African Journal of Plant Science Vol. 7(1), pp. 1-8, January 2013
Available online at http://www.academicjournals.org/AJPS
DOI: 10.5897/AJPS12.057
ISSN 1996-0824 ©2013 Academic Journals

Full Length Research Paper

In vitro propagation studies of *Albizia amara* (Roxb.) Boiv.

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Accepted 13 December, 2012

***Albizia amara*, belonging to the family Fabaceae is a valuable economic, medicinal and multipurpose drought tolerant tree commonly found in dry forests of India. Keeping view of its economic importance, a protocol was developed for rapid clonal multiplication of *A.amara* by means of plant tissue culture. In the present study, development of complete plantlets via induction of multiple shoots from seedling derived cotyledonary node explant and their successful rooting and acclimatization is reported. Cotyledonary node explants were excised from 12-15 day old aseptic seedlings to initiate culture for multiple shoot induction on MS medium. Among the different plant growth regulators tested either in single or in combination, BAP 1 mg/l, BAP 1 mg/l + Kn 2 mg/l, BAP 1 mg/l + NAA 1 mg/l were effective in inducing multiple shoots from cotyledonary node explants. Shoot elongation was prominent at Kn 0.25 mg/l concentration. *In vitro* rooting experiments were successful on half strength MS medium fortified with NAA 1 mg/l.**

Key words: *Albizia amara*, tree, micropropagation, axillary bud multiplication.

INTRODUCTION

Albizia amara (Roxb.) Boiv., belonging to the family Fabaceae, is a valuable economic, medicinal and multipurpose drought tolerant tree commonly found in dry forests of India. The wood of *Albizia amara* is purplish brown with lighter bands, very hard and strong, used for cabinets in building and agriculture purpose. The bark of the tree yields gum, which is used for ulcers (Kashyapa and Ramesh, 1992) and molluscidal activity (Ayoub and Yankov, 1986). Besides these, the leaves contain a flavanol glycoside namely 4'O–menthylrutin and they are extensively used as herbal cosmetic for hair maintenance. Leaves are also useful in ophthalmia. Budmunchiamines, spermine macrocyclic alkaloid (Mar et al., 1991) extracted from the seeds of *A. amara* were found to interact with DNA by inhibiting the catalytic activity of DNA polymerase, RNA Polymerase, and HIV reverse transcriptase. *Albizia* is also one among the 10 major species, which is widely used in plantation forestry programme in India. Kumar et al. (1998) described regeneration of plants from leaflet explants in *A. procera.* Sinha et al. (2000) described *in vitro* differentiation and plant regeneration in *A. chinensis.* Ramamurthy and Savithramma (2003) described shoot bud regeneration from leaf explants. Micropropagation via axillary bud proliferation was not described in *A. amara*, hence this study was undertaken. This paper describes a reproducible and efficient protocol to regenerate true to type plantlets directly from seedling explants.

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MATERIALS AND METHODS

Healthy seeds of *A. amara* after mechanical separation were washed in an agitated detergent solution (0.1% laboline Merck, India) for 5 min followed by thorough washing under a jet flow of tap water for half an hour. Seeds were then scarified with conc. H_2SO_4 (98%) for 3 min. After scarification, seeds were subjected to repeated washing for 3 to 4 times with sterilized double distilled water (DDW), the seeds were soaked in sterile DDW and incubated at 25 $\pm$ 2°C for 24 h. Surface sterilized seeds were inoculated singly on different nutrient media. Various types of surface sterilants like mercuric chloride ($HgCl_2$), Sodium hypochlorite (NaOCl) and

Figure 1. Morphogenetic response of cotyledonary node of *A. amara* on MS medium.

Hydrogen peroxide (H_2O_2) were used for sterilizing the seeds in order to raise healthy seedlings. Various experiments were performed to standardize a low cost media for high percent of germination. Tap water + agar, Tap water + agar + 2% sucrose, MS microsalts, MS macrosalts, ½ MS alone, MS alone were tested for aseptic seed germination. These experiments were conducted sequentially after the thorough study of remarks obtained in the previous experiment.

