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CYTOLOGICAL INVESTIGATIONS OF THE IN VITRO PLANTS OF *SEDUM TELEPHIUM* SSP. MAXIMUM L. AT THE ROOT APEX

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ABSTRACT. While analyzing the results regarding the reaction of plant inoculum of *Sedum telephium ssp. maximum L.* produced by the *propagules* cultivated in the medium of *Murashige - Skoog* (1962) (MB - MS) with an input of different growth regulators, which stood at the basis of the experiments carried out within the monitorisation of the reactions of the explants of *Sedum telephium ssp. maximum L.*, at the presence in the cultivation sub-layer that we modified, we have noticed -at the root apexes of the plants cultivated in MB – MS mediums with input of different growth regulators, using 1,5 mg/l from each of them-, a raspberry-red-coloration of the apexes of the small roots regenerated at the level of different types of explants, either propagules, or callus regenerated from the propagules. These particular reactions observed at the level of plant inoculum of *Sedum telephium ssp. maximum L.* determined us to lead our investigations towards the examination of these cells with an optical microscope. After the examination with an optical microscope of the root apexes of the in vitro plants of *Sedum telephium ssp. maximum L.* or of the small roots regenerated at the level of callus, we inferred the fact that, both the calyptra cells and the meristem (situated under the apex) have been coloured in red, especially in the case of the plant inoculum cultivated in the MB – MS medium, with a supplement of the mixture KIN and ANA (1,5mg/l from each of them).
We highlighted the presence of these anthocyanins only at the level of plant inoculum of *Sedum telephium ssp. maximum L.*, as this interesting phenomenon was not observed in the case of the organs belonging to plants cultivated in natural conditions. The presence of anthocyanins in the root apexes is a novelty in the specialty literature.

Keywords: *Sedum telephium ssp. Maximum* L., root, in vitro, plants, cytology

INTRODUCTION

Sedum gender belongs to the *Crassulaceae family* (Ştefan & Oprea, 2007; Metcalfe & Chalk, 1972) and consists of almost 400 species with succulent leaves. *Sedum telephium ssp. maximum* (L.) Krock. is frequently spread in the Romanian flora as a spontaneous species, as well as an ornamentally cultivated species. More than that/ In addition, the Romanian traditional medicine considers that this plant might have therapeutic (vulnerary, antiseptic, wounds) effects.

In the middle of the sixteenth century, Hieronymus Bock reported that extracts of *Sedum telephium ssp. maximum* were used in the Rhine valley to treat internal injuries like lung ulcers. Now/today, medical researchers isolate the active ingredients from those traditional medicine plants and test their efficacy. In the early 1990's, some researchers in Munich have identified two polysaccharides in *Sedum telephium ssp. maximum* that were anti-inflammatory (Mulinacci & al., 1993). A few years later, some Italian scientists observed the ways in which the polysaccharides and flavonols operated on cells during wound healing. The vegetable from the *in vitro* culture is part of modern biotechnology industry that focuses on various areas, including a special interest in plant biotechnology presents, that in vitro cultivation of physiotherapeutic interest.

In general, many herbs micro propagated *in vitro*, were used as starting material in the popular culture media that are filled with bioreactors, and the biomass collected from a number of days in vitro culture pass extraction and condition the compounds of pharmaceutical interest (Cachiţă et al., 2004).

As the in vitro cultivation of species of Sedum is concerned, the specialty literature does not have scientific papers regarding the gathering of this type of material that could be exploited as a source of active principles.

