HISTO-ANATOMICAL SPECIFIC FEATURES OF RED BEET AND SUGAR BEET VITROPLANTLETS

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Abstract

In this study we aimed to identify natural colour beetroot red (E 162) from the tissues of vegetative organs of red beet (Beta vulgaris var. Conditiva), and in vitro cultivated plant structure changes of this species, generated by the specific tissues cultures conditions, compared with those of sugar beet (Beta vulgaris var. Saccharifera). Besides root, beetroot red has been identified in red beet hypocotyl, including epidermal cells, to the fundamental parenchyma in the upper vascular bundles of petiole and in his epidermis, as well as to the level of a single cells layer around the vascular bundles of foliar limb. From a structural point of view, plants grown in natural conditions had no starch deposits in chloroplast stroma, as was noticed, especially in hypocotyls cells, from vitrocultures conditions, some of these cells having even a beginning of cellular degradation, a hyperhydricity specific issue, but also an entry into senescence of the organs.

Key words: *Beta vulgaris*, betaine, structure, vitroplantelts

INTRODUCTIONS

Red beet and sugar beet species, belonging to the *Beta* genus, *Chenopodiaceae* family, are cultivated mainly for roots, which - through processing - is obtained either beetroot red colorant or sugar as main product, both of them having an important role in food industry. The red beet contain all types of pigments from white to green (yellow, orange, red and purple), and specific phenotypes can be obtained only through sequences of specific induction, these cells can then be stabilized by cultivation on a maintenance medium (Girod and Zryd, 1991). Leathers and collaborators (1992) concluded that betalaine (betaxantine and betacianine) were found in throughout plant of *Beta vulgaris* L. var. Bikores. Beetroot red (E 162) contains significant amounts of betacianine (Leathers et al., 1992). In human body, betaines have lipotrophic and antioxidant effects (Jones and Storey, 1981). But this colorant is a normal food component and because of that the FAO / OMS considered that it is not necessary to set a daily dose (Directive 2008/128/CE, 2009).

In vitro cultures, especially cell cultures, produced in bioreactors, and "hairy roots" cultures are modern methods to obtain secondary products and are used in the beetroot red production, a natural colorant useful in the food industry. The biotechnologist and geneticists trying to identify specific requirements to such procedures and methods to improve the vitroculture medium and conditions (Jesudian and Bose, 1983, Reid et al., 1987), to find new varieties (Goldman, 1996), to optimize the color production, to increase quantity production with low-cost, but maintaining its quality for a long time, compliance rules of ecosanogenesis. There is an increasing need for betalaine quantitative concentration to improve these commercial applications as food colorant. Beets with high pigment concentrations make the preparation of natural beetroot red colorant much easier and less costly. To this line were produced seeds, plants, populations and hybrids of beet with high quality (Goldman, 1996). In this regard, Akita and collaborators (2002) added - to the

culture medium Linsmaier-Skoog (LS), of beet (*Beta vulgaris* L.) cell suspensions - high concentrations of iron and zinc studying the effect on betacianine production. Betacianine production was higher than that in any previous research.

Sucrose effects on the betacianine accumulation and increasing their concentration in *Phytolacca americana* L. cell suspensions was studied by Sakuta and collaborators (2006). Betacianine maximum accumulations were observed at 88mM and 175mM sucrose concentrations. This situation was caused because the cell size decreased as the initial sucrose concentration increased. Further studies using mannitol showed that sucrose itself caused an increase of cell number and cell size was affected by both sucrose concentration and water potential. Betacianine accumulations, per cell and per fresh weight, increase either with sugar concentration in culture medium increasing or water potential increasing.

Yamada and collaborators (2009) emphasized that glycinebetaine (a betaine) is synthesized in beet as response to abiotic stress by two-step choline oxidation stages, involving cholinmonooxygenize (CMO) and betaine-aldehyde-dehydrogenase (BADH). Significant amounts of betaine (higher than 20 micromol/GFW) are accumulated in young leaves of *Beta vulgaris*, even under normal growth conditions, while the old leaves, cotyledons, hypocotyls and roots betaine were low. Under the same conditions, CMO was accumulated exclusively in old leaves and difficult to detect in young leaves. By contrast, BADH levels were high in all tissues. In response to high salinity stress, betaine levels increased in all tissues, but more significantly in leaves

