

Abstract

Elicitation is one of possible methods for increasing the secondary metabolites production in plant cell cultures. This paper explores the potential effect of methylviologene on isoflavonoids production in *Genista tinctoria* L. suspension and callus cultures.

Schenk and Hildebrandt nutrient medium was used with the addition of growth regulators 2,4-D in concentration of 5 mg/L and kinetin of 1 mg /L of medium. Elicitor was added in the form of ethanol solution at concentration of $2.19 \cdot 10^{-2} \text{ mol l}^{-1}$, $2.19 \cdot 10^{-3} \text{ mol l}^{-1}$, $2.19 \cdot 10^{-4} \text{ mol l}^{-1}$. Samples were taken after 6, 12, 24, 48, 72 and 168 hours of elicitor exposure. Control samples were collected at 6, 24, 72 and 168 hours. The isoflavonoid content in suspension and callus cultures and in nutrient medium was evaluated by high performance liquid chromatography.

The biggest increase of isoflavonoid content in callus culture was reached in daidzein content after 48 hours elicitor treatment in concentration of $2.19 \cdot 10^{-3} \text{ mol l}^{-1}$ (8.5 mg/g DW) and $2.19 \cdot 10^{-4} \text{ mol l}^{-1}$ (1.6 mg/g DW). The production of genistin, genistein and formononetin was slightly increased or zero compared to controls. Biochanin A was almost absent in the samples.

The highest level of isoflavonoids was measured in the suspension culture after 48 hours elicitor application in the concentration of $2.19 \cdot 10^{-4} \text{ mol l}^{-1}$ (25.6 mg/g DW). In the suspension culture there the highest genistein production was observed after 48 hours of methylviologene application in concentration of $2.19 \cdot 10^{-3} \text{ mol l}^{-1}$ (22.8 mg/g DW) and $2.19 \cdot 10^{-4} \text{ mol l}^{-1}$ (21.1 mg /g DW). Furthermore, the suspension culture produced daidzein after 24 hours of elicitor addition in concentration of $2.19 \cdot 10^{-4} \text{ mol l}^{-1}$ (6.4 mg/g DW). Genistein and formononetin was also detected, but not biochanin A.

Both callus and suspension culture released genistin and daidzein into the medium. The highest content of genistin and daidzeine was excreted in to the medium after 168 hours of elicitor application in concentration of $2.19 \cdot 10^{-3} \text{ mol l}^{-1}$ (0.43 and 0.02 mg /100 ml) by suspension culture.

Methylviologene elicitation could be recommended for increasing production of secondary metabolites in *G. tinctoria* callus and suspension cultures.