Micropropagation of *Terminalia arjuna* Roxb. from cotyledonary nodes

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Cotyledonary node explants excised from 21 day old seedlings of *T. arjuna* produced multiple shoots when cultured on full strength MS or modified MS (1/2 strength major salts and Fe-EDTA) medium supplemented with different concentrations (0.1-1.0 mg/l) of BAP. Maximum 8.9 shoots/explant could be recorded after 30 days of inoculation on modified MS medium supplemented with BAP (0.5 mg/l). A proliferating shoot culture was established by reculturing the original cotyledonary nodes (2-3 times) on shoot multiplication medium after each harvest of the newly formed shoots. Shoots (each having 2-3 nodes/shoot) thus obtained were also used as a source of nodal explant that gave rise to 1-2 shoots when cultured on modified MS+BAP (0.5 mg/l) medium. Thus, 45-55 shoots could be obtained after 60 days of culture initiation from a single cotyledonary node. About 88% shoots rooted well after 15 hr pulse treatment with IBA (1 mg/l) in liquid MS medium followed by transfer to modified MS medium without IBA. About 80% of these plantlets were successfully acclimatized in plastic pots containing sand and soil mixture and 70% plantlets transferred in the field those survived even after 6 months of transplantation.

In recent years use of tissue culture technique for clonal propagation of forest trees has increased considerably by using juvenile as well as mature plant parts as starting material. Seedling explants are in general more responsive than derived from mature trees. Many tree species have been propagated successfully through *in vitro* shoot proliferation from cotyledonary node explant of seedlings.

*Terminalia arjuna* Roxb. is one of the economically important trees of tropical and subtropical forests. Its wood has multiple uses and bark is utilized in tanning and dyeing industries and ayurveda system of medicine. The leaves are fed to tasar silk worms [*Antheraea mylitta* (Druy)]

Low percentage of germination and difficulty in rooting restricts the conventional propagation of *Terminalia arjuna*. Therefore, the present work has been undertaken to develop an efficient protocol for regeneration of *T. arjuna* for high frequency of shoot proliferation through cotyledonary node so that plants selected with higher medicinal and increased nutrient value in leaves could be multiplied for commercial purpose.

Materials and Methods

Mature and dried fruits of *T. arjuna* were collected from 20-30 year old trees growing at International House, BHU campus, Varanasi. Since it was difficult to separate out the seeds from mature fruits, the entire fruits were kept for germination after surface sterilization by washing under running tap water for 1 hr followed by immersion in aqueous solution of 8% (v/v) sodium hypochlorite along with teepol (50 ml teepol/100 ml water) for half an hour, then washed thoroughly with tap water for removal of detergents.

The surface sterilized fruits were germinated on moist cotton bed covered with filter paper in petri dishes in dark at 30°-35°C. Cotyledonary nodes were isolated from 7, 14, 21 and 28 day old seedlings and surface sterilized for 10-15 min with 2% (v/v) cetrimide (ICI, India) having 1-2 drops tween-20. After thorough washing with tap water the explants were taken to Laminar Flow and dipped with 70% alcohol for 30 sec followed by sterilization with 0.05% (w/v) HgCl2 solution for 1-2 min and washed 3-4 times with autoclaved double distilled water.

The cotyledonary nodes (1 to 1.5 cm) were inserted vertically in to the culture medium consisting of full strength MS* or modified MS (half strength major salts and Fe-EDTA) with 3% (w/v) sucrose (Himedia), 0.6% (w/v) agar (Himedia) and supplemented with (0.01, 0.1, 0.5 or 1.0 mg/l) or without BAP for shoot proliferation. The pH of the media was adjusted to 5.8±0.02 before autoclaving at 121°C for 15-20 min. The cultures were grown at 25°±2°C under 16 hr photoperiod of 60 μEm²/s¹ light intensity. Twelve replicates were taken for each treatment. Each experiment was repeated thrice. Data for shoot proliferation were collected after 30 days of culture initiation.
For shoot multiplication the original cotyledonary nodes after each harvest of the newly formed microshoots were recultured for 2-3 times in fresh shoot multiplication medium [modified MS+BAP 0.5 mg/l]]. The microshoots having 2-3 nodes obtained from the cotyledonary node after 30 days of inoculation were cut into single node pieces and recultured on fresh multiplication medium. Atleast 12 explants were taken per experiment and each explant served as an experimental unit. Experiments were repeated more than three times.

