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IN VITRO SHOOT MULTIPLICATION FROM SHOOT TIP AND NODAL EXPLANTS OF *Rauvolfia serpentina* (L.) Benth. ex. Kurz, AN ENDANGERED MEDICINAL PLANT

RANJUSHA AP*, RADHAMANY PM, GANGAPRASAD A AND NAIR GM

Corresponding Author: E Mail: ranjushaap@gmail.com

Department of Botany, University of Kerala, Kariavattom, Thiruvananthapuram – 695 581, Kerala, India

ABSTRACT

Presence of pharmaceutically important alkaloids in various parts of the plant body made *Rauvolfia serpentina* an overexploited one and led to its inclusion in Red Data Book of India as an endangered species. Conventional propagation is beset with problems like poor seed viability, low seed germination rate and delayed rooting of cuttings. *In vitro* clonal propagation may therefore be beneficial for the production of elite plants and subsequent germplasm conservation. Multiple shoots were obtained from shoot tip and nodal explants of *Rauvolfia serpentina* on MS media supplemented with different concentrations of BAP. Maximum number of shoots was obtained from shoot tip and nodal explants at 13.32µM BAP. Shoot multiplication and their development was better on MS medium supplemented with 8.87µM BAP and 2.85µM IAA for shoot tip explants and 8.87µM BAP with 5.71µM IAA for nodal explants. *In vitro* regenerated shoots were rooted in half strength MS medium fortified with 7.38µM IBA. The regenerated plantlets were acclimatized and successfully transferred to field condition.

Keywords: *In Vitro*, Shoot Tip, Node, Multiple Shoot, *Rauvolfia serpentina*

INTRODUCTION

Rauvolfia serpentina (L.) Benth.ex.Kurz, of family Apocynaceae is an evergreen, erect, perennating under shrub. It is a rich source of indole alkaloids of high medicinal value such as ajmalicine, reserpine, serpentine etc. which are used in the treatment of circulatory disorders **[1]**. The root is bitter, acrid, pungent, anthelminthic and cures the

poisonous effects of scorpion sting and snake bite **[2, 3]**. The antihypertensive properties of *Rauvolfia* roots are attributed to the presence of reserpine, which is a weak tertiary base occurring in the oleoresin fraction of the roots. For centuries, the drug *Rauvolfia* has been used in the Ayurvedic system of medicine in India. The germination percentage of seeds is very poor and variable (25-50%) and is often as low as 10 %. This is partly due to the adverse influence of the stony endocarp **[4]**. Indiscriminate collection of roots along with several other factors caused their decline in the wild habitat. *Rauvolfia serpentina* is categorized as critically endangered one by IUCN 2000. So there is an urgent need to develop a non conventional method for the propagation and conservation of this valuable medicinal plant. Micropropagation is a proven method for an efficient *in vitro* propagation of medicinal plants and also for the conservation of rare and endangered plant species **[5, 6]**. Clonal propagation of *R. serpentina* has been previously reported by several workers using shoot tip and nodal explants **[7-10]**. Propagation through axillary buds and indirect regeneration from leaf callus has also been reported **[7, 11]**. The protocol discussed here, however, describes a more efficient propagation system for *R. serpentina* by culturing shoot tip explants on medium containing combinations of BAP and IAA.

MATERIALS AND METHODS

Explants such as shoot tip and nodal segments were taken from 6-7 month old plants grown in the green house of Botany Department, University of Kerala, Kariavattom Campus. The young shoot cuttings with 5-6 node length were washed thoroughly in running tap water and subsequently washed with 5% (v/v) Teepol for 8 minutes and then in running tap water for 30 minutes. For surface decontamination, they were immersed in 0.1% (w/v) mercuric chloride for 8 minutes and rinsed with sterile distilled water for 4-6 times. The shoots were cut in pieces of 0.5-1cm containing single node with dormant axillary bud and 0.5cm shoot tip inoculated on to the nutrient MS medium **[12]** with 3% (w/v) of sucrose and varied concentrations of auxins (IAA and IBA) and cytokinins (BAP and KIN) either alone or in combinations.