MATERIALS AND METHODS

For the vegetal material, we used two categories of root apexes. The first category was represented by the apexes sampled from young roots of about 3 cm, harvested from plants cultivated in natural conditions in the forest of Milova, in the Arad County. The second category was represented by root apexes sampled from in vitro plants cultivated in the *Murashige - Skoog* (1962) (MB - MS) medium with add-ons of different growth regulators. Unlike the previous experiments, in which we produced primary cultures of *Sedum*

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telephium ssp. Maximum, starting from callus apexes or germinated seeds in an aseptic system, this experiment presumed the creation of a subculture of *Sedum telephium ssp. maximum L.,* using as inoculum the propagules detached from the cultures of leaves and stems which made the scrubs that have regenerated from the seed embryos of *Sedum telephium ssp. maximum L.,* germinated in the culture medium of *Murashige - Skoog* (1962), with agar-agar, modified by us as the vitamin input is concerned, an input supplemented with different growth regulators, and administrated separately or blended, that is BA (benzyladenine) with a concentration of 1,5 mg/l, or AIB (indolil butyric acid) with a concentration of 1,5 mg/l; in addition, we also used a variant that we discovered from a blend of BA with a concentration of 1,5 mg/l and AIB with a concentration of 1,5 mg/l, or another blend of KIN (kinetin) with a concentration of 1,5 mg/l and ANA (α-naftil-acetic-acid) administrated with a concentration of 1,5 mg/l. In order to analyse the reaction of these propagules at the presence of different growth regulators in the culture mediums of *Murashige - Skoog* (1962), we proceeded as it follows: from the scrubs generated from zygotic embryos – in the 30th day of germination, we sampled the propagules (Fig. 1B) that had been sub-cultured in the same types of mediums like the ones they had been taken from. The only difference was that they were recently prepared. For this reason, the scrubs were dismembered in propagules that were selected so that they had three leaves and the approximate height of 1-2 cm. The experimental variants constituted for the sub-cultivation of propagules were the following:

VM – the witness variant consists of the root apexes of the plants cultivated in a natural medium;

V0 – the witness variant in a MB – MS medium deprived of growth regulators;

V1 – the experimental variant in a MB – MS medium with BA, with a concentration of 1,5 mg/l;

V1, - the experimental variant in a MB – MS medium with KIN, with a concentration of 1,5 mg/l;

V2 – the experimental variant in a MB – MS medium with AIB, with a concentration of 1,5 mg/l;

V2,- the experimental variant in a MB – MS medium with ANA, with a concentration of 1,5 mg/l;

V3- the experimental variant in a MB – MS medium with BA, with a concentration of 1,5 mg/l and with AIB, with a concentration of 1,5 mg/l;

V4 - the experimental variant in a MB – MS medium with KIN, with a concentration of 1,5 mg/l and with ANA, with a concentration of 1,5 mg/l;

After 60 days of in vitro culture, while observing with the naked eye the root apexes of the plant inoculum cultivated in a MB – MS medium with BA and AIB, as growth regulators, but also of those cultivated in vitro in a MB – MS medium with an input of KIN and ANA, we have noticed some interesting phenomena consisting in the differentiation of the colouring in red of some root cells probably because of the presence of anthocyanins in their vacuoles, phenomena that has not been detected in the case of the organs belonging to the plants cultivated in natural conditions.

