Indian J. Plant Physiol., Vol. 12, No. 4, (N.S.) pp. 360-365 (Oct.-Dec., 2007)

MULTIPLE SHOOT INDUCTION IN INTACT SHOOT TIP, EXCISED SHOOT TIP AND NODAL SEGMENT EXPLANTS OF *RAUWOLFIA SERPENTINA*

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Received on 3 Oct., 2007, Revised on 30 Dec., 2007

SUMMARY

The effect of different plant growth regulators alone or in combination on multiple shoots production from different explants of *Rauwolfia serpentina* L. was studied. Multiple shoots were obtained from intact or excised shoot tip, and nodal segment in MS medium supplemented with BAP (2.0 mg l^{-1}) and NAA (0.5 mg l^{-1}). Maximum number of shoots (16-24 per explant) was obtained from the excised shoot tips.

***Key words*: Axillary buds, meristematic zone, node, plant growth regulators, *Rauwolfia serpentina*, shoot tips.**

INTRODUCTION

Rauwolfia serpentina, a medicinal plant of tropical origin, is a rich source of indole alkaloids, *viz.* reserpine, recinnamine, serpentine, ajamaline, ajmalicine, etc. According to Ayurveda, its root and whole plant is used for the treatment of cardiovascular disorder, snake bite, rheumatism, hypertension, insanity, epilepsy, eczema, and leaves are used in removal of opacities of the cornea (Joshi and Kumar 2000, Manuchair 2002). Natural reserves of this plant are declining due to overexploitation by local people and tribals who harvest the plants for medicinal uses (Rajendra and D' souza 1999). This has led to listing of this species as "endangered" by the International Union for the Conservation of Nature and Natural Resources (IUCN) (Jain *et al*. 2003). Thus, there have been various attempts to develop *in vitro* propagation techniques for rapid and large scale propagation of this plant to meet the industrial demand and also for its sustainable conservation. Multiple shoots were developed from axillary meristem explants of *Rauwolfia serpentina* on MS medium supplemented with BAP (1.0 mg l^{-1}) and NAA (0.1 mg l^{-1}) (Roja *et al.* 1987) or shoot tips in MS medium containing BAP (2.0 mg l^{-1}) and NAA (0.5 mg l^{-1}) and 15 – 20 shoots were obtained from a single shoot tip explant (Mukhopadhyay *et al.* 1991). Multiple shoots were also obtained from axillary meristems of *R. serpentina* cultured in MS medium supplemented with BA (4.44 µM) and NAA (0.54 µM) (Roja and Heble 1996). Multiple shoot induction depends on different hormonal concentration and combinations. Kinetin and BAP were found to be more effective while 2,4-D, IAA and GA did not have any significant role in multiple shoot induction (Sarma *et al.* 1999). Multiple shoots were also regenerated from nodal segments and shoot tip apices in MS medium supplemented with BA (1.0 mg l^{-1}) and NAA (0.1 mg l^{-1}) with shoot proliferation rate of 79% and 6.4 shoots per explant were obtained (Sarker *et al.* 1996). Different explants of *R. serpentina* were tested and shoot apices inoculated in MS medium supplemented with different concentration of BA and NAA gave best response (Sehrawat *et al.* 2002, and Ahmad *et al.* 2002). Nodal section along with axillary buds were cultured in MS, B_5 and WRC medium and best response was observed in MS medium supplemented with BAP and NAA (Tiwari *et al.* 2003). The present study was aimed at enhancing both the regeneration efficiency and the

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multiple shoot production for mass multiplication of *Rauwolfia serpentina*.

MATERIALS AND METHODS

Young meristematic shoot tips (1.0 – 1.5 cm long) and nodal segments (1.0 cm long containing at least one node with the axillary buds) were collected from one year old field grown plants of *Rauwolfia serpentina*. These explants were washed in running tap water for 30 minutes followed by washing with 1.0% solution of Twene-20 for 25 minutes. The explants were washed thoroughly thereafter until the detergent was completely washed out. To prevent fungal contamination, shoot tip and nodal segment explants were treated for 30 minutes with 6 and 10% Bavistin solution, respectively. Finally, the explants were treated with 70% ethanol for one minute followed by 0.1% $HgCl_2$ for 5-10 minutes in a laminar flow chamber. After each treatment, explants were washed thrice with autoclaved double distilled water. The explants were then cultured on MS medium supplemented with various concentrations of the growth regulators BAP, kinetin, NAA and IBA, separately or in combination. The pH of the medium was adjusted to 5.8 ± 1.0 before autoclaving.

