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High frequency shoot regeneration from nodal and shoot tip explants in *Holarrhena antidysenterica* L.

Golam Ahmed*, P K Roy & A N K Mamun

Plant Biotechnology and Genetic Engineering Division, Institute of Food and Radiation Biology, AERE, G.P.O. Box 3787, Dhaka-1000, Bangladesh

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Shoot tip and nodal segment explants of *Holarrhena antidysenterica* when cultured on MS medium containing BAP (1.0-3.0 mg/l) with NAA (0.2-1.0 mg/l) and BAP (1.0-3.0 mg/l) with Kn. (0.2-1.0 mg/l) produced multiple shoots. Maximum multiple shoots was found in MS medium supplemented with BAP (2.0 mg/l) and NAA (0.5 mg/l). Subculture on the same medium resulted in rapid shoot multiplication at an average rate of 16 new shoots per subculture. Addition of urea (100 mg/l) in the medium increased the number of shoots up to 22 per culture. For best rooting, the shoots were excised from the culture flask and implanted individually on half strength MS medium with 0.5 mg/l each of IBA, IAA and NAA. After 20 days of transfer on root induction medium 95% rooting was achieved. Regenerated plantlets were successfully acclimatized and established in soil. About 90% of plantlets survived under open field conditions.

Holarrhena antidysenterica, commonly known as kurchi, is used for extraction of therapeutically active alkaloids. An important medicine of amoebic dysentery and diarrhoea can be made from the extraction of the bark. The bark has astringent, antidysenteric, anthelmintic, stomachic, febrifugal and tonic properties. Eighteen kinds of alkaloids are found in this medicinal plant. Conessine is the most important and principal alkaloid present in it. *Holarrhena antidysenterica* is propagated by seed, but the seed viability is poor and has very low germination percentage. Micropropagation method is specifically applicable to species in which clonal propagation is needed[1]. Clonal propagation through conventional methods like cutting or grafting has not been successful in this plant. The present study was undertaken to develop a protocol for large-scale propagation of *Holarrhena antidysenterica* through *in vitro* culture.

Holarrhena antidysenterica plants (2-3 years old) were chosen for the collection of shoot tip and nodal segment explants. These were washed with running tap water; kept in water for 45 min to remove dirt on the surface, rinsed several times with distilled water, surface sterilized with an aqueous solution of $HgCl_2$ (0.1%) for 5 min and rinsed 5 times with sterile distilled water. These explants were cut into small pieces (2 cm) and kept in MS medium[2] supplemented with different concentrations and combinations of cytokinin and auxin such as 6-benzyl aminopurine (BAP), kinetin (Kn) and α-naphthalene acetic acid (NAA) for shoot regeneration and half strength MS medium supplemented with auxins such as NAA, indole-3-acetic acid (IAA), indole-3-butyric acid (IBA) was used for root induction. Urea (75-150 mg/l) was used in the medium for development of shoot multiplication. The *p*H of the medium was adjusted to 5.8 before adding agar and the medium was autoclaved at 1.06 kg cm^{-1} for 20 min at 120°C. All medium were gelled with agar (0.6%). Cultures were incubated at 26°±1°C under cool white fluorescent light for 16 hr photoperiod. The well rooted *in vitro* regenerated plantlets (about 8-10cm long) were taken out from the test tubes, washed thoroughly to remove the agar medium from the roots and transplanted in to small earthen pots containing mixture of soil, sand and composed (2:1:1 v/v) for hardening. The pots were covered with transparent polythene bags to maintain the relative humidity. The bags were removed periodically for aeration. After the emergence of a new leaf that occurred within 2-3 weeks, the plantlets were transferred to larger pots and gradually acclimatized to outdoor conditions. After 2 months, fully acclimatized plants were transferred to the net house. The whole experiment was repeated three times.

*Corespondent author: Fax. 88-02-8613051,
Email: aere@bangla.net

Table 1—Response of different concentrations of growth regulators supplemented in MS medium on proliferation and multiplication from nodal segment explants of *Holarrhena antidysenterica*. (Data were taken after 27 days of culture.)

