

Full Length Research Paper

## ***Rauwolfia serpentina*: Protocol optimization for *in vitro* propagation**

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***Rauwolfia serpentina* L. Benth. ex. Kurz. (Apocynaceae) a woody perennial shrub, possess alkaloids namely reserpine, reserpinamine and yohmbine, and used to cure various neurological ailments. The present investigation is an effort to establish *Rauwolfia* for micro-propagation and sub-culturing. The shoots and leaves were used as explants and cultured on MS media supplemented with three phytohormones in two combinations; 2,4-dichlorophenoxyacetic acid (2,4-D) + 2-benzyl amino purine (BAP) and indole-3-butiric acid (IBA) + BAP. These combinations were examined for callus induction and direct regeneration. Induction of callus was established from leaf and stem tissues and direct regeneration from apical and nodal explants. Combination of IBA (0.125 mg/L.) + BAP (1.0 mg/L.) produced better results for both callus induction and direct regeneration.**

**Keywords:** *Rauwolfia serpentina*, medicinal plant, micro-propagation, phytohormone, tissue culture

### **INTRODUCTION**

*Rauwolfia serpentina* L. Benth. ex. Kurz (family: Apocynaceae) is a woody perennial shrub, commonly known with different names; sarpagandha, snake root plant, chotachand, chandrika, etc. The roots of this plant have been used for centuries in ayurvedic medicines under the name sarpagandha and nakuli for the treatment of mental disorders. It has been stated that the drug is useful in mental disease, epilepsy, sleeplessness and several other ailments (Ojha and Mishra, 1985). Due to its medicinal values, the root of this plant has been popular both in India and Malaya-peninsula, from ancient times as an antidote to the stings of insects and poisonous reptile. It has also been used as febrifuge and stimulant to uterine contraction for insomnia and most of all for insanity (Vakil, 1949).

The plant is vegetatively propagated by root cutting because of poor seed viability and low germination percentage that may be largely ascribed to the presence of cinnamic acid and derivatives in the seed (Mitra, 1976; Sahu, 1979). Further *Rauwolfia* is threatened with extinction due to its limited cultivation and over exploitation by local people, government agencies and various pharmaceutical houses (Mamgain et al., 1998). Due to the prevailing reasons there is a huge need for *in vitro* propagation of *Rauwolfia serpentina* to satisfy the growing commercial demand of the plant for the production of life supporting alkaloids and conservation of this valuable endangered plant itself. Hence improvements in plant tissue culture techniques for the mass propagation of *R. serpentina* are highly desirable. The present study deals with the *in vitro* propagation including callus induction from leaf and stem tissues and direct regeneration from apical and nodal tissues of field grown *R. serpentina*.

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**Table 1.** Effect of different phytohormonal combinations (2,4-D + BAP and IBA + BAP) on callus induction in leaf and stem tissues of *R. serpentina*.

Explant(s)	Phytohormones (mg.L <sup>-1</sup> )		Regeneration	Phytohormones (mg.L <sup>-1</sup> )		Regeneration
	2,4-D	BAP		IBA	BAP	
Leaf	-	1.0	-	-	1.0	-
	-	1.5	-	-	1.5	-
	0.125	1.0	++	0.125	1.0	+++
	0.125	1.5	++	0.125	1.5	++
	0.250	1.0	+	0.250	1.0	+
	0.250	1.5	+	0.250	1.5	-
Stem	-	1.0	-	-	1.0	-
	-	1.5	-	-	1.5	+
	0.125	1.0	++	0.125	1.0	++
	0.125	1.5	-	0.125	1.5	+
	0.250	1.0	-	0.250	1.0	-
	0.250	1.5	-	0.250	1.5	-

(-) Indicates no regeneration, and (+) indicates status of callus induction.  
+ = Poor, ++ = fair, and +++ = good.

