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"HAIRY" ROOT CULTURE OF *Scutellaria altissima* L. FOR HIGH-LEVEL FLAVONOID PRODUCTION

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The study is devoted to the obtaining of *Scutellaria* "hairy" root culture and the analysis of the level of accumulation of pharmacologically valuable substances in *Scutellaria* plants.

The aim was to obtain the transgenic root culture of *Scutellaria altissima* L. and *Scutellaria albida* L. via *Agrobacterium rhizogenes*-mediated transformation and to compare the level of flavonoid accumulation in the obtained cultures.

Methods: surface sterilization of seeds, *A. rhizogenes*-mediated genetic transformation, polymerase chain reaction, spectrophotometric method for determination of flavonoids by rutin.

As a result of *Agrobacterium*-mediated transformation we obtained the "hairy" root cultures of these plants, and PCR-analysis proved the transgenic nature of the obtained cultures. A significantly higher flavonoid content was shown for *Ri*-root culture compared to the roots of plants grown *in vitro* plants and came up to ±0,12 mg/g and ±0,09 mg/g respectively.

Conclusions. The transgenic root culture of *Scutellaria altissima* was characterized by a high content of flavonoids and can be considered a pharmacologically promising material.

Keywords: *Scutellaria* L., genetic transformation, *Agrobacterium rhizogenes*, hairy root culture, flavonoids, antioxidant activity.

The modern pharmaceutical industry offers a wide range of chemical-based medicines that are characterized by their rapid effect on the human body. Still, at the same time, they are known for their side effects and contraindications. The search for herbal medicines that would be effective and have no side effects is becoming increasingly important.

Recent studies of the antibacterial and antioxidant effects of *Scutellaria altissima* extracts have shown these mechanisms being based on the effect of the flavonoid substances (scutellarin, vogonin, baikalin, and baikalein) [1, 2]. These substances were shown to accumulate mainly in the root tissues of these plants. Therefore, the obtaining of hairy root culture, which is known for its relatively low-cost cultivation and hormone-free rapid growth, is promising. This way, *A. rhizogenes*-meadiated genetic transformation can be used in order to obtain "hairy" root cultures of *S. altissima* and *S. albida*, which are considered promising producers of compounds with antioxidant and antiviral properties [3]. However, the problem of genetic transformation's effect on the accumulation levels of biologically active compounds in plants remains unstudied.

Our study aimed to obtain *Scutellaria* transgenic root culture and compare the level of accumulation of biologically active compounds for the obtained transgenic root and intact root cultures.

All the studies were carried out at the Institute of Cell Biology and Genetic Engineering NAS of Ukraine.

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Scutellaria plants were introduced into *in vitro* culture by surface sterilization of seeds of two *Scutellaria* L. species (*S. albida*, *S. altissima*) by rinsing them in 70% ethanol solution for 1 minute and in 25% hydrogen peroxide solution for 20 minutes. The plants were cultivated on Murashige-Skoog medium [4] at 18–20 °C, 16-hour photoperiod. We used 6–12-week plants for further genetic transformation. The overnight bacterial suspension culture (*Agrobacterium rhizogenes* A4 strain) was sedimented by centrifugation for 10 min. (4000 rpm), then, the precipitate was resuspended in a liquid MS medium. The plant (*S. albida* and *S. altissima*) explants were cultivated with bacterial suspension in an orbital shaker (150 rpm) for 48 hours at 28 °C. Subsequently, the explants were transferred to an MS agar medium with the addition of 400 mg/L of antibiotic cefotaxime for bacteria elimination.

To confirm the transgenic nature of the obtained root cultures, we performed PCR analysis for the presence of *Agrobacterium rolB* gene (fragment size 780 bp, nucleotide sequence of primers: *5′-atggatcccaaattgctattccttccacga-3′*, *5′-ttaggcttctttcttcaggtttactgcagc-3′*). The amplification was carried out under the following conditions: denaturation 94 °C/ 5 min, 30 cycles (denaturation 94 °C/ 30 s, annealing 55 °C/ 30 s, synthesis 72 °C/ 50 s), final synthesis 72 °C/ 5 min. The reaction products were separated by electrophoresis in a 1.5% agarose gel in a TAE buffer system.

The plant material was weighed, homogenized in 96% ethanol, and centrifuged, and then the supernatant was used for biochemical analysis. In order to analyze the flavonoid content in the transgenic root culture and intact root lines, we used the spectrophotometric method (wavelength = 510 nm) based on the property of flavonoids to form a colored complex with an alcoholic solution of aluminum chloride [5]. The study was performed three times, and the t-test was used to analyze the significance of the difference in the obtained results.

