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In-Vitro Platlet Regeneration In *Rauwolfia Serpentina*

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ABSTRACT

Shoot tips and nodal segment explants of *Rauwolfia serpentina* when cultured on MS medium containing varying concentration of benzyl adenine purine (BAP) and benzyl adenine purine in combination with indole butyric acid (IBA) produced multiple shoots. Maximum multiple shoots (86.7%) were found in static MS medium supplemented with 5.0mg/l BAP and 0.5mg/l IBA along with 2.5% sucrose and 0.85% agar. The developed shoots after one month were excised from the cul ture tube and implanted individually on static MS medium with varying concentration of IBA and indole acetic acid (I.A.A). Maximum rooting (76.3%) was observed in 0.5 mg/l IBA after 27 days. Regenerated pla tlets were successfully acclimatized and established in soil. About 75 % of plantlets survived under open field conditions.

KEYWORDS

Rauwolfia serpentina;
Tissue culture;
Multiple shoot generation.

INTRODUCTION

Plant tissue culture techniques have become a powerful tool for studying basic and applied problems in plant biotechnology. The latest researches have witnessed a dramatic increase in our ability to manipulate the plant cells in culture. The potential of plant tissue culture for plant propogation and bioproduction of secondary metabolites has itself provided a substantial impetus for research. It is apparent that cell culture is keystone to progress in plant biotechnology and the application and development of current propogation techniques are opening the door to a second green revolution. Rauwolfia com-

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monly known as serpagandha consists of dried roots of *Rauwolfia serpentina* family *Apocynaceae*. It contains about 30 indole alkaloids (0.7-2.4%), phytosterols, unsaturated alcohols and sugars. Among various alkaloids, reserpine is the active constituent and is well known for its anti-hypertensive action. It depletes the stores of catecholamines at nerve endings. The other important alkaloids are ajmalicine, rescinnamine, yohimbine and serpentine[2-5]. A large number of reports have been indicated on tissue culture of *R.serpentine*[6-10]. The present study was undertaken for multiple shoot generation and then transferring to rooting media (using different cytokinins and auxins alone and in combination of different ratio).

EXPERIMENTAL

Material and methods

The plant of *Rauwolfia serpentina* was obtained and identified from K.N.K.Agriculture College, Mandsaur. The entire chemical procured from Sigma-Aldrich GmbH, CDH, Calcutta, India.

Stem tips and nodal explants were collected form young plant. Surface sterilization of explants (both stem tips and nodal segment) were done by washing with running tap water for 15 min, then with an antifungal agent bvastin (0.2%) for 7-8 min, followed by 0.1% mercuric chloride treatment of 2-3 min. The explants were then washed thrice with sterile double distilled water. Cutted explants of 1×1 cm size were cultured on to the MS medium containing 2.5% sucrose[11]. The medium was solidified using 0.8% agar.

The effect of 6-benzyl amino purine (BAP) at different concentrations and combinations of BAP with indole butyric acid (IBA) were studied on the induction of multiple shoot formation in MS media.

The regenerated shoots were transferred after one month to MS medium with 3% sucrose and containing different concentration of IBA and IAA separately. The number of stem explants responded to rooting was measured after one month.

The pH of the medium was adjusted to 5.75 before autoclaving at 12°C,15 lbs for 15 min. The cultures were incubated at 25°C under white fluorescent light with 12 hrs photoperiod and RH of 55-60%[12-14].

Each treatment included 15 replicates. The results were determined after 4 weeks.

RESULTS AND DISCUSSION

In medium containing only BAP frequency of multiple shoot formation from both nodal segment and stem explant was less. Shoot multiplication from nodal segment explant was better when cultured in static MS medium supplemented with BAP in combination with IBA. Maximum shoot initiation (86.7%) was observed in MS+BAP (5.0 mg/l)+ IBA (0.5 mg/l). In the same medium multiple shoot induction from stem tip explant was low (Figure.1 and TABLE 1).

Well-developed and elongated shoots were excised and cultured on root induction medium. Different concentration of IBA and IAA were used in

Figure 1 : Multiple shoots generated in static Ms media containing 5.0mg/l BAP + 0.5 mg/l IBA

TABLE 1: Response of different concentration of growth regulators supplemented in MS medium on proliferation and multiplication from nodal segment explant of *R.serpentina*

Hormone (mg/l)		% Responded explant	No.of shoots /explant
BAP	IBA		
1.0	--	11.3	2.9±0.5
2.0	--	13.4	2.7±0.3
5.0	--	26.7	4.2±0.2
10.0	--	10.1	2.3±0.2
1.0	0.1	24.0	4.7±1.2
1.0	0.5	13.7	4.4±1.2
1.0	1.0	15.2	4.1±0.3
2.0	0.1	36.2	5.5±0.9
2.0	0.5	22.9	5.1±0.3
2.0	1.0	18.8	3.9±0.4
5.0	0.1	58.5	8.2±0.6
5.0	0.5	86.7	13.2±1.5
5.0	1.0	44.2	6.2±0.4
10.0	0.1	42.4	5.1±0.6
10.0	0.5	34.1	3.2±0.5
10.0	1.0	23.7	3.1±0.5

Response was measured after 30 days.

TABLE 2 : Effect of different auxins in full strength MS medium with 3% sucrose on root formation from regenerated shoots of *R.serpentina*

Growth regulator (mg/l)	% Responded stem explant
0.1 IAA	37.2±2.5
0.25 IAA	26.9±1.7
0.5 IAA	24.7±1.6
1.0 IAA	25.1±0.9
0.1 IBA	29.2±2.5
0.25 IBA	43.1±1.6
0.5 IBA	76.3±3.4
1.0 IBA	52.4±2.1

Response was measured after 27 days.

full strength MS medium for root regeneration. Best response (76.3%) was observed in 0.5mg/l IBA, while indole acetic acid has shown poor growth (TABLE 2).

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