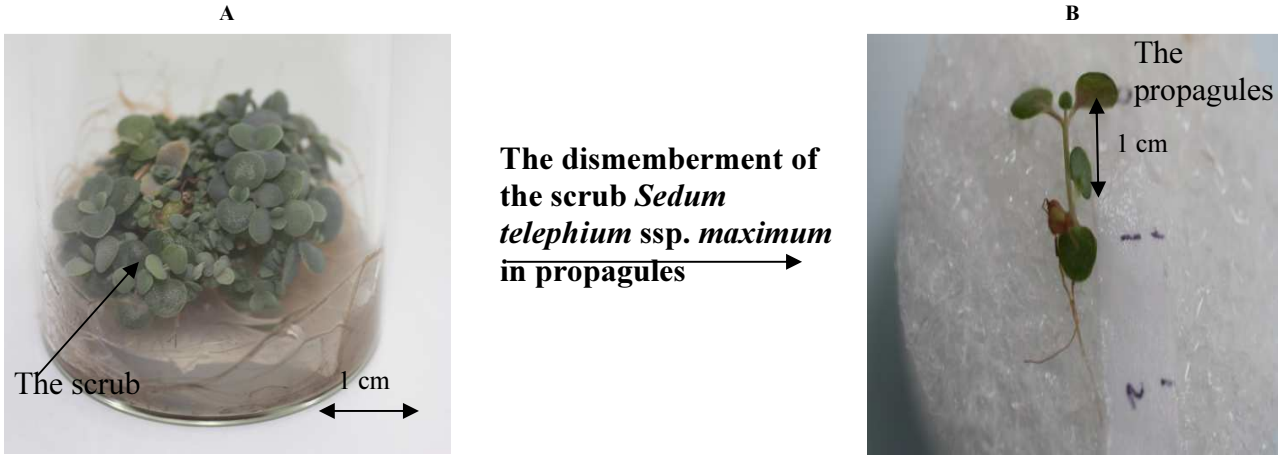

Fig. 1. Scrub belonging to a zygotic embryo of a seed of *Sedum telephium* ssp. *maximum* L. after 30 days from the inoculation of the seed in the culture medium of ***Murashige – Skoog*** (1962) (**A**); the dismemberment of the scrub in propagules in order to inoculate them in recently-made culture mediums (**B**); the plant *Sedum telephium* ssp. *maximum* L. which will be planted *ex vitro* for acclimatization (**C**).

For the examination of these cells at an optical microscope, we prepared freshly-made ones, from the smashing of the root apexes – about 1 cm long- on a microscope slide, in a water drop, the pressure being applied perpendicularly, from top to bottom, which allowed for the easy display of the cells and their observation with ease, without any cell deterioration, like in the case of the studies about mitosis or karyotype.

RESULTS AND DISCUSSIONS

It is interesting the fact that, in the case of the newly-formed roots of the plant inoculum (propagules, plants and callus) with rhizogenesis, their apexes were coloured in red, - a fact visible with the naked eye-, a phenomenon which we did not find mentioned in the specialty literature (Fig.2 B).

The natural colouring in red, especially of the cells of the root meristems, but also of the most recent calyptra cells is extremely surprising and we cannot accurately interpret this phenomenon, all the more when it has not yet been singled out in the specialty literature.

We mention that the very presence of a blend between a cytokine (most of all KIN) and an auxin (most of all ANA- Fig. 3 A-D), has determined the more extensive colouring of the cells in the meristem, while the growth area through extension of the cells situated in the close vicinity of the meristem apex was left totally uncoloured (Pl.1-3).

