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THE HISTO-ANATOMICAL ASPECTS OF THE LOCAL POTATO VARIETIES PLANTLETS MAINTAINED *IN VITRO* BY THE SLOW GROWTH

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Abstract. The authors had investigated the structure of vegetative organs (roots, stolon, shoots, microtubers and ramifications thickened) to five local populations of potato maintained in the *in vitro* collection of the Suceava Genebank, Romania, by the slow growth method**.** A comparative analysis of samples obtained by the explants subculturing on four formulas of culture medium (a medium of micromultiplication – MS (1962) medium supplemented with 40 g/l sucrose and 6 mg/l daminozide, control medium – and three conservation medium, with the addition of 30 mg/l daminozide, 40 g/l mannitol and 40 g/l sorbitol) for 2, 7, 12 and respectively 17 months, have revealed, especially, quantitative differences: number of tector hairs and/or secretory hairs, number and distribution of cells with oxalifer sand, number of xylem and phloem vessels into bundles conductive. Cortical parenchyma of the root, to meatic type, has the cells whose size decreases inwards. In the internal cortex of the ramifications thickened the cells shows walls transverse of division.
Keywords: potato, *in vitro* cultures, slow growth, histo-anatomy.

Introduction

In terms of taxonomy, potato belongs to the family *Solanaceae*, the genus *Solanum*, subgenus Potatoe (once Pachystermonum), section Petota (once Tuberarium), subsection Potatos (once Hyperbasarthum) (Hanneman, 1989).

The potato (*Solanum tuberosum* L.) represent one of main cultivated plants of the temperate zone, and as worldwide harvest is the 4-th food crop after rice (*Oryza sativa*), wheat (*Triticum aestivum*) and maize (*Zea mays*) (Dowling, 1995; Jones, 1973). Is grown on all continents for its tubers, use for human and animal food, as seed and as important raw material for the spirits industry, starch, glucose and dextrin (Berar, 2010). Being the most important crop by the tubers expect to be a solution that to ensure food security for the next decades (Chiru et al., 2009).

The market economy and increasing of consumers requirements has led, unfortunately, indirectly, to the replacement of traditional varieties, called and “local populations” or “local varieties” with new varieties, genetically homogeneous, more productive but far more vulnerable to an attack phytopathogenic. Risk of loss or damage to local varieties, which have a high heterogeneity, had imposed preserving native genetic material in collections for using to it subsequent (Cristea, 1985).

In the gene banks, in parallel with maintaining a collection in classic form, by the annual planting of existing genotypes, the potato germplasm is conserved and by introduction in *in vitro* collection, on various preservation media. Maintaining local varieties of the potato, through *in vitro* culture technologies ensures the rescue of some old genotypes with resistance characteristics to the many of unfavorable factors, biotic and abiotic, from areas of origin, well as a high quality culinary.

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Investigation and knowledge of the behavior of these local varieties of the potato to conditions of maintaining in *in vitro* cultures can be very useful for the preservation their proper in germplasm fund.

In our country, *in vitro* culture of potato is practiced either as a secure means of the genotypes regeneration affected by viral infections, either as a simple technique that contributes quickly to obtaining seed potato and/or as an alternative for the medium and long term conservation of this species (Baciu et al., 2009; Chiru and Antofie, 1997). Anatomy of potato plants, healthy or viral infected and of those grown *in vitro* was described by a series of authors, between which Constantinescu et al. (1969), Zanoschi and Toma (1985), Salas et al. (2003).

Use of osmotically active substances and of growth inhibitors is a simple and relatively inexpensive mode to modify a growth medium in a conservation medium, this method being effective *in vitro* conservation of local varieties of potato (Ciobanu et al., 2011; Ciobanu and Constantinovici, 2012).

