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IN VITRO MICROPROPAGATION OF *RAUVOLFIA SERPENTINA* (L.) BENTH THROUGH INDUCTION OF DIRECT AND INDIRECT ORGANOGENESIS

S. K. BHADRA, T. K. BHOWMIK AND P. SINGH *
Department of Botany, University of Chittagong, Chittagong - 4331, Bangladesh

ABSTRACT

Leaf and nodal segments of two months old field grown seedlings of *Rauvolfia serpentina* (L.) Benth were aseptically cultured on agar solidified MS medium supplemented with various combinations and concentrations of auxins (NAA, IAA, 2,4-D and picloram) and cytokinins (BAP and Kn). The nodal segments produced highest number of multiple shoot buds (5.85/explant) on MS medium supplemented with 2.0 mgl^{-1} BAP + 0.2 mgl^{-1} NAA or 2.0 mgl^{-1} BAP + 0.1 mgl^{-1} IAA. Whereas nodal segment produced callus tissue of different nature on MS medium supplemented with 1.5 mgl^{-1} BAP + 0.5 mgl^{-1} IAA+ 1.5 mgl^{-1} 2,4-D; 3.0 mgl^{-1} BAP + 1.0 mgl^{-1} NAA + 1.5 mgl^{-1} Kn and 0.1 mgl^{-1} Pic + 1.0 mgl^{-1} Kn. The callus tissue of light green and nodular nature on further subculture in a wide range of plant growth regulators (PGRs) supplemented media, differentiated into multiple shoot buds that underwent rapid elongation on 2.0 mg/l BAP and 0.2 mg/l NAA supplemented media. The elongated shoot buds on further subculture in rooting media produced strong and stout roots. Half strength MS with 1.5% (w/v) sucrose was most effective for enhancing rooting. Finally those plantlets were acclimatized in field. Thus a protocol was established for rapid micropropagation of this medicinal plant through induction of direct and indirect organogenesis from nodal explant.

Key words: Micropropagation, *Rauvolfia Serpentina,* Organogenesis

INTRODUCTION

R. serpentina (L.) Benth commonly known as 'Sarpagandha' is a small erect shrub with pale bark. Roots of this plant are used for medicinal purposes as they contain some important alkaloids such as reserpine, deserpidine, ajmaline, ajmalinine, serpentine and serpentinine. The roots are used as a valuable remedy for high blood pressure, insomnia, anxiety, insanity, epilepsy and other disorders of the central nervous system (Kirtikar and Basu 1975). This plant species is becoming rare in

* Corresponding author.

Bangladesh and, therefore, needs to be propagated rapidly to meet up our medicinal demand and also for conservation purposes. Tissue culture technique has been proved very efficient in rapid propagation of many medicinal plants (Erdei *et al*. 1981, Tsay and Huang 1989, Fay 1992, Sagare *et al*. 2000, Lakshmi and Mythili 2003). This research study was undertaken with a view to develop a reliable, reproducible and efficient protocol for *in vitro* propagation of this medicinal plant species.

MATERIALS AND METHODS

Two months old seedlings *were* collected from the nursery of BCSIR, Chittagong, and were established in garden pots of Botany Deparment of Chittagong University. Leaf and nodal segments of approximate 0.5 - 1.0 cm size were used as explant. Surface sterilization of the leaves and nodes was done by dipping in 70% ethanol for 30 sec. and submerging them in 0.2 % (w/v) $HgCl_2$ solution for 10 min. The segments were then washed three times with sterile distilled water and finally cultured in test tube (2.5 × 15 cm) and conical flask (100 ml). MS basal medium (Murashige and Skoog 1962) supplemented with various combinations of plant growth regulators (PGRs), *viz.*, auxins (NAA, IAA, 2,4-D and Picloram) and cytokinins (BAP and Kn) at different concentrations were used for culture of the explants. All the media were made 0.8% (w/v) agar solidified and pH of the media was adjusted to 5.8 prior to autoclaving for 20 min. at 121^0C under 1.9 kg/cm^2 pressure. Culture vessels with inoculated explants were maintained in a culture room under a regular cycle of 14h light and 10h dark at 25 ± 2^0C. The nodal segments underwent direct or indirect organogenesis in some of the media combinations giving rise to multiple shoot buds. The multiple shoot buds were further grown on elongation media and finally the elongated shoot buds were individually rooted on rooting media. The complete plantlets were finally transferred and established in outside natural environment through successive phases of acclimatization.