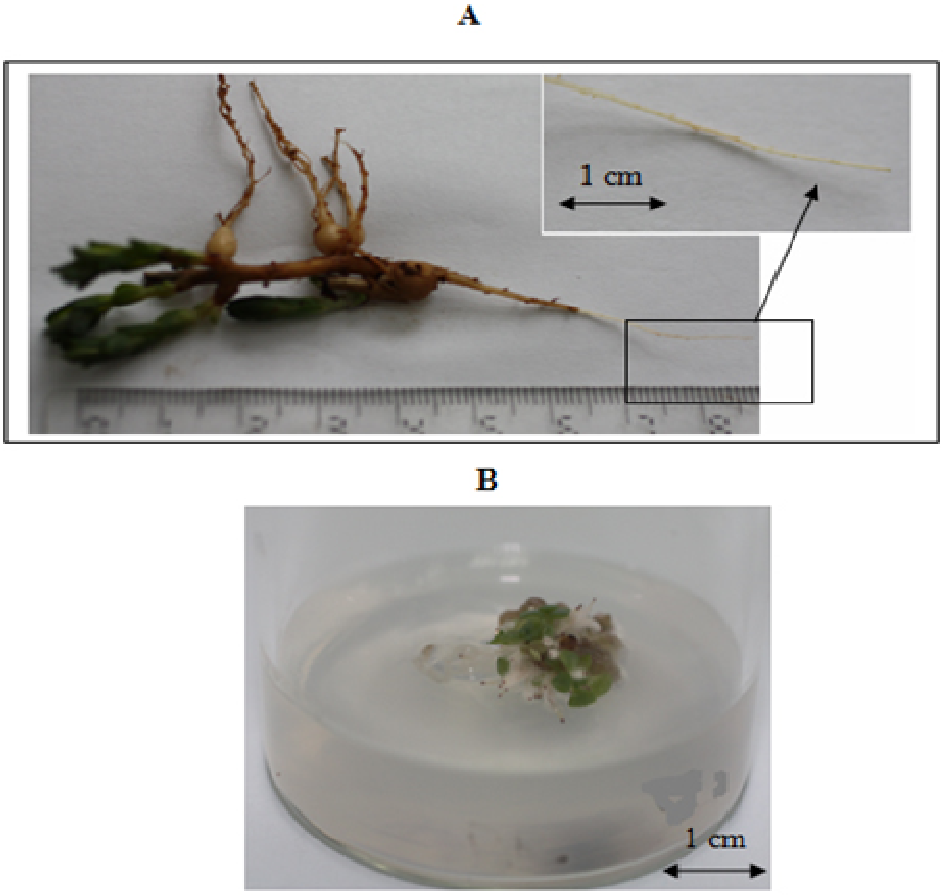

Fig. 2. The root apexes of the plants of *Sedum telephium ssp. maximum L.* cultivated in the natural medium **(A)** and of the plant inoculum cultivated in vitro in the medium MB - MS with an input **KIN** with a concentration of de 1,5 mg/l plus **ANA** with a concentration of 1,5 mg/l **(V_4) (B).**

The role of anthocyanins, respectively of those carrying the compounds in vacuoles is still unknown (Lev - Yadum et al, 2009 and Qu J et al, 2011). The anthocyanins are specific for the secondary, vegetal metabolism, forming a part of the category of pigments which are spread mainly in the epidermal cells of petals, of sepals, of some leaves and stems, but it is not mentioned that they can be present in roots as we have discovered in the in vitro cultures of Sedum telephium ssp. maximum. According to some authors in the specialty literature (Waniska, 2000; Moţa et al, 2002; Rodrigues et al, 2009, etc.), the role of anthocyanins in the life of plants is complex, just like their chemical structure. As vegetal pigments, they can be found under various chemical forms, being phenolic compounds, that is phenolic pigments which fall in the category of flavonoids, which are subdivided (according to Moţa et al, 2002) into six types of derivatives: flavans, flavones, flavanones, calcons, aurones and anthocyanidins, the anthocyanins being anthocyanidins, under the form of glycoside, in anthocyanins the monosaccharides and the disaccharides bonding with the anthocyanidins.