Since substances that have of growth inhibition effect can cause structural changes, physiological or can generate mutations (Hughes, 1981; Lazarraga et al., 1989; Wescott, 1981b), this study aimed to describe anatomical of the structures vegetative of the plantlets of some local varieties of potato maintained in *in vitro* collection at Suceava Genebank, Romania, for 2, 7, 12 and 17 months, to establish the influence of some growth inhibitors (daminozide, mannitol and sorbitol) on the structural characteristics of these plantlets. In this experiment were used media slow growth used in the aforementioned institution, in combination with reducing temperature and light intensity, for the medium-term conservation of local potato varieties.

Materials and methods

The researches were initiated by using minicuttings with 1-2 nodes prelevated from of five local potato populations (SVGB-14376, SVGB-15079, SVGB-15102, SVGB-15140 and SVGB-15446), maintained in *in vitro* collection of the Suceava Genebank, by the slow growth method. Local varieties studied were selected both for their way of reaction on the storage medium *in vitro*, and for the fact that in the collection of living plants of experimental field the five genotypes manifests morphological differentiation of the aerial part and tubers.

The culture medium used was based on Murashige-Skoog formulation (1962) in ½ dilutions, with low concentrations of growth regulators, sucrose 2-3%, with the addition of daminozide, mannitol and sorbitol. Control samples were maintained in the same ambient conditions, like the conservation samples, on MS medium supplemented with 40 g/l sucrose and 6 mg/l daminozide (Table I). Knowing the positive effect of kinetin on the tolerance of plantlets to low temperatures and long periods of subculture (Kotkas, 2004), in order to help the control samples to overcome the storage conditions, the amount of this growth regulator in the medium was 50 times higher. Thereby was favored the growth and development of potato plantlets during the experiment.

After inoculation and flask closing, they were transferred into growth chamber, where they were placed under the light of 2000-2500 lx. with a photoperiod of 16/24 hours, at a temperature of 20-22^{0}C. The ambient humidity was about 60 -70%. After 4 weeks, the samples were transferred to storage room, where cultures were maintained at temperatures

between 6-12^{0}C, in white fluorescent light with an intensity of 1000 lx. and photoperiod 10/24 hours.

Table 1. Variants of cultured media used for *in vitro* preservation local potato genotypes

Culture medium variant	Basal culture medium and hormonal balance	Other components
M_{14} (control)	MS[1] + 1mg/l K[2] + 0,02mg/l ANA[3] + 6mg/l daminozide	40 g/l sucrose
C_{22}	MS ½ + 0,02 mg/l K + 0,02 mg/l BA[4] + 0,02 mg/l ANA	30 g/l sucrose + 30 mg/l daminozide
C_{23}	MS ½ + 0,02 mg/l K + 0,02 mg/l BA + 0,02 mg/l ANA	20 g/l sucrose + 40 g/l mannitol
C_{24}	MS ½ + 0,02 mg/l K + 0,02 mg/l BA + 0,02 mg/l ANA	20 g/l sucrose + 40 g/l sorbitol

[1] Murashige-Skoog; [2]Kinetina; [3] α naphthyl acetic acid; [4] Benziladenina

The histo-anatomical observations were conducted on roots, stolon, shoots, microtubers and thickened ramifications to the local potato varieties studied, for experimental periods of 2, 7, 12 and 17 months. For to highlighting the anatomy of organs plantlets were conducted cross-sections on the vegetal material conserved in 70^0 ethanol, by means of the microtome of hand and razor anatomically, using as a backing marrow of elder (Tarnavschi et al., 1974; Toma et al., 2003).

The sections finest were analyzed immediately on a slide dry and defatted in a drop of distilled water. The preparation was analyzed to the Olympus microscope with camera incorporated, found out in the endowment Laboratory of Vegetal Morphology and Anatomy from the Faculty of Biology of the University "Alexandru Ioan Cuza" of Iaşi.