Properly sterilized nodal segment explants (containing axillary buds) and intact or excised shoot tip (2.0–3.0 mm apical portion removed) were inoculated in glass bottles containing MS medium supplemented with different concentrations of growth hormones. Each treatment was repeated thrice. The regenerated plants were sub-cultured every three weeks interval.

RESULTS

Intact and excised shoot tips inoculated in basal MS medium failed to produce multiple shoots. Intact shoot tips inoculated in MS medium supplemented with either BAP (1.0-2.5 mg l^{-1}) or Kinetin (1.0-2.5 mg l^{-1}) alone could produce multiple shoots. The best response for multiple shoot induction was obtained in MS medium supplemented with both BAP (2.0 mg l^{-1}) and NAA (0.5 mg l^{-1}) wherein 85.58 % of the cultures produced multiple shoots. Excised shoot tips inoculated in MS medium supplemented with either BAP (1.0-2.5 mg l^{-1}) or kinetin (1.0-2.5 mg l^{-1}) alone also produced multiple shoots, but in the presence of both BAP (2.0 mg l^{-1}) and NAA (0.5 mg l^{-1}), the multiple shoot regeneration effiency was higher (91.40 %) (Table-1, Plate-1, and Plate-2).

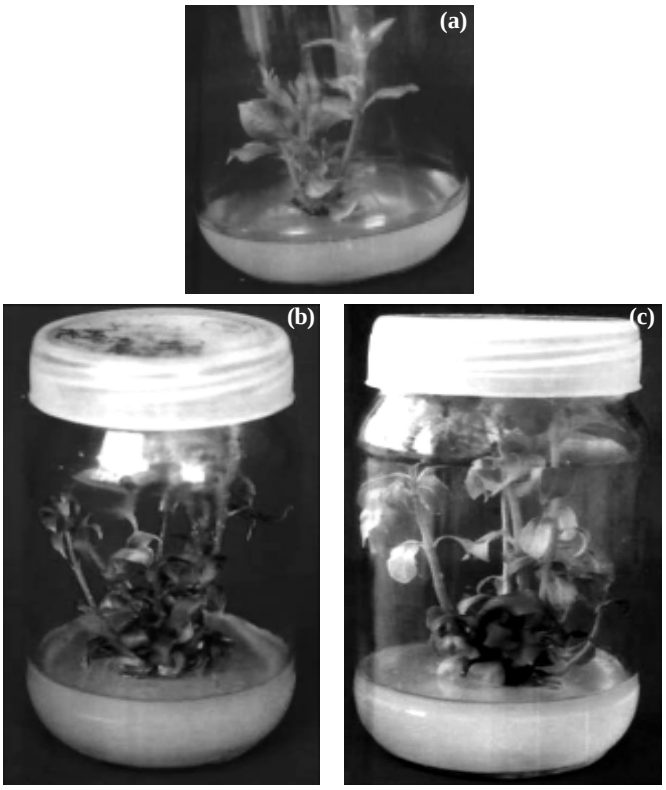

Plate 1. Induction of multiple shoots in *R. serpentina* by using shoot tip explants on MS medium supplemented with BAP (2.0 mg l^{-1}) and NAA (0.5 mg l^{-1}): (a) 5 DAI, (b) 15 DAI, (c) 25 DAI

Nodal segments inoculated in basal MS medium showed very low percent shooting (15%) and only two shoots per culture were obtained. MS medium supplemented with either BAP (0.1-2.5 mg l^{-1}) or kinetin (0.1-2.5 mg l^{-1}) alone showed variation in the induction of multiple shoots which ranged between 25 to 66 % in case of BAP and 17 to 56.35 % in case of kinetin alone. The highest percent of shoot induction (86.68 %) was obtained in MS medium supplemented with BAP (2.0 mg l^{-1}) and NAA (0.5 mg l^{-1}) and 5.0 shoots per culture were obtained (Table-2, Plate-3).

