

Grindelic Acid Production in *Grindelia Pulchella* Cell Suspension Cultures Elicited with CuSO_4

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Introduction

Abiotic elicitors affect both, biomass and secondary metabolite production in cell suspension cultures. In this work we have studied CuSO_4 effect on the accumulation of grindelic acid in *Grindelia pulchella* cell suspension cultures.

Material and Methods

Cell suspension cultures

MS media supplemented with indolbutiric acid and bencilamine purine was employed. Samples of 20 ml were taken, after filtration biomass dry weight was evaluated.

Grindelic acid production evaluation

Liquid media was acidified to pH 5.00 with HCl 10% and submitted to liquid-liquid extraction procedure with Et_2O (x3). Cells were extracted by reflux in MeOH 4h (x3). The methanolic extract was dried, recovered with distilled acidified water (pH 5.00) and extracted with Et_2O (x3). Samples were methylated with CH_3N_2 and evaluated by GC. Each assay was repeated three times.

Elicitation with CuSO_4

CuSO_4 (final concentration 1 y 2 mM sterilized by filtration) was added at day 7.

Results and Discussion

Cu SO_4 addition in both concentrations inhibited the biomass production. This effect may be attributed to the fact that heavy metals (Cu or Cd) cause an increment in the catabolic activity or suppress the lipid biosynthesis.

Grindelic acid accumulation, in cultures elicited with CuSO_4 (1mM), was completely inhibited. On the other hand the addition of CuSO_4 (2mM) induced a grindelic acid accumulation dismissing at the early times of the cell cycle but increased the production in the stationary phase.

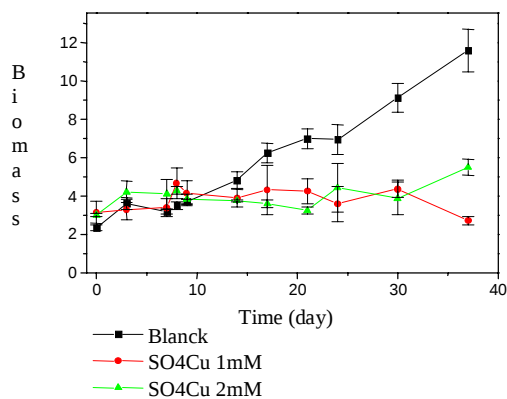


Figure 1. Cu SO_4 effect on biomass production [Biomass (g dry weight/ml)].

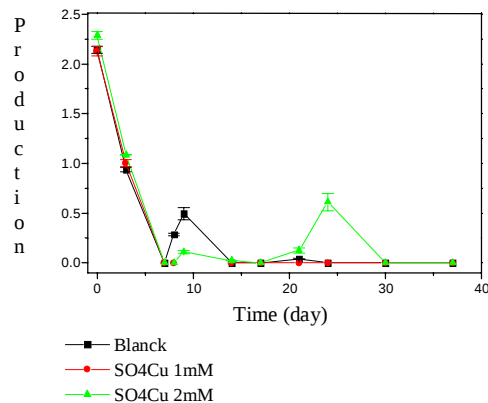


Figure 2. CuSO_4 effect on grindelic acid accumulation [Production (mg/ g cell)].

References and Notes

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