Results and discussions

The root structure

On all the samples analyzed, regardless of the culture medium tested and the time elapsed since the initiation of the experiment (2, 7, 12 or 17 months), the primary root structure is typical with of the three anatomical regions, namely: rizodermis, cortex and central cylinder. Cortical parenchyma, usually tristratified, is of type meatic, with parenchymal cells whose size decreases towards the inside, some of them amiliferous. The last layer of the cortex is a primary endoderm with well individualized bands Caspary (Fig.1, Pl. I).

Central cylinder may be triarch, with a large vessel of metaxylem that replaces pith (Fig. 2, Pl. I) in case roots adventitous or diarch (two large vessels of metaxylem which replaced pith) in case radicles (Fig. 3, Pl. I).

The between chemicals investigated, mannitol had a more pronounced inhibitory effect on the process of rhizogenesis, shown by the existence of roots much younger, with diarch structure that still presents pith, after 12 months of subculture of the explants on C_{23} medium (Fig. 4, Pl. I).

The stolon structure

To the samples grown on the micromultiplication medium M_{14}, at the level epidermis is observed uniseriate pluricellular tector hairs and very rare secretory hairs (with unicellular base, unicellular pedicle and unicellular gland) (Fig. 5, Pl. I). In the central cylinder, bundles conductive are arranged in a circle type bicolateral opened, heteromorphy.

We notice a procambium bi-tristratified well individualized, with tangentially the flattened cells, arranged more or less in radial series, that formed towards the inside xylem vessels (of caliber similar in diameter within each fascicle) and very little xylem parenchyma cells, and outwards very little phloem (riddled tubes and appendices cells) (Fig. 6, Pl. I). Mention that the in each fascicle there is a quantitative disproportion between phloem primary and xylem primary, in favor of the latter.

To the samples grown on culture medium supplemented with 40 g/l sorbitol it is noted a large number of very long uniseriate pluricellular tector hairs and rare secretory hairs, with tetracellular gland. (Fig.7, Pl. I). Procambium uni-bistratified has produced especially towards the inside primary xylem elements; primary phloem is very little quantitative, or is missing. The last xylem vessels have not walls impregnated with lignin (Fig. 8, Pl. I).

In case of using the culture medium supplemented with 40 g/l mannitol is observed the cortical parenchyma with large cell in all its content (Fig. 9, Pl. I). Sometimes, procambium produces some elements of primary phloem (riddled tubes, appendices cells) without form towards the inside and primary xylem (Fig. 10, Pl. II). At the level of epidermis is observed secretory hairs with multicellular storeyed gland (2-3 storeys of cells) (Fig. 11, Pl. II) or very long secretory hairs, with multicellular pedicle (4-5 cells), uniseriate and pyriform gland (Fig. 12, Pl. II).

To cross-sections performed at samples obtained by cultivating explants on the culture medium with 30 mg/l daminozide is noted conductive fascicle greater, primary xylem having more vessels (Fig.13, Pl. II). More cells in the cortex have oxalifer sand.

Like the samples grown on the M_{14} medium, on the external surface of cell walls forming tector hairs is observed centrifugal irregular deposition of calcium oxalate (Fig.14, Pl. II). From place to place at the level epidermis is observed stomata located slightly above the level external of its (Fig.15, Pl. II).

The shoot structure

Compared to stolon, to experimental variants analyzed after 7 months of subculture, are distinguished:

- a layer tangential collenchyma in the hypodermic position (that is well observed in polarized light) (Fig.16, Pl. II);
- elements more or less solitary of sclerenchyma in periphloemic position (Fig. 17, Pl. III).

At the level epidermis are present rare secretory hairs shorts, with unicellular gland. In some of cortex and pith cells is observed amyloplasts, but is noticed, too, the presence of cells with oxalifer sand (Fig.18, Pl. III). The pith has the tendency disorganization after 12 months of subculture (Fig. 19, Pl. III).