Multiple shoots were obtained from all the three explants though in different proportions. Higher numbers of multiple shoots were obtained from excised shoot tip explants (16-24 shoots per explant) followed by that

Table 1. Multiple shoot induction in shoot tip and excised shoot tip explants of *R. serpentina* L. in MS medium supplemented with different concentration of BAP, kinetin, NAA and IBA in combination or alone.

Growth regulators	% Shoot formation*		Average shoots per culture*		Average length of shoots (cm)*	
(mg/l)	Shoot tip	Excised shoot tip	Shoot tip	Excised shoot tip	Shoot tip	Excised shoot tip
MS_0	-	-	-	-	-	
BAP						
0.1	15.00	17.00	2.3	2.6	2.4	2.5
0.5	38.00	39.00	3.1	3.3	3.4	3.6
1.0	4200	44.00	3.3	3.6	3.5	3.8
1.5	46.00	4800	3.6	3.8	3.6	4.0
2.0	61.00	65.00	5.4	5.6	4.1	4.3
2.5	51.00	53.00	3.4	3.7	3.7	3.8
3.0	21.00	27.00	2.2	2.4	2.1	2.3
Kinetin						
0.1	10.00	12.00	2.0	2.3	1.4	1.3
0.5	34.00	36.00	2.8	2.9	1.8	1.9
1.0	40.00	42.00	3.0	3.1	2.1	2.3
1.5	51.00	53.00	3.3	3.4	2.6	2.8
2.0	58.00	60.35	4.8	6.4	3.1	3.7
2.5	49.00	51.00	3.1	3.3	2.4	2.4
3.0	21.00	23.00	2.2	2.4	2.1	2.3
BAP + NAA						
0.1 + 0.1	30.33	35.33	2.6	2.8	2.6	2.8
0.5 + 0.2	35.00	39.00	3.2	4.5	3.2	3.4
1.0 + 0.3	48.22	48.22	4.8	6.9	3.4	3.6
1.5 + 0.4	68.00	68.00	9.8	16.25	4.6	4.8
2.0 + 0.5	85.58	91.40	14.0	24.00	5.1	5.7
BAP + IBA						
0.1 + 0.1	25.33	27.15	1.6	1.6	1.5	1.5
0.5 + 0.2	32.00	30.54	2.2	2.2	2.1	2.4
1.0 + 0.3	42.22	44.28	2.8	2.8	2.6	2.8
1.5 + 0.4	62.00	63.00	3.5	3.5	3.1	3.3
2.0 + 0.5	66.58	68.37	5.1	5.9	3.6	4.2
Kin + NAA						
0.1 + 0.1	26.43	28.31	1.8	1.8	1.9	1.9
0.5 + 0.2	31.34	34.38	2.1	2.1	2.3	2.4
1.0 + 0.3	47.56	48.14	2.6	2.6	2.5	2.7
1.5 + 0.4	63.67	64.13	3.1	3.1	3.1	3.3
2.0 + 0.5	71.32	72.00	6.6	8.3	3.3	3.7
Kin + IBA						
0.1 + 0.1	16.73	18.73	1.3	1.3	1.4	1.5
0.5 + 0.2	28.84	25.84	1.8	1.8	1.6	1.8
1.0 + 0.3	42.56	43.59	2.7	2.7	2.1	2.4
1.5 + 0.4	52.67	53.23	2.9	2.9	2.5	2.6
2.0 + 0.5	54.19	58.19	4.1	5.1	3.1	4.1

* Mean of three replicates of shoot tip explants (intact and excised)