We carried out *A. rhizogenes*-mediated genetic transformation for *S. altissima* and *S. albida* plants. The initiation of root culture was observed on *S. altissima* and *S. albida* plant explants three weeks after transformation. The obtained roots were characterized by *Ri*-phenotype (such as rapid growth, hairness, and lack of geotropism) (Fig. 1). Confirmation of the transgenic nature of the

Fig. 1. **Initiation of hairy root formation in *S. altissima* and *S. albida* explants**

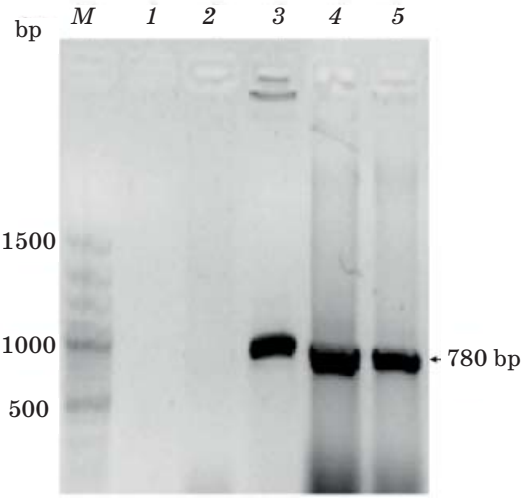

Fig. 2. **Electrophoregram of PCR analysis for the presence of rolB gene:**
M — Marker (1 kb Plus DNA Ladder, Fermentas); *1* — negative control (sample without DNA); *2* — negative control (DNA of a non-transformed plant); *3* — positive control (plasmid DNA (A4)); *4–5* — DNA of the analyzed samples of the “hairy” root culture

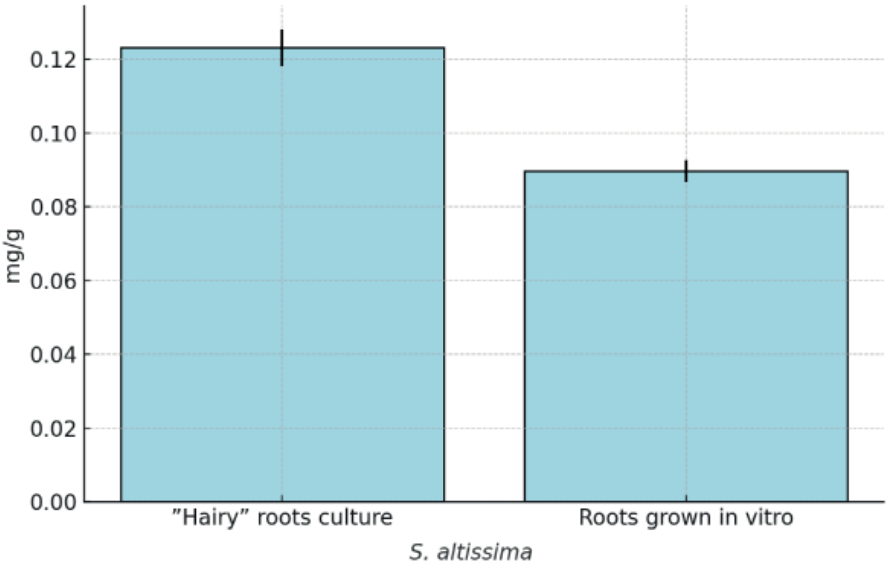

Fig. 3. **Flavonoid content in extracts of transgenic roots and roots of plants grown *in vitro***

obtained root cultures was carried out using the PCR analysis (Fig. 2). For this purpose, a 780 bp fragment of the *Agrobacterium rolB* gene was amplified. The presence of the desired fragment was confirmed for 80% of the analyzed cultures.

In order to analyze the content of flavonoids in the transgenic root and intact root cultures of *S. altissima* plants, the spectrophotometric method was used [3]. This way, the significantly higher flavonoid content was proved for the *Ri*-root culture compared to the roots of plants grown *in vitro* for *S. altissima* plants (Fig. 3).

Conclusions. The obtained data allow us to consider the transgenic root culture of *Scutellaria altissima* L. as a pharmacologically promising one as the high content of flavonoids and beneficial characteristics of cultivation characterized it.

Authors' contribution

Koshchavko K. — planning, data analysis, introduction of plants into *in vitro* culture, genetic transformation of the obtained plants, molecular biological analysis of the obtained cultures, analysis of the flavonoid content in the studied samples, writing article.

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