RESULTS AND DISCUSSION

Within 25 - 30 d of culture both leaf and nodal segments gave different responses to different PGRs combinations and the type of response was dependent on both explant and PGRs. Nodal segments underwent direct organogenesis producing multiple shoot buds when cultured on (i) 2.0 mg/l BAP + 2.0 mg/l NAA (ii) 1.0 mg/l NAA + 0.5 mg/l BAP (iii) 2.0 mg/l BAP + 0.2 mg/l NAA (iv) 2.0 mg/l BAP (v) 1.0 mg/l Kn + 3.0 mg/l BAP + 1.0 mg/l NAA and (vi) 0.1 mg/l

IAA + 2.0 mg/l BAP supplemented media (Table 1). The highest number of multiple shoot buds was, however, produced on the medium fortified with (i) 0.1 mg/l IAA + 2.0 mg/l BAP and (ii) 2.0 mg/l BAP + 0.2 mg/l NAA (Fig. 1a). This finding indicates that BAP supplemented media was more effective in induction of multiple shoot buds. Mao *et al*. (1995) reported that BAP was superior to other cytokinins for inducing multiple shoots in *Clerodendrum colebrookianum*. Induction of multiple shoot buds directly from nodal explant has also been reported in a wide range of plant species (Murashige 1974, Miller *et al*. 1991). Nodal segments, however, produced callus tissue on a range of PGR supplemented media. Green and granular callus was produced on 3.0 mg/l BAP + 1.0 mg/l NAA + 1.5 mg/l Kn supplemented medium (Fig. 1b). After 2 - 3 subcultures on the same medium, these green callii multiplied along with differentiation of shoot buds**.** Thus indirect organogenesis took place in the course of culture. This finding indicates that combination of BAP, Kn and NAA was effective for inducing indirect organogenesis. Sarker *et al*. (1996) reported that BA and NAA combination was effective for shoot bud differentiation from callus of *R. serpentina*. Nodal segments produced white and nodular callus on the medium fortified with 1.5 mg/l BAP, 0.5 mg/l IAA and 1.5 mg/l 2,4-D. Whereas, loose and friable callus was produced on 0.1 mg/l Pic and 1.0 mg/l Kn containing medium (Table 2 & Fig. 1c). After 2 - 3 subcultures on the same media, the white callus differentiated producing rhizoids.

TABLE 1: MULTIPLE SHOOT BUDS DEVELOPMENT FROM NODAL SEGMENTS OF *R. SERPENTINA* ON MS MEDIUM SUPPLEMENTED WITH DIFFERENT PGR COMBINATIONS.

PGR combination	Explants*	Time (d) required for multiple shoot buds initiation	Average number of multiple shoot buds/explant
2.0 mgl^{-1} BAP + 2.0 mgl^{-1} NAA	NS	15 - 25	2.71
	LS	-	-
1.0 mgl^{-1} NAA + 0.5 mgl^{-1} BAP	NS	20 - 25	1.00
	LS	-	-
2.0 mgl^{-1} BAP + 1.0 mgl^{-1} IAA	NS	-	-
	LS	-	-
2.0 mgl^{-1} BAP + 0.2 mgl^{-1} NAA	NS	15 - 20	5.85
	LS	-	-

2.0 mgl^{-1} BAP	NS	15 - 20	2.28
	LS	-	-
3.0 mgl^{-1} BAP + 1.0 mgl^{-1} NAA + 1.0 mgl^{-1} Kn	NS	20 - 25	3.28
	LS	-	-
1.0 mgl^{-1} BAP + 0.1 mgl^{-1} IBA	NS	-	-
	LS	-	-
1.0 mgl^{-1} BAP + 0.1 mgl^{-1} NAA	NS	-	-
	LS	-	-
2.0 mgl^{-1} BAP + 0.1 mgl^{-1} IAA	NS	15 - 20	5.85
	LS	-	-
0.1 mgl^{-1} Pic + 1.0 mgl^{-1} BAP	NS	-	-
	LS	-	-

*NS = Nodal segment, LS = Leaf segment

TABLE 2: CALLUS INDUCTION FROM NODAL AND LEAF SEGMENTS OF *R. SERPENTINA* ON MS MEDIUM SUPPLEMENTED WITH DIFFERENT PGR COMBINATIONS.

PGR combination	Explants*	No. of explants used	No. of explants responsed	% of response	Types of responses	Time (d) required for callus induction
1.5 mgl^{-1} BAP + 0.5 mgl^{-1} IAA+ 1.5 mgl^{-1} 2,4-D	NS	10	8	80	White granular callus	15 - 20
	LS	10	3	30		15 - 20
2.0 mgl^{-1} BAP + 0.2 mgl^{-1} IAA	NS	10	-	-	-	-
	LS	10	-	-	-	-
0.1 mgl^{-1} BAP + 0.1 mgl^{-1} Pic	NS	10	-	-	White and compact callus	-
	LS	10	2	20		15 – 20
2.0 mgl^{-1} BAP + 0.5 mgl^{-1} Pic	NS	10	-	-	White and compact	-
	LS	10	2	20		20 – 25
3.0 mgl^{-1} BAP + 1.0 mgl^{-1} NAA + 1.5 mgl^{-1} Kn	NS	10	6	60	Green granular callus	20 - 25
	LS	10	-	-		-
0.1 mgl^{-1} Pic + 1.0 mgl^{-1} Kn	NS	10	4	40	White, Soft	20 - 25
	LS	10	1	10		20 - 25

*NS = Nodal segment, LS = Leaf segment, '-' = indicates no response.

