Studii şi Cercetări	Martie 2019	Biologie	28/1	94-100	Universitatea"Vasile Alecsandri" din Bacău

A COMPARATIVE MORPHO - ANATOMICAL RESEARCH IN *RHODIOLA ROSEA* L. (GOLDEN ROOT) IN CONVENTIONAL AND *IN VITRO* CULTURES

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Key words: *Rhodiola rosea, morpho-anatomical, in vitro, conventional cultures*

INTRODUCTION

Rhodiola rosea L., known as the golden root or the rose root, is a medicinal plant species, a part of the Crassulaceae family. The etymology of its name is due to the therapeutical properties of the root compounds, which turn the species into an exquisite remedy. Our previous published scientific articles described the chemical composition, the healing properties and biotechnological studies on *Rhodiola* – the *in vitro* research.

This present scientific contribution represents a detailed, thorough sequel of our research for the past 8 years, focussing on the comparative analysis on the histo-anatomical structures (plants from their native habitat versus plants provided *in vitro*).

MATERIAL AND METHODS

In order to run the histo-anatomical tests, the biological material was primarily chosen. It was represented by axial vegetative organs (such as: root, rhizome/tuber, aerial stem), and lateral organs (leaves) of plants in various stages of development.

The vegetal material used in the anatomical, morpho-physiological and biochemical tests in *Rhodiola rosea* L. originated in the Ceahlău mountains and it was represented by plants in various stages of development. The vegetal material provided *in vitro* came from the Genetics Laboratory of the University „Vasile Alecsandri" of Bacău and consisted of neoplantlets grown on several variants of culture medium [4, 5].

The *in vitro* neoplantlets were obtained starting from shoot tips and uninodal fragments, following the classic methods described by the references in this fields. In order to evince the histo-anatomical structure, cross sections were effected through the root, rhizome/tuber, stem and leaves, using the botanical scalpel, elder pith and hand microtome.

The laboratory methods we used during the entire research are the classic ones, according to the references, as it follows:

The vegetative organs were sectioned by means of the hand microtome and of the botanic scalpel.

The lab protocol used in the making of the microscopic slides followed the successive stages: the harvested plants were fixed and preserved in alcohol 70%, subsequently cross sections were effected through each vegetative organ. The cell content is removed from the plant tissues (the plant sections are immerged into some drops of sodium hypochlorite, and maintained for various periods of time depending on the vegetative organ where they were sampled from (underground organs – 20 minutes; aerial stem - 15 minutes; leaf lamina - 10 minutes). The emptied sections are washed in acetic water, and then rinsed in tap water (twice). The surface sections through the leaf lamina and aerial stem will not be submitted to cell content removal. The sections were stained using one of the following reagents depending on the vegetative organ where they were sampled from: ruthenium red, iodine green, Carr solution, or acetic orcein. The surface sections through the leaf lamina and aerial stem were stained either with iodine green or ruthenium red.

The stained sections were transferred onto glass slides in a few drops of glicero-gelatin (sligthly pre-heated at the flame of an alcohol lamp) and covered with coverglass. The cross sections were subsequently analyzed using a photonic microscope (Olympus CX31) at the objectives 4X, 10X, 40X, and 100X, then photographed by means of a digital camera (Olympus C 5060).

RESULTS AND DISCUSSIONS

Regarding *Rhodiola rosea* L., this species was thoroughly studied from the pharmaceutical viewpoint, but the histo-anatomical studies are scarce. As a consequence, the golden root is not described in the plant anatomy treaties, or in other recent scientific papers.

In 2007, COSTICĂ M. et. al. Published a scientific contribution ('Phytocoenological, histo-anatomical and biochimical aspects in *Rhodiola rosea* L. species from Romania'), that comprised some histo-anatomical aspects in this valuable plant species.