The p^H of the medium was adjusted to 5.8 before adding agar. To solidify the medium 0.8% (w/v) of agar (SRL, India) was used. Medium was dispensed in 15ml aliquots into 25x150mm culture tubes and autoclaved at121^0C and $1.1kgcm^2$ pressure for 15 minutes. Cultures were incubated in a culture room maintained at 25 ± 2^0C, RH 50-60% and 12/12h photoperiod provided by cool white

fluorescent tubes (Philips India Ltd., Mumbai). The numbers of shoots were counted as an average for 8 replicates and subculture was done at 30 days interval.

Node and shoot tip explants, isolated from the *in vitro* developed axillary smaller shoots were sub cultured on MS medium containing BAP (13.32µM) in combination with Kinetin (2.32 - 18.6µM). Different concentrations of IAA (0.57 – 39.97µM) and IBA (0.49 – 34.3µM) along with 8.87µM BAP were also tried. To induce root, micro shoots (6 cm) were cut and placed vertically on to MS half and full strength agar or liquid medium with filter paper bridges containing varied concentrations of IBA (0.49 – 9.8µM) and NAA (0.54 – 10.74µM) individually. The well developed plantlets with roots were recovered from the culture tubes, washed thoroughly to remove agar residue and transferred to plastic cups for hardening. After three weeks hardened plants were potted in community pots and transferred to green house conditions.

Every treatment composed of three replications and each replication is represented by 8 culture tubes. Data on various parameters were evaluated by analysis of variance (ANOVA) and mean values were compared with Duncan's New Multiple Range Test (DNMRT).

RESULTS AND DISCUSSION

Initiation of shoots started from shoot tip **(Figure 1)** and nodal explants on 5^{th} and 8^{th} day respectively after inoculation. BAP at 13.32µM concentration elicited the maximum number of shoots from shoot tip and nodal explants (8.8±0.35) (8.1±0.29) **(Table 1)**. This superiority of BAP over other cytokinins in shoot initiation was reported in the same plant by many workers **[9, 11, 13]**. BAP (13.32µM) in combination with KIN (4.65µM) produced multiple shoots from shoot tip (6.7±0.25) **(Figure 2)** and nodal explants (4.2±0.25) **(Figure 3) (Table 2)**. MS medium supplemented with BAP (8.87µM) in combination with different concentrations of IAA (0.57-39.97µM)/IBA (0.49-34.3µM) was used to improve the rate of shoot proliferation **(Table 3)**.

In the present study MS medium supplemented with BAP (8.87µM) and IAA (2.85µM) elicited the maximum shoot proliferation (24.5±0.32) from shoot tip explants **(Figure 4)** while for nodal explants, the maximum shoot proliferation (11.8±0.29) was achieved on 8.87µM BAP and 28.54µM IAA. It appears from the results that shoot tip explants are more responsive than nodal explants. Hence this combination proved to be ideal for multiple shoot production from shoot tip explants. The synergistic effect of BAP

and IAA on shoot proliferation has also been reported in niger **[14]**.

Micro shoots developed under *in vitro* conditions failed to develop roots on half strength MS basal medium and auxin supplemented MS full strength medium. However, root initiation was achieved in half strength MS medium supplemented with IBA and NAA **(Table 4)**. Maximum number of roots (5.0±0.26) was observed in half strength MS medium supplemented with 7.38µM IBA after 30 days of inoculation **(Figure 5)**. Rooting efficiency was poor on NAA supplemented medium. It could be concluded from the result that at low as well as higher concentrations of IBA, the percentage of root induction was low, while at a medium concentration the rooting percentage was highest **[15]**. The rooted plantlets were transferred to filter paper bridges in liquid medium supplemented with 3% sucrose, devoid of growth regulators **(Figure 6)**. After two weeks they were planted in plastic cups containing sterile soil rite for 4 weeks **(Figure 7 and 8)**. Finally the plants were transferred to 15cm diameter clay pots with 75% establishment rate. The induction of multiple shoot through axillary branching and shoot tip multiplication is now recognized as useful technique for the propagation of rare and endangered species. The present investigation thus demonstrates an efficient protocol for the multiplication of *R. serpentina* through axillary shoot tip culture.