## MATERIALS AND METHODS

The explants were obtained from Medicinal Research and Development Centre, Haldi, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar. Endophytic microflora is a major cause of contamination for *in vitro* culture of *R. serpentina*. Hence, a proper sterilization was needed for successful culture. Various explants were taken from field growing plant were thoroughly washed with running water for 30 min. Washed explants were treated with 1.0% mild detergent (Tween-20) for 5 min and rinsed off thoroughly under tap water. This is followed by mercuric chloride treatment (0.1% w/v) for 2 min, and washing 2 - 3 times with distilled water. Thereafter, the explants were treated with sodium hypochlorite (50%, v/v) for 2 min, and washing with distilled water 2 - 3 times. Finally they were washed 4 - 5 times properly with sterile double distilled water and then placed on sterile filter paper sheets to remove moisture. Both mercuric chloride and sodium hypochlorite treatments were given inside the Laminar flow chamber.

Murashige and Skoog (1962) medium was used as basal medium fortified with 3% (w/v) sucrose, 100 mg.L<sup>-1</sup> myoinositol, 0.5 mg.L<sup>-1</sup> nicotinic acid, 0.5 mg.L<sup>-1</sup> pyridoxine-HCl, 2.0 mg.L<sup>-1</sup> glycine, 0.1 mg.L<sup>-1</sup> thiamine along with auxins, that is, 2-benzyl amino purine (BAP), indole-3-butyric acid (IBA) and 2,4-dichlorophenoxyacetic acid (2,4-D) in concentrations ranging from 0.00 - 0.25 mg.L<sup>-1</sup> and solidified with 0.725% agar.

Leaf and stem segments of 0.5 - 1.0 cm for callus induction were properly sterilized and inoculated in culture bottles containing MS media supplemented with different combinations of hormones; 2,4-D, BAP and IAA in different concentrations. For direct regeneration, both nodal and apical segments of 1 - 2 cm size were taken from field grown plant and after following proper sterilization, were transferred to MS media supplemented with hormones under aseptic conditions. The regenerated plants were sub-cultured at every 4 week interval. The inoculated culture bottles then transferred from Laminar chamber to Culture Room at 20 - 25°C for 16/8 h light and dark conditions under 3000 lux light intensity.

## RESULTS AND DISCUSSION

Two different combinations of hormones, namely 2, 4-D + BAP and IBA + BAP (Figure 1) with different concentra-



**Figure 1.** *R. serpentina* callus from leaf explant in MS medium supplemented with IBA (0.125 mg.L<sup>-1</sup>) along with BAP (1.0 mg.L<sup>-1</sup>) after 7 weeks of culture.

tions were applied for callus induction. No callus induction was observed when only BAP was applied either to stem or leaf tissues (Table 1). The callus was induced with the combination of 2,4-D and BAP in all the four applied concentrations (mg.L<sup>-1</sup>); 0.125 + 1.0, 0.125 + 1.5, 0.250 + 1.0 and 0.250 + 1.5, respectively, on leaf tissues (Table 1). However, better results of callus induction were observed with two combinations (mg.L<sup>-1</sup>); 0.125 + 1.0 and 0.125 + 1.5, respectively. Callus induction was also observed in stem tissues with combination of 2,4-D and BAP hormones at the rate of 0.125 + 1.0, respectively (Table 1). Sehrawat et al. (2001) also reported induction of callus from various explants on MS medium has also been observed with various levels of 2,4-D + BAP, 2,4-D + Kin and NAA + BAP.

**Table 2.** Effect of different combinations of phytohormones (2,4-D + BAP and IBA + BAP) in direct regeneration from apical and nodal explants of *R. serpentina*.

