

Medicinal plant cultures in vitro – XVI

The subject of this diploma thesis is the evaluation of secondary metabolites production in *Silybum marianum*, (L.) Gaertn. cultures *in vitro* after elicitor treatment. The aim of the study was to find if an abiotic elicitor 5-*tert*-butyl-*N*-(4-chlorobenzyl)pyrazine-2-carboxamide increases the flavonolignan production *Silybum marianum* cultures *in vitro*. Experiment was carried out in callus and suspension cultures of *Silybum marianum* using Murashige – Skoog nutrient medium supplemented with 10 mg/l α -naphthylacetic acid. The elicitor was added in the form of solution in three different concentrations ($C_1 = 3.292 \cdot 10^{-3}$ mol/l, $C_2 = 3.292 \cdot 10^{-4}$ mol/l and $C_3 = 3.292 \cdot 10^{-5}$ mol/l) and it was affecting 6, 12, 24, 48, 72 and 168 hours. The content of flavonolignans was determined by HPLC. The maximum flavonolignan production (0.280 mg.g⁻¹ DW) in callus culture was observed after 24 hours of elicitor application in concentration of $C_2 = 3.292 \cdot 10^{-4}$ mol/l, when the highest content of silychristin was detected. The second significant increase in flavonolignan production (0.271 mg.g⁻¹ DW) in callus culture was noticeable after 12 hours of elicitor treatment in concentration of $C_3 = 3.292 \cdot 10^{-5}$ mol/l, when the highest increase in silydianin and silybin B accumulation was found. The maximum content of flavonolignan (1.871 mg.g⁻¹ DW) in suspension culture was detected after 48 hours of elicitor treatment in concentration of $C_2 = 3.292 \cdot 10^{-4}$ mol/l, when the maximum production of silychristin and silydianin was observed. Flavonolignan release into the nutrient medium was also detected. In the case of callus cultures, the silydianin release was obvious especially after 168 hours of elicitor addition in concentration of $C_2 = 3.292 \cdot 10^{-4}$ mol/l and silybin A after 24 hours of elicitor treatment in concentration of $C_1 = 3.292 \cdot 10^{-3}$ mol/l. The most significant increase of flavonolignan release from suspension culture into nutrient medium was found after 12 hours of elicitor treatment in concentration of $C_3 = 3.292 \cdot 10^{-5}$ mol/l, when the presence of silychristin, silydianin and silybin A was detected.