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EFFECT OF AMINO ACIDS ON EPHEDRINE PRODUCTION IN *EPHEDRA GERARDIANA* CALLUS CULTURES

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Key Word Index—*Ephedra gerardiana*; Gnetales; plant tissue culture; precursor amino acids; IBA; ephedrine.

INTRODUCTION

Exogenenous amino acids have proved useful in improving the yield of some cell products, particularly alkaloids. Effects of precursor amino acids have been reported in *Datura* [1–3], *Scopolia* [2], *Phaseolus vulgaris* [4] and *Lithospermum* [5] cultures. The addition of phenylalanine to the cultures of *Datura* [3] and *Lithospermum* [5] caused a two to three-fold increase in the amounts of secondary metabolites whereas the use of tryptophan in *Phaseolus vulgaris* [4] cell suspensions produced two new alkaloids, harman and norharman.

Results obtained from previous studies indicated that *Ephedra gerardiana* produced ephedrine in callus cultures while *E. foliata* was devoid of it [6]. Studies with *E. gerardiana* callus tissues showed that indole butyric acid (IBA) was the best auxin for ephedrine production amongst the growth regulators used [7].

In this note we describe the effects of some precursor amino acids (phenylalanine, methionine and glycine) used with NAA or IBA in Murashige and Skoogs (MS) medium [8]. Tissues grown on media supplemented with aspartic acid, serine or leucine could not be analysed due to poor growth or complete failure of tissues to grow on such media.

RESULTS AND DISCUSSION

Effect of amino acids in combination with NAA

Results obtained with precursor amino acids on ephedrine yield in *E. gerardiana* callus tissue are shown in Table 1. Maximum yield of ephedrine was recorded in the callus tissues grown on MS medium supplemented with 0.1 g/l. L-phenylalanine. Moderately high ephedrine contents were recorded with L-phenylalanine (0.4 g/l.), DL-methionine (0.1 and 0.4 g/l.) and glycine (0.1 g/l.), respectively. Tissues grew well on such treatments and growth of tissues did not correlate with ephedrine production.

Effects of amino acids in combination with IBA

On the basis of experience obtained with previous results, the optimal concentration of IBA (10 mg/l.) was used in place of NAA (10 mg/l.) and various amino acids were incorporated in the medium. The results obtained are shown in Table 2. A synergistic effect of IBA and L-phenylalanine and DL-methionine was observed on the yield of ephedrine. Maximum yield of ephedrine was obtained from tissues grown on media supplemented with IBA (10 mg/l.) and phenylalanine (0.1 g/l.). NAA

Table 1. Effect of precursor amino acids on ephedrine production in *E. gerardiana* callus tissues at 8-weeks growth in 16 hr light (1000 lx) at 26 ± 2°

MS + amino acid	Concn g/l. medium	Dry wt/culture in mg	% Ephedrine
*MS + L-phenylalanine	0.1	250	0.50
	0.4	230	0.40
MS + DL-methionine	0.1	230	0.43
	0.4	210	0.35
MS + glycine	0.1	225	0.30
	0.4	220	0.21
MS + serine	0.1	60	—
MS + aspartic acid	0.1	50	—
MS alone	—	190	0.17

* MS = Medium with kinetin (0.5 mg/l.) and NAA (10 mg/l.).

Table 2. Synergistic effect of IBA and amino acids on ephedrine production in *E. gerardiana* callus tissues at 8-weeks growth in 16 hr light (1000 lx) at 26 ± 2°.

Medium		Dry wt/culture mg	% Ephedrine
*BM alone		195	0.30
BM + phenylalanine	0·1 g/l.	250	0.60
	0.4 g/l.	225	0.45
BM + methionine	0.1 g/l.	230	0.40
	0.4 g/l.	230	0.40
BM + glycine	0.1 g/l.	225	0.30
	0.4 g/l.	225	0.30

* BM = Medium with kinetin 0.5 mg/l. and IBA 10 mg/l.

and IBA proved equally effective in stimulating growth (Tables 1 and 2).

Phenylalanine, methionine and glycine (0.1–0.4 g/l.) increased the alkaloid yield in *E. gerardiana* callus tissues. As precursors, these amino acids play an important in ephedrine biosynthesis [9]. Two possible pathways have been suggested [9] for ephedrine synthesis in plant tissues. Phenylalanine is directly incorporated into the nitrogen of ephedrine and showed a profound effect on cultured tissues grown on phenylalanine supplemented medium. Furthermore, methionine and glycine are partially incorporated in the biosynthesis of ephedrine. This may be the reason why these amino acids increased ephedrine production markedly in cultured *E. gerardiana* tissues.

The effects of various precursor amino acids in increasing yield of metabolites have been reported in several cultured tissues [10]. Both phenylalanine and tyrosine increased the total alkaloid contents of *Datura tatula* [3]. Recently, it has also been reported that L-phenylalanine stimulated shikonin derivatives markedly (more than 3 times over control) when added to culture media during the 4th week of culture [5]. It may be that when the levels of auxin and nitrogen are high, phenylalanine, methionine and glycine are converted to proteins. However, after auxin and nitrogen are exhausted, and protein synthesis has ceased, these amino acids are used for ephedrine synthesis [11].

The results obtained with *E. gerardiana* tissues are supported by those obtained with *Lithosphermum* callus cultures [5]. Our results and those obtained with *Lithospermum* showed that biosynthesis of ephedrine and shikonin derivatives proceeds when protein synthesis in cultured cells has ceased. The carbon to nitrogen ratio in cells may be an important regulatory factor for the production of these metabolites. Similar results were also obtained for tannin synthesis in *Acer* suspension cultures [12].

A synergistic effect of IBA and precursor amino acids on alkaloid production has been observed with *E. gerardiana* tissues. The superiority of IBA over NAA in alkaloid synthesis is well established yet the explanation is still to be found. The inability of glycine to further increase the alkaloid yield may be due to the fact that it supports the synthesis of proteins [11] and is a less effective precursor of ephedrine [9].

As a result of experimentation, a fairly high ephedrine (0.6 %) callus strain has been developed after experimental manipulation which could be used as a natural source for commercial production of ephedrine. The callus strain may also be differentiated to produce better plant germplasm with high capability to produce ephedrine.

EXPERIMENTAL

Callus culture. Seeds of *E. gerardiana* Wall. were collected from Leh (Jammu and Kashmir). Callus tissues were raised and maintained on MS medium [8] as described earlier [7]. L-Phenylalanine, DL-methionine and glycine (0.1 and 0.4 g/l.) were incorporated in MS medium (pH 6), before autoclaving it at 1.4 kg/cm^2 for 20 min.

Extraction of ephederine as described earlier [7] by the method of ref. [9].

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