African Journal of Biotechnology Vol. 8 (17), pp. 4048-4051, 1 September 2009
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2009 Academic Journals

Full Length Research Paper

Callus regeneration from stem explants of *Pseudarthira viscida* (L.) Wight and Arn. – a vulnerable medicinal plant

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Accepted 14 September, 2006

An *in vitro* propagation protocol has been developed for *Pseudarthira viscida* (L.) Wight and Arn. (Papilionaideae), a vulnerable medicinal plant from stem callus was used for axillary shoot multiplication. The plant was standardized by using MS (Murashige and Skoog) medium containing 3% (w/v) sucrose, supplemented with a cytokinin (BAP, KN) maximum number of shoot (29.87±5.34) were observed on the medium containing 0.6 mg/l BAP after three weeks of culture. Single shoots were induced and elongated on MS medium supplemented with BAP+NAA (0.4 + 0.04 mg/l). The elongated shoots were rooted in half MS medium with 0.4 mg/l IBA, and then were successfully hardened and transferred to the field.

Key words: *In vitro* regeneration, micropropagation, vulnerable, *Pseudarthria viscida*, plantlets.

INTRODUCTION

Tissue culture, an important aspect of biotechnology can be used to improve the productivity of planting material through enhanced availability of identified planting stock with desired traits. Significant advances in increasing regeneration frequency have come from tissue culture work done in cereals (Inove and Maeda, 1980; Jawahar et al., 1997; Jawahar et al., 1988; and Nabors et al., 1982). *Pseudarthria viscida* of Paplionoideae is a perennial sub shrub, distributed throughout India especially found in river basins and in hills up to above 900 m. The plant has several medicinal uses in indigenous system of medicine. The roots are astringent, sweet, bitter and digestive. They are useful in conditions of cough, bronchitis asthma, tuberculosis, diarrhea and diabetes (Ogura and Shimada, 1978). This work is chiefly aim at developing a micropropagation method in through the induction of multiple shoot from stem derived calli.

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This paper deals with indirect regeneration of from stem callus of *in vitro* seedlings.

MATERIALS AND METHODS

Seeds were collected from natural habitats. Healthy seeds were soaked for 24 h in distilled water. Later they were kept in running tap water for 30 min. Seeds were then treated with liquid detergent (Teepol 1 ml per 100 ml of sterilized distilled water) for 5 min followed by thorough washing. They were surface sterilized with 0.1% mercuric chloride (w/v) for 5 min washed 5 – 6 times in sterile double distilled water and then implanted aseptically on half strength MS basal medium (Murashige and Skoog, 1962) containing sucrose and gelled with 0.8% (w/v) agar. When the seedlings grew up to 6 cm, the stem explants derived from 25 days old aseptic seedlings were excised and used to initiate culture.

Culture media and condition

The MS medium (Murashige and Skoog, 1962) was variously supplemented with cytokinin (BAP, KN) and auxin (NAA, IBA) whether singly or in combination. The pH was adjusted to 5.7 - 5.8 using 0.1% NaOH or 0.1% HCl prior autoclaving at 121ºC for 20 min. All cultures were incubated at 25±2ºC in a photoperiod for 16 h

Table 1. *In vitro* responses from stem callus of *Psudarthria viscida* Wight & Arn.

Growth Hormones	Concentration (mg/l)	Number of Culture	Number of responded %	Mean no. of shoot (cm)	Mean no. of shoot length (cm)	Mean no. of root (cm)	Mean no. of root length (cm)
BAP							
	0.2	25	76	11.95±0.38	2.66±0.57	-	-
	0.4	25	84	14.38±3.72	3.90±0.43	-	-
	0.6	25	96	29.87±5.34	4.94±0.38	-	-
	0.8	25	84	17.38±4.98	3.21±0.70	-	-
	1.0	25	72	11.16±1.77	2.62±0.29	-	-
KN							
	0.2	25	80	11.75±0.45	2.22±0.32	-	-
	0.4	25	88	13.09±2.85	2.90±0.24	-	-
	0.6	25	92	14.78±4.04	3.10±0.32	-	-
	0.8	25	76	12.15±0.58	3.64±0.57	-	-
	1.0	25	68	11.76±0.54	3.39±0.65	-	-
BAP+NAA							
	0.2+0.02	25	76	11.31±0.46	3.96±0.65	-	-
	0.4+0.04	25	84	11.61±0.48	6.78±0.73	-	-
	0.6+0.06	25	88	13.00±0.85	4.45±1.14	-	-
	0.8+0.08	25	80	12.60±0.58	4.57±0.57	-	-
	1.0+0.1	25	68	11.76±0.54	4.11±0.57	-	-
KN+NAA							
	0.2+0.02	25	68	11.38±0.48	2.93±0.23	-	-
	0.4+0.04	25	72	11.89±0.44	3.65±0.39	-	-
	0.6+0.06	25	88	12.31±0.55	4.41±0.66	-	-
	0.8+0.08	25	68	11.77±0.41	3.81±0.77	-	-
	1.0+0.1	25	60	11.41±0.49	3.12±0.41	-	-
IBA							
	0.2	25	80	-	-	2.20±0.60	8.26±1.15
	0.4	25	88	-	-	3.68±0.46	11.27±0.82
	0.6	25	80	-	-	2.95±0.58	9.53±0.88
	0.8	25	72	-	-	2.27±0.44	8.03±0.98
	1.0	25	68	-	-	1.47±0.49	6.83±1.10