Table 1: Effect of BAP/KIN on *In vitro* Shoot Multiplication from Shoot Tip and Nodal Explants of *R. serpentina*

BAP (µM)	KIN (µM)	No. of shoots	
		Shoot tip*	Node*
2.22		1.62±0.18^{def}	2.00±0.26^{e}
4.44		2.00±0.00^{de}	3.50±0.18^{cd}
8.87		5.25±0.16^{b}	4.25±0.25^{bc}
13.32		8.87±0.35^{a}	8.12±0.29^{a}
17.74		3.37±0.18c	5.00±0.32^{b}
22.2		2.37±0.32^{cd}	3.00±0.46^{d}
	2.32	0.37±0.18^{f}	0.50±0.18^{f}
	4.65	0.75±0.25^{ef}	0.87±0.22^{f}
	9.29	1.50±0.18^{def}	2.00±0.18^{e}
	13.95	1.62±0.32^{def}	2.87±0.39^{d}
	18.6	2.25±1.20^{cd}	1.25±0.25^{ef}
	23.25	0.75±0.25^{ef}	1.00±0.18^{f}
Main effect F Df (n-1) = 11		30.691***	63.648***

***Means within a column followed by same letters are not significantly different as determined by DNMRT (P<0.05). **Significant at P<0.01 level; ***Significant at P<0.001 level**

Table 2: Effect of BAP and KIN on *In vitro* Shoot Multiplication from Shoot Tip and Nodal Explants of *R. serpentina*

KIN (µM)	BAP (µM)	No. of shoots	
		Shoot tip*	Node*
2.32	13.32	0.50±0.18[c]	0.62±0.18[c]
4.65		6.75±0.25[a]	4.25±0.25[a]
9.29		3.37±0.37[b]	2.62±0.32[b]
13.95		1.25±0.25[c]	0.50±0.18[c]
18.6		0.87±0.29[c]	0.50±0.26[c]
Main effect F Df (n-1) = 4		86.957***	46.236***

***Means within a column followed by same letters are not significantly different as determined by DNMRT (P<0.05). **Significant at P<0.01 level; ***Significant at P<0.001 level**

Table 3: Effect of BAP, IAA and IBA on *In vitro* Shoot Proliferation from Shoot Tip and Nodal Explants of *R. serpentina*

Hormonal treatment (µM)			No. of shoots	
IAA	IBA	BAP	Shoot tip*	Node*
0.57		8.87	9.75±0.31[b]	10.62±0.32[c]
2.85			24.5±0.32[a]	10.87±0.29[c]
5.71			6.25±0.31[c]	13.00±0.37[a]
28.54			1.50±0.26[f]	11.87±0.29[b]
39.97			1.25±0.25[f]	7.50±0.32[d]
	0.49		1.50±0.26[f]	5.25±0.25[e]
	2.46		6.00±0.18[c]	5.00±0.26[ef]
	4.90		4.62±0.37[d]	7.62±0.32[d]
	24.6		3.75±0.31[e]	5.25±0.25[e]
	34.3		1.62±0.32[f]	4.25±0.25[f]
Main effect F Df (n-1) = 9		557.155***	116.472***	

***Means within a column followed by same letters are not significantly different as determined by DNMRT (P<0.05). **Significant at P<0.01 level; ***Significant at P<0.001 level**

Table 4: Effect of NAA and IBA on Rooting of *In vitro* derived shoots of *R. serpentina*

Hormonal treatment (µM)		No. of roots per shoot*
NAA	IBA	
0.54		1.62±0.26[b]
2.69		1.25±0.25[b]
5.37		0.50±0.18[c]
8.07		---
10.74		---
	0.49	0.50±0.18[c]
	2.46	0.62±0.18[c]
	4.9	1.25±0.25[b]
	7.38	5.00±0.26[a]
	9.80	1.37±0.26[b]
Main effect F Df (n-1) = 9		47.618***

***Means within a column followed by same letters are not significantly different as determined by DNMRT (P<0.05). **Significant at P<0.01 level; ***Significant at P<0.001 level**