To many plants the pigment synthesis may be influenced by various factors as light, temperature, source of nutrients, enzymes, growth regulators and/or light quality (Boo et al., 2010). The authors have also studied the effect of light emitting diodes (LEDs) on the betalaine synthesis in hairy root cultures of red beet (*Beta vulgaris* L.) cultivated in a bioreactor of 1 litter, the ¼ MS liquid medium for 14 days. Hairy roots were grown in a growth chamber at a controlled temperature air 25 °C and a relative humidity of 70%. Lights were used varied nature, namely: blue (A), red (R) and mixed by A + R (AR), A + Far-red (Bfrs), Far-red and red (Rfar) with a flow photosynthetic photon (FFF) of 50 μmol $m^{-2}s^{-1}$. Hairy root growth illuminated with different light presented a dependence of biomass formation (fresh and dry weight) dependent by the quality of light. Sugar concentrations were higher in hairy roots grown under light R + A and A, and lower in those maintained in light of R and R + F. Betacianine concentration per fresh weight of hairy roots, after 14 days, was 4.2 times higher than treatment with Afr comparatively with R light treatment, while its concentration was not significantly different from A, AR and treatments with fluorescent lamps (Boo et al., 2010).

MATERIAL AND METHOD

Young seedlings of sugar beet (*Beta vulgaris* var. Saccharifera) and red beet (*Beta vulgaris* var. Conditiva), were obtained from seeds germinated in septic medium, in peat substrate type, placed in pots and *in vitro,* on Murashige-Skoog culture medium (1962) ½, with thiamine HCl, pyridoxine HCl and nicotinic acid, each 0.1 mg/l, without glycine, with 20 g/l sucrose, whit 7 g/l agar-agar, without grown regulators and with 5.5 pH. When seedlings and *in vitro* plantlets had 60 days, from the roots, hypocotyls and leaves were made sections, which was applied manually to highlight the natural colours, or were included in EPONE 812 (Hayat, 2002) and the blocks with samples were cut with a Leica UC6 ultramicrotome, to structural details observations. The natural colorant identifying in hypocotyls epidermis was highlighted by skinning technique (Andrei and Paraschivoiu, 2003). Only sections manually applied through sugar beet organs were colored with iodine green and ´Congo` red. The best 10 sections per sample were chosen. These were

immediately analyzed using an optical microscope Leitz brand, Webster M and the most representative images were taken with an adapted digital camera.

RESULTS AND DISCUSSIONS

To emphasize the natural beetroot red dye, and certain particularities in the structure of red beet hypocotyls we made transverse sections of vegetative organs of the plant by hand and we presented the same issues compared to sugar beet, highlighting differences emerged, either from plants grown natural conditions or to those from vitroculture, depending on purpose and importance of the results.

a. Identification of beetroot red colorant

Roots structure of red beet and sugar beet

In the case of roots, at red beet, the cell vacuoles of cortical parenchyma, but also the pith, are coloured in a deep red (Fig.1). Rhizodermis and conductive vessel are not coloured. Towards sugar beet, red beet roots do not contain starch.

There is a secondary structure of roots, which contain concentric circle consisting of conductive tissues, penetrated by parenchyma as wider or narrower rays, formed from cells with cellulose walls (Fig. 1A). The pith is smaller, specifically to roots (Toma and Rugină, 1998), and the root structure is likewise the stem, because the secondary formations are comparable.

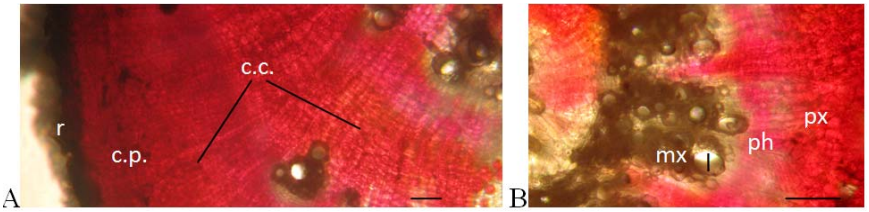

Fig. 1. Histo-anatomical aspects of red beet (*Beta vulgaris* var. Conditiva) root tip, cultivated *in vitro,* at 60 days from culture initiation from seeds (c – cambium; c.c. – concentric circle; l – lumen of xylem vessel; r – rhizodermis; c.p. –cortical parenchyma; ph – phloem; px – protoxylem; mx – metaxylem) (bars means 50 μm).