The microtuber structure

On all the tested medium culture, including on the control variant (the micromultiplication medium M_{14}), the cross-sectional contour is more or less ellipsoidal. On the outside is observed soft suber formed of the 7-9 layers of cells strongly the flattened tangentially, unequal, thin-walled, suberisation, arranged in radial series. Sometimes, is noted, remnants of exfoliated epidermis (Fig. 20, Pl. III), and in some places appear small structures the type *Sambucus* similar with the lenticels, with tissue filling, having rounded cells in progress exfoliation (Fig. 21, Pl. III).

In the fundamental parenchyma, at the polarization microscope is observed the presence of numerous large starch granules (Fig. 22, Pl. III), in general simple, with centrically or

eccentrically hilum, rare composed (Fig. 23, Pl. IV). Amyloplasts size from cells increases as we move forward towards the inside of fundamental parenchyma. (Fig. 24, Pl. IV). In the cross-section through a microtuber next to an "eye" is observed a small bud accompanied by a foliar primordium, and at the periphery of the microtuber, soft suber (Fig. 25, Pl. IV).

The ramifications thickened structure

The internal cortex cells are very large, are elongated radial and at their level occur transverse walls of division (Fig. 26, Pl. IV); on the internal portions of the cortex occur and the phenomena of suberisation of cores. No it is noted a primary endoderm. In the thickness of cortex and pith, numerous cells have oxalifer sand.

In the central cylinder is observed three conductive large bundles oriented in V, having very well developed primary xylem compared with primary phloem (Fig. 27, Pl. IV). At the periphery phloem are present isolated sclerenchyma elements (Fig. 28, Pl. IV).

Conclusions

The histo-anatomical observations had not revealed significant changes in the vegetative organs of the plantlets developed during explants cultivation on the formulas tested of culture medium.

The bundles conductive of vegetative organs studied presents more xylem and phloem less, probably due to the culture conditions in which explants were cultured. The large cells from internal cortex of the ramifications thickened suggest a process water accumulation in vacuoles. Vegetative organs developed to the surface of the culture medium, on all the samples analyzed, are covered on addition to tector hairs and of rare secretory hairs unicellular or multicellular, with gland spherical, oval or pyriform.

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Explanation of the plates

Plate I

Figure1. Cross-section by the root to the genotype SVGB-14376 after 12 months of subculture on medium M_{14}

Figures 2, 3. Cross-section by the root to the genotype SVGB-15446 after 7 months of subculture on medium C_{22}

Figure 4. Cross-section by the type diarh root to the genotype SVGB-15102 after 12 months of subculture on medium C_{23}

Figure 5. Cross-section by the stolon to the genotype SVGB-14376 after 7 months of subculture on medium M_{14}

Figure 6. Cross-section by the stolon to the genotype SVGB-15140 after 7 months of subculture on medium M_{14}

Figure 7. Cross-section by the stolon to the genotype SVGB-14376 after 7 months of subculture on medium C_{24}

Figure 8. Cross-section by the stolon to the genotype SVGB-15079 after 17 months of subculture on medium C_{24}

Plate II

Figure 9. Cross-section by the stolon to the genotype SVGB-15102 after 12 months of subculture on medium C_{23}

Figure 10. Cross-section by the stolon to the genotype SVGB-15140 after 17 months of subculture on medium C_{23}

Figure 11. Secretory hair with multicellular storeyed gland (2-3 storeys of cells) to the genotype SVGB-15079 after 7 months of subculture on medium C_{23}

Figure 12. Secretory hair with pyriform gland to the genotype SVGB-14376 after 12 months of subculture on medium C_{23}

Figure 13. Cross-section by the stolon to the genotype SVGB-14376 after 2 months of subculture on medium C_{22}

Figure 14. Cross-section by the stolon to the genotype SVGB-15446 after 7 months of subculture on medium C_{22}

Figure 15. Cross-section by the stolon to the genotype SVGB-15102 after 7 months of subculture on medium C_{24}

Figure 16. Cross-section by the shoot to the genotype SVGB-15446 after 7 months of subculture on medium C_{22} (col – collenchyma)

