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Short Communication

Stimulation of reserpine biosynthesis in the callus of *Rauvolfia tetraphylla* L. by precursor feeding

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Reserpine is an important indole alkaloid that is used to treat hypertension and various psychiatric diseases by acting as a tranquilizing agent. In pharmaceutical industries, reserpine is in great demand. Chemical synthesis of reserpine is costlier than extracting it from natural resources. So enhancing this alkaloid in the already available system is a beneficial approach. Tryptophan is the starting material in the biosynthesis of reserpine. Callus was induced from leaf explants of *Rauvolfia tetraphylla* L. on MS medium supplemented with the combination of 9 µM 2,4- D and 25, 50, 75 and 100 mg/l tryptophan. An increase in the reserpine content was observed at 50 mg/l tryptophan than in other concentrations.

Key words: Callus, MS medium, *Rauvolfia tetraphylla* L., reserpine, tryptophan.

INTRODUCTION

Rauvolfia tetraphylla L. is an endangered woody shrub belongs to the family Apocynaceae. The plant is medicinally important in the treatment of cardiovascular diseases, hypertension and various psychiatric diseases (Faisal and Anis, 2002). The plant is reported to contain various indole group alkaloids that are localized in the root. Among these alkaloids, reserpine, a 36 carbon compound is present in high amount. Reports indicate that the isolated reserpine possesses thousand times better hypotensive activity than the crude extract of the plant and it is one of the natural tranquilizers (Pullaiah, 2002). *Rauvolfia* roots are reported to contain 0.7-3% of total alkaloids in the dry mass and the amount varies depending upon the time and source of collection (Kokate et al., 1998).

Even though the chemical synthesis of reserpine is possible, it costs more than extracting it from natural resources (Farooqi and Sreeramu, 2001). Tryptophan is the starting material in the biosynthetic pathway of reserpine, and is converted to tryptamine by tryptophan decarboxylase enzyme. Tryptamine is combined with secologanin in the presence of strictosidine synthetase enzyme and yields strictosidine. Various enzymatic conversion reactions lead to the synthesis of reserpine from strictosidine (Ramawat et al, 1999). For enhancing the secondary metabolite production of the cultured cells, there is a possibility of feeding the precursor or one of the intermediates in the metabolic pathway for its subsequent conversion to derive final product. Reports are available on the enhancement of economically or medicinally important secondary metabolite production by feeding with a precursor. For example, geraniol and pinine enhanced the production of the anticancer compound taxol in *Taxus buccata* suspension culture (Hirasuna et al., 1996), and phenylalanine increased the alkaloid content in the cultures of *Capsicum frutescens* (Yeoman et al., 1990).

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MATERIALS AND METHODS

Callus induction

Leaf explants of field grown plants were collected and surface sterilized with 0.1% (w/v) mercuric chloride for 4 min. The sterile explants were grown on callus induction medium consisting MS (Murashige and Skoog, 1962) salts with B_5 (Gamborg et al., 1968) vitamins, 9 µM 2,4-D, 40 g/l sucrose and 8 g/l agar. The medium pH was adjusted to 5.7 and sterilized at 15 lb for 15 min. The cultures were exposed to 16/8 h light/dark condition by using cool white fluorescent tubes (40 µM $m^{-2}s^{-1}$). The effect of tryptophan on callus induction, growth and alkaloid accumulation was determined by adding 25, 50, 75 and 100 mg/l tryptophan in the callus induction medium.

Table 1. Effect of tryptophan on callus induction and growth in *Rauvolfia tetraphylla* L.

Treatment	Percentage of callus induction	Percentage of callus growth
2,4-D 9 M (Control)	95	100
2,4-D+ tryptophan		
9 M +25 mg/l	90	90
9 M +50 mg/l	78	85
9 M +75 mg/l	23	48
9 M +100 mg/l	-	-

Table 2. Effect of tryptophan (trp) on moisture content and growth value in the callus of *Rauvolfia tetraphylla* L.

Treatment	F. Wt. of the callus (g)	D. Wt. of the callus (g)	Moisture content (%)	Growth value (%)
Control (9 M 2,4-D)	0.3354	0.0826	75.37	46
9 M 2,4-D+25 mg/l trp	0.3128	0.0825	73.62	34
9 M 2,4-D+50 mg/l trp	0.2955	0.0820	72.25	25
9 M 2,4-D +75 mg/l trp	0.1554	0.0581	62.61	11

Table 3. Effect of tryptophan (trp) on crude alkaloid and reserpine content in the callus of *Rauvolfia tetraphylla* L.

Treatment	Crude alkaloid content (mg / g D. Wt.)	Reserpine content (mg/g D. Wt.)
Reserpine (standard)	-	0.1 mg –3.0 mg/ml
Control (9 M 2,4-D)	5.0	0.9
9 M 2,4-D+25 mg/l trp	6.2	1.7
9 M 2,4-D+50 mg/l trp	7.1	2.1
9 M 2,4-D +75 mg/l trp	3.1	0.3

Alkaloid extraction and analysis

The dried callus was extracted with methanol, acidified with 0.1 N HCl and then neutralized with NH_4OH (Sheludo et al., 1998) . The content was evaporated under vacuum and the weight was recorded as crude alkaloid content. Crude alkaloid was dissolved in few drops of methanol and subjected to TLC and reverse phase HPLC analysis by using reserpine (SRL products, India) as standard. Reserpine content in the crude extract was calculated and tabulated.

RESULTS AND DISCUSSION

Callus was induced from leaf explants of *Rauvolfia tetraphylla* L. on MS medium supplemented with the combination of 9 µM 2,4-D and 25, 50, 75 and 100 mg/l tryptophan (Tables 1 and 2). Enhancement of alkaloid content was noticed with the addition of tryptophan in the callus induction medium. An increase in the reserpine content was observed at 50 mg/l tryptophan than in other concentrations (Table 3). Cost of precursor relative to that of product, inability of the cultures for the precursor uptake, toxic nature of the precursor at the concentration levels used for feeding and the possibility of exogenous precursor for being diverted to pathways other than the desired product are the limiting factors in this approach (Pars, 1992). This has been supported by various reports that the utilization of tryptamine for alkaloid biosynthesis enhances metabolic flux through the indole pathway in *Catharanthus roseus* (Whitmer et al., 1998) . Zenk et al. (1977) also reported that exogenous tryptophan increased the tryptamine content and it has also been reported to cause a nearly 3-fold increase in alkaloid production in one cell line. The enzymes, tryptophan decarboxylase (tdc) and strictosidine synthetase (sss) have been reported to control tryptamine and secologanin metabolism involved in *Catharanthus* alkaloids (Pasquali et al., 1992). In contrast, Goddijn et al. (1995) reported that in *Catharanthus roseus* crown gall callus, overexpression of tdc cDNA occurred that resulted in increased tdc activity, protein and tryptamine content but no significant increase in terpenoid indole alkaloid. Addition of secologanin increased the alkaloid ajmaline production ten folds in *Rauvolfia serpentina* (Merillon et al., 1986). Vanadyl sulphate also increased the production of the alkaloids catharanthine, serpentine and tryptamine in the suspension cultures of *Catharanthus roseus* (Tallevi and DiCosmo, 1988).

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