The vegetal material used in the anatomical, morpho-physiological and biochemical tests in *Rhodiola rosea* L. originated in the Ceahlău

mountains and it was represented by plants in various stages of development. The vegetal material provided *in vitro* came from the Genetics Laboratory of the University „Vasile Alecsandri" of Bacău and was represented by neoplantlets grown on several variants of culture medium

The root. The cross sections evinced the fact the root has a round shape, (photo 1). The periderm was thin, comprising 5-7 layers of flat cork cells, with thin cell walls. The cork, of various size on the root circumference, is made of cells with slightly suberized walls, partially exfoliated to the outer side. The periderm structure was similar for both types of analyzed plants – the ones from their native habitat, and the ones provided by means of *in vitro* cultures.

The cortex was thick and compact, non-differentiated into an exoderm, a cortex parenchyma and an endoderm. It comprised 7 - 8 layers of oval cells with thin cellulosic walls, and with small air spaces of various sizes (photo 4).

If the *in vitro* provided plants were acclimatized and then transfered on the Ceahlău mountain (the case study area), the histo-anatomical structure would be no different of the one in the native habitat plants. Either the individuals from the native habitat, or the ones provided *in vitro* the wood vessels display slightly lignified walls. vasele lemnoase au pereții uşor lignificați. The phloem generates small isles of few conductive elements (sieve tubes and their associated elements)(photo 6, 7).

The central cylinder from the root is compact and displays a much smaller number of wood vessels in the *in vitro* provided plants than in the plants from Ceahlău mountains, although the plants were almost of the same age. The explanation is that the culture medium is deprived of its essential elements after some time in the case of *in vitro* plants, which leads to the cease of plant growth and development.

The underground stem (the rhizome)

At an early phase we aimed to analyze (from the histo-anatomical viewpoint) the rhizomes of the *Rhodiola rosea* plants grown in their native environment (Ceahlău mountains) compared to the rhizomes of some plants of the same species grown *in vitro* and subsequently acclimatised in their native environment. The lab analysis proved a similar histo-anatomical structure for the two types of plants.

The aerial stem. The stem displays a round shape in cross section for both types of the studies plants (from native habitat and from the *in vitro* cultures)(photo 3, 7).

For both types of the studied golden root plants, the epidermis contains isodiametric cells, with a protruded outer wall, covered by a thin cuticle; the cells lack chlorophyll and the deposit substances.

The cortex (the cortical parenchyma) displayed 9-11 layers of round cells, with cellulosic thin walls, within the plants harvested from mount Ceahlău (photo 4); in case of the *in vitro* provided plants, the number of layers varies from 6 to 9; the first layer of cells beneath the epidermis had thicker walls than those in the inner layers (photo 8).

For both types of the studied golden root plants, there are small air spaces in between the cells of the cortical parenchyma.

The central cylinder. In the first third and also in the medium level stem, the conductive tissues compose two concentric rings of xylem and of phloem in the mature plants harvested from the Ceahlău mountain (photo 9), and in the upper third, the conductive tissues consist of many xylemic-phloemic bundles separated by cellulosic parenchyma cells of the pith rays; as for the *in vitro* plants, one may observe only isles of conductive tissues, that will subsequently join together (photo 10). Although the analyzed plants were of the same age, the *in vitro* provided individuals had a slower growth, possibly as a consequence of a lack in culture medium. For the both typed of studied plants, the phloem consisted of small – size conductive elements (sieve tubes and their associated elements). The xylem was made of larger vessels, with thick lignified walls..

The xylem of the plants harvested from their native habitat displayed more vessels (3-5) on surface unit, compared to the *in vitro* plants (2-4). Moreover, the pith parenchyma is much more developed in the plants originating from Ceahlău, compared to the *in vitro* plants. In both cases, the pith parenchyma had large cells, with thin cellulosic walls; the plants from the native habitat had intercellular air spaces of various sizes, they seldom generated aeriferous lacunae (photo 5). The pith within the *in vitro* provided plants display small air spaces between the cells (photo 7).

