Effect of Magnesium on the Growth and Alkaloid Production of Hairy Root Cultures

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Abstract

In vitro cultures of *Atropa belladonna* L. (Solanaceae) cultivated on a half dilution MS (Murashige and Skoog) medium containing 3% sucrose, was infected by microinjection with *Agrobacterium rhizogenes* strain R1601. The hairy roots formed within 2 months were isolated, subcultured on B_5 (Gamborg) medium and selected from the point of view of growth and tropane alkaloid production. Determination of alkaloids was performed by HPLC methods. Atropine, scopolamine and 6-OH-hyoscyamine were identified and measured by this technique. We have studied the effect of $MgSO_4$ on the growth and alkaloid production of genetically modified hairy roots as well.

INTRODUCTION

Atropa belladonna L. is a perennial plant, native to Central and Southern Europe and cultivated worldwide. It contains tropane alkaloids, possessing anticholinergic activity. The plant is used in stomach mixtures and powders as a sedative, in bronchial conditions as an antispasmodic, and for colds and fevers to reduce nasal secretions.

The application of genetically modified hairy root cultures obtained by different biotechnological methods has several advantages. These cultures are genetically stable, have stable universal and specific metabolism, unlimited growth on media without plant growth regulators and their metabolite production reaches that of the corresponding plant or may exceed it in some cases. Moreover, the administration of biotechnological methods gives the opportunity (e.g. through suspensions cultures) to select only one cell by which further increase in secondary metabolite production would be possible. Different types of cell and tissue culture can be transformed into each other and it is possible for example to obtain an organized plant from hairy root culture.

In vitro cultures of *A. belladonna* were infected by microinjection of *Agrobacterium rhizogenes* strain R1601. 10 hairy root clones have been isolated and selected from the point of view of growth and alkaloid production. Determination of alkaloids (e.g. atropine and scopolamine) in the culture material was performed using HPLC methods.

Previous results indicated the positive effects of elevated magnesium concentrations on the growth and metabolite production of different tissue cultures of Solanaceae, and therefore we have investigated these effects on genetically modified *A. belladonna* tissues (Bálványos et al. 1998).

MATERIALS AND METHODS

Cultivation

An in vitro culture of *Atropa belladonna* cultivated on a half dilution MS medium with 3% sucrose, was infected by microinjection of *Agrobacterium rhizogenes* strain R1601. 10 hairy root clones were isolated and cultured in solid MS medium containing antibiotics (cefotaxim 250 mg/L and ampicillin 1000 mg/L). After the elimination of bacteria, the clones were transferred to antibiotics free solid B5 media supplemented with

2% sucrose. Hairy roots were cultivated in B5 liquid medium to increase the alkaloid production.

The effects of $MgSO_4$ on the alkaloid production of hairy roots (clone K8 and K9) were studied (Bálványos et al. 2001). The cultures were grown in B5 liquid media (in the dark) containing 0,125,250,500,1000 mg/L $MgSO_4$. Fresh and dry weight was measured at the end of the cultivation period (4 weeks). For the determination of dry weight, cultures were freeze dried.

Sample Preparation

Lyophilized and powered hairy root samples (1000 mg) were extracted with chloroform: methanol: 25% ammonium hydroxide (15: 5: 1, v/v/v) for 3x10 minutes using an ultrasound device (Braun Labsonic U). The filtration and unification of the fractions were followed by evaporation to dryness**.** The residues were made up in 10 mL chloroform and extracted with 0.2 M H_2SO_4 (3×15 mL). Combined acidic fractions were alkalized (pH 9) and were extracted again with chloroform (3×15 mL). Finally the organic fractions were concentrated to dryness under vacuum.

HPLC Analysis of Tropane Alkaloids

The evaporated samples were redissolved in an appropriate volume of acetonitrile: 0.1% trifluoracetic acid (2:4, v/v). The HPLC system used consisted of a Spectra Physics P4000 quaternary gradient pump, a FOCUS scanning UV-VIS detector in combination with a Rheodyne 7125 injector. HPLC separation of tropane alkaloids was performed on a LUNA octyl column (C_8, Phenomenex) (250 x 4.6 mm i.d., 5 μm particle size). An isocratic mixture of acetonitrile: methanol: 0.1% trifluoracetic acid (12.5 : 8.5 : 78.8, v/v/v) was used as eluent at a flow rate of 1 mL/min. Hyoscyamine, scopolamine, and 6β-hydroxyhyoscyamine were identified by addition of authentic standards and by diode-array detection. Hyoscyamine and scopolamine in the range of 16-80 μg/mL were determined by an external standard method at 210 nm (Kursinszki et al. 2000).

RESULTS AND DISCUSSION

The best of the growing hairy root clones (K9, K8) were selected and cultivated further on B5 liquid media. To achieve higher biomass and tropane alkaloid production the effect of magnesium sulfate on the hairy roots has been studied. The fresh and dry weights were measured at the end of the cultivation period. The highest biomass production was observed on media supplemented with 500 mg/L $MgSO_4$ with the clone K9 (fw.: 4,18g; dw.:0,23g) and on media supplemented with 125 mg/L $MgSO_4$ with K8 hairy root culture (fw.:13,63g; dw.: 0,73g) (Fig.1), which were not significantly different from the biomass of clones growing on media containing 250 mg/L (control) or 1000 mg/L $MgSO_4$. The most disadvantageous was the administration of the lowest quantity of $MgSO_4$.