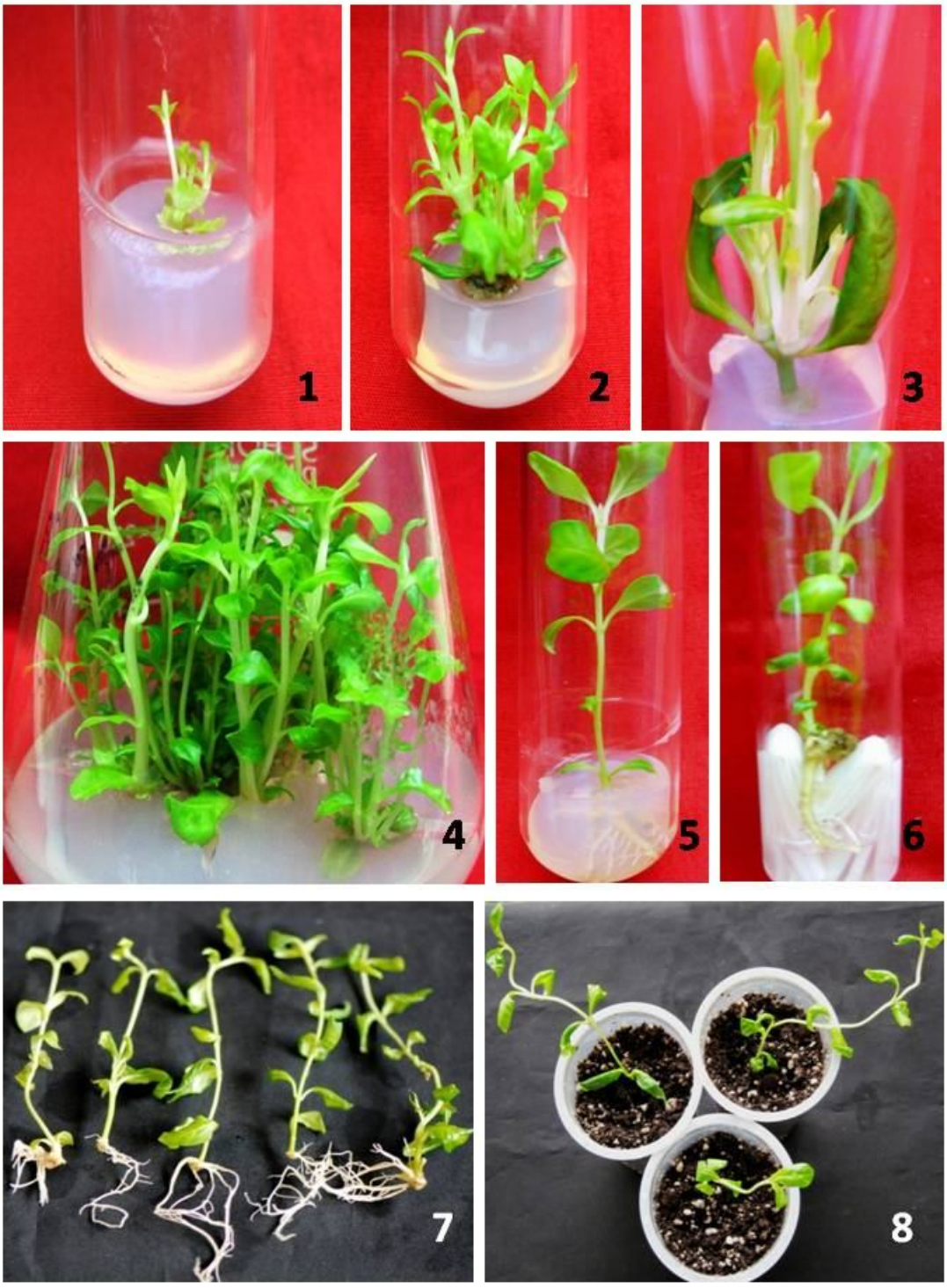

Figure (1 – 8): *In vitro* Shoot Multiplication of *Rauvolfia serpentina* from Shoot Tip and Nodal Explants; 1: Shoot Initiation from Shoot Tip Explants on MS Medium with BAP (13.32µM); 2: Multiple Shoots from Shoot Tip Explant after 30 Days of Culture; 3: Multiple Shoots from Nodal Explants after 30 Days of Culture; 4: Shoot Proliferation and Elongation on MS Medium with BAP (8.87µM) and IAA (2.85µM): 5: Rooting of Shoot on ½ Strength MS Medium with 7.38µM IBA; 6: Rooted Plantlets Transferred to ½ Strength MS Liquid Medium Provided with Filter Paper Bridge; 7: *In vitro* Derived Plantlets Ready for hardening; 8: Potted Plant for acclimatization