Two to three weeks old aseptic seedlings were used as explant source. Different seedling explants were excised form aseptically grown seedlings and planted on different media. These were subcultured for every 20 days. The results were recorded after the second passage and cultures that were contaminated were excluded. In the present investigation, all the experiments were repeated thrice and fifteen replicates per treatment were taken. The cultures were incubated at 2000 lux light under cool white, fluorescent tubes (Philips, India Make, 40 watt). The photo period regime was 16 h light/8 h dark diurnal cycles maintained at a constant temperature of 25 ± 2°C. Morphogenetic potentiality was examined by culturing different seedling explants such as roots, hypocotyl, cotyledons, cotyledonary nodes, nodal explants, internodes, leaves, shoot tips etc. on MS medium supplemented with 2% sucrose and BAP 0.1 to 3 mg/l. The morphogenic responses of the explants were recorded. Best explants for shoot regeneration were identified based on the number of shoots, shoot length and percentage of response. MS, B_5, WPM media supplemented with 2 mg/l BAP and 2% sucrose were used for screening the efficient medium on the organogenesis of various seedling explants. Criteria for medium selection include percentage of explants responded, average number of shoots, and shoot length per explant.

Morphogenic influence of different plant growth regulators and other organic supplements was further tested after the selection of best medium and best explant for shoot multiplication. Single shoot with 2 to 3 nodes were excised from multiple shoot cultures and transferred to elongation medium. MS ½ strength, supplemented with auxins such as IAA, IBA, NAA (0.1 to 3 mg/l) were used for rhizogenesis of regenerated shoots. After root induction, the plantlets were removed carefully from the test tubes and washed thoroughly under running tap water to remove the traces of agar adhering the plantlets. The plantlets were transferred to pots containing coarse sand and vermiculate (1:3) covered with polythene bags and incubated at 25 ± 2°C for 10 to 15 days to ensure high humidity. MS quarter strength basal liquid medium was used during the period of acclimatization. Later small holes were made on the polythene bag to reduce the relative humidity. Gradually the pots were transferred to room temperature having diffuse light and finally plants were exposed to sunlight.

RESULTS AND DISCUSSION

In vitro seed germination studies

Among the three surface sterilants used, 0.1% $HgCl_2$ for 3 min was found to be an effective disinfectant and was also good at promoting germination. Of the different treatments, high percent frequency of germination was obtained by placing uniform, healthy seeds on ½ strength MS medium. A germination frequency of 80% was recorded with in a period of 24 h after inoculation on medium. Within 14 days of culture, seedlings attained a height of 6.8 cm with 3 to 4 nodes. In the present study, it was observed that ½ strength MS medium was effective for seed germination, as they do not require much ion supplementation for its further development.

Multiple shoot induction

Explant evaluation

Different seedling explants were tested for their ability to regenerate shoot buds by culturing on MS medium fortified with BAP and Kn separately with 2% sucrose and the results are tabulated. To regenerate shoot buds, maximum bud proliferation was observed on cotyledonary nodes (Figure 1). Axillary bud sprouting was observed from the 9th day of inoculation, shoot elongation started after 12 days of inoculation. Cotyledonary nodes cultured on MS medium fortified with BAP 1 mg/l showed highest number of multiple shoots (6.84 ± 0.30 shoots/ explant) with average shoot length of 3.20 ± 0.54 (Table 1). The cotyledonary nodes proved to be better even on Kn 0.5 mg/l (3.42 ± 0.30 shoots/ explant) than other explants for regeneration of shoots. Since the highest number of shoots was observed from cotyledonary nodes in all harmonal media, further shoot multiplication experiments were carried out with cotyledonary nodes. Regeneration potentiality of explants in the present study is in the following order: Cotyledonary node > node > Shoot tip.

Media evaluation

Media evaluation is a prerequisite step for efficient organogenesis in plant tissue culture. Bhojwani and Razdan (1983) suggested that in order to formulate

Table 1. Effect of BAP and Kn on shoot initiation from seedling explants of *A. amara*.