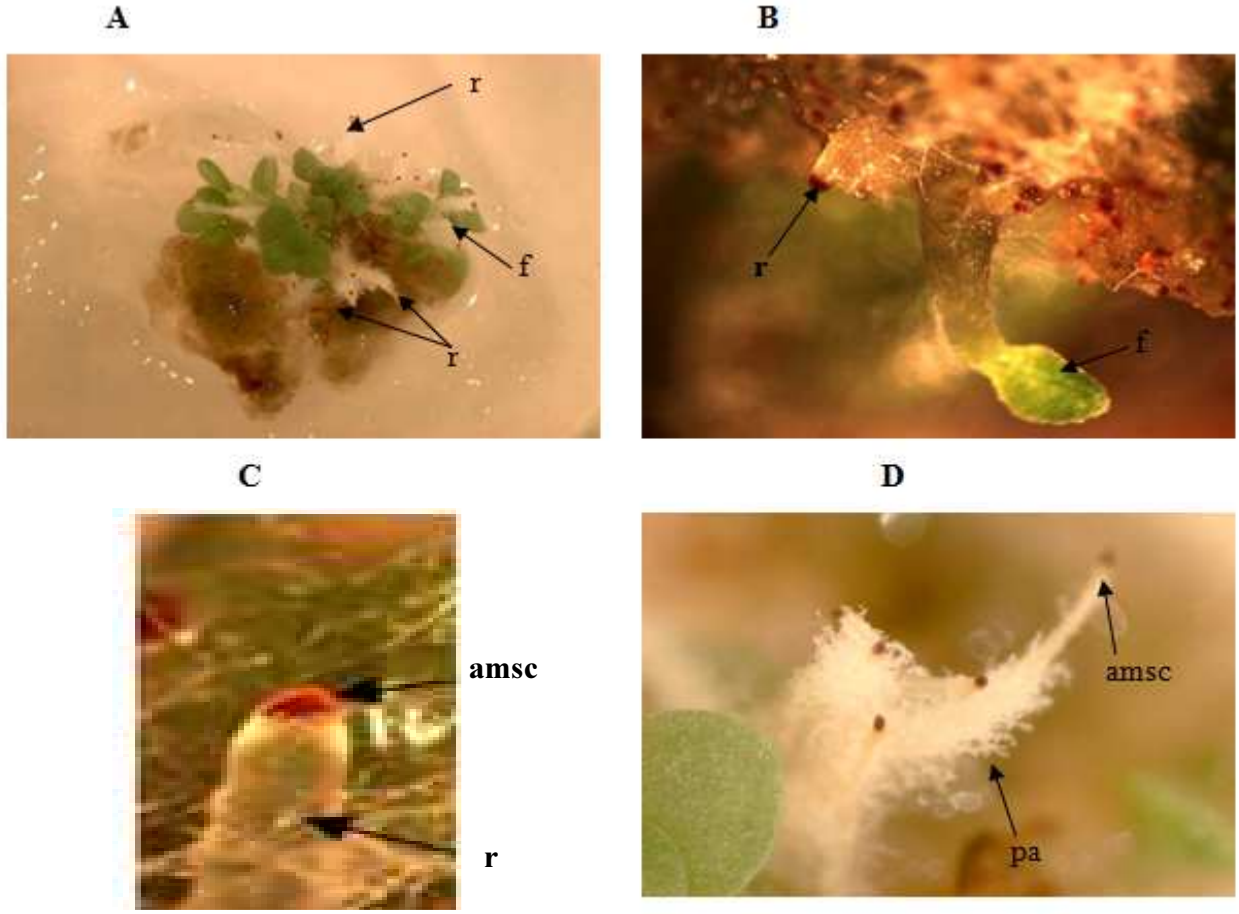

Fig. 3. A-D – Morphological aspects of the small roots of the in vitro plants of *Sedum telephium ssp. maximum L.* cultivated in the MB – MS medium with 1,5 mg/l **KIN** and 1,5 mg/l **ANA (V_4)**, generated from the propagules detached from the detached from the cultures of leaves and stems (scrubs) regenerated from the seed embryos of the seeds germinated in the culture medium of ***Murashige – Skoog***, supplemented with various growth regulators, after 60 days from the in vitro inoculation that have a red apex, where f- leaf; r- small root; amsc- meristem apex and calyptra cells; pa- absorbing root hairs, examined with a stereoscope.

As vegetal pigments, they can be found under various chemical forms, being phenolic compounds, that is phenolic pigments which fall in the category of flavonoids, which are subdivided (according to Moţa et al, 2002) into six types of derivatives: flavans, flavones, flavanones, calcons, aurones and anthocyanidins, the *anthocyanins* being anthocyanidins, under the form of glycoside, in anthocyanins the monosaccharides and the disaccharides bonding with the anthocyanidins. According to Chung and his collaborators (1998), the anthocyanidins are derived from the anthocyanins, which are very instable from the chemical point of view. The *flavonoids* are in their majority *flavans*, among which a part is represented by the anthocyanidins (also called *anthocyanins*).