Plate III

Figure 17. Cross-section by the shoot to the genotype SVGB-15446 after 7 months of subculture on medium C_{23}

Figure 18. Cross-section by the shoot to the genotype SVGB-15140 after 2 months of subculture on medium C_{24}

Figure 19. Cross-section by the shoot to the genotype SVGB-14376 after 12 months of subculture on medium C_{22}

Figure 20. Cross-section by the microtuber to the genotype SVGB-15102 after 12 months of subculture on medium M_{14}

Figure 21. Cross-section by the microtuber to the genotype SVGB-15140 after 12 months of subculture on medium M_{14}

Figure 22.Cross-section by the microtuber to the genotype SVGB-14376 after 7 months of subculture on medium C_{22}

Plate IV

Figure 23. Starch granules to the genotype SVGB-14446 after 17 months of subculture on medium C_{22}

Figure 24. Cross-section by the microtuber to the genotype SVGB-15079 after 7 months of subculture on medium M_{14}

Figure 25. Cross-section by the microtuber in the right of a "eye" to the genotype SVGB-15079 after 17 months of subculture on medium C_{24}

Figure 26. Cross-section by the ramifications thickened to the genotype SVGB-15140 after 12 months of subculture on medium C_{24}

Figure 27. Cross-section by the ramifications thickened to the genotype SVGB-15102 after 12 months of subculture on medium C_{23}

Figure 28. Cross-section by the ramifications thickened to the genotype SVGB-14376 after 17 months of subculture on medium C_{23}

Iustina Brîndușa Ciobanu, Lăcrămioara Ivănescu, C. Toma **PLATE I**

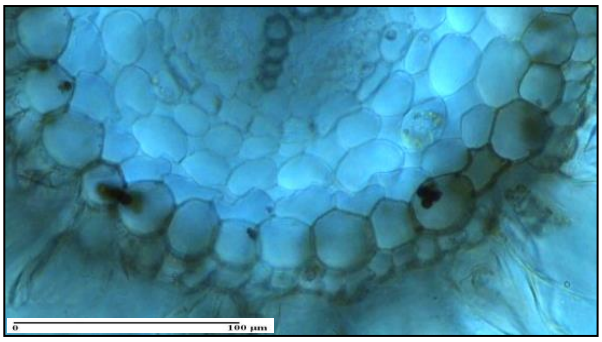
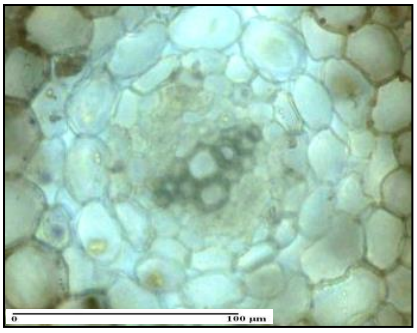


Figure 1

Figure 2



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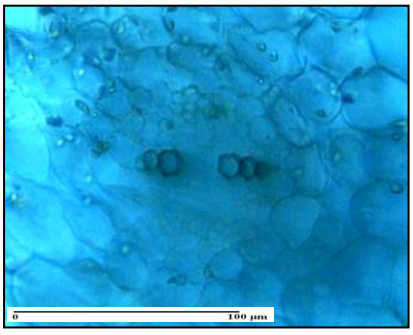

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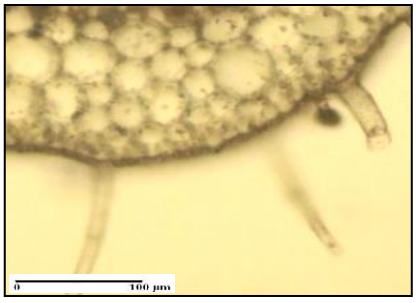