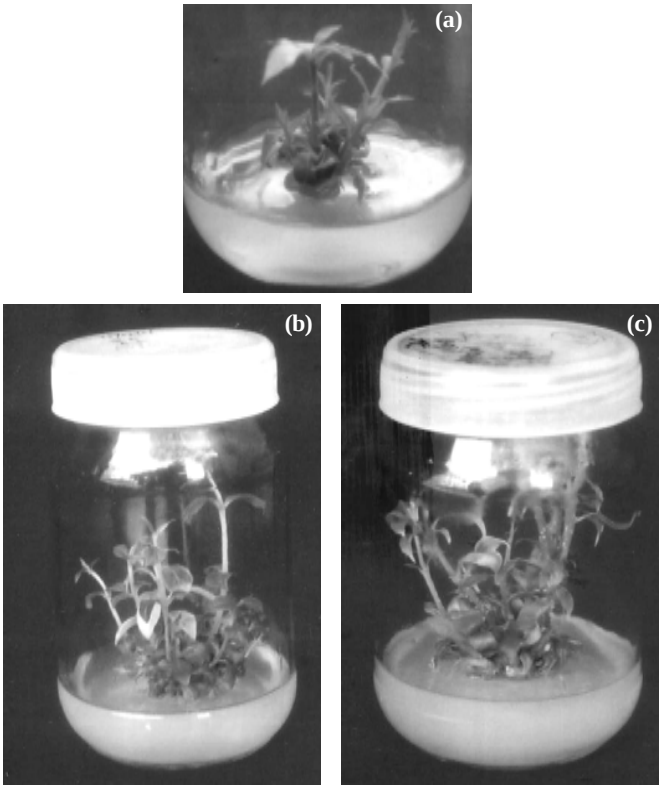

Plate 2. Induction of multiple shoots in excised shoot tip explants of *R. serpentina* in MS medium supplemented with BAP (2.0 mg l^{-1}) and NAA (0.5 mg l^{-1}): (a) 5 DAI, (b) 15 DAI, (c) 25 DAI

from intact shoot tip explants (14 shoots per explant). Nodal segment explants produced the least number of multiple shoot (five per explant).

DISCUSSION

From intact shoot tip, shoot tip with excised apical portion and nodal segment explants, about 5-24 shoots per culture were obtained. Shoot tip, excised shoot tip and nodal segment explants produced 85.58 %, 91.40 %, and 86.68 % multiple shoots respectively inoculated in MS medium. BAP (2.0 mg l^{-1}) and NAA (0.5 mg l^{-1}) was found to be the best combination for multiple shoot induction. The least responsive combination for multiple shooting in shoot tips, excised shoot tip and axillary bud was kinetin (0.1 mg l^{-1}) and the multiple shoots obtained were 10.00, 12.00 and 16.00%, respectively. When all these explants except nodal segment explants, were inoculated in basal MS medium shoots were not

Table 2. Multiple shoot induction in nodal segment explants of *R. serpentina* L. in MS medium supplemented with different concentration of BAP, kinetin, NAA and IBA in combination or alone.

Growth regulators (mg/l)	% of Shoot formation*	Average no. of shoots per culture*	Average length of shoots (cm)*
MS_0	15.0	2.0	2.4
BAP			
0.1	25.00	2.4	4.4
0.5	38.25	2.8	4.6
1.0	48.00	3.1	4.7
1.5	58.00	3.4	4.7
2.0	66.00	3.9	4.8
Kinetin			
0.1	16.00	2.0	1.4
0.5	36.00	2.4	1.8
1.0	46.00	2.5	2.1
1.5	54.00	2.6	2.6
2.0	56.35	3.2	3.7
2.5	49.00	3.1	2.4
3.0	21.00	2.2	2.1
BAP + NAA			
2.0 + 0.1	46.00	3.1	4.3
2.0 + 0.2	55.00	4.2	6.1
2.0 + 0.3	62.22	3.9	5.2
2.0 + 0.4	72.00	4.2	5.6
2.0 + 0.5	86.68	5.0	5.8
BAP + IBA			
0.1 + 0.1	28.15	1.6	1.5
0.5 + 0.2	32.31	1.8	2.1
1.0 + 0.3	46.21	2.8	2.8
1.5 + 0.4	61.28	4.0	3.1
2.0 + 0.5	72.24	4.2	4.6
Kin + NAA			
0.1 + 0.1	30.31	1.8	1.9
0.5 + 0.2	38.38	2.1	2.3
1.0 + 0.3	51.37	2.6	2.5
1.5 + 0.4	66.49	3.1	3.1
2.0 + 0.5	74.00	4.3	3.4
Kin + IBA			
0.1 + 0.1	17.79	1.3	1.4
0.5 + 0.2	26.84	1.8	1.8
1.0 + 0.3	47.13	2.7	2.1
1.5 + 0.4	55.23	2.9	2.6
2.0 + 0.5	61.19	3.5	4.5