Plant growth regulator (mg/l)	Cotyledonary node			Node			Shoot Tip		
	% of explants responded	No. of shoots Mean ± S.D	Shoot length(cm) Mean + S.D	% of explants responded	No. of shoots Mean + S.D	Shoot length(cm) Mean + S.D	% of explants responded	No. of shoots Mean + S.D	Shoot length(cm) Mean + S.D
BAP 0.25	45	1.75±0.55	1.50±0.35	35	2.00±0.30	1.20±0.40	30	2.00±0.10	1.50±1.42
BAP 0.50	60	3.75±0.22	2.60±0.25	40	2.60±0.20	1.25±0.35	50	2.50±0.70	2.00±0.20
BAP 1.0	90	6.84±0.30	3.20±0.54	50	2.95±0.10	1.60±0.20	45	2.20±0.60	1.80±0.38
BAP 2.0	70	4.60±0.55	3.00±0.31	60	3.80±0.35	1.80±0.45	55	2.80±0.45	2.10±0.25
Kn 0.25	60	2.00±0.45	4.80±0.35	30	1.25±0.45	1.10±0.30	35	1.30±0.40	2.35±0.05
Kn 0.50	70	3.42±0.30	2.00±0.60	40	1.90±0.25	1.20±0.50	50	1.75±0.35	2.90±0.40
Kn 1.0	50	1.80±0.36	1.75±0.30	55	2.60±0.20	1.40±0.30	40	1.50±0.32	2.50±0.45
Kn 2.0	45	1.65±0.25	1.00±0.40	45	2.20±0.50	1.20±0.35	30	1.10±0.30	2.10±0.10

a suitable medium for a new system, it would be better to start with well known basal media such as MS, B_5 and WPM. In the present study, MS medium was found to be superior for the induction of more number of multiple shoots with high percent frequency. The percentage of response was highest in MS (90%) followed by B_5 (70%) and WPM (60%).

Effect of different carbon sources on morphogenesis

Organogenesis may be influenced by the type of carbon source used in the media and this was tested by using 2% of different carbon source like glucose, fructose, sucrose and maltose. Though, the average number and average length of regenerated shoots was not much influenced by the type of carbon source, nature of response varied greatly. Among different carbon sources used, sucrose was unique in producing healthy and sturdy shoots with fast growth, showing 90% response. About 90% of cultures responded well at 2% sucrose concentration with a maximum of 3.60±0.35 shoots / explant with an average length of 3.52 ± 0.40 cm.

Effect of different plant growth regulators on shoot multiplication

The highest rate of micropropagation will often depend not only on the selection of the most suitable explant and medium, but also on the discovery of correct combination of growth regulators and modulation of hormone levels.

Role of cytokinins on shoot multiplication

Transversely, sectioned cotyledonary node explants were inoculated into each culture tube containing 15 ml of the medium. Each culture contained a single explant and the mouth was covered with aluminum foil. In the present study, four cytokinins BAP (0.1 to 3 mg/l), Kn (0.1 to 2 mg/l) TDZ (0.01 to 1 mg/l) and Zeatin (0.1 to 1 mg/l) were employed either singly or in various combinations for multiple shoot induction from cotyledonary nodes of *A. amara* (Table 2). Among the various concentrations of BAP tested for shoot proliferation at 1mg/l cotyledonary nodes showed 80% of response with an average of 6.84 ± 0.30 shoots/explants and attained a length of 3.20 ± 0.54 cm with 4 to 5 nodes with little basal callus (Figure 2a). It was observed that increase in concentration of BAP increased basal callusing, with retardation of shoot growth.

Among different concentrations of Kn used for shoot multiplication, better response (70%) was observed at 0.5 mg/l with a maximum of 3.42 ± 0.30 shoots/explant and a length of 2.00 ± 0.60 cm. Among various cytokinins tested, TDZ (0.01-1 mg/l) show a differential response. The concentration at which TDZ is most effective is 10 to 1000 times lower than other PGRs. Low concentrate of TDZ at 0.05 mg/l show fasciated shoots, which differ markedly from normal shoots, appearing as though several shoots are fused together to form a flattened stem. Shoot regeneration efficiency of cotyledonary node was 65% at 0.25 mg/l Zeatin. On the whole Kn, TDZ and Zeatin were inferior to BAP in terms of percentage of response, number of shoots/explant and shoot length.