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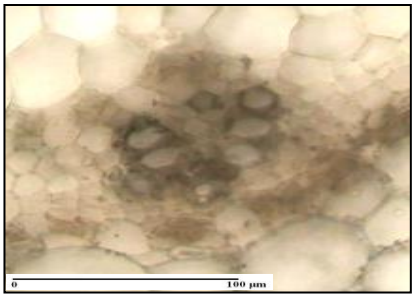
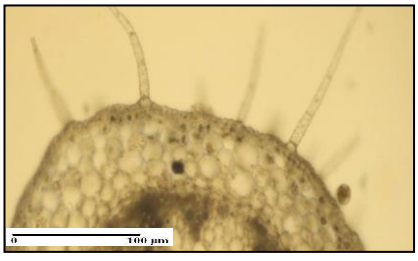


Figure 6



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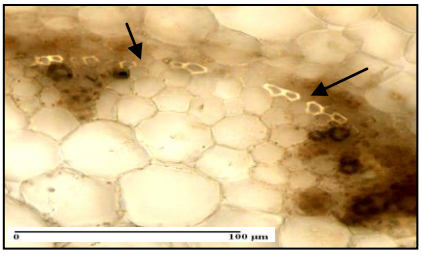

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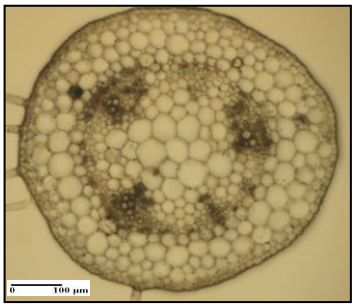

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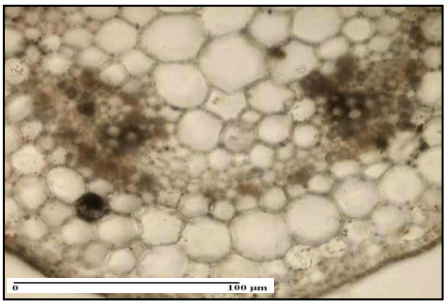

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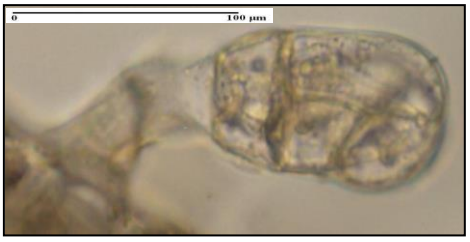

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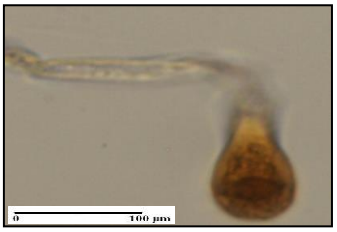

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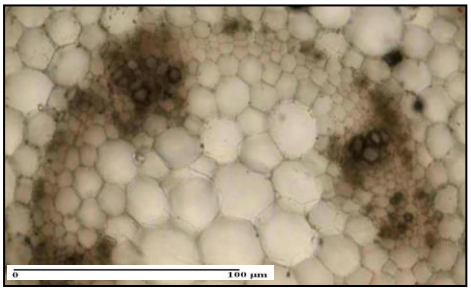
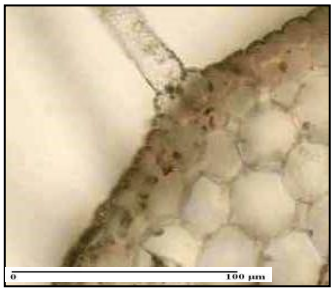


Figure 13



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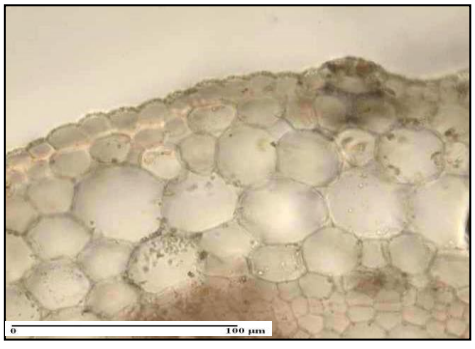