The leaf. It was ascertained that there are several morphological differences concerning the leaf – the photoassimilative organ: at the mature plants harvested from Ceahlău, the leaves are fleshy, densely arranged, sessile, oblongue-ovate, with pointed tips, with a length of 3-5 cm; for the *in vitro* - provided neoplantlets, the leaves are petiolated, with roundish tips (photo 11, 12).

a. The petiole of the in vitro provided plants. The cross section is almost crescent- shaped, with a ventral slot, wide and shallow. The epidermis is made of cells slightly elongated , with thin walls, only the outer wall is protruded, thicker than the other walls. During our histo-anatomical research at this level, no type of hair was detected.

The main parenchyma was rather homogenous, comprising oval cells, with cellulosic walls, and with small air spaces in between the cells. A bundle of vascular vessels was observed in this parenchyma; they were arranged in the median region, and continued with secondary smaller ones, disposed sideways, at the distal end of the petiole. The xylem was made of thickened walls vessels,

situated towards the outer side of the petiole; the phloem consisted of sieve tubes and their associated cells (of smaller size, arranged towards the inferior side)(photo 14).

b. Tha lamina. The epidermis viewed frontally: the superior epidermis displayed cells with sinuous side walls and anisocytic stomata. The inferior epidermis had a similar structure to the superior one, nevertheless its cells displayed side walls more sinuous, and the stomata are more numerous on surface unit (photo 15, 16).

Both the adaxial and the abaxial epidermises, in cross section, had similar cells (in the plants harvested from Ceahlău mountain, and in the ones provided *in vitro*). The epidermic cells of both epidermis (superior and inferior) were made of large cells, the outer wall covered by a thin cuticle.

The leaf parenchyma was homogenous, lacunar, therefore the lamina had a bifacial-isofacial structure. There are many vascular bundles within the leaf (up to 23 in the plants harvested from nature), of collateral type, the central vascular bundle was larger than the side ones (fig. 17, 18).

The xylem displayed vessels with thick lignified walls. The phloem comprised sieve tubes and associated cells. The conductive tissues are less developed in the *in vitro* plants, compared to those plants harvested from the native habitat (Ceahlău). The secondary vascular bundles (the smallest ones) seldom display only phloemic elements).

CONCLUSIONS

For the species *R. rosea,* the histo-anatomical structure displays only quantitative differences (and not qualitative ones). At the root, stem, and leaf level, the vascular system is more developed (there are numerous wood vessels) in the plants harvested from their natural habitat, compared to the ones provided *in vitro*.

The axillary roots are of endogenous origin, resul from the pericycle's activity. The central cylinder is compact, with much fewer xylemic bundles in the in vitro plants, compared to the plants harvested from Ceahlău.

The cortex (the cortical parenchyma) displayed 9-11 layers of round cells, with cellulosic thin walls, within the plants harvested from mount Ceahlău; in case of the *in vitro* provided plants, the number of layers varies from 6 to 9; the first layer of cells beneath the epidermis had thicker walls than those in the inner layers.

The xylem of the plants harvested from their native habitat displayed more vessels (3-5) on surface unit, compared to the *in vitro* plants (2-4). Moreover, the pith parenchyma is much more developed in the plants originating from Ceahlău, compared to the *in vitro* plants.

There are some morphological differences within the leaves of *Rhodiola rosea* L.: the mature plants harvested from Ceahlău displayed fleshy leaves, densely arranged, sessile, elongated-ovate, pointed, with a 3-5 cm length; for the *in vitro* neoplantlets, the leaves have a roundish tip and petiole.

The mechanical tissue from stem and leaves is less developed or even absent in the *in vitro* plantlets, compared to the conventional cultures plants. At the same time, the xylemic-phloemic conductive fascicles are more developed in the plants from conventional cultures than in the plants grown *in vitro*.

The conductive tissues are less developed in the *in vitro* plants, compared to those plants harvested from the native habitat (Ceahlău).