For induction of embryogenesis/ organogenesis the callus that was induced from leaf and nodal segments was further cultured on a broad spectrum of PGRs supplemented media (Table 3). White callus failed to undergo any kind of differentiation and finally died. When green and granular callus was cultured on the medium supplemented with 3.0 mg/l BAP + 1.0 mg/l Kn + 1.0 mg/l NAA, it multiplied and differentiated into shoot buds (Fig. 1d). This observation suggests that only the green and nodular callus had morphogenic competence for regeneration. Similar result was reported by Sarker *et al*. (1996). In order to induce rapid elongation, multiple shoot buds originated either through direct organogenesis or through the intervention of callus were grown on different PGRs supplemented media (Table 4). MS Medium fortified with 2.0 mg/l BAP + 0.2 mg/l NAA was proved efficient for rapid elongation (Fig. 1e)**.** Ahmed *et al*. (2002) also noted similar findings in this plant species. On elongation media shoot buds did not produce any root.

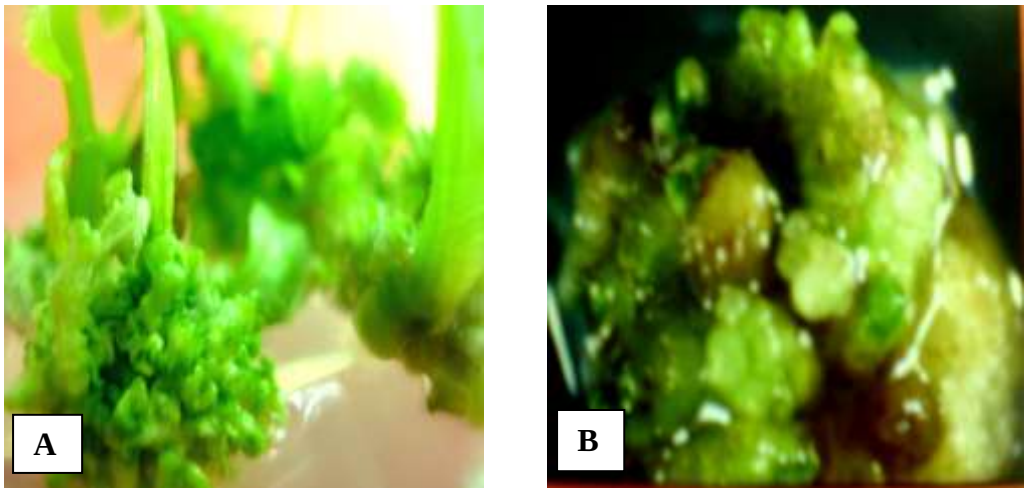
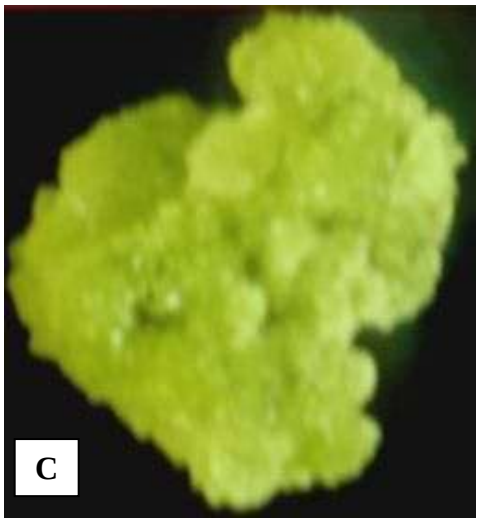
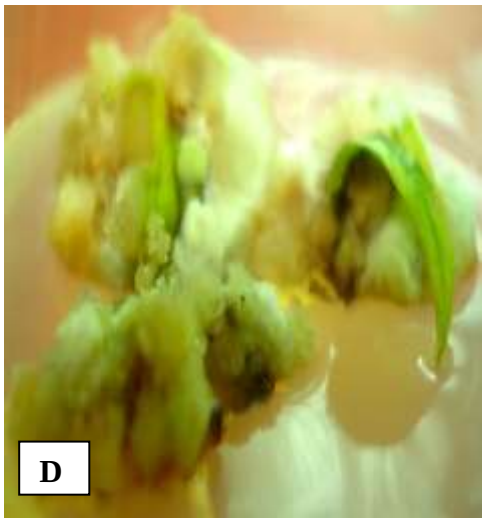


