

## IN-VITRO MICROPROPAGATION OF RAUWOLFIA SERPENTINA THROUGH MULTIPLE SHOOT GENERATION

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**ABSTRACT :** Shoot tips and nodal segment explants to *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 (85.6%) were found in static MS medium supplemented with 5.0 mg/ l BAP and 0.5 mg / l IBA along with 2.5% sucrose and 0.85% agar. The developed shoots after one month were excised from the culture tube and implanted individually on static MS medium with varying concentration of IBA and Indole butyric acid (IBA). Maximum rooting (76.6%) was observed in 0.5 mg/l IBA after 27 days. Regenerated plantlets were successfully acclimatized and established in soil. About 74% of plantlets survived under open field conditions.

### INTRODUCTION

*Rauwolfia* commonly known as sarpagandha 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<sup>1-5</sup>. Micro propagation is specifically used for the species in which clone propagation is needed. Clonal propagation through conventional methods like cutting or grafting has not been successful in this plant<sup>6</sup>. A large number of reports have been indicated on tissue culture of *R. serpentina*<sup>7-10</sup>. The present study was undertaken to propagate the plant by multiple shoot generation and then transferring to rooting media (using different cytokinins and auxins alone and in

combination of different ratio), finally planting the rooted plant to the open field.

### MATERIAL AND METHODS

The plant of *rauwolfia* was obtained from medicinal garden of B.R.N. College of Pharmacy, Mandsaur (M.P.) and was identified by Agriculture College, Mandsaur. Stem tips and nodal explants were collected from 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. Cut explants of 1\*1 cm size were cultured on to the MS

medium containing 2.5% sucrose<sup>11</sup>. 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 static 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 121<sup>0</sup>C, 15 lbs for 15 min. The cultures were incubated at 25<sup>0</sup>C under white fluorescent light with 12 hrs photoperiod and RH of 55-60%<sup>12-15</sup>.

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

In medium containing only BAP frequency of multiple shoot formation from both nodal segment and stem explants was less. Shoot multiplication from nodal segment explants was better when cultured in static MS medium supplemented with BAP in combination with IBA. Maximum shoot initiation (85.6%) was observed in MS+BAP (5.0 mg/l) + IBA (0.5 mg/l). In the same medium multiple shoot induction from stem tip explants was low.

Well-developed and elongated shoots were excised and cultured on root induction medium. Different concentration of IBA and IAA were used in full strength MS medium for root regeneration. Best response (75.4%) was observed in 0.5 mg/l IBA while Indole Acetic Acid has shown poor growth.

The plant exhibited normal development with out gross anatomical changes. The well-rooted plantlets were transferred in small earthen pots. After acclimatization 74% plantlets were successfully established in the open field.

## **RESULTS AND DISCUSSION**

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**Table :1**

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

Hormone (mg/l)		% Responded explants	No. of shoots / explants
BAP	IBA		
1.0	--	10.3	2.0 ± 0.5
2.0	--	12.4	2.7 ± 0.3
5.0	--	25.7	4.0 ± 0.2
10.0	--	9.1	2.1 ± 0.2
1.0	0.1	23.0	4.2 ± 1.2
1.0	0.5	12.7	4.0 ± 1.2
1.0	1.0	14.2	3.7 ± 0.3

2.0	0.1	35.2	5.1 ± 0.9
2.0	0.5	23.9	4.7 ± 0.3
2.0	1.0	17.8	3.9 ± 0.4
5.0	0.1	56.5	7.1 ± 0.6
5.0	0.5	85.6	13.0 ± 1.5
5.0	1.0	43.2	5.4 ± 0.4
10.0	0.1	41.4	5.1 ± 0.6
10.0	0.5	32.1	2.3 ± 0.5
10.0	1.0	21.7	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***

<b>Growth regulator (mg/l)</b>	<b>% Responded stem explants</b>
0.1 IAA	36.2 ± 2.5
0.25 IAA	25.9 ± 1.7
0.5 IAA	23.7 ± 1.6
1.0 IAA	24.1 ± 0.9
0.1 IBA	28.2 ± 2.5
0.25 IBA	42.1 ± 1.6
0.5 IBA	76.6 ± 3.4
1.0 IBA	51.4 ± 2.1

\* Response was measured after 27 days.

