ABSTRACT

The Effect of Abiotic Elicitors on the Content of Secondary Metabolites in In vitro Plant Cultures – II.

The method of elicitation is used for the increased production of secondary metabolites in *in vitro* plant cultures. In this study, there the effect of pyridine derivate *N*-(5-chlorpyridin-2-yl)-4-ethylbenzamide in three concentration - $3.845.10^{-3}$ mol/l; $3.845.10^{-4}$ mol/l and $3.845.10^{-5}$ mol/l for the production of flavonolignans in callus and suspension cultures of *Silybum marianum* was tested. The evaluation of elicitation effect proceeded after 6, 24, 48, 72 a 168 hours against control samples without the elicitor treatment after 24 and 168 hours. In the same intervals the samples of culture medium were taken and assessed. The cells were cultivated on Murashige and Skoog medium with 10 mg/ml of α-naphtylacetic acid as growth regulator. The obtained and adjusted samples were analysed by the HPLC method. From the parts of silymarin complex were detected silychristin, silybin A, isosylibin A, isosilybin B. Flavonoid taxifolin wasn' t detected in any case. In callus culture, there was achieved a maximal production of all detected components of silymarin complex (30.508 μg/g DW) after 168 hours of elicitor treatment in concentration of $3.845.10^{-4}$ mol/l, it was about only substance – silychristin. The concentrations of elicitor $3.845.10^{-3}$ mol/l and $3.845.10^{-4}$ mol/l had a positive influence for the production of individual parts of silymarin complex in callus culture in 50 % of cases, the concentration $3.845.10^{-5}$ mol/l increased their production in less cases. After the contents results of detected parts of silymarin complex, there was found its most pronounced increasing after 24 hours of elicitor application in concentration of $3.845.10^{-5}$ mol/l, it was 73.426 μg/g DW (51.915 μg/g DW silychristin + 21.511 μg/g DW isosilybin A) in the suspension culture. The concentrations of elicitor $3.845.10^{-3}$ mol/l and $3.845.10^{-4}$ mol/l made again statisticaly significant increased production of silymarin complex substances in half cases. The elicitor in the concentration of $3.845.10^{-5}$ mol/l positive influenced a small part of samples. It was monitored a release of components of silymarin complex into nutrient medium. In the medium of cultures without elicitor treatment, there wasn' t detected the present of this one. In other samples there were detected mostly in all cases at least trace amount of some components of silymarin complex.

Key words: abiotic elicitation, explant culture, *Silybum marianum*, silymarin complex, pyridine derivate, *N*-(5-chlorpyridin-2-yl)-4-ethylbenzamide