Growth regulators (mg/l)	Percentage of shoot inducing explants	No. of shoots per explants.
1.0 BAP	15.6	2.0±0.5
2.0 BAP	40.3	3.8±0.2
3.0 BAP	20.4	2.0±0.2
1.0 BAP + 0.2 NAA	22.3	4.0±1.4
1.0 BAP + 0.5 NAA	44.8	6.2±0.5
1.0 BAP + 1.0 NAA	38.6	3.0±0.3
2.0 BAP + 0.2 NAA	66.1	7.0±0.5
2.0 BAP + 0.5 NAA	88.3	16.0±1.5
2.0 BAP + 1.0 NAA	60.1	8.0±0.2
3.0 BAP + 0.2 NAA	32.4	4.2±0.4
3.0 BAP +.05 NAA	28.2	2.4±1.5
3.0 BAP + 1.0 NAA	25.7	2.0±1.2
3.0 BAP + 0.2 Kn.	40.5	4.2±0.2
3.0 BAP + 0.5 Kn.	44.2	5.1±0.2
3.0 BAP + 1.0 Kn.	38.9	3.2±1.1

Shoot multiplication from nodal segment explant was observed when cultured in MS medium supplemented with BAP singly or in combination with Kn or NAA. Maximum (88.3%) shoot initiation was observed in MS + BAP (2.0 mg/l) + NAA (0.5 mg/l) medium (Table 1, Fig. 1a). The regenerated shoots increased when they were subcultured in the same medium within 15 days. In the same medium, multiple shoot induction from shoot tip explant was low. In medium containing only BAP or in combination with Kn, frequency of multiple shoots was less. On the medium containing high concentrations of BAP and low concentrations of NAA, both types of explants responded well and produced more shoots than the medium containing only different concentrations of BAP or in combination with Kn. Brand and Lineberger[3] have reported that adventitious shoot formed when shoot tip explants of mature *Liquidambar styraciflua* L. cultured on medium supplemented with BAP (2.5 mg/l) + NAA (0.1 mg/l). Rahman *et al.*[4] have reported that multiple shoot regeneration is possible from nodal segment explants of *Emblica officinalis* on BAP (1.0 mg/l)+NAA (0.25 mg/l) supplemented medium. From nodal segment explants of *Rauwolfia serpentina* similar results have been reported[5] when BAP (1.5mg/l)+NAA (0.5 mg/l) are supplemented in the medium. For further development of the medium and enhance shoot proliferation, urea (75-150 mg/l) was added in the medium. Addition of urea (100 mg/l) to the medium increased the number of shoot (nodal explant, 22; shoot tip explant, 15) per culture (Fig. 2). Thus the most effective medium for high frequency regeneration of multiple shoots with proper length was determined as MS + BAP (2.0 mg/l) + NAA (0.5 mg/l) + 100 mg/l of urea (Fig. 1b). Well developed and elongated shoots were excised and cultured on root induction medium. Different c oncentrations of

