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Shoot induction and multiplication of an endangered medicinal plant *Rauvolfia serpentina*

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Abstract

***Rauvolfia serpentina* is an endangered medicinal plant which is used to treat various diseases. Shoot induction and multiplication was achieved from nodal, apical and leaf explants. MS media supplemented with IAA and BAP was found suitable for shoot induction and multiplication. The apical portions of shoot segment gave good results in multiplication. The regenerated shoot when subcultured to same medium shown better proliferation.**

Keywords: *Endangered*, *Medicinal plant*, *Micropropagation*, *Subculture*

Introduction

Rauvolfia serpentina belongs to family Apocynaceae represented by 200 genera representing 2000 species. Its common name is Sarpagandha. It is small undershrub generally about 45cm height (George and Sherrington,1984). It is widely distributed within tropical Himalaya and plains near the foothills from Sirkind, edge worth, Muradabad to Sikkim. Rarely found in forest of Bastar, Raipur and Amarkantak.

Family: Apocynaceae
Genus: *Rauvolfia*
Species: *serpentina*

Active compounds, ajmaline, ajmalinine azamalicine, serpentine, serpentinine, reserpine, raupine, sarpagine, reserpinine can be extracted from roots of the plant (Bhojwani, 1990).Yield of alkaloid. 0.8- 2.29 % (standard as per international pharmaceutical codex). Leaves stem and seeds also contain alkaloid. IUCN (International Union for Conservation of Nature and Natural Resources) kept *Rauvolfia serpentina* in endangered species. Besides this it has great importance in treating high blood pressure, hypertension, neuropsychiatric condition, gynological disorder and insomnia Because of the above reasons and it's high medicinal values it is propagated by both conventional and tissue culture method.

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Materials and Method

Glasswares like test tubes, bottles, petriplates, conical flasks, pipettes, beakers were washed with chromic acid or Labolene (Neutral liquid detergent). Washed glassware were sterilized in vertical autoclave at 121˚C and 15 pressure for 30 minutes then transferred to hot air oven for drying at 60˚C for 15-20 minutes. Along with glassware, equipment like scalpels, forceps, scissors, distilled water were also sterilized.Explant like apical portion, nodal portion and leaf were collected from nursery grown plant of *Rauvolfia serpentina*. Explants were washed with DDW (Double distilled water) for 4-5 times and then the explants were treated with 5% solution of Extran and 1% Bavistin. In LAF (Laminar Air Flow) the explant were washed by presterilized DDW, then surface sterilization was done with 0.1% solution of $HgCl_2$. In the present study, Murashige and Skoog's media referred as MS media was used. Different plant growth regulators like, Auxin-IAA, IBA and Cytokinin-BAP were supplemented in low concentration (0.5-5.0mg/l.). pH was adjusted to 5.7 to 5.8 with 1N HCl and 1NaOH. The semi solid growth medium was prepared with the addition of 0.8 % agar-agar powder in the basal media. All stock solutions were prepared in double distilled water and were stored in refrigerator. The cultures were maintained in culture room at 25 ± 2˚C for less than 16 hours photo period in presence of florescent light (1000 lux). Relative humidity *i.e.* 70-80% was also maintained.

Results and Discussion

Growth media were standardized for micropropagation by adding different concentration and combination of plant growth regulators. **Multiplication**: Incorporation of BAP with IAA showed good morphogenetic response for shoot multiplication when the level of IAA (1.0 mg/l) and BAP (2.0 mg/l) was low, better shoot proliferation occurred with 1mg/l IAA and 3.0 mg/l BAP and 2.0 mg/l IAA and 3.0 mg/l BAP also gave good result in case of shoot multiplication (Table-2 and Fig. 2). Shoot length increase when MS media supplemented with 0.5 mg/l of IAA and 2.0 mg/l of BAP excellent growth were observed (Table-1 and Fig. 1). In just 3-weeks shoot get elongated to 3.0-3.5 cm in length.

Interaction of Cytokinin and Auxin on *R. Serpentina* for shoot Induction: High multiplication rate was observed when medium was supplemented with 1.0 mg/l + 2.0 mg/l of BAP and optimal growth was observed in medium containing 0.5 mg/l IAA and 2.0 mg/l BAP. Whereas no morphogenetic response was observed in control medium. Similar observations has been recorded by Kataria *et al*. (2005), Sarkar *et al*. (1996), Bhuya *et al*. (2000) and Kirillova and Komov (2002).

On the basis of this work following PGR combinations and concentrations were recommended for –

Shoot proliferation

IAA 1.0 mg/lit + BAP 2.0 mg/lit

Shoot elongation

(a) IAA 0.5 mg/lit + BAP 2.0 mg/lit

(b) IAA 2.0 mg/lit + BAP 3.0 mg/lit

Table- 1: Morphogenetic resposnse for growth in *Rauvolfia serpentina*

Media composition (in mg/l)	Morphogenetic response				Remark
		After 10 days	After 20 days	After 30 days	
IAA:BAP	Growth				
1:2		+	++	+++	
1:3		-	++	++	
1:4		+	++	+++	
2:3		++	+++	+++	Swelling observed at base after 18-20 days (Fig 1)
2:4		-	++	+++	
5:2		+	+++	+++	
Control		_	+	+	

Table-2: Morphogenetic resposnse for Shoot multiplication in *Rauvolfia serpentine*

Media composition (In mg/l)	MORPHOGENETIC RESPONSE				REMARK
		After 10 days	After 20 days	After 30 days	
IAA:BAP	Shoot multiplication				
1:2		+	++	+++	
1:3		-	+++	+++	
1:4		-	+	+	
2:3		-	+	+++	
2:4		++	+++	+++	
5:2		+	+++	+++	Elongation in internode was observed after subculturing (Fig. 2)
Control		_	+	+	

Now from the above result it is concluded that plant tissue culture of *R. Seprentina* can be done under aseptic condition and according to the need one can obtain the shoot height and more number of plant by applying different plant growth regulators.

Fig. 1: Growth response

Fig. 2: Shoot multiplication

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