Like to the red beet, the sugar beet roots showed in the cross section well represented xylem vessel, revealing to the root center (to pith) a protoxylem, outwards associated with a metaxylem. Under the phloem is the cambium, provided from the pith rays, pericycle or pith. Most of the root thickness due to this cambium, which produces more secondary xylem than secondary phloem, because this cambium from the phloem produces more secondary xylem on its internal face. This xylem is oriented outwards (because inwards is arranged the primary metaxylem).

Root structure of the *Beta* genus is similar to the stem because of certain structural anomalies (Toma and Rugina, 1998), namely the formation of supernumerary cambium, condition when, normal cambium early ceases activity, after the normal secondary structure formation. At this time it appears, in pericyclic position, a supernumerary cambium, which produces fundamental parenchyma, in which vascular bundle are organized, arranged in a circle, like the stem structure. After a while, this cambium cease his function, another cambium being formed (from fundamental parenchyma), which works just like the first one, and the process continues. Thus, is forming to the beet, during the same growing season, 6-8 supernumerary cambium zones, resulting in as many vascular bundle rings, separated both radial and tangential by fundamental cellulosic parenchyma, composed of cells rich in sucrose, at sugar beet (Fig. 2). Red beet has several supernumerary cambium compared with sugar beet, but less emphasized because the beetroot colorant.

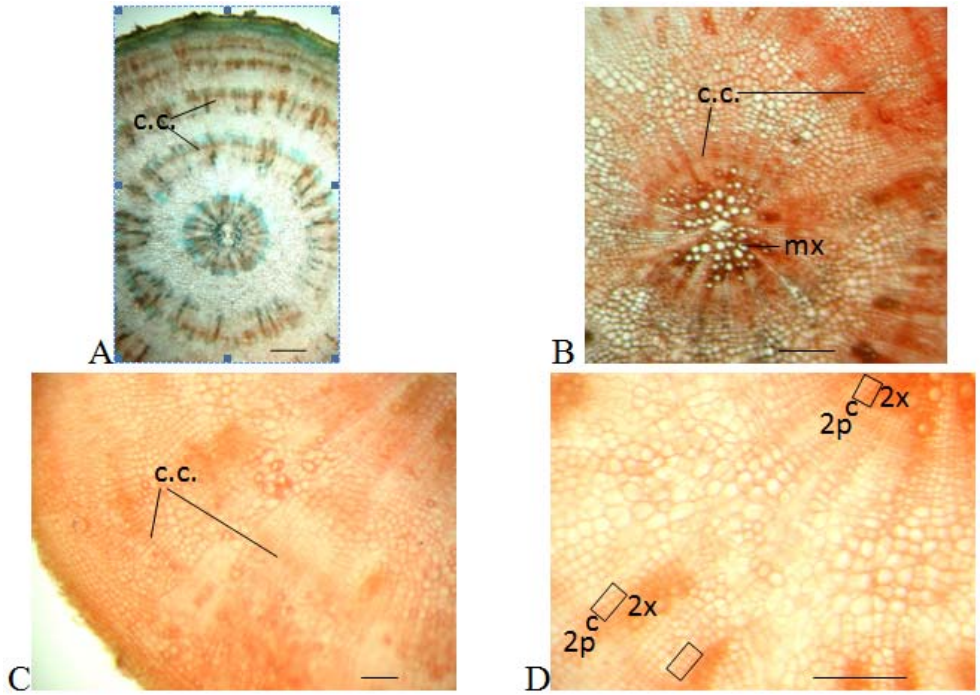

Fig. 2. Histo-anatomical aspects of sugar beet (*Beta vulgaris* var. Saccharifera) roots cultivated *in vivo*, at 60 days from culture initiation from seeds (2p – secondary phloem; 2X – secondary xylem; c – cambium; c.c. –concentric circle; mx - metaxylem) (in the microphotographs of the rectangles are highlighted supernumerary cambia) (bars means 100 µm).

Beet vascular bundles are collateral type, the phloem is located in the back. Phloem comes from procambium. Between two primary tissues persists meristematic tissues namely cambium, from which secondary xylem and phloem are forming.

Hypocotyl aspects of red beet

Generally, in our studies, identification of natural colorant localization in plant tissues encountered difficulties because of the ease with which it leaves cell vacuoles, while achieving biological preparations. This was much more intensely observed in hypocotyls. In red beet hypocotyls, the colorant was highlighted, including skin, by skinning technique (Fig. 2).