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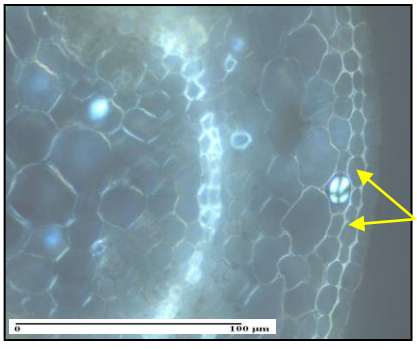

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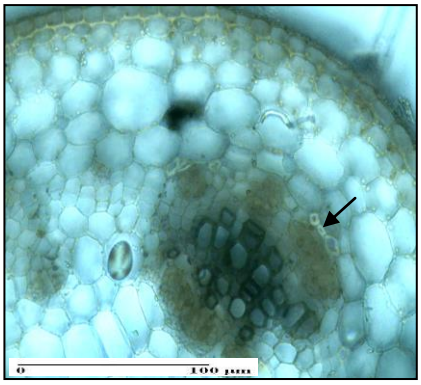

Figure 17

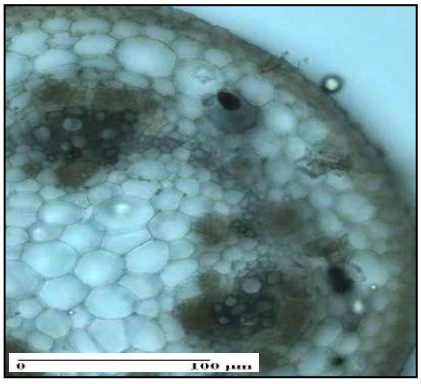

Figure 18

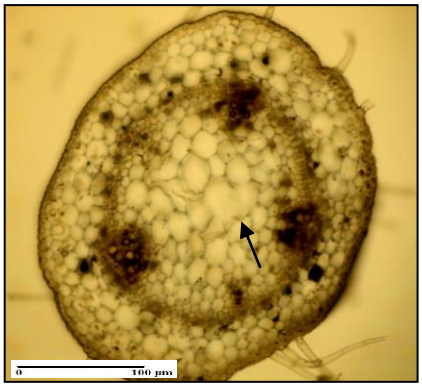

Figure 19

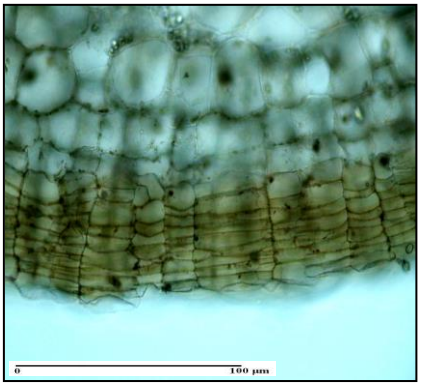

Figure 20

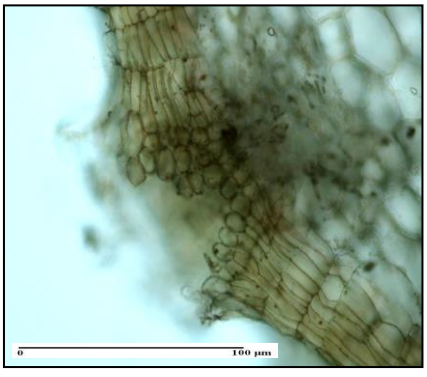

Figure 21