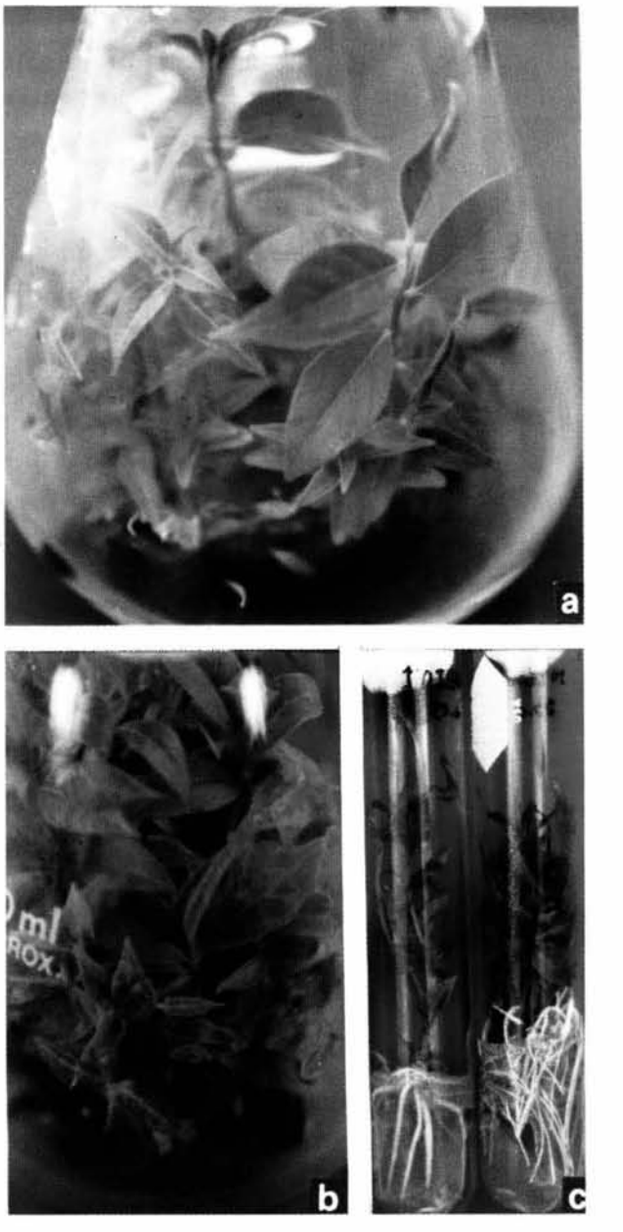

Fig. 1—(a) Regeneration of multiple shoots from nodal segments on MS + BAP (2.0 mg/l) + NAA (0.5 mg/l) medium. (b) Positive effect of urea (100mg/l) on rapid multiplication of shoots. (c) *In vitro* root induction on half strength of MS supplemented with 0.5 mg/l each of IBA, IAA and NAA.

Table 2—Effect of auxins in half strength of MS medium with 3% sucrose on root formation in regenerated shoots of *Holarrhena antidysenterica*. (Data were taken after 20 days of culture).

Growth regulators (mg/l)	Shoots rooted (%)
0.25 IAA	38.2±2.5
0.5 IAA	30.4±3.2
1.0 IAA	24.2±1.5
0.25 NAA	25.1±2.2
0.5 NAA	38.3±3.2
1.0 NAA	35.4±3.4
0.25 IBA	55.7±4.3
0.5 IBA	80.2±5.5
1.0 IBA	62.8±4.2
0.25 IAA+0.25 NAA	42.3±3.6
0.5 IAA+0.5NAA	65.3±4.5
1.0 IAA+1.0NAA	50.6±5.3
2.5 IAA+0.5NAA+0.25IBA	82.4±4.7
0.5 IAA+0.5NAA+0.5IBA	95.1±3.5
1.0 IAA+1.0NAA+1.0IBA	72.5±4.8

IBA, IAA and NAA were used in half strength MS medium for root formation. Best response (95%) was observed when 0.5 mg/l each of IBA, IAA and NAA were added in half strength MS medium (Table 2, Fig. 1c). Within 20 days of culture, shoots were rooted. On this medium root formation was 95%. Roy *et al.*[7] have reported that IBA and NAA are essential for root induction in *Artocarpus heterophyllus*. The well rooted plantlets were transplanted in small earthen pots. After acclimatization, 90% Plantlets were successfully established in the net house.

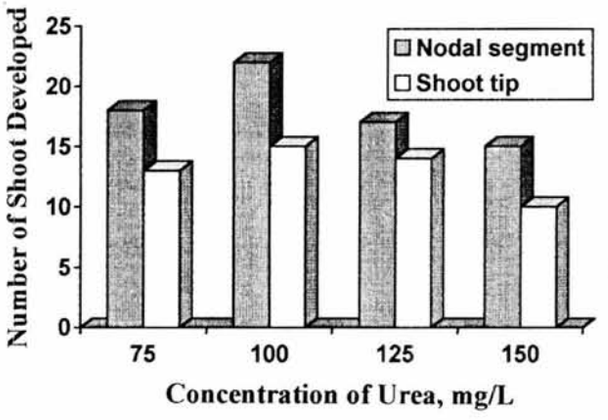

Fig. 2—Effect of different concentrations of urea (75-100 mg/l) along with BAP (2.0mg/l) + NAA (0.5mg/l) in MS medium on number of shoot development.

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