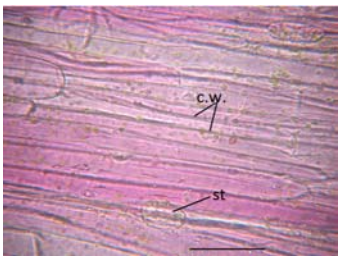

Fig. 3. Epidermis of red beet (*Beta vulgaris* var. Conditiva) hypocotyls with cells natural coloured with betaine (c.w. – cell wall; st - stomata) (bars means 50 µm).

Petiole structure of red beet and sugar beet

Both red beet and sugar beet, in cross-section, the petiole are a semi-circular contour, with an adaxial ditch, with latero-adaxial wings. In the cellulosic fundamental parenchyma are stuck vascular bundles, located on an arc, with mechanical elements on the phloem periphery.

To red beet, from anatomical point of view, the petiole has a natural coloration in the fundamental parenchyma cells arranged upstairs of vascular bundles, and in epidermis cells (Fig. 4).

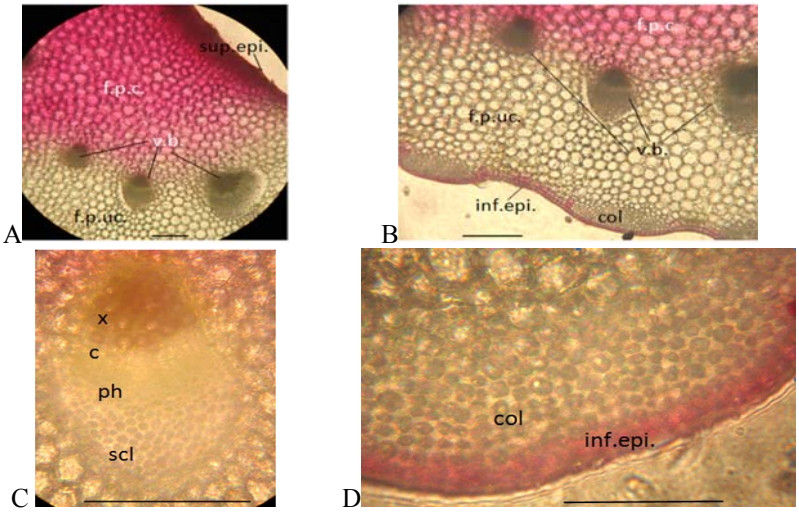

Fig. 4. Histo-anatomical aspects of red beet (*Beta vulgaris* var. Conditiva) petiole, cultivated *in vivo*, at 60 days from culture initiation from seeds (c – cambium; col – colenchyma; f.p.c. – fundamental parenchyma colored; f.p.uc. – fundamental parenchyma uncolored; inf.epi. – inferior epidermis; ph – phloem; scl – sclerenchyma arcs; sup.epi. – superior epidermis; v.b. – vascular bundle; x –xylem) (bars means 100 µm).

To sugar beet, the petiole - at this stage of plant development - has 3-4 mature vascular bundles and 3-6 young, one side of the petiole center, to the two future wings of leaf. In figure 4 C is presented a vascular bundle separation. Periphloemic mechanical tissue is sclerenchyma (sclerenchyma springs) very well developed in the petiole. Sclerenchyma is the last tissue which is separated, after the xylem, the cambium and phloem are already separated. At the petiole level, except natural colouring of red beet fundamental parenchyma (Fig. 4) and its smaller cells than in sugar beet (Fig. 5) there are no significant differences from histo-anatomical point of view.

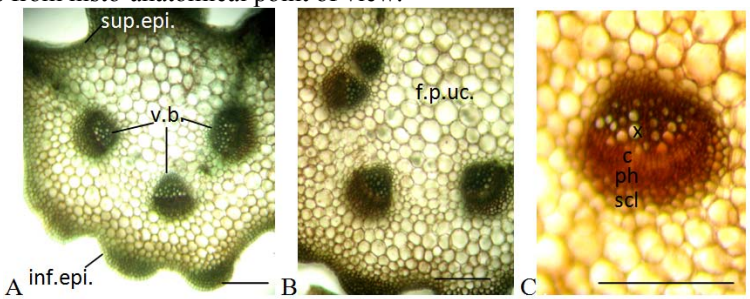

Fig. 5. Histo-anatomical aspects of sugar beet (*Beta vulgaris* var. Saccharifera) petiole, cultivated *in vivo*, at 60 days from culture initiation from seeds (c – cambium; col –

colenchyma; f.p.uc. – fundamental parenchyma uncolored; inf.epi. – inferior epidermis; ph – phloem; scl – sclerenchyma arcs; sup.epi. – superior epidermis; v.b. – vascular bundle; x –xylem) (bars means 100 μm).