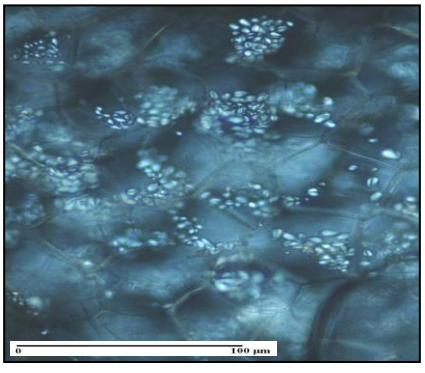

Figure 22

Iustina Brînduşa Ciobanu, Lăcrămioara Ivănescu, C. Toma **PLATE IV**

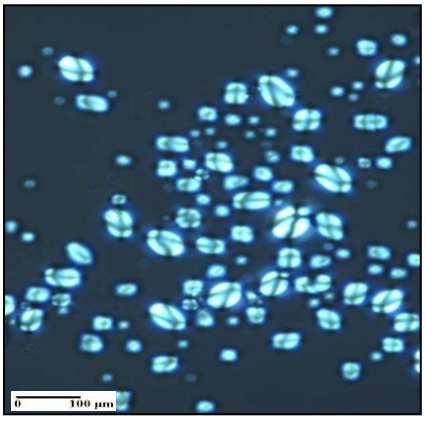

Figure 23

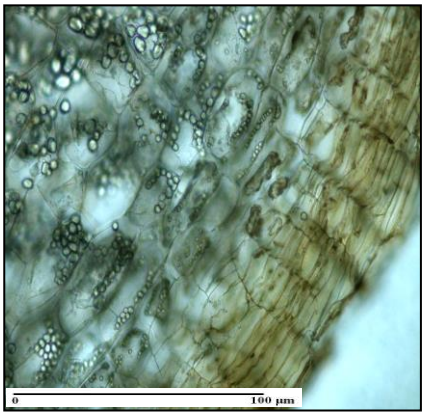

Figure 24

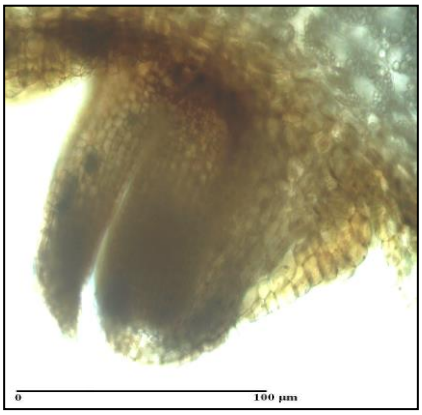

Figure 25

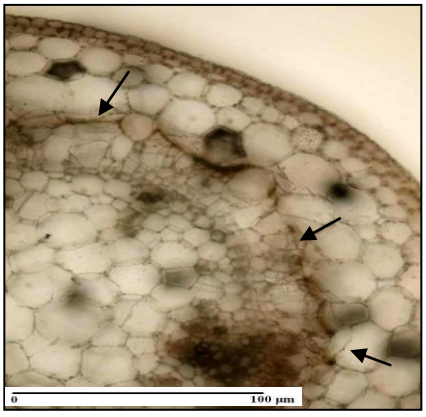

Figure 26

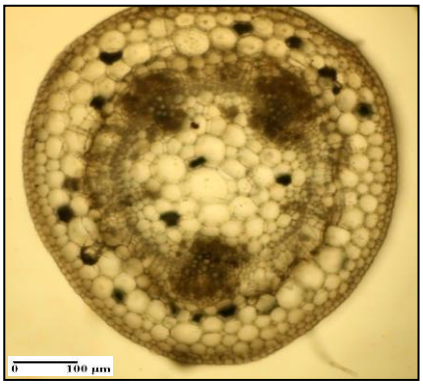

Figure 27

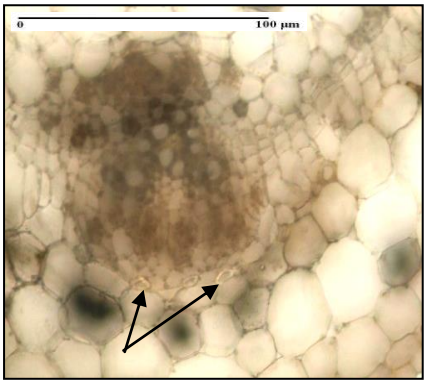

Figure 28