But the polymeric compounds also fall in the category of the phenolic compounds, and they are also known as tannins (Oberthur, 1983), some of the condensed tannins being proanthocyanidins (according to Hahn, 1984; Hahn et al, 1983; Hahn et al, 1986), occasionally the phenolic compounds being categorized as tannins; according to Chung and collaborators (1998), they can be subdivided into three large categories, that is phenolic acids, flavonoids and tannins, the flavonoids –in most cases- being *flavans*, among which a part are anthocyanidins, a great amount of the flavans being represented by *leukoanthocyanidins.*

In our opinion, in the cells of many plants the vacuolar sap contains *leukoanthocyanin*, which, in some stressful conditions, in situations of photo-protection- converts into a coloured anthocyanin, fact that bestows on those cells (or tissue, or organ) a beneficial effect for the plant that experiences such a phenomenon.

Future research will contribute to the clarification of this interesting, yet difficult to explain phenomenon.

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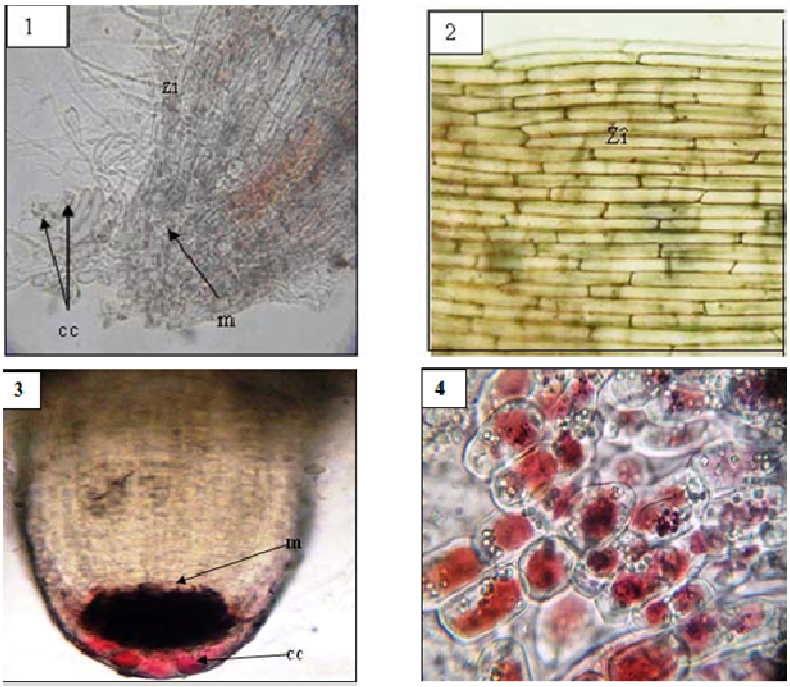

Plate 1, Fig. 1 - 4: Apex of the small roots regenerated at the level of the in vitro plants of *Sedum telephium ssp. maximum L.* generated from propagules (Fig. 1), cultivated in the medium of ***Murashige – Skoog*** (1962), deprived of growth regulators, after 60 days from the in vitro inoculation, noticed at the optical microscope, on freshly-made ones; Fig. 2- ob. 40x – growth area through extension of the small root where: cc- calyptra cells; m- meristem; zî- growth area through extension of the small root at the level of the in vitro plants of *Sedum telephium ssp. maximum L.* generated from propagules cultivated in the medium of ***Murashige – Skoog*** (1962), with an input of 1,5 mg/l **KIN** and **ANA**. Osmiophil compounds, of different sizes, identified in the calyptra cells at the root apexes of *Sedum telephium ssp. maximum L.* regenerated in mediums with which bonded in their structures the anthocyanins that were present in the vacuolar sap (Fig. 4 - ob. x40).

