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Production of Bioactive Substances with Antiviral Activity in *Nitraria schoberi* Hairy Roots Culture

Tatiana ZHELEZNİCHENKO[1], Tatyana NOVİKOVA[1], Evgeny BANAEV[1], Sergey ASBAGANOV[1], Maria VORONKOVA[1], Tatiana KUKUSHKİNA[1], Natalia MAZURKOVA[2], Ekaterina FİLİPPOVA[2]
[1]Central Siberian Botanical Garden, SB RAS, Russia
[2]State Research Center of Virology and Biotechnology "Vector", Russia
zhelez05@mail.ru

Aims of the study were: A. rhizogenes-mediated transformation of *Nitraria schoberi* plantlet parts, obtaining intensively growing on hormone-free media hairy root cultures producing biologically active substances and subsequent evaluation of culture extracts for antiviral activity, in particular against the influenza virus.

Material and Methods: Hairy roots were obtained by agrobacterial transformation of sterile seedling hypocotyls and primary leaves using A. rhizogenes straines A4-RT, R-1601, 8196-RT, 15834 SWISS. After the transformation the explants were placed on agar-solidified BDS medium supplemented with cefotaxime. The developed hairy roots were transferred to hormone-free BDS liquid media. To prove the transformed nature of hairy root culture, PCR-analysis was performed with the use of primers detecting rol-genes, and to exclude possible contamination of agrobacterial DNA, the preparations were checked by amplification of the vir-gene sequence. Analysis of the secondary metabolite content of *N. schoberi* hairy roots was performed by spectrophotometric methods (Belikov, Shraiber, 1970; Ermakov et al., 1987; Kiseleva et al., 1991). The quantitative and qualitative compositions of catechins was studied using an analytical HPLC system consisting of an «Agilent 1200» chromatograph with a diode array detector and a ChemStation system. The roots of the intact five-year-old plant were used as a control. Cytotoxicity and antiviral activity against RNA-containing A influenza virus of H3N2 and H5N1 subtypes were determined in aqueous solutions of ethanol extracts on MDCK culture and laboratory mice.

Results: Hairy roots were formed when using all four strains of agrobacteria, however, the most effective were 8196-RT and 15834 SWISS. PCR analysis showed presence of rol-genes in hairy roots, which indicated the successful agrotransformation. As a result of the analysis, no agrobacterial DNA contamination of the samples was detected. Biochemical study showed that the content of flavonols, tannins, catechins, pectins, protopectins, and saponins in the genetically modified roots was considerably higher than that of the same components in the control. In the extracts of the hairy roots, 13 compounds of the catechin nature were found. Gallic acid, ± catechin, and L-epicatechin were identified. It was found that aqueous solutions of plant extracts under study in a concentration of ≤ 0.01 mg / ml did not cause toxic effects on the cell culture of MDCK. The aqueous solution of the ethanol extract of *N. schoberi* hairy roots was shown to have antiviral activity against the RNA genome virus of human A influenza of H3N2 subtype and the highly pathogenic avian influenza virus of H5N1 subtype at a concentration of no less than 0.01 mg/ml in the cell culture of MDCK and 1.0 mg/ml in the model of experimental influenza infection in mice. Thus, the data obtained favour the prospect of further study of *N. schoberi* hairy roots.

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