Effect of cytokinin combination on shoot multiplication

Different combinations of cytokinins were used to

Table 2. Effect of various cytokinins on multiple shoot induction from cotyledonary nodes of *A. amara* on MS medium.

Growth regulators (mg/l)	% of response	No. of shoots / explants (Mean ± S.D)	Shoot length (cm) (Mean ± S.D)	Basal Callus
BAP				
0.1	30	1.60±0.40	1.00±0.30	+
0.25	45	1.75±0.55	1.50±0.35	+
0.50	60	3.75±0.22	2.60±0.25	+
1.0	90	6.84±0.30	3.20±0.54	++
2.0	70	4.60±0.55	3.00+0.31	+++
3.0	50	2.80±0.50	2.00±0.10	+++
Kn				
0.1	40	1.45±0.20	2.60±0.25	+
0.25	60	2.00±0.45	4.80±0.35	+
0.5	70	3.42±0.30	2.00±0.60	+
1.0	50	1.80±0.36	1.75±0.30	+
2.0	45	1.65±0.25	1.00±0.40	+
TDZ				
0.05	50	1.30±0.72		
0.1	40	1.00±0.65	0.65±0.10	+
0.25	35	CP	CP	+++
0.5	30	CP	CP	+++
1.0	30	CP	CP	+++
Zeatin				
0.1	3	2.10±0.45	1.50±0.20	+
0.25	65	2.80±0.24	1.75±0.40	+
0.5	30	1.90±0.55	1.28±0.40	+
1.0		NR	NR	

+, less; ++, Moderate; +++, Profuse; CP, Callus production; NR, No response.

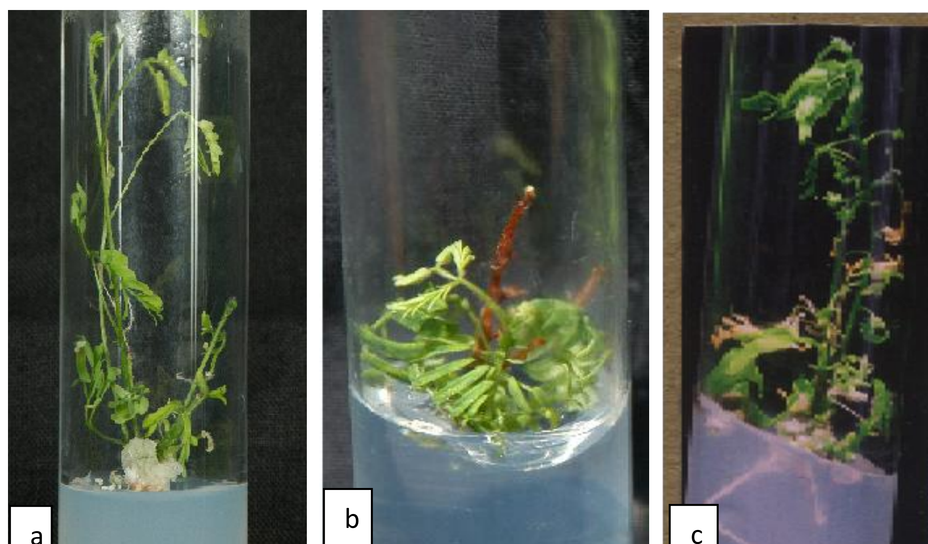

Figure 2. Multiple shoot induction of cotyledonary node on MS medium with **a**, BAP 1 mg/l; **b**, BAP 1 mg/l + Kn 2 mg/l; **c**, BAP 1 mg/l + NAA 1 mg/l.

Table 3. Effect of various cytokinin combination on multiple shoot induction from cotyledonary nodes of *A. amara* on MS medium.