Analyzing the alkaloid production of the clones, hyoscyamine, scopolamine and 6-OH-hyoscyamine were identified. The maximum hyoscyamine content was measured in K8 and K9 clones on media containing 125 mg/L $MgSO_4$. The maximum scopolamine content was measured in clones K8 and K9 on media containing 1000 mg/L and 125 mg/L $MgSO_4$ concentrations, respectively (Fig.2).

Literature Cited

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Figures

Fig. 1. *Atropa belladonna* hairy root clone #K8 cultivated in the dark on Gamborg B5 solid medium

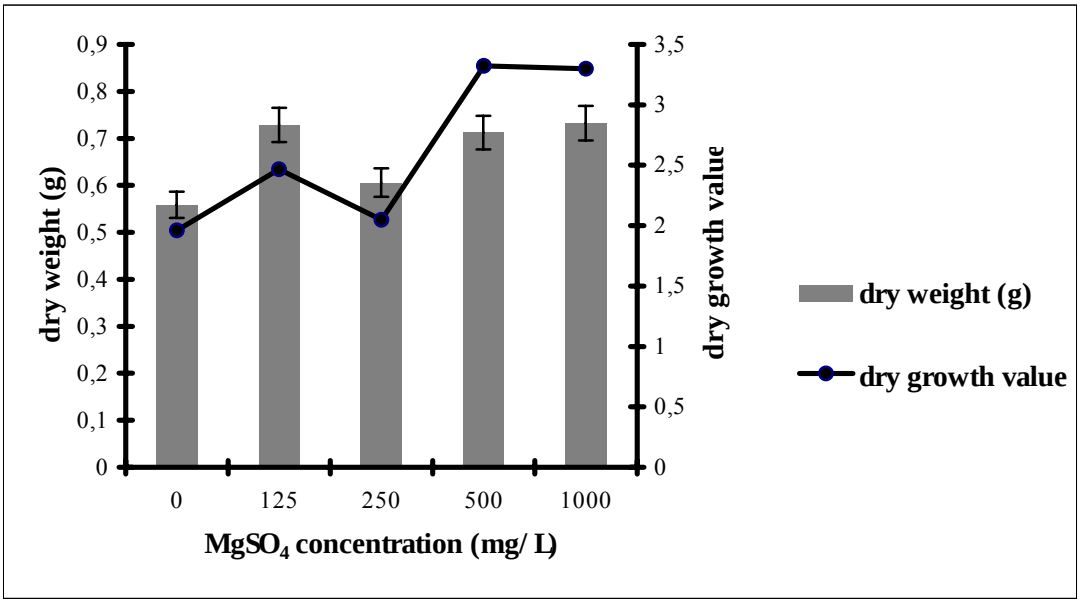

Fig. 2. Dry weight of hairy root culture #K8 cultivated on B5 liquid medium supplemented with $MgSO_4$ (0-1000 mg/L)

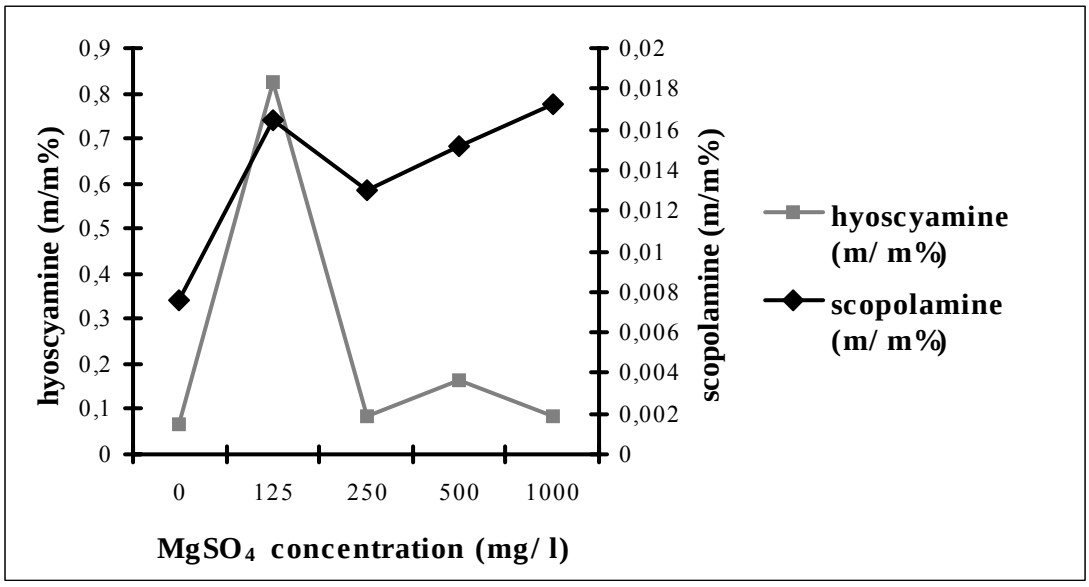

Fig. 3. Alkaloid content (atropine and scopolamine, m/m%) of hairy root culture #K8 cultivated on B5 liquid medium supplemented with $MgSO_4$ (0-1000 mg/L)