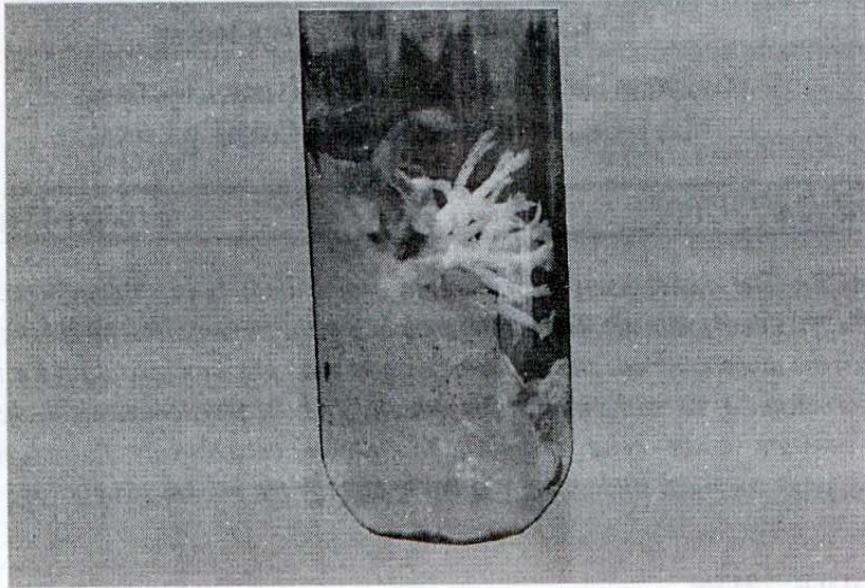


Fig 1: Multiple shoots generated in static MS media containing 5.0mg/L Benzyl Adenine Purine (BAP) + 0.5 mg/L IBA

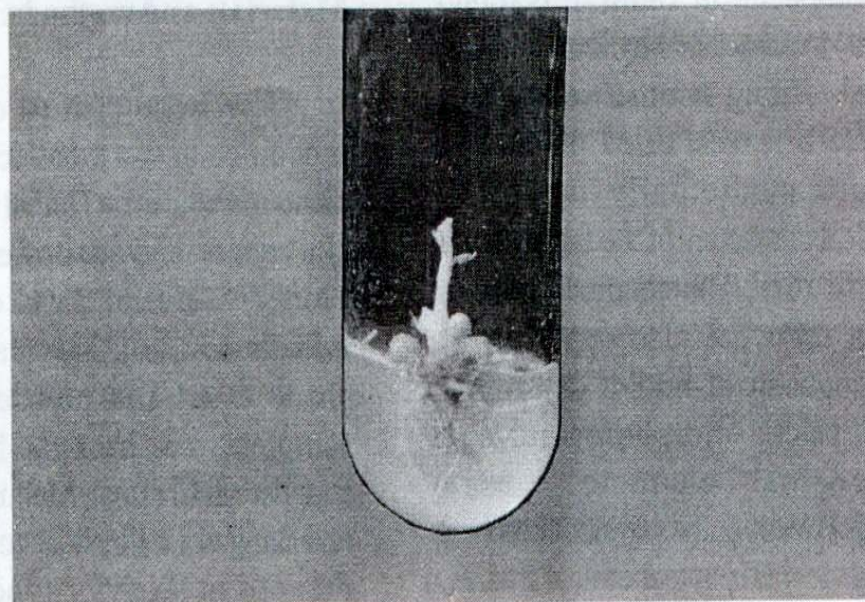


Fig 2 : Generated shoots showing rooting in static MS media containing 0.5 mg/L Indole Butyric Acid (IBA)