The anatomical structure of the *in vitro* provided plants of *Rhodiola rosea* L., post acclimatisation and embedding in their native habitat, is similar to the one of the individuals from the spontaneous flora, their physiological activity being regular.

ABSTRACT

Rhodiola rosea L. was thoroughly studied from the pharmaceutical viewpoint, though the histo-anatomical research is scarce. In view of evincing the histo-anatomical structure of *Rhodiola rosea* L., cross sections were effected through roots, rhizomes, stems and leaves. The comparative research was effected both on plants originating in their native habitat (Ceahlău mountains), and on plantlets provided *in vitro*.

The *Rhodiola rosea* L. plants regenerated *in vitro* displayed, after acclimatization and cultivation in their native environment, an anatomical structure similar to the plants from spontaneous flora, their physiological activity being normal.

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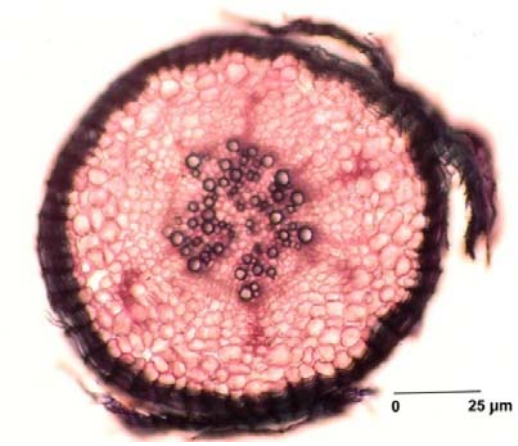

Photo 1. Cross section through the root of *Rhodiola rosea* L. - plants harvested from conventional cultures

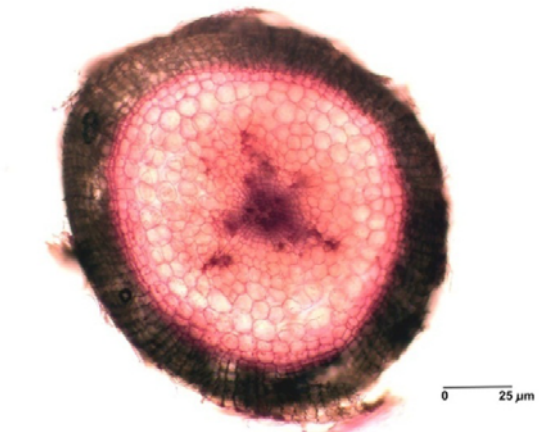

Photo 2. Cross section through the root of *Rhodiola rosea* L. – plants cultivated *in vitro*

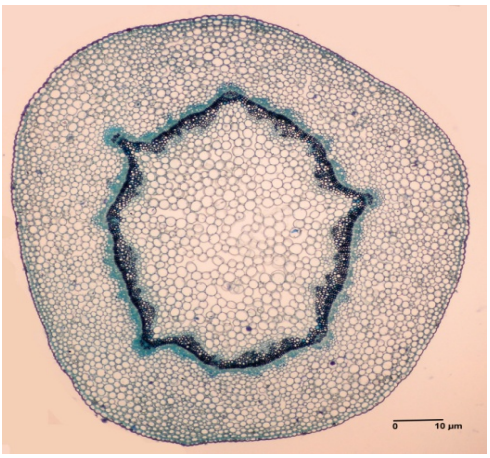

Photo 3. Cross section through the inferior third of the *Rhodiola rosea* L. stem – plants from their natural environment