Foliar limb structure of red beet and sugar beet

Foliar limb of red beet leaves have a natural color at the nervure level, specifically of vacuoles of a single cell layer that ”wrap” the vascular bundle (Fig. 6 A and B) and a few isolated cells from fundamental parenchyma of principal nervure, but not at the epidermal layer. After Sepulveda-Jimenez and collaborators (2004), red beet accumulate betacianine, in specially betanine, in storage roots, and in leaves, the betacianines synthesis is induced by damage or infiltration of virulent *Pseudomonas syringae* pv. Tabaci or *Agrobacterium tumefaciens*. In our case, the colouring is natural.

To the figure 6 A and B can highlight the work of supernumerary cambium, similar to those mentioned in the root, by the appearance of phloem which surrounds the xylem at the rib level. By this way, the xylem appears muffled in phloem.

To sugar beet, besides beetroot red colorant lack, were not found significant changes in leaf structure (Fig. 6 C and D). Highlighted in figure 6 C is a homogeneous foliar mesophyll of leaf and how is separated the two vascular bundles in foliar limb (Fig. 6 D), the mechanical tissue sclerenchyma spring type is the last tissue that separates.

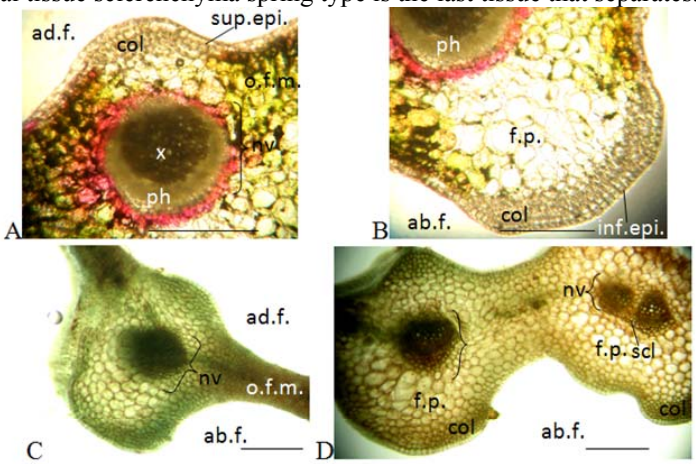

Fig. 6. Histo-anatomical aspects of red beet (*Beta vulgaris* var. Conditiva) (A and B) and sugar beet (*Beta vulgaris* var. Saccharifera) (C and D) foliar limb, cultivated *in vivo*, at 60 days from culture initiation from seeds (ab.f. – abaxial face; ad.f. – adaxial face; col – colenchyma; f.p. – fundamental parenchyma; inf.epi. – inferior epidermis; nv – nervure; o.f.m. – homogeny foliar mesophyll; ph – phloem; scl – sclerenchyma arcs; sup.epi. – superior epidermis; x –xylem) (bars means 100 μm).

b. Structural changes of red beet plant organs, *in vitro* cultivated

Because, in vitrocultures conditions, had appeared some morphological changes at red beet ***hypocotyls*** level, namely blowing it and appearance of unpigmented swelling, we decided to study the structure of this vegetative organ, by advanced optical microscopy analyze.

Changes in structure of hypocotyl

In the images from Figure 7, can be seen cells belonging hypocotyls, in a state of cellular degradation. These cytological issues are specific to hyperhydricity phenomenon, described in sugar beet by the Cachi□ă and collaborators (2008 a and b), but the same cellular changes may occur in case of early degeneration, in involution of a plant organ (for example in the case of stem which pith is distorted, such as dandelion, or cereals). Given

that in our case is studied seedlings obtained from seeds germinated in aseptic conditions and hyperhydricity is a tumour manifests especially in subculture, and to the leaflets (an organ which greatly suffering from hyperhydricity) was not observed tumour phenomenon, no phenotypic or structural, we tend to consider that state of degradation is a physiological, entry into senescence.

