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PRELIMINARY HISTO-ANATOMICAL RESEARCH ON *RHODIOLA ROSEA* L. IN CONVENTIONAL AND *IN VITRO* CULTURES

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

INTRODUCTION

By means of *in vitro* cultures one can put into practice the following aspects: plant breeding (micropropagation or microbreeding), creating new geneotypes (speculating genetic variability or using mutagene agents or somatic hybridizations), providing pathogen-free plants using meristematic cultures, obtaining haploids, selecting and cultivating some cell lines capable to synthesize secondary metabolites, preserving the gene pool [1, 7, 10, 11, 12].

An extremely important field of plant biotechnologies is represented by cell, tissue and organ cultures. Very diverse species from the taxonomical view point were cultured *in vitro* under perfect aseptic conditions. Fragments of these plants were cultivated on complex nutritive medium variants, some of them supplemented with growth regulators.

Depending on the explant type and culture conditions, the cells either differentiate, or resume their stage of young embryogenetic non-differentiated cells. In the first case, there are started tisue and organ cultures (anthers, roots, floral buds etc.), and in the second case callus cultures and cell suspension cultures are initiated.

Sistematic classification and phyto-pharmaceutical importance of the species *Rhodiola rosea* L. (Photo 1)

Regnum: *Plantae*
Phyllum: *Magnoliophyta*
Class: *Magnoliopsida*
Order: *Rosales*
Family: *Crassulaceae*
Genre: *Rhodiola*
Species: *Rhodiola rosea*
Subspecies: *R. rosea*, ssp. *atropurpurea*
R. rosea, ssp. *borealis*
R. rosea, ssp. *elongata*
R. rosea, ssp. *integrifolia*
R. rosea, ssp. *krivochizhinii*
R. rosea, ssp. *neomexicana*
R. rosea, ssp. *polygama*
R. rosea, ssp. *roanensis*
R. rosea, ssp. *sachalinensis*
R. rosea, ssp. *tachiri*
Varieties:
R. rosea, var. *alaskana*
R. rosea, var. *alpina*
R. rosea, var. *integrifolia*
R. rosea, var. *scopolii*
R. rosea, var. *subalpina*
Formes: *R. rosea*, f. *purpurascens*

Photo 1. *Rhodiola rosea* L. collected from the Călimani mountains (original)

Rhodiola species are well-known and used by the traditional Tibetan medecine for over 1000 years. LINNÉ stated (in his works from 1748 and 1749) that *R. rosea* is used as an astringent and also to cure hernia, leuchorrea, hysteria and head aches. According to the data provided by, Galambosi [9], the plant is known and used in the various regions of its spreading area, to improve physical endurance, work productivity, longevity, resistance to altitude sickness, to remove fatigue, treat depression, anemia, impotence, infections, gastro-intestinal and nervous system disorders etc.

The benefits of this plant in the treatment of pain (including head aches), scurvy, hemorrhoids, as a stimulant and anti-inflammatory were described in Germany.

In Middle Asia, the tea of *R. rosea* is the most efficient remedy to fight cold and influenza during very harsh winters characteristic to this region. In Mongolia it is recommended to fight cancer and tuberculosis [25].

MATERIAL AND METHODS

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 [10, 11, 12, 14, 22].

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 used methods are the classic ones, according to the references, and may be studied thoroughly during the present scientific paper [6, 19, 20, 21, 23].

RESULTS AND DISCUSSIONS

In view of evincing the histo-anatomical structure of *Rhodiola rosea* L., cross sections were effected through roots, rhizomes, stems and leaves. 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 (Photo 2 and 3).

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 stem cortex (the cortical parenchyma) displays a number of 9-11 layers of roundish cells, with thin cellulosic walls, at the plants harvested from Ceahlău mountains; in case of the *in vitro* provided plants, the number of layers is reduced to 6-9; the under-epidermic cells (the first layer) display thicker walls than those from the inner layers (Photo 4 and 5).

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 6 and 7).

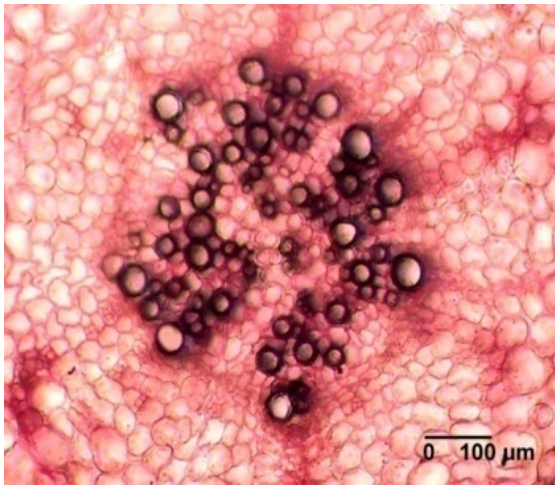

Photo 2. Cross section through the root of *Rhodiola rosea* L. - plants harvested from conventional cultures – detail of the central cylinder