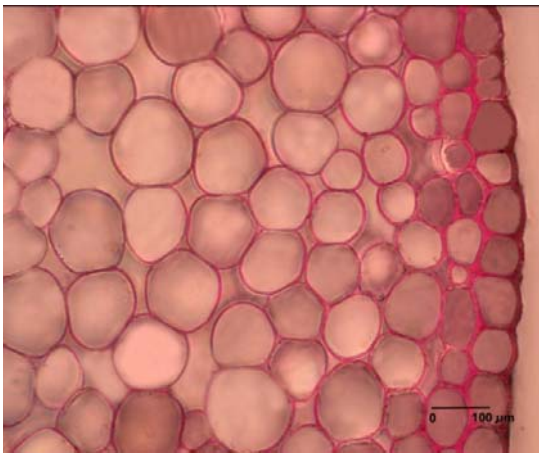

Photo 4. Cross section through the inferior third of the *Rhodiola rosea* L. stem – plants from their natural environment – detail of the epidermis and cortex

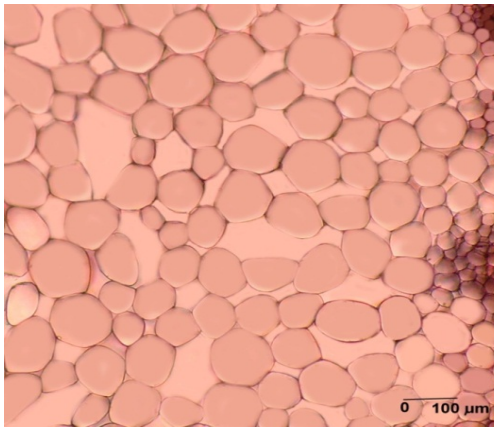

Photo 5. Cross section through the inferior third of the *Rhodiola rosea* L. stem – plants from their natural environment – detail of the pith parenchyma

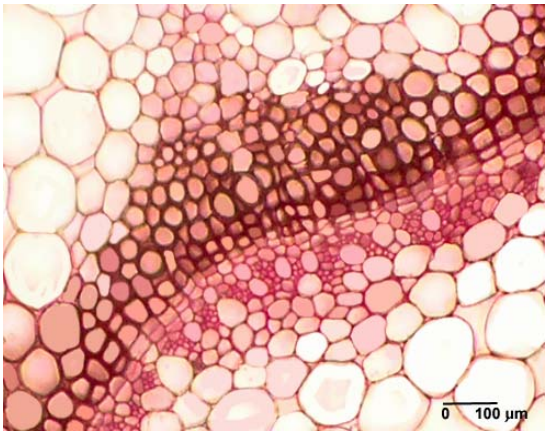

Photo 6. Cross section through the inferior third of the *Rhodiola rosea* L. stem – plants from their natural environment – detail of the vascular bundles

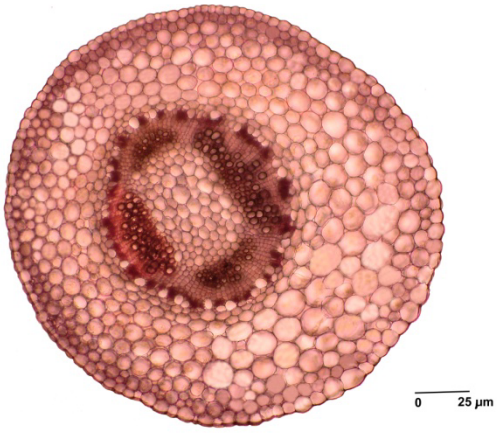

Photo 7. Cross section through the stem of *Rhodiola rosea* L. – *in vitro* plants

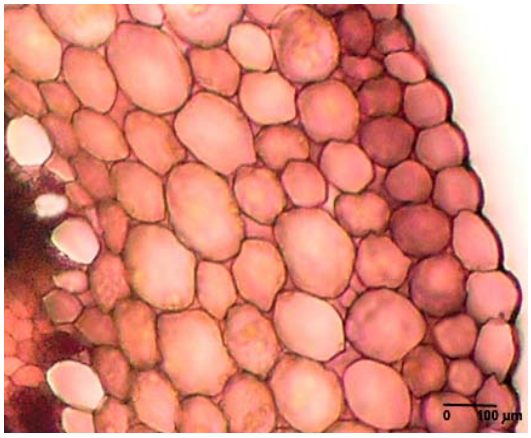

Photo 8. Cross section through the stem of *Rhodiola rosea* L. – *in vitro* plants – detail of the epidermis and cortex