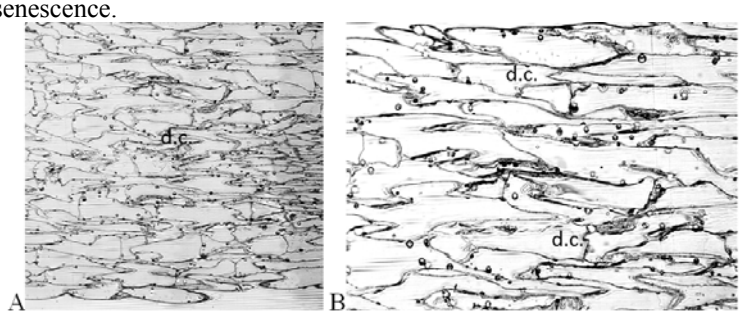

Fig. 7. The structure of red beet (*Beta vulgaris* var. Conditiva) hypocotyls, from *in vitro*, at 60 days from culture initiation from seeds (d.c. – disorganized cell), (A and B - 20X).

Changes in structure of foliar limb

To reveal structural foliar limb details of red beet leafs, without the possibility to distinguished any natural colored tissue, we can study the images in figure 8-9. Foliar limb structure and ultrastructure of sugar beet have been studied by us (Cachi□ă et al., 2008 a and b) in previous research.

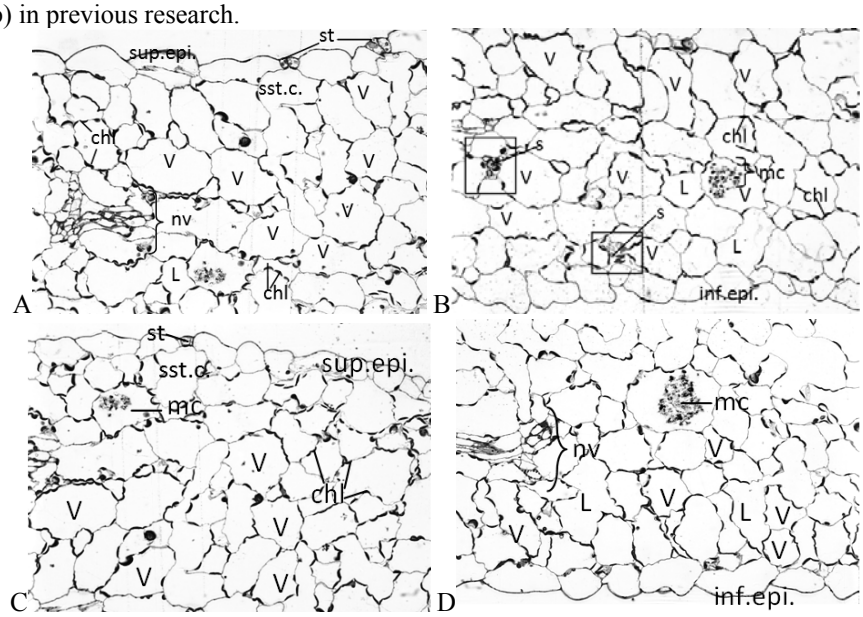

Fig. 8. Structure of red beet foliar limb (*Beta vulgaris* var. Conditiva), at 60 days from culture initiation from seeds *in vitro* (A and B – 40X) or *in vivo* (C and D – 40 X) (chl – chloroplast; inf.epi. –inferior epidermis; L – lacuna; mc – macle; nv – nervure; V – vacuole; st – stomata; sst.c. – substomatic camera; s – starch in chloroplast stroma; sup.epi. – superior epidermis).

From a structural point of view, at the foliar level of red beet, besides common components found at this genus, namely homogeneous foliar mesophyll, macle type formations (Fig. 8 A - D), composed from calcium oxalate crystals, ribs consist of vessel

riddled, with lignin deposition (Fig. 9 A), surrounded by parenchyma cells, with a especially role comparative with those from fundamental parenchyma, at vitroplantlets was identify large chloroplast, deformed due accumulation of starch in increased quantities in their stroma (Fig. 8 A and B and Fig. 9 B - D).

Riddled tubes in sugar beet are highly specialized cells with complex development. Phloem parenchyma cells are, as well as other parenchyma cells, less specialized (Esau, 1934). By meristematic renewed activity, they can function in the cambium initiation, or by simply division can add phloem or vascular bundles. Cambium activity is initiated in young leaves and continues throughout the growth period. The obstruction of riddled tubes and annex cells start early and continue while new phloem elements are formed. Moreover, the chloroplasts degeneration by grane disappearance, abnormal stroma aspect, with large starch granules, with increased density and size of osmiophylic grain was reported by Tomlinson and Webb (1978) in the structure of lettuce leaves infected with beet yellow virus *Claytonia perfoliata,* under natural conditions of life.