Plant growth regulators (mg/l)			% of response	No. of shoots / explants (Mean ± S.D)	Shoot length(cm) (Mean ± S.D)	Basal Callus
BAP	KN	Zeatin				
1.0	0.5	-	50	3.25±0.60	1.50±0.30	+
1.0	1.0	-	60	5.35±0.50	1.80±0.25	+
1.0	2.0	-	80	8.30±0.20	2.00±0.40	+
2.0	0.5	-	45	3.35±0.30	2.15±0.25	++
2.0	1.0	-	70	6.20±0.50	3.45±0.35	+
2.0	2.0	-	55	4.45±0.90	2.20±0.20	++
-	0.5	0.1	50	2.80±0.45	1.50±0.20	-
-	1.0	0.25	65	4.20±0.70	1.95±0.35	-
-	2.0	0.5	40	2.50±0.25	1.35±0.45	-
1.0	-	0.1	40	3.50±0.20	1.00±0.30	+
1.0	-	0.25	45	4.10±0.35	1.00±0.45	+
1.0	-	0.5	60	6.80±0.20	1.85±0.20	+
1.0	-	1.0	50	4.35±0.50	1.28±0.40	+
2.0	-	0.10	40	2.20±0.45	1.00±0.25	++
2.0	-	0.3	55	2.80±0.50	1.25±0.50	+
2.0	-	0.5	40	2.50±0.30	0.90±0.35	++

find out a suitable nutrient medium that would promote high rate of shoot multiplication without the intervention of callus. The explants were cultured on MS medium fortified with different combinations of BAP + Kn, BAP + Zeatin, Kn + Zeatin, and 2% sucrose. Fifteen and thirty days after placement of the explants of cultures exhibiting shoot proliferation and number of shoots/explant were determined. Multiple shoot induction from cotyledonary nodes occurred maximum on MS medium fortified with BAP 1 mg/l + Kn 2 mg/l (Figure 2b). The percentage of response was 80 and about 8.30 ± 0.20 shoots/explant were formed and the shoot length was 2.00 ± 0.40 cm. Increase in concentration of BAP to 2 mg/l in combination with Kn 1 mg/l enhanced shoot length to 3.45 ± 0.35 cm and reduced shoot number with little basal callusing (Table 3). Among the different combinations of cytokinins observed, the synergistic effect of BAP and Kn was effective than either use of single harmone. This study was further weighed by previous survey of literature (Benneth and Davies, 1986; Rahman et al., 1993; Bhat et al., 1995; Anuradha and Pullaiah, 2001).

Effect of auxin and cytokinin on multiple shoot induction

Many aspects of cell growth, cell differentiation and organogenesis in tissue and organ cultures have been found to be controlled by an interaction between cytokinins and auxins. In the present study, various combinations of cytokinins and auxins were used at different concentrations for rapid shoot proliferation (Table 4). However among the different combinations used, BAP 1 mg/l + NAA 1 mg/l was crucial for multiple shoot induction, as it produced 6.28 ± 0.25 shoots with a length of 2.50 ± 0.35 cm (Figure 2c). Media containing BAP 1 mg/l + IAA 0.5 mg/l responded well with a maximum of 3.44 ± 0.65 cm. About 60% shoot induction was observed on medium supplemented with BAP 1 mg/l + 2.4 D 0.5 mg/l. Among different combinations of auxins tested with kinetin, Kn 0.5 mg/l + NAA 1mg/l was effective producing 4.00 ± 0.70 shoots/explant with a maximum length of 2.35 ± 0.20 cm (Table 5). Media containing Zeatin 0.25 mg/l + NAA 2 mg/l induced 5.10 ± 0.15 shoots/explant with 2.15 ± 0.15 cm of shoot length. On the whole, cytokinin in combination with NAA was found to be more effective in rapid shoot proliferation and also root induction. Further increase or decrease in the concentration of either BAP or NAA did not enhance the shoot number. But the addition of NAA with optimal concentration of BAP significantly reduced the frequency of shoot formation compared to BAP alone. This result was supported by Kumar et al. (1998) in *Albizia procera,* Sinha et al. (2000) in *Albizia chinensis* and Ramamurthy and Savithramma (2003) in *Albizia amara*.

