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(22nd SPPS Meeting)

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Institute for Biological Research "Siniša Stanković", University of Belgrade

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The effect of sucrose on the density and distribution of leaf glandular trichomes of *in vitro*-grown *Inula britannica* L.

PP1-12

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Inula britannica (family Asteraceae) belongs to a group of aromatic plants used in traditional medicine, horticulture, chemical, food and cosmetic industry. As with many aromatic plant species, adaxial and abaxial surfaces of *I. britannica* leaves are covered with glandular and non-glandular trichomes. Trichomes, hair-like structures, which originate from the epidermal cells, are genetically determined, but external factors also have important influence on their development. The glandular trichomes contain chemical compounds with various functions, including defense. Changes in number/density of trichomes and in composition/concentrations of their compounds may have important consequences for plant adaptation to different external factors. *In vitro* cultures may be an alternative choice to manipulate and provide a better control of abiotic factors that may affect the type, size, number and density of leaf trichomes as well as synthesis of secondary metabolites. Sucrose used in growth medium is required by plant cells as energy and carbon source, and can also act as an osmotic agent that may cause osmotic stress and modifications in growth and development.

The aim of present study was to analyze the effect of different sucrose concentration in the growth medium on the density and distribution of leaf glandular trichomes of *in vitro* grown *I. britannica*. It was observed that all applied concentrations of sucrose have effect on density and distribution of glandular trichomes. The highest density of glandular trichomes was noticed on the abaxial side of the leaf of plants cultivated at the sucrose concentration of 0.3 M.

Keywords: *Inula britannica*, aromatic plants, micropropagation, glandular trichomes, osmotic stress

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Morphogenesis of *Artemisia alba* Turra as a key for targeting secondary metabolism *in vitro*

PP1-13

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Artemisia alba Turra is a fragrant shrub, whose essential oil and non-volatile constituents have been utilized in traditional medicine and explored scientifically. The effects of morphogenic changes on several biochemical parameters of the plant have been studied in tissue culture conditions. Auxin and cytokinin treatments resulted in the development of two main morphotypes. While

PGR-free control and IBA treated plants were characterized with the development of both aerial and root tissue, the combined IBA and BA treatments resulted in root inhibition and callusogenesis at the explant base. The root-suppressed morphotype expressed inhibition of some antioxidant enzymes, as compared with normally rooted plants. Root suppression was also characterized by a drop in the content of salicylic acid, as well as abscisic acid and its catabolites. This effect was accompanied by elevation of the levels of jasmonic acid and its conjugate jasmonic acid-isoleucine, as well as of the polyphenolics content, thus leading to a preservation of lipid peroxidation levels, as compared with non-treated control. In addition to polyphenolics, the terpenoid profile of *in vitro* cultivated plants was also affected, as rooting was related to domination of monoterpenoids, and root inhibition – to domination of sesquiterpenoids in the oils from aerial parts. The obtained results imply that morphogenesis plays an important role in the interplay between enzymatic and non-enzymatic defense of *A. alba in vitro* and seems to be an important factor for targeting the biosynthesis of secondary metabolites in this species.

Keywords: *Artemisia alba* Turra tissue culture, *in vitro* morphotypes, essential oil profile, polyphenolics production, antioxidant defense

This work was supported by the bilateral agreement between the Czech and Bulgarian Academies of Sciences.

Selection of stable reference genes in *Centaurium erythraea* Rafn during *in vitro* somatic embryogenesis and mechanical wounding

PP1-14

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Centaurium erythraea Rafn (Gentianaceae) is a medicinal plant, rich in secondary metabolites, mainly secoiridoid glucosides and xanthones, known for numerous beneficial effects on human health. In addition, *C. erythraea* possesses remarkable developmental plasticity and is easily cultured *in vitro*, so it represents a suitable model system for studying developmental biology. One of the most notable illustrations of plant cell totipotency is the capability of certain somatic plant cells to initiate embryogenic development through somatic embryogenesis (SE). It has been proposed that mechanical wounding during *in vitro* manipulations of plant material can promote cell differentiation and somatic embryo development. Our recent research is based on elucidating the gene expression profiles of centaury tissues in response to wounding and in different SE stages using quantitative real-time PCR. Considering the fact that these processes exhibit a dynamic genetic activity, the selection of stable reference genes is paramount in order to obtain unbiased conclusions. Hereby we report selection of stable reference genes in *C. erythraea* for studying gene expression during somatic embryogenesis and wounding. Thirteen frequently used reference genes were selected and their expression stability was assessed in different developmental stages, including globular and cotyledonary stages of embryos, as well as wounded tissues. Specific sets of primers were designed relying on previously obtained next-generation RNA sequencing data. The results were interpreted using two algorithmic approaches - geNorm and NormFinder - and the most stable reference genes from a set of tested candidate genes were determined.

Keywords: reference genes, gene expression, somatic embryogenesis, wounding

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