For rooting, the microshoots (2-4 cm in length) were selected for rooting. These microshoots were transferred to modified MS medium either directly or after giving 15 hr pulse treatment in different concentrations (1, 3 or 5 mg/l) of IBA or NAA in MS liquid medium having 3% (w/v) sucrose. Number and length of roots were recorded after 30 days of transfer to modified MS medium. The well rooted shoots were washed gently with tap water to remove agar and then transferred to plastic pots containing autoclaved sand and soil mixture in 3:1 ratio. The potted plantlets were covered with polythene bags and kept in culture room under 16 hr photoperiod (60μEm²s⁻¹ light intensity) at 25°±2°C. After 3 weeks the pots containing plantlets were kept outside the culture room (25°-30°C). Thereafter polythene bags were removed on the 1st day for 15 min, then time was increased gradually and polythene bags were removed completely after 4 weeks of transplantation. The well established plantlets were transferred to the earthen pots containing garden soil.

Results and Discussion

Cotyledonary nodes excised from one and two week old seedlings showed low percentage of survival whereas explant taken from four week old seedlings exhibited delayed responses. Cotyledonary nodes excised from 3 week old seedlings cultured in full strength MS medium containing different concentrations of BAP were found better for shoot bud induction (data not shown). However, they failed to give any response to medium without BAP. Shoot initiation was observed at all concentration of BAP. BAP has been also found effective in induction of shoot in many forest trees 20. ANOVA revealed that per cent responses, shoot number and shoot length were significantly affected by BAP in MS full and modified strength medium. Per cent response for shoot multiplication, the number and length of shoots increased with increase in BAP concentration and was maximum on 0.5 mg/l of BAP (Fig. 1A). Concentration higher than 0.5 mg/l of BAP showed a slight decrease in shoot proliferation rate (Table 1). Higher concentration of BAP has been found inhibitory by others as
As compared to BAP in full strength MS medium, the modified MS medium, having different concentration of BAP showed better response for shoot proliferation. Low salt concentration has also been reported effective for shoot proliferation in other plants. About 58% explants produced a few shoots (1.99) in modified MS medium without incorporation of BAP, but these shoots did not elongate beyond 4.9 mm in length (Table 1).

Shoot culture established by reculturing the original cotyledonary nodes after harvest of newly formed shoots on shoot multiplication medium (0.5 mg/l, BAP supplemented modified MS medium) produced multiple shoots at 1st and 2nd subcultures, but at 3rd subculture, only few microshoot developed (data not shown). Nodes excised from microshoots and cultured on shoot multiplication medium also showed shoot multiplication. About 80% of these nodes responded and produced 1-2 shoots from each node. Thus, from a single cotyledonary node about 45-55 shoots could be obtained after 60 days of culture initiation.

Only about 2% microshoots rooted, when cultured on modified MS medium without auxin. From these microshoots, roots (1-2) measuring 5-6 mm in length were produced as recorded after 30 days of culture. Whereas, shoots cultured in modified MS medium after 15 hr pulse treatment in different concentrations (1, 3 or 5 mg/l) of IBA or NAA showed better response for rooting. ANOVA results showed that rooting of microshoots was affected significantly by different concentrations of IBA and NAA. Among the two auxins (IBA and NAA) tested for rooting, IBA proved better (Table 2) confirming the observations of others. The pulse treatment with IBA (1 mg/l)
showed better response for rooting of microshoots (88%) as compared to the pulse treatment with other concentrations of IBA. Number of roots per microshoot and average length of roots were also found maximum with 1mg/l, IBA pulse treatment (Table 2). About 40-48 shoots of the 45-55 shoots, obtained from the single cotyledonary nodes, could be well rooted within 30 days (Fig. 1B) and of these about 32-38 plantlets acclimatized successfully in the pots containing sand and soil (3:1) in 7-8 weeks (Fig. 1C). Per cent survival in the field was 70% and these plantlets attained a height of 60 cm after 6 months of transplantation. The protocol standardized in the present study enabled high rate of shoot multiplication without any callusing at any stage and could be applied for afforestation purpose of this valuable tree.

References