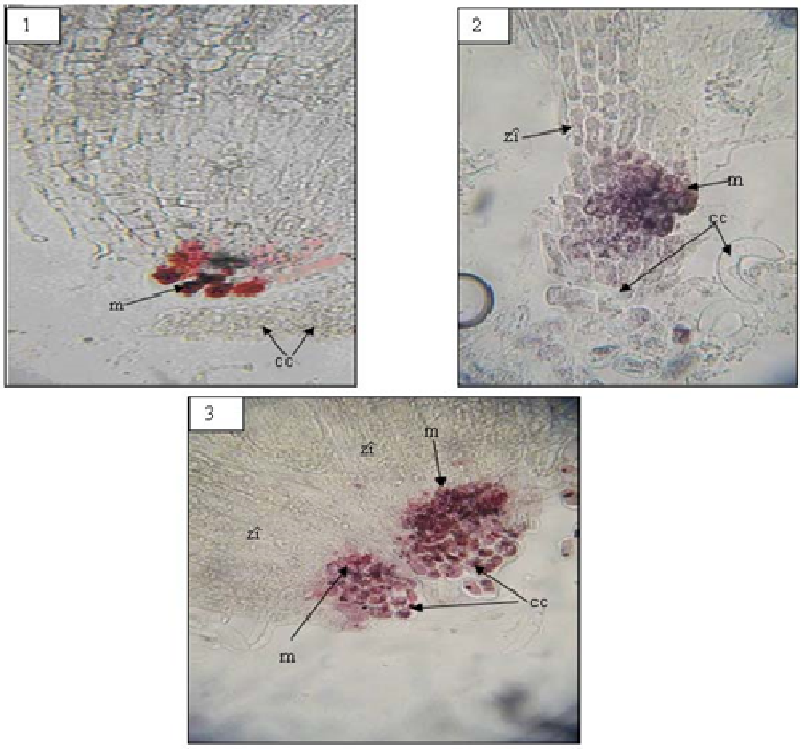

Plate 2, Fig. 1 - 3: Histological aspects observed at the optical microscope on freshly-prepared root apexes of the in vitro plants *Sedum telephium ssp. maximum L.* cultivated in the medium ***Murashige–Skoog*** (1962), with agar-agar, modified by us an with an add-on of either **BA** (Fig. 1), or **AIB** (Fig. 2), or a blend of **BA** and **AIB**, 1,5 mg/l from each of them **(V_3)** (Fig. 1 - ob. 20x; Fig. 2 - ob. 20x; Fig. 3 - ob. 20x).