25 explants were used for each treatment and data (Standard Deviation) recorded three-four weeks after culture.

for a day under white fluorescent light and with 55-60% relative humidity. Each experiment was repeated at least two times with 25 cultures per treatment. The mean percentage of cultures producing multiple shoots and mean number of shoots per culture were recorded after two weeks. The percentages of rooting and average number of roots per shoot were recorded after 10 days. The data were analyzed statistically.

RESULTS AND DISCUSSION

Stem segments produced callus efficiently on the MS medium (Murashige and Skoog, 1962) augmented with various concentration of cytokinin (BAP 0.2, 0.4, 0.8, 1.0 mg/l, KN 0.2, 0.4, 0.8, 1.0 mg/l). Significant callus formation was observed within 10 – 15 days at the cut ends and surface of the explants. Compact green nodular callus was observed in stem explants within 4 weeks. The buds like calli were observed after one month of inoculation. Initially buds were light green color on MS medium (Murashige and Skoog, 1962) supplemented with 0.2 mg/l BAP. Among the different concentrations of cytokinin used for callus induction, optimal result was observed in MS medium with 0.2 mg/l BAP (Figure 1a,b).

For shoot differentiation light green compact calli of stem explants were subcultured in MS medium supplemented with different concentrations of BAP or KN. The highest percentage of shoot multiplication was observed on MS medium with 0.6 mg/l BAP (Table 1). At low con-

Figure 1. *In vitro* multiplication of *Psuedarthria viscida* through indirect regeneration. a,b) callus induction from stem explant in MS+BAP (0.2mg/l); c) Shoots multiplication in MS+BAP (0.6mg/l), d) Shoot elongation in MS+(BAP 0.4+NAA 0.04mg/l); e)Root development in half MS+ (IBA 0.4mg/l); f,g) Hardening (sand, garden soil and farmyard manure ratio of 1:1:1); h) Transferred to plastic pages.

centration of BAP, the callus did not produce multiple shoots. Therefore, increasing concentration of BAP resulted in production of more amount of callus with buds. Notable shoots multiplication was observed on MS medium with 0.6 mg/l BAP (Table 1, Figure 1c). 24 explants responded out of 25 inoculated explants. 29.87±5.34 multiple shoots of 29.87±5.34 were observed in same concentration. When treated with various concentrations of cytokinin, less amount of multiple shoots were observed compared to BAP. Highest percentage (92%) was observed on MS medium supplemented with KN 0.6 mg/l (Table 1). The maximum number of shoot multiplication was observed in the concentration 14.78±4.04 (Table 1). When treated with high concentration of KN, the callus did not produce multiple shoots, hence, color was changed from light green to brown color. In the same concentration of KN the callus produced very low multiple shoots (Shimada and Yamada, 1979). The regenerated shoot buds were grown in MS medium containing different concentrations of cytokinin (BAP or KN) singly or in combination with auxin (NAA or IAA) for shoot elongation. The highest shoot elongation was achieved by culturing on MS medium supplemented with the combination of cytokinin and auxin (BAP 0.4+NAA 0.04 mg/l) maximum shoot length 6.78 cm was observed in this concentration (Figure 1d).

Cytokinin treatment alone did not elongate shoot but produced multiple shoots. KN with NAA treated explants produced basal callus on surface of the explants. The best result was observed on MS medium supplemented with BAP with NAA (0.4 mg/l + 0.04 mg/l).Healthy shoots were transferred to rooting medium containing half strength MS medium supplemented with different concentration of auxin (IBA 0.2 - 1.0 mg/l). Roots emerged from the cut end of the shoots within one week. The roots are white, long and slender with hairs. Jawahar et al. (1998) have reported on *Lycopersicon esculentum* species roots were white and long slender with hairs. Maximum numbers of roots were observed on half MS medium supplemented with auxin (IBA 0.4 mg/l (Figure 1e). Maximum root length (1.27±0.82) per shoot was

recorded on 0.4 mg/l IBA (Table 1).

Well developed plantlets were transferred to *in vivo* conditions. The plantlets were carefully taken out and then washed with tap water to remove the traces of agar stick into the roots. Care was taken not to injure the roots while washing. The plants were transferred to paper cups containing sand, garden soil and farmyard manure in the ratio of 1:1:1 (Figure 1f, g) and covered with plastic bag for one month in culture room half or quarter strength MS medium was added twice a week. After one month, plants were transferred to plastic bags, and maintained at root temperature for 10 days and later moved to the green house (Figure 1h).

ACKNOWLEDGEMENTS

We thank Ministry of Environment Forest Division, New Delhi, India for financial support. Our thanks are also due to Dr. S. Soosairaj for the helpful discussion and criticism of the manuscript.

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