EFFECT OF L-DOPA AND L- METHIONINE SUPPLEMENTATION ON BIOPRODUCTION OF EMENTINE IN CALLUS CULTURES OF CEPHAELIS IPECACUANHA

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ABSTRACT: Callus cultures were induced from leaf segments of cephaelis ipecacuanha on modified gamborg medium containing 2,4 – dichlorophenoxy acetic acid (2mg/l) Indole acetic acid (1mg/l), kinetin (1mg/l) and sucrose (20g/l). The biogenetic profile for alkaloids was established. The established callus cultures were transferred to modified gamborg medium supplemented with L- DOPA (40 mg/l) and L methionine (3mg/l). After four weeks of their growth, the biomass and medium were extracted for emetine alkaloid and analysed by HPLC. The bioproduction of emetine was found to be extracellular in nature. The emetine content had increased to 0.587% mg FW as compared to control cultured (0.0245% mg FW).

INTRODUCTION

Cephaelis ipecacuanha (Family: Rubiaceae) known as lpecac, the roots of which are used as an expectorant, emetic and amoebicide, contains emetine, cephaeline psychotrine, O-methyl psychotrine, emetamine, ipecoside and ipecacuanhin (1).

An exogenous supply of a biosynthetic precursor to the culture medium may increase the final yield of alkaloid content. Tabata et al (2) reported that the addition of tropic acid increased the alkaloid content in scopolia and Datura callus cultures. Sairam and Khanna (3) observed the effect of L-phenylalnine and tyrosine on growth and total alkaloid production in seed callus tissue of Datura tatula. Zenk et al (4) reported that the production of anthraquinone glycosides increased two folds by the supply of precursor o-succinyl benzoic acid in cell cultures of Morinda citrifolia. Veeresham etal (5) & (6) reported the influence of 1 phenylalanine, shilimic acid, and DL-tryptophan supplementation on synthesis of isoquinoline alkaloids in callus culture of C. ipecacuanha and L- tyrosine supplementation on bioproduction of emetic alkaloids in static cultures of C. ipecacuanha. In this communication we report influence of L-DOPA and L.methionine supplementation on bioproduction of emetic alkaloids in static cultures of C. ipecacuanha.

METHODS

The callus cultures were initiated on modified Gamborg medium from leaf segments on C. ipecacuanha. Leaf segments of ipecac were cultured on modified Gamborg medium supplemented with 2,4-dichloro phenoxy acetic acid (mg/l). Indole acetic acid (1mg/l), kinetin (1mg/l) and sucrose (20g/l). The cultures were allowed to grow in the dark at 27o ±2oC for 4 weeks. The callus was subcultured onto the same medium after 4 weeks. The established biomass was then transferred onto modified
Gamborg medium supplemented with L-DOPA (40mg/1) and L-methionine (3mg/1) and allowed to grow at 27°C +2°C for a weeks. The control callus cultures were also run simultaneously. At the end of 4th week, both medium and callus were used for extraction of emetic alkaloids.

The alkaloids were extracted from 4 weeks old callus grown on L-DOPA and L-methionine supplemented medium and the medium itself. The nutrient medium (20ml) was treated with 30ml of 10% ammonia solution. It was then extracted thrice with 50ml of solvent ether. The combined ethereal extracts were concentrated in vacuo and the residue was weighed and dissolved in 1ml of HPLC grade methanol and subjected to TLC and HPLC analysis.

The extracts obtained from cultures were applied on silica gel-H plates and the mobile phase used was chloroform-methanol 10% ammonia solution in the ratio of 100:10:1 the alkaloids were detected under UV light and dragendorff’s reagent (7).

The extracts were analysed quantitatively by HPLC using shimadzu liquid chromatography (Model SCL- 6A) equipped with a variable wave length shimadzu UV spectrophotometer (Model SPC- 6AV). Analysis was carried out using a hypersil silica gel column (4mm i.d x 15 cm) with chloroform- methanol triethylamine (273:27:0.05) as the mobile phase and maintaining the absorbance at285 nm. The flow rate was 1ml/min throughout the analysis. The authentic samples of emetine and cephaeline were run with retention times of 3.362 and 4.015 minutes respectively. Emetine and cephaeline were quantitatively analysed by the methods adopted by veeresham et al (6). The percentage of alkaloids in biomass and medium was calculated using this standard graph.

RESULT AND DISCUSSION

The investigations were aimed at observing the influence of L-DOPA and L-methionine supplementation on bioproduction of emetic alkaloids. The thin layer chromatographic investigations on silica gel H pates with solvent system of chloroform – methanol – 10% ammonia solution (100:10:1) under UV light and spraying with dragendorff’ s reagent revealed the presence of emetic alkaloids (cephaeline Rf = 0.18 and emetine Rf= 0.38). The observation pertaining to influence of L – DOPA and L- methionine on bioproduction of emetic alkaloids as estimated by HPLC are recorded in Table I.

The exogenous feeding of medium with L-DOPA (40mg/1) and L- methionine (3mg/1) did not influence bioproduction of cephaeline. However the extract derived from medium was found to be rich in emetine (0.587 ± 0.007). The emetine content of L- DOPA and L- methionine – fed medium was over 24 fold, as compared to that of control group. (0.0245 ± 0.002 % mg) Zenk et al (4) reported increased production of indole alkaloids in cell cultures of Catharanthus roseus due to supplementation of DL-tryptophan. Sairam and Khanna (3) reported the identical results due to supplementation of L- phenylalanine and tyrosine in Datura tatula seed callus cultures.

The overall in vitro increased bioproduction of emetine on supplementation of L-DOPA and L-methionine might be due to positive role of L-DOPA and L- methionine in biogenesis of alkaloids, as emetine is synthesized from shikimic acid pathway which generates dopamine, which inturn combines with secologanin and results in production of emetine (8). The bioproduction of emetine under the influence of L-DOPA and L-methionine was
mostly extracellular in nature. Accumulation of alkaloids was found only in the nutrient medium. However, concentration of emetine detected was less as compared to the root powder (9). It is important that the supplementation of L-DOPA and L-methionine enhanced only the bioproduction of emetine, but not cephaeline.

**Table I**

Effect of L-DOPA and L-Methionine supplementation on production of isoquinoline alkaloids in callus cultures of C. ipecacuanha four weeks after supplementation

|                      | Cephaeline %mg | Emetine % mg FW |
|----------------------|---------------|-----------------|
|                      | Medium | Callus       | Medium | Callus       |
| L-DOPA and L-Methionine Control | Not detected | Not detected | 0.587 ± 0.007 | Not detected |
|                        | 0.082 ± 0.005 | 0.016 ± 0.003 | 0.0245 ± 0.002 | 0.0018 ± 0.0002 |
| Root powder           | 0.696 ± 0.024 | -              | 1.392 ± 0.022 | -              |

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