CONCLUSION

The study herein suggests a rapid multiplication method for the propagation of *R. serpentina*, the critically endangered plant. Even though so many protocols have been reported, the protocol described here initiated an average of 24 shoots from single shoot tip in MS medium containing a combination of BAP and IAA, in first culture itself. By sub culturing these shoots, a large number of micro shoots are produced. MS medium augmented with 8.87µM BAP along with 2.85µM IAA can thus be selected as the best medium for shoot multiplication. Rooting is achieved in ½ strength MS medium containing 7.38µM IBA without basal callusing, which increased the survival percentage of the *in vitro* derived plantlets. Moreover, this simple and rapid propagation protocol is reproducible and can be useful in the future conservation programmes of *R. serpentina*.

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REFERENCES

[1] Tyler VE, Brady LR and Robbers RE, Pharmacognosy, Lea & Febiger, Philadelphia, 1981, 233-237.

[2] Kirtikar KR and Basu BD, Indian Medicinal Plants, Volume 2, Lalit Mohan Basu, Allahabad, 1998.

[3] Nadkarni KM, Indian Materia Medica, Volume 2, Popular Prakashan Pvt. Ltd., Bombay, 1976.

[4] Wealth of India, Volume 8, Publications & Information Directorate, CSIR, New Delhi, 1973, 376-390.

[5] Almeida R, Goncalves S and Romano A, *In vitro* micropropagation of endangered *Rhododendron ponticum* L. subsp. *baeticum* (Boissier & Reuter) Handel-Mazzetti, Biodivers Conserv, 14(5), 2005, 1059-1069.

[6] Faizal A, Lambert E, Foubert K, Apers S and Geelen D, *In vitro* propagation of four saponin producing *Maesa* species, Plant Cell Tiss Organ Cult, 106(2), 2011, 215-223.

[7] Ahmad S, Amin MN, Azad MAK and Ashik Mosaddit M, Micropropagation and plant regeneration of *Rauvolfia serpentina* by tissue culture technique, Pak J Biol Sci, 5(1), 2002, 75-79.

[8] Baksha R, Jahan MAA, Khatun R and Munshi JL, *In vitro* rapid clonal

propagation of *Rauvolfia serpentina* (Linn.) Benth, Bangladesh J Sci Ind Res, 42(1), 2007, 37-44.

[9] Goel MK, Kukreja AK, Khanuja SPS, Cost effective approaches for *in vitro* mass propagation of *Rauvolfia serpentina* Benth.Ex.Kurz, Asian J Pl Sci, 6(6), 2007, 957-961.

[10] Salma U, Rahman MSM, Islam S, Haque N, Khatun M, Jubair TA and Paul BC, Mass propagation of *Rauwolfia serpentina* L. Benth, Pak J Biol Sci, 11(9), 2008, 1273-1277.

[11] Roja G and Heble MR, Indole alkaloids in clonal propagules of *Rauvolfia serpentina* benthe ex kurz, Plant Cell Tiss Organ Cult, 44(2), 1996, 111-115.

[12] Murashige T and Skoog F, A revised medium for rapid growth and bioassays with tobacco tissue cultures, Physiol Plant, 15, 1962, 473-497.

[13] Pant KK and Joshi SD, Rapid multiplication of *Rauvolfia serpentina* Benth.ex.kurz through tissue culture, Scientific world, 6, 2008, 58-62.

[14] Sarvesh A, Reddy TP and Kavikishore PB, Plant regeneration from cotyledons of niger, Plant Cell Tissue Organ Cult., 32(2), 1993, 131-135.

[15] Illahi I, Rahim F and Jabeen M, Enhanced clonal propagation and alkaloid biosynthesis in cultures of *Rauvolfia*, Pak J Pl Sci, 13, 2007, 45-56.