Explant(s)	Phytohormones (mg.L <sup>-1</sup> )		Regeneration	Phytohormones (mg.L <sup>-1</sup> )		Regeneration
	2,4-D	BAP		IBA	BAP	
Apical	-	1.0	-	-	1.0	+
	-	1.5	+	-	1.5	+
	0.125	1.0	++	0.125	1.0	+++
	0.125	1.5	+	0.125	1.5	++
	0.250	1.0	-	0.250	1.0	-
	0.250	1.5	+	0.250	1.5	+
Nodal	-	1.0	-	-	1.0	-
	-	1.5	+	-	1.5	-
	0.125	1.0	+++	0.125	1.0	++++
	0.125	1.5	+++	0.125	1.5	+++
	0.250	1.0	++	0.250	1.0	++
	0.250	1.5	++	0.250	1.5	-

(-) Indicates no regeneration, and (+) indicates status of callus induction.

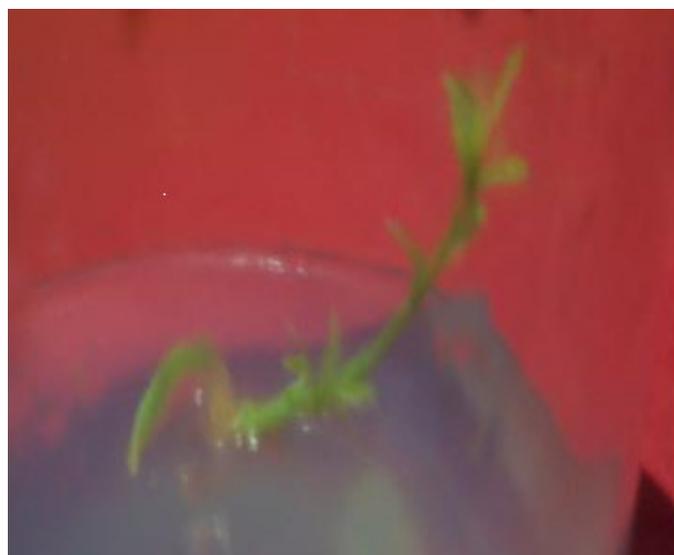
+ = Poor, ++ = fair, and +++ = good.

Two combinations of hormones namely 2,4-D + BAP and IBA + BAP in different concentrations were applied to induce direct regeneration in apical as well as nodal explants. The regeneration was totally absent when only BAP (1.0 mg.L<sup>-1</sup>) was applied either for apical or nodal explants (Table 2). The regeneration was observed with the combinations of 2,4-D and BAP at all concentrations (mg.L<sup>-1</sup>); 0.125 + 1.0, 0.125 + 1.5 and 0.250 + 1.5 which showed good response except for 0.250 + 1.0 in case of apical explants, which showed no response. While in case of nodal explants, all five combinations (mg.L<sup>-1</sup>); 0.00 + 1.5, 0.125 + 1.0, 0.125 + 1.5, 0.250 + 1.0, and 0.250 + 1.5 showed positive response (Table 2).

The regeneration observed in case of combination of IBA and BAP (0.125 + 1.0 mg.L<sup>-1</sup>) with nodal explants was the best compared to others. Two combinations (mg.L<sup>-1</sup>); 0.125 + 1.0 and 0.125 + 1.5 of IBA + BAP, respectively, showed positive response in both the explants. Regeneration was also observed with (in mg.L<sup>-1</sup>) 0.0 + 1.0, 0.00 + 1.5 and 0.250 + 1.5 combinations, respectively, for IBA + BAP in apical explant and with 0.250 + 1.0 in case of nodal explant and no response was observed in rest of the combinations (Table 2). Among all the combinations of phytohormones in both apical and nodal explants (Figure 2) the best results of direct regeneration were observed with 0.125 mg.L<sup>-1</sup> IBA + 1.0 mg.L<sup>-1</sup> BAP. Among these, the results of nodal explants were far better than those for apical explants.

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**Figure 2.** *R. serpentina* direct regeneration from nodal explants in MS medium supplemented with IBA (0.125 mg.L<sup>-1</sup>) along with BAP (1.0 mg.L<sup>-1</sup>) after 7 week of culture.

plant materials.

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