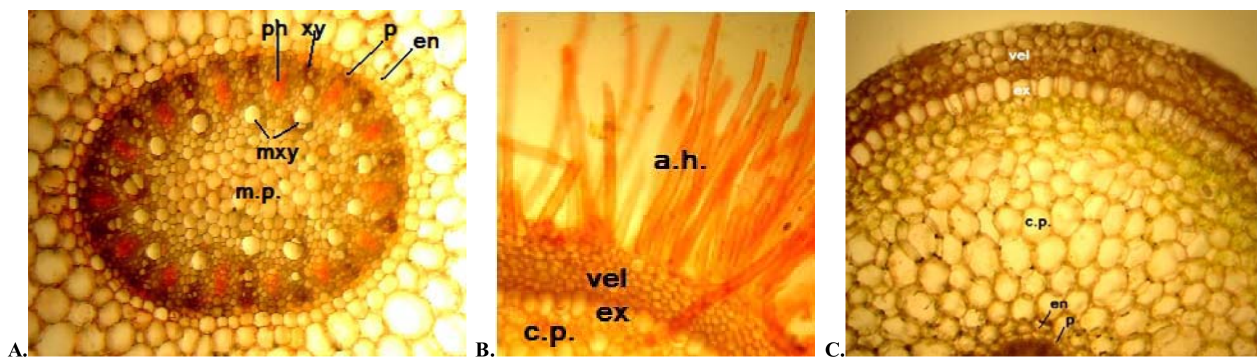


**Figure 1.** Hysto-anatomical aspects of *Cymbidium hybridum* roots which was „in vivo"(A), "in vitro" (B) or "ex vitro" (C) cultivated (a.h. – absorbed hairs; c.p. – cortical parenchyma; ex. – exodermis; en.– endodermis; ph. - phloem; m.p. – medullar parenchyma; mxy – methaxylem; p. – pericycle; vel. – velamenum radicum; xy. – xylem) (A, B - 100X; C - 400X).

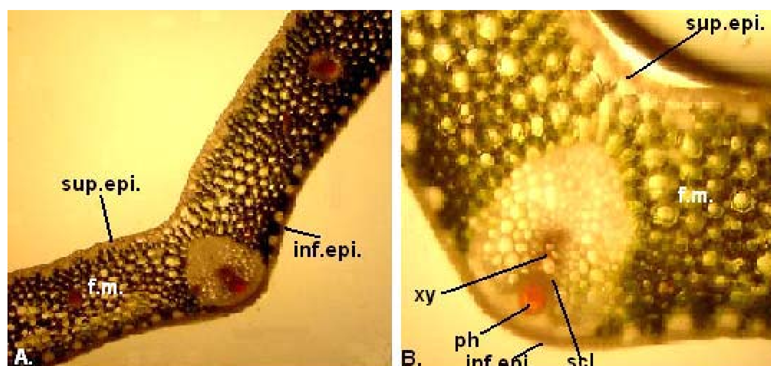


**Figure 2.** Hysto-anatomical aspects of *Cymbidium hybridum* foliar limb which was „in vivo"(A and B) cultivated (f.m. – foliar mesophyll; inf.epi. – inferior epidermis; nv. – nervure; ph - phloem; scl. – sclerenchyma; sup.epi. – superior epidermis; xy. – xylem) (A – 200X; B – 400X).

## DISCUSSIONS

At *Cymbidium hybridum* vitroplantlets, exvitroplantlets and greenhouse plants no observed significant differences between the three categories of cultures regarding the roots or leaves structure, except to the normal changes of ontogenetic order, situation which was found at *Chrysanthemums* and *Saintpaulia* cultivated "in vitro" and the end of the acclimatization to "ex vitro" conditions, comparatively with plants grown under natural conditions, in greenhouse, by Petruş and collaborators [15, 16].

Moreover, the at red maple (*Acer rubrum* Red Sunset) plantlets, [19] compared the anatomy of "in vitro" rootlets with that of similar organs but generated "ex vitro", in the substrate consisting in sterile sand, and author found that vitrorootlets - when vitroplantlets was transferring in septic medium - contains fewer hairs sinks and very thin, the cortex tissue excess and drive less weak. After 8 to 10 weeks in exvitroculture, 50% of vitrorootlets died, with cortical and vascular tissue destroyed. After 16 to 20 weeks, most anatomical differences that existed between "in vitro" and "ex vitro" rootlets - in plantlets who survived - had disappeared. Also, other authors [10, 14] sustain that in the moment of exvitroplantlets transferring in septic medium, the defects in the rootlets functioning are the main reasons for raising the mortality rate at the end of the acclimatization process, which was not revealed by us, at *Cymbidium*. Regarding leaflet hysto-anatomy, as well as in our research, the histological studies [5, 6] to plum and apple vitroleaflets, or those made by Smith and collaborators [20] to birch vitroplantlets, reported a much less developed palisade mesophyll, than that existing in the leaves of plants grown in natural conditions. On this issue, both Wardle and collaborators [21] and Reuther [17] found that there is a correlation between the decrease in the number of mesophyll cells and modifying the structure and function of stomatas.

Observing the foliar limb structure changes that occur during the *Aralia elata* and *Phellodendron amurense* exvitroplantlet acclimatization periods, [22] concluded that the post-acclimatization survival rate depended on the differentiation of mesophyll cells, especially those palisades and vascular connections established at this level. This latter process is dependent on the "in vitro" rooting degree, for this reason, the authors recommended to make efforts, already during the vitroculture period, to find and implement solutions to induce differentiation of anatomical structures of leaves, as close to state of normality, and selection for acclimatization just exvitroplantlets which is appropriate in this aspect.

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