FIGURE 1 PAGE 6 CONTINUE





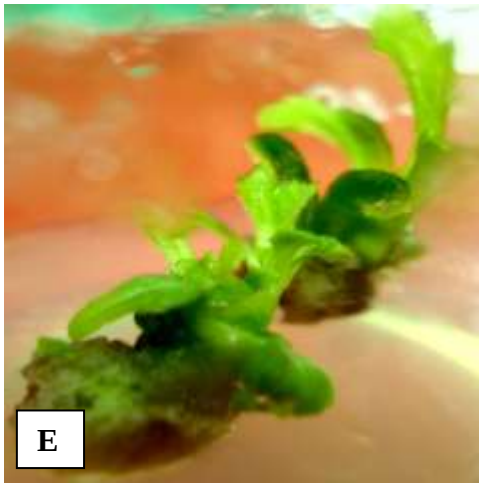


FIGURE 1 A: MULTIPLE SHOOT BUDS DEVELOPED FROM NODAL SEGMENT OF *RAUVOLFIA SERPENTINA*; B, COMPACT GRANULAR AND GREEN TISSUE DEVELOPED FROM NODAL SEGMENT; C, LOOSE AND FRIABLE TISSUE INDUCED IN NODAL SEGMENTS; D, GREEN AND GRANULAR TISSUE UNDERGOING DIFFERENTIATION TO FORM SHOOT BUDS; E, SHOOT BUDS UNDERGOING RAPID ELONGATION IN ELONGATION MEDIUM.

TABLE 3 : RESULTS OF FURTHER CULTURE OF INDUCED CALLUS OF *R. SERPENTINA* ON MS MEDIUM SUPPLEMENTED WITH DIFFERENT PGR COMBINATIONS.

PGR combination	Initial colour of callus tissue	Nature of response after 45d of culture on the medium
1.5 mgl^{-1} BAP + 0.2 mgl^{-1} NAA	White	Multiplied them became brownish and death
1.0 mgl^{-1} BAP + 0.1 mgl^{-1} NAA	White	Multiplied and then died
2.0 mgl^{-1} BAP + 0.1 mgl^{-1} NAA	Green	Multiplied, become light green and produced granular callus
2.0 mgl^{-1} BAP + 2.0 mgl^{-1} NAA	White	Multiplied and then died
2.0 mgl^{-1} BAP + 1.0 mgl^{-1} IAA	White	No multiplication occurred, became brown and finally died
1.0 mgl^{-1} BAP + 2.0 mgl^{-1} Kn	White	No multiplication occurred, became brown and finally died
3.0 mgl^{-1} BAP + 1.0 mgl^{-1} NAA + 1.0 mgl^{-1} Kn	Green	Multiplied along with organogenesis producing shoot buds

TABLE 4 : ELONGATION OF MULTIPLE SHOOT BUDS DEVELOPED FROM NODAL SEGMENTS OF *R. SERPENTINA* ON MS MEDIUM SUPPLEMENTED WITH DIFFERENT PGR COMBINATIONS.

PGR combination	Average initial length (cm)* of individual multiple shoot bud	Average length (cm) of individual multiple shoot bud after 45d of culture on elongation medium	Increased in length (cm) of shoot bud within 45d of culture on elongation medium
2.0 mgl^{-1} BAP + 2.0 mgl^{-1} NAA	1.06	2.43	1.37
0.2 mgl^{-1} NAA + 2.0 mgl^{-1} BAP	1.16	3.96	2.80
3.0 mgl^{-1} BAP + 1.0 mgl^{-1} NAA + 1.0 mgl^{-1} Kn	1.29	2.98	1.69
0.1 mgl^{-1} IAA + 2.0 mgl^{-1} BAP	1.25	2.84	***1.59***

* Only the length of shoot system.

For induction of strong and stout root system the elongated shoots were individually grown on four different rooting media, *viz*., (i) half strength MS + 1.5% (w/v) sucrose (ii) half strength MS + 1.5% (w/v) sucrose + 0.5 mg/l IAA + 0.5 mg/l IBA (iii) half strength MS + 1.5% (w/v) sucrose + 0.5 mg/l NAA and (iv) full strength MS + 3% (w/v) sucrose + 0.5 mg/l IAA. Half strength MS with 1.5% (w/v) sucrose was found most effective for rooting. It proved, the auxin free medium was best for rooting. Such result was also reported by Azad *et al*. (1999). Well developed plantlets were then transferred to outside under natural environment through sequential phases of acclimatization. The protocol thus developed for mass production of seedlings of *R. serpentina* can be used reliably for conservation of this important medicinal plant species of Bangladesh.

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