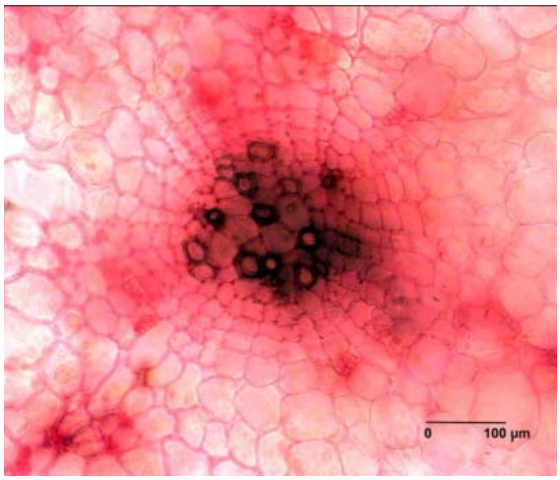

Photo 3. Cross section through the root of *Rhodiola rosea* L. – plants cultivated *in vitro* - detail of the central cylinder

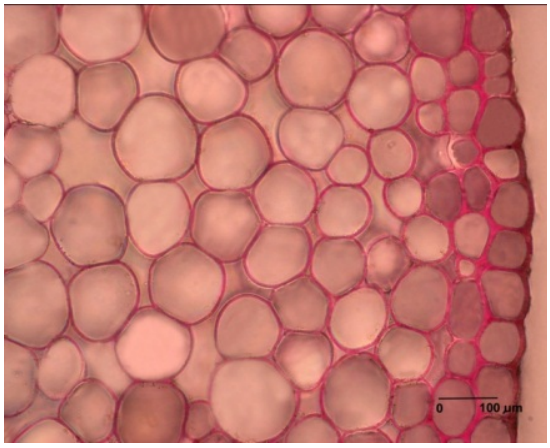

Photo 4. Cross section through the inferior third of the *Rhodiola rosea* L. stem – plants from their natural environment – detail of the epidermis and cortex

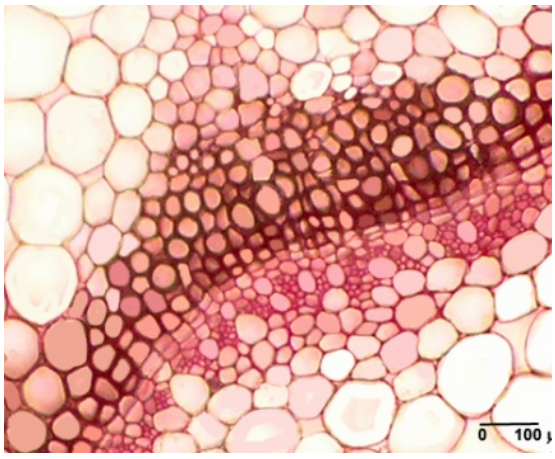

Photo 5. Cross section through the inferior third of the *Rhodiola rosea* L. stem – plants from their natural environment – detail of the vascular system

Photo 6. Morphologic aspect of the *Rhodiola rosea* L. leaf - plants from their natural environment

Photo 7. Morphologic aspect of the *Rhodiola rosea* L. leaf - plants provided *in vitro*

CONCLUSIONS

Our investigations regarding the morphogenetic response of the studied species (*Rhodiola rosea* L.), aiming the elaboration of a micropropagation protocol, the disclosure of its anatomical structure, and of other morpho-physiological and biochemical features, led to the following more important conclusions:

- The *R. rosea* explants' *in vitro* growth is slow, allowing their maintenance in the same culture vial for a long time (even up to one year) with no defacement of the biological material, an important aspect for preserving the cultures in this growth and cultivation system.
- The best medium variants in the micropropagation of the *R. rosea* species were: Kin+2.4-D, BAP+IAA, BAP+IBA, hormone-free MS, and Kin + NAA, Zt + IAA, NAA, as well.
- The acclimatization of the *in vitro* - provided neoplantlets to the *ex vitro* environment was easily accomplished, in a hydroponic system, in about 7 days, without any significant loss of biological material. There was not any loss of *in vitro* regenerants at the transfer moment into soil pots.
- 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*.
- 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*.
- In case of the *Rhodiola rosea* species, it was ascertained that some regenerants' age between 100 and 360 days, interval in which they lived and fed with the ingredients from the nutritive medium from the culture vials, without any sign of senescence or degenerescence.

ABSTRACT

Rhodiola species are well-known and used by the traditional Tibetan medicine for over 1000 years [9]. LINNÉ stated (in his works from 1748 and 1749) that *R. rosea* is used as an astringent and also to cure hernia, leuchorrea, hysteria and head aches [12].

Rhodiola rosea L. was thoroughly studied from the pharmaceutical viewpoint, though the histo-anatomical research is scarce. This is the reason for the species was not included in the histo-anatomical treaties or specialty papers.

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 on plants 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 [2, 3, 4, 18].