*Mean of three replicates of nodal segment explants

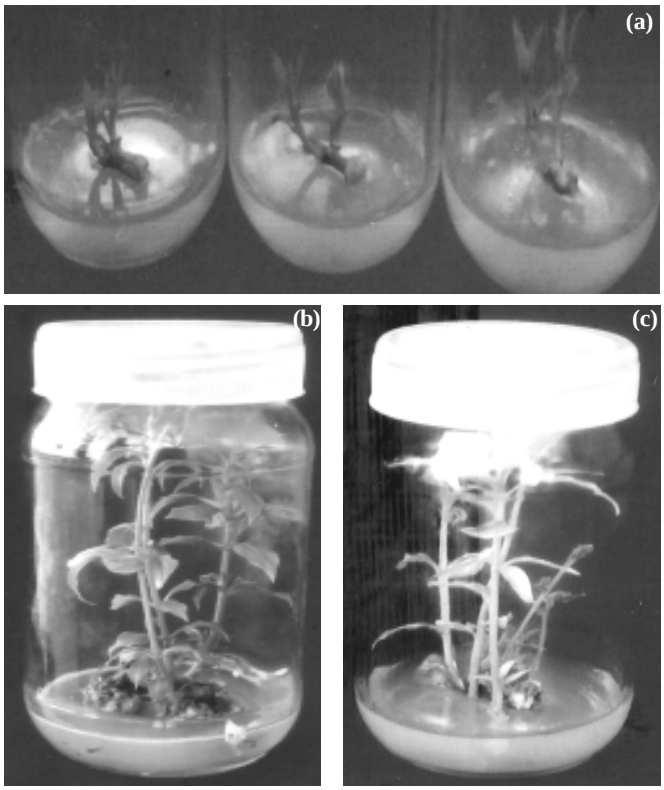

Plate 3. Induction of multiple shoots in *R. serpentina* by using nodal segment explants on MS medium supplemented with BAP (2.0 mg l^{-1}) and NAA (0.5 mg l^{-1}): (a) 5 DAI, (b) 15 DAI, (c) fully developed multiple shoots 25 DAI

produced. MS medium supplemented with cytokinins alone (BAP or Kinetin) produced shoots in all the explants. But by supplementing the medium with cytokinins (BAP or Kinetin) and auxins (NAA & IBA), both the percent shoot regeneration as well as number of shoots per culture was increased which shows that auxin (NAA) also plays significant role in multiple shoot induction and contradict the earlier reports of Sarma *et al.*, 1999. The 2:1 ratio of auxin and cytokinin was also found effective in terms of shoot formation.

The enhanced rate of multiple shoot induction in cultures supplemented with BAP and NAA may be largely ascribed due to increased rates of cell division induced by cytokinin (BAP) in the terminal and axillary meristematic zone of explant tissues. Cells in this zone divide with the faster pace and thus, produce large number of shoots. The increased multiple shooting in excised shoot tip explants may be due to rapid division of cells in the excised tip and production of several primordial outgrowths which eventually develop into shoots. Removal of terminal portion of the shoot tip explants also subverts the phenomenon of "apical dominance".

In the present investigation higher numbers of multiple shoots (16-24 shoots per explant) were obtained from excised shoot tip explants of *R. serpentina L.* There are no earlier reports in which excised shoot tip explants were used for the multiple shoot induction in *R. serpentina*. Thus, this protocol can be suitably exploited for the mass multiplication on a larger scale and may be worthful in the conservation of natural reserves of *R. serpentina*.

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