Shoot elongation

Single shoot was excised from multiple shoot culture and transferred to MS medium supplemented with low concentration of Kn. Maximum shoot length of 4.80 ± 0.35 cm was attained with 3 to 4 nodes on MS medium fortified with Kn 0.25 mg/l. Elongated shoots after one sub-culture were subjected to different concentrations of auxins to induce rhizogenesis.

Rhizogenesis

Elongated shoots with 3 to 4 nodes were transferred to different rooting media to induce rhizogenesis. Root

Table 4. Effect of BAP with various concentrations of auxin on multiple shoot induction from cotyledonary node of *A. amara* on MS medium.

Plant growth regulators (mg/l)				% of response	No. of shoots / explants (Mean ± S.D)	Shoot length(cm) (Mean ± S.D)	Basal Callus
BAP	IAA	NAA	2,4D				
1	-	0.10	-	50	4.20±0.20	1.25±0.35	+
1	-	0.25	-	55	4.80±0.75	2.00±0.15	+
1	-	0.50	-	60	5.75±0.38	2.20±0.45	+
1	-	1.00	-	80	6.28±0.25	2.50±0.35	+
1	-	2.00	-	50	4.15±0.68	1.50±0.58	++
1	0.10	-	-	30	1.80±0.60	1.00±0.25	+
1	0.25	-	-	40	2.50±0.45	1.40±0.30	+
1	0.50	-	-	45	3.40±0.65	1.85±0.75	+
1	1.00	-	-	35	2.20±0.28	1.25±0.35	+
1	-	-	0.10	30	2.55±0.25	1.06±0.20	+
1	-	-	0.25	40	3.00±0.75	1.25±0.37	+
1	-	-	0.50	60	4.10±0.15	1.50±0.30	+
1	-	-	1.00	50	3.75±0.22	1.28±0.38	++

Table 5. Effect of Kn and Zeatin with various concentrations of auxin on multiple shoot induction from cotyledonary nodes of *A. amara* on MS medium.

Plant growth regulators (mg/l)					% of response	No. of shoots / explants (Mean ±S.D)	Shoot length(cm) (Mean ± S.D)	Basal Callus
Kn	ZEA	IAA	IBA	NAA				
0.5		0.25			30	2.15±0.68	1.90±0.35	+
0.5		0.50			20	1.55±0.70	1.25±0.37	+
0.5			1.0		70	4.00±0.70	2.35±0.20	+
0.5			2.0		50	3.75±0.22	2.00±0.45	+
0.5				0.5	40	2.15±0.15	2.45±0.25	++
0.5				1.0	55	2.50±0.25	2.80±0.30	++
	0.25	0.25			35	1.85±0.10	1.28±0.38	+
	0.25	0.50			20	1.25±0.65	1.00±0.10	+
	0.25		1.0		50	3.95±.50	1.85±0.10	+
	0.25		2.0		60	5.10±0.15	2.15±0.15	+
	0.25			0.5	40	2.45±0.10	1.95±0.50	++

induction experiments from *in vitro* raised shoots of *Albizia amara* were performed at different concentrations of MS and B_5 salts individually supplemented with NAA 1 mg/l. High frequency of rooting with 80% was observed on half strength MS medium on an average of 3.50 ± 0.45 roots/culture and a length of 5.25 ± 0.12 cm (Figure 3) (Table 6). Other concentrations of MS salts that is, full strength and quarter strength was less effective. However, all the three concentrations of B_5 salts (¼, ½ and full strength) promoted low percent of rhizogenesis. Rooting assay was performed by transferring healthy and sturdy shoots (4 to 5 cm long) on ½ strength MS medium fortified with different auxins (IAA, IBA and NAA) applied singly at various concentrations (Table 7). Of this half strength MS medium containing NAA 1 mg/l induced rooting in 80% of the shoots within 15 days. Each shoot developed 3 to 4 roots after 20 days. Even lateral root induction was also observed. Rooting efficiency decreased at a concentration above and below 1 mg/l NAA; about 3.50 ± 0.45 roots/shoot with a average length of 5.25 ± 0.12cm was observed.