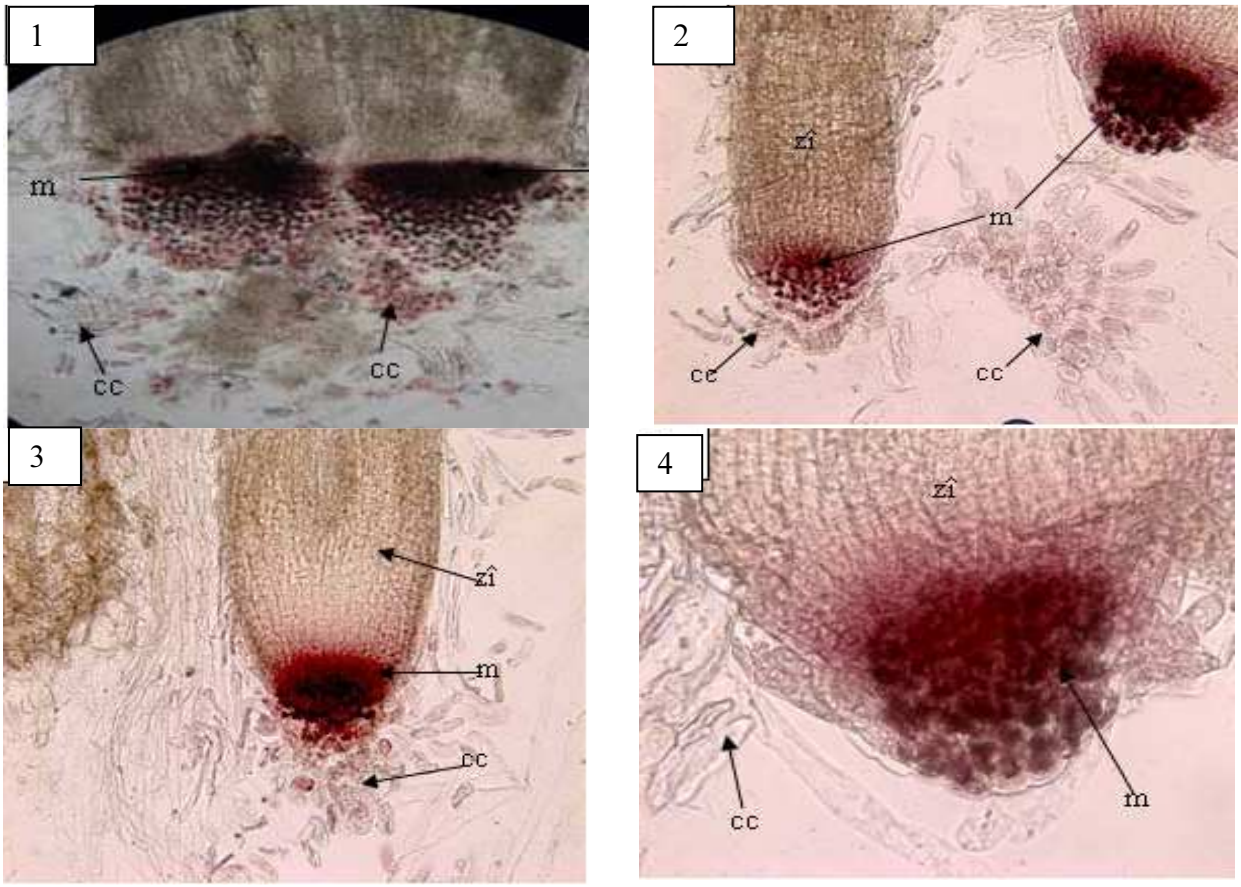

Plate 3, Fig. 1 - 4: Histological aspects observed at the optical microscope on freshly-prepared *Sedum telephium ssp. maximum L.* made of small root apexes regenerated at the level of in vitro plants generated from propagules cultivated in a medium ***Murashige–Skoog*** (1962), with a blend of 1,5 mg/l **KIN** and 1,5 mg/l **ANA (V_4)**, after 60 days from the in vitro inoculation, where cc- calyptra cells; m- meristem; zî- growth area through extension of the small root (Fig. 1 - ob. 10x; Fig. 2 - ob. 10x; Fig. 3 - ob. 20x; Fig. 4 - ob. 20x).

CONCLUSIONS

The observation with the naked eye of the root apexes of the in vitro plants of *Sedum telephium ssp. maximum L.,* especially those cultivated in vitro in the medium *Murashige-Skoog* (1962), with agar-agar, with an input of KIN and ANA, 1,5 mg/l from each of them, led to the discovery of a process of arbitrary colouration in red of some epidermal cells, while the cells which formed the meristem tissue of the small roots apexes and the calyptra cells, in their vicinity, have been coloured uniformly in raspberry red; this phenomenon has not been mentioned before in the specialty literature and has not been observed at other plant inoculum, nor at the small root apexes of plants cultivated in natural or germinate conditions. This colouration could have appeared due to the accumulation in vacuoles of some cells of anthoycianins at the *in vitro* plants (especially at those cultivated *in vitro* in the medium mentioned above), which presented a more intense red colouration even at the examination with the naked eye.

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