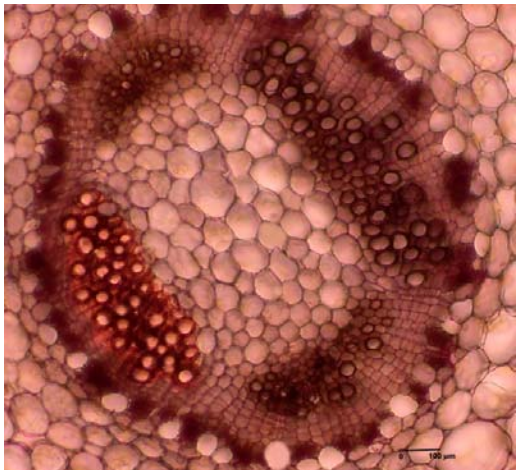

Photo 9. Cross section - stem of the *Rhodiola rosea* L. – *in vitro* plants, detail of the central cylinder

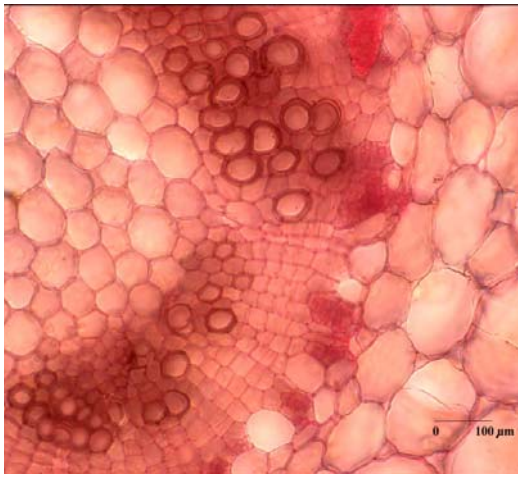

Photo 10. Cross section through the stem of *Rhodiola rosea* L. – *in vitro* plants – detail

Photo 11. Morphological aspect of the leaf (plants from their natural environment)

Photo 12. Morphological aspect of the leaf (plants provided *in vitro*)

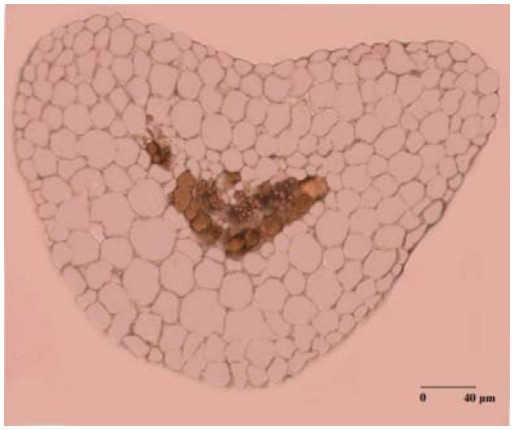

Photo 13. Cross section through the petiole of the *in vitro* provided plants

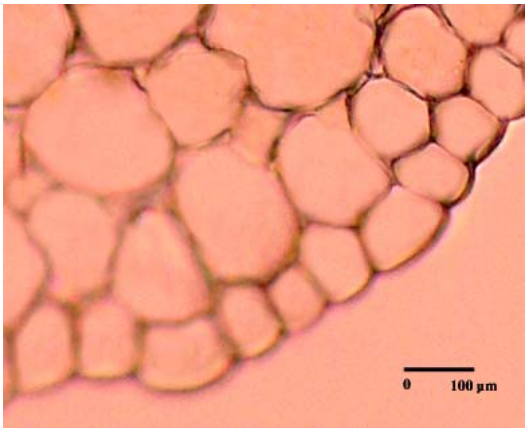

Photo 14. Cross section through the petiole of the *in vitro* provided plants – detail