Acclimatization of rooted plantlets

Acclimatization and hardening are the most important aspects for micropropagated tree species. *In vitro* hardening can be achieved by decreasing the water potential of the medium and reducing humidity in culture vessel. *In vitro* regenerated complete plantlets of *A. amara* were taken out of the rooting media and agar traces were removed by washing with distilled water for 10 to 15 min. Fungal growth rate was checked by

Figure 3. Root induction on ½ strength MS medium with NAA 1 mg/l.

Table 6. Rooting efficiency of *in vitro* raised shoots of *A. amara* cultured on different media with NAA (1 mg/l).

Types of medium	% of response	No. of roots / shoots Mean ± S.D.	Length of roots (cm) Mean ± S.D.	Days of rooting
MS full strength	55	2.50±0.15	3.50±0.33	20
MS 1/2 strength	80	3.50±0.45	5.25±0.12	12
MS 1/4 strength	45	2.35±0.25	3.15±0.15	25
B5 full strength	30	1.00±0.25	0.90±0.28	35
B5 1/2 strength	50	1.35±0.32	1.00±0.15	30
B5 1/4 strength	20	1.00±0.00	0.85±0.18	40

Table 7. -Effect of various auxins on root induction from shoots of *A. amara* on half strength MS medium.

Auxins	Concentrations (mg/l)	% of response	Mean No. of roots / culture (Mean ± S.D.)	Length of roots(cm) (Mean + S.D.)	Nature of response
IAA	0.10	30	1.55±0.15	1.45±0.35	Thin and short roots
	0.25	50	2.35±0.28	2.00±0.28	Thin and medium roots with nodules
	0.50	20	1.20±0.30	1.25±0.20	No response
	1.00	0	NR	NR	No response
	2.00	0	NR	NR	No response
IBA	0.10	0	NR	NR	No response
	0.25	20	1.10±0.25	1.00±0.25	Thin and very short roots
	0.50	30	2.20±0.12	1.05±0.50	Thick and short roots
	1.00	25	1.35±0.25	1.00±0.60	Thick and very short roots
	2.00	0	NR	NR	No response
NAA	0.10	30	1.80±0.40	2.85±0.15	Thick and long roots
	0.25	40	2.00±0.35	3.30±0.27	Thick and long roots
	0.50	65	2.90±0.37	4.50±0.33	Thick and long roots
	1.00	80	3.50±0.45	5.25±0.12	Thick and long tap root system with lateral roots
	2.00	60	2.35±0.35	4.30±0.05	Thick and long roots

Figure 4. Acclimatized plant after two weeks.

spraying 0.5% (w/v) Benolate fungicide. Then plantlets were transferred to pots of size 7.6 cm^2 containing sterile peat moss and vermiculate (1:1) and incubated for two weeks at 25 ± 2°C (2000 lux) for 16 h photoperiod. The pots were enclosed in plastic covers and small holes were punched to decrease the relative humidity. During the period, liquid quarter strength MS basal nutrient medium was provided. Gradually, pots were transferred to room temperature having diffuse light. Later, pots were shifted to green house and size of the holes was increased. Covers were removed after 6 weeks and plants were acclimatized in normal soil (Figure 4). About eight to nine true-to-type plantlets of *A. amara* were raised *in vitro* from single cotyledonary node explant. But successful transfer of *in vitro* raised plantlets to soil through hardening is poor (50%) due to premature defoliation of leaflets.

Conclusion

We have established a protocol for direct *in vitro* regeneration system for conservation and micro-propagation of *A. amara* from cotyledonary nodal explants. MS medium fortified with BAP 1 mg/l + Kn 2 mg/l, was effective in inducing multiple shoots from cotyledonary node explants. *In vitro* rooting experiments were successful on half strength MS medium fortified with NAA 1mg/l.

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