REFERENCES

1. ABIDOV M., GRACHEV S., SEIFULLA R.D., ZIEGENFUSS T.N., 2004 – *Extract of Rhodiola rosea radix reduces the level of C-reactive protein and creatinine kinase in the blood,* Bull. of Exp. Biol. and Med., 7, 63-64;
2. ALM T., 2004 - *Etnobotany of Rhodiola rosea (Crassulaceae) in Norway*. Brit.Org.SIDA, 21, 1, 321-344;
3. ANDREI M., 1978 – *Anatomia plantelor*. Edit. Did. şi Pedag., Bucureşti;
4. ANDREI M., 1997 – *Morfologia generală a plantelor*. Edit. Enciclopedică, Bucureşti, 247;
5. BOXUS P., JEMMALI A., PIERON S., 1995 – *Multiplication vegetative – la micropropagation*, Biotechnologies végétales, CNED, Inst. De Rennes, France, 5-116;
6. COSTICĂ M. , COSTICĂ N., TOMA O., 2007 - *Phytocoenological, histo-anatomical and biochemical aspects in Rhodiola rosea L. species from Romania*. An. Şt. Univ. „Al. I. Cuza" Iaş, Secţ. Genetică şi Biologie Moleculară, VIII, 119-121;
7. EVSTATIEVA L., TODOROVA M., ANTONOVA D., STANEVA J., 2010 - *Chemical composition of the essential oils of Rhodiola rosea L. of three different origins*. Pharmacogn. Mag., 6, 24, 256-258;
8. FAHN A., 1982 – *Plant Anatomy* (ed. a 3-a), Pergamon Press, London;
9. GALAMBOSI B., 2005 - *Rhodiola rosea L., from wild collection to field production. Medicinal plant conservation*. Newsletter of Medicinal plant specialist Group of the IUCN, 11, Silphion, 31-35;
10. GHIORGHIŢĂ G., NICUŢĂ D., 2005 – *Biotehnologiile azi*, Edit. Junimea, Iaşi, 149-173;
11. GHIORGHIŢĂ G., HÂRŢAN M., NICUŢĂ D., MAFTEI D.E., 2008 - *Preliminary data regarding the in vitro reaction of Rhodiola rosea* L., Proceed. 5-th CMAPSEEC, 2-5 Sept., Brno, 1-7;
12. GHIORGHIŢĂ G., HÂRŢAN M., MAFTEI D.E., NICUŢĂ D., 2011 - *Some considerations regarding the in vitro culture of Rhodiola rosea* L., Romanian Biotechnol. Letters, 16, 1, 5902-5908;
13. KEAN CH. I., 1924 (1927) – *The Morphology and Physiology of the Leaves of Some Crassulaceae*, Trans. Bot. Soc. Edinburgh, 29:96-104;
14. MAFTEI D.- E., 2007 – *Studii morfogenetice, citogenetice şi biochimice în culturi in vitro şi ex vitro la unele plante aromatice*, Teză de doctorat, Univ. „Al I Cuza" Iaşi, 263 p.;
15. RAMAZANOV Z., *Phytochemistry, Pharmacology and Standardization of Rhodiola rosea root extract*, http://rhodiolarosea.org.;
16. RĂVĂRUŢ M., 1956 – *Fam. Crassulaceae*, În Flora R.P. Române, 4: 46-85, Edit. Acad. Rom., Bucureşti;
17. SALIKHOVA R.A., ALEKSANDROVA I.V., MAZURIC V.K., MIKHAILOV V.F., USHENKOVA L.N., POROSHENKO G.G., 1997 - *Effect of Rhodiola rosea on the yield of mutation alterations and DNA repair in bone marrow cells*. Patol Fiziol. Eksp. Ter., 4, 22-24;
18. ŞERBĂNESCU – JITARIU G., TOMA C., 1980 – *Morfologia şi anatomia plantelor*, Edit. Did. şi Pedag., Bucureşti;
19. TOMA C., GOSTIN I., 2000 – *Histologie vegetală*, Edit. Junimea, Iaşi;
20. TOMA C., NIŢĂ M., 1995 - *Celula vegetală*, Edit. „Al. I. Cuza" Iaşi;
21. TOMA C., RUGINĂ R., 1998 - *Anatomia plantelor medicinale.* Atlas, Edit. Acad. Rom, Bucureşti, 310-320;
22. TOMA O., COSTICĂ M., COSTICĂ NAELA , 2008 - *Contributions to Rhodiola rosea* L. *ecological characteristics interpretation and biochemical matrix of soluble proteins*

identification, Zilele Universitatii "Alexandru Ioan Cuza" Iasi, Sesiunea Ştiinţifică "Biochimie si biologie moleculară - prezent □i perspective", 24-25 oct. 2008;
23. ZYCH M., FURMANOVA M., KRAJEWSKA-PATAN A., LOWICKA A., DREGER M., MENDLEWSKA S., 2005 - *Micropropagation of Rhodiola kirilowii plants using encapsulated axillary buds and callus*. Acta Biol. Cracoviensia, Ser. Bot., 47, 2, 83-87;
24. http://rhodiolarosea.org/rhodiola-rosea-taxonomy-rhodiola-geographical-distribution
25. http://www.hippocratus.com/metasite/web_site/1/contenu/public/pdf/memoires/avril2011/LARIVE_*Rhodiola_rosea*.pdf

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