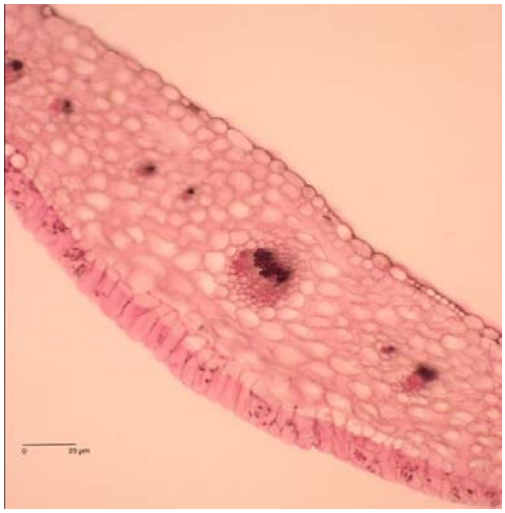

Photo 15. Cross section through the lamina of the *golden root* plants from their native habitat

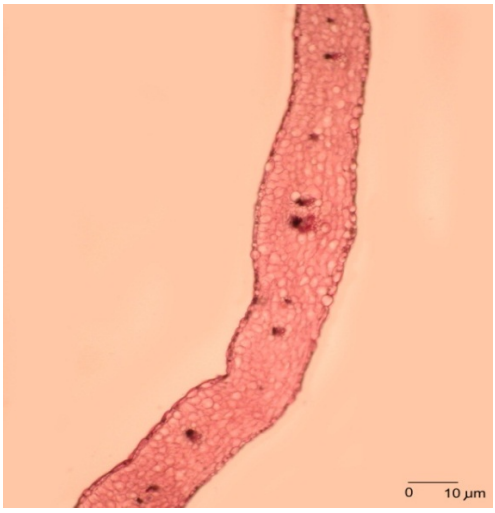

Photo 16. Cross section through the lamina of the *golden root* plants provided *in vitro*

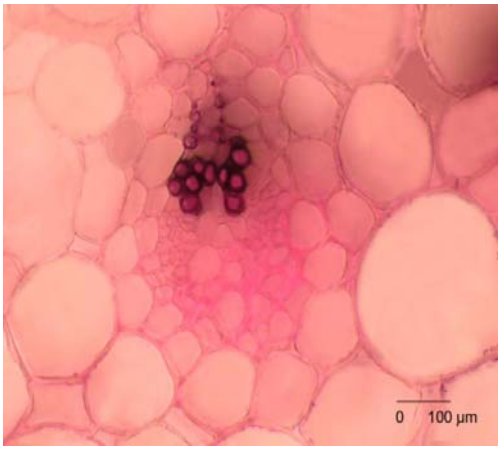

Photo 17. Cross section through the lamina of the *golden root* plants from their native habitat - detail of a vascular bundle

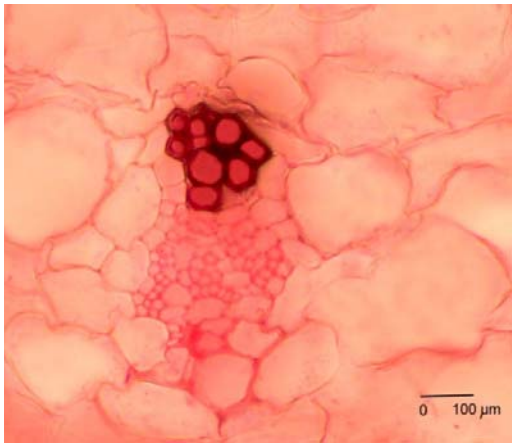

Photo 18. Cross section through the lamina of the *golden root* - plants provided *in vitro* – detail of a vascular bundle