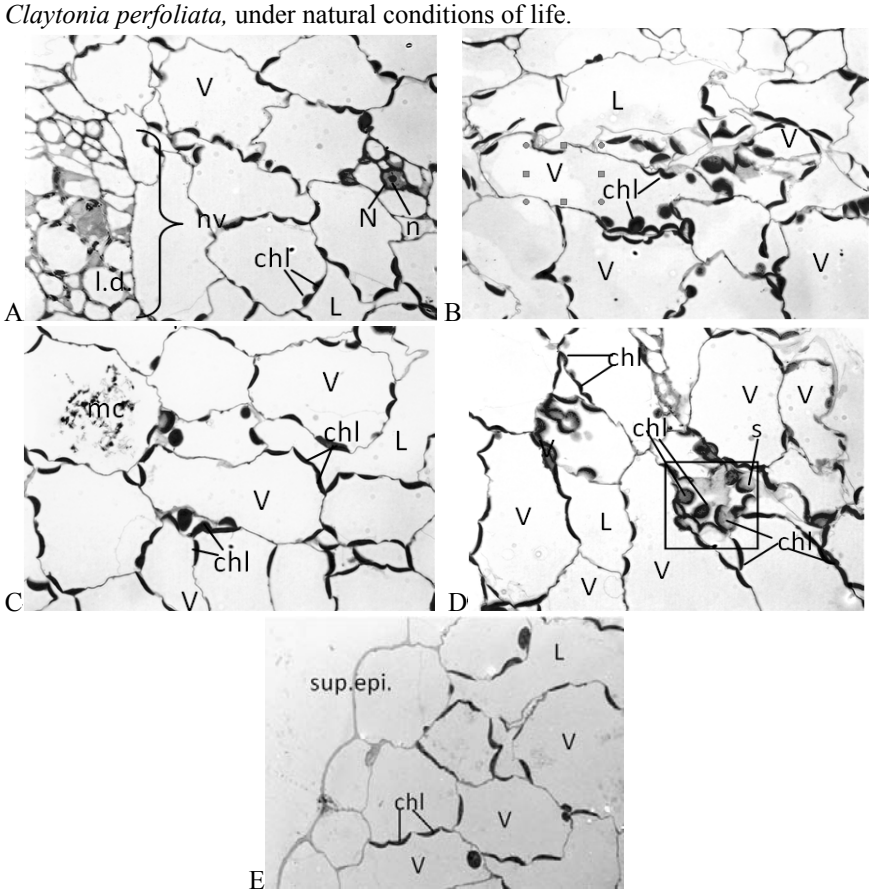

Fig. 9. Structure details of red beet foliar limb (*Beta vulgaris* var. Conditiva), at 60 days from culture initiation from seeds *in vitro* (A - D – 100X) or *in vivo* (E – 100 X) (chl – chloroplast; L – lacuna; l.d. – lignin deposits; mc – macle; N – nucleus; n – nucleolus; nv – nervure; sup.epi. - superior epidermis; st – stomata; sst.c. – substomatic camera; s – starch in chloroplast stroma; V – vacuole).

CONCLUSIONS

1. Beetroot red colorant from red beet is located particularly in the roots, but it is present also in the hypocotyls, including epidermis cell, in the parenchyma of the vascular bundle upper from petiole, and to the level of a single layer cells which wrap the leaf vascular bundle.
2. By studies of anatomical structure were not detected betacianine in vegetative organs of sugar beet.
3. The presence of supernumerary cambium, both at roots level of beet, regardless of variety, and even in the stem or leafs is a genus particularity.
4. From a structural standpoint, in the chloroplast stroma of foliar mesophyll cells of *in vitro* newformated leafs were highlighted large amounts of starch, which gives abnormal aspects of this organite, comparative to that found in plant leaves grown in natural conditions life, which is caused by the presence of sucrose in the vitroculture medium and myxotrophic nutrition of vitroplantlets.
5. Red beet hypocotyl, at 60 days of vitroculture, it's entering in a phase of degradation, observed phenotypic and structural, because of hyperhydricity, or because of its entry into a senescence stage.

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