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In vitro Clonal Propagation of *Vitex negundo* L. - An Important Medicinal Plant

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Vitex negundo L., a perennial shrub belonging to the family Verbenaceae, is an important medicinal plant. It grows abundantly in St. Martin's Island and commonly known as Nishinda. Generally leaf is used for medicinal purpose but root, flower and fruit also have the medicinal values (Hasan 1982). Leaf of the plant contains essential oil, an alkaloid, nishindin. Stem and bark contain flavonoid glycosides. Leaves of nishinda very effectively reduce the inflammatory swellings of joints in rheumatic attacks. Juice of fresh leaf removes fetid discharges and worms from ulcers. Flower oil is applied to sinuses and scrofulous sores. Root juice is tonic, expectorant and diuretic (Ghani 1998).

Tissue culture has greatly enhanced the scope and potentiality of mass propagation by exploiting the regenerative behavior in a wide range of selected horticultural and agricultural plants including the medicinal ones (Roy et al. 1994, Thiruvengadam and Jayabalan 2000, Islam et al. 2001 and Jawahar et al. 2008). Keeping in mind the medicinal importance of all the parts of this plant, an attempt has been made to formulate its regeneration system through *in vitro* culture.

Plant materials (twigs) of field-grown plants were collected from Kolakupabandura village under Nababganj thana of Dhaka district. Sample was washed thoroughly under running tap water and then with distilled water. Shoot apex of the twigs were then excised and surface sterilized with 0.1% $HgCl_2$ solution for about 7 minutes followed by three to five washes with sterilized distilled water to remove the traces of $HgCl_2$ solution. Shoot tip explants were then cultured in MS supplemented with different concentrations (0.5, 1.0, 1.5 and 2.0 mg/l) of BA, 2iP and Kn singly or in combination with fixed concentration (0.1 mg/l) of NAA. For root induction, excised shoots of *in vitro* regenerated plants were cultured on half strength of MS supplemented with various concentrations of IBA ranging from 0.5 - 1.5 mg/l. In all the cases pH of the media

was adjusted to 5.8 before autoclaving and gelled with 3.6% phytagel. Following inoculation the cultures were maintained at 26 ± 2°C under 16 hr photoperiod having light intensity of 1500 lux (approx.) emitted from fluorescent tubes. Subcultures were carried out at regular intervals. Data on shoot morphogenesis were recorded after six weeks of culture and rooting performance was noted after five weeks of culture.

Table 1. Effects of different concentrations and combinations of Kn, BA, 2ip and NAA on shoot multiplication in Nishinda from shoot apex.

Growth regulators (mg/l)	% of explants producing shoot	Number of shoots/explant	Mean length of shoot (cm)	Callus formation*
BA				
0.5	73	6.2 ± 0.50	5.4 ± 0.52	–
1.0	73	5.9 ± 0.88	5.1 ± 0.65	–
1.5	80	6.5 ± 0.73	5.6 ± 0.60	–
2.0	93	7.2 ± 0.58	6.8 ± 0.59	–
Kn				
0.5	40	4.2 ± 0.46	5.4 ± 0.55	–
1.0	33	4.8 ± 0.45	6.4 ± 0.59	–
1.5	47	4.5 ± 0.48	7.1 ± 0.68	–
2.0	40	4.1 ± 0.39	7.6 ± 0.79	–
2iP				
0.5	80	3.1 ± 0.38	3.6 ± 0.32	–
1.0	73	3.7 ± 0.52	4.8 ± 0.38	–
1.5	80	4.1 ± 0.55	4.9 ± 0.46	–
2.0	87	3.2 ± 0.40	4.4 ± 0.49	–
BA + NAA				
0.5 + 0.1	87	6.2 ± 0.61	6.0 ± 0.61	++
1.0 + 0.1	95	6.8 ± 0.75	6.1 ± 0.75	++
1.5 + 0.1	80	6.8 ± 0.71	6.3 ± 0.71	+++
2.0 + 0.1	80	5.8 ± 0.53	5.8 ± 0.53	+
2iP + NAA				
0.5 + 0.1	67	3.2 ± 0.31	4.2 ± 0.41	+
1.0 + 0.1	73	4.8 ± 0.45	4.4 ± 0.45	+
1.5 + 0.1	60	3.8 ± 0.41	5.4 ± 0.51	+
2.0 + 0.1	67	4.1 ± 0.43	5.8 ± 0.53	+

* – = No callus. + = Slight callusing. ++ = Moderate callusing. +++ = Profuse callusing.

The effect of different concentrations of BA, Kn and 2iP singly or in combination with NAA on multiple shoot induction is presented in Table 1. All the treatments except Kn resulted in variable responses to induce multiple shoot from shoot apex. Among the three cytokinins used, BA responded well (Fig. 2A)

with regard to percentage of explants producing shoot (90%) as well as number of shoots per explant (6.5) followed by 2iP while Kn failed to respond in organogenesis. Among cytokinin-auxin combinations BA-NAA was found to produce good response compared to 2iP-NAA. Among all the combinations, best response was recorded under BA-NAA (1.0 + 0.1 mg/l) combination which accounted to 95% response of explants towards multiple shoot induction as well as maximum number of shoots (6.8) per explant. No callus was produced when cytokinins were used alone but slight to moderate callusing was noticed when NAA was added to cytokinin (Table 1). Although BA alone or BA-NAA combination produced good response towards multiple shoot induction, 2iP + NAA at a concentration of 0.5 and 0.1 mg/l was found to produce shoots with

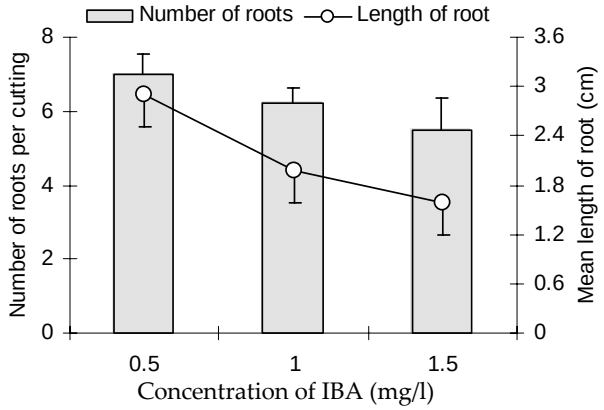

Fig. 1. Effects of different concentrations of IBA in half-strength of MS on rooting of excised shoots. Each treatment consisted of 15 replicates.

higher elongation (8.2 cm). Good responses on shoot morphogenesis using supplements of cytokinin and auxin were also reported by Hossain et al. (1994) and Morshed et al. (2001). Cultures at the age of eight - ten weeks produced flower spikes in almost all the shoot induction medium. Maximum flowering was recorded (80%) in the combination of 1.0 mg/l BA + 0.1 mg/l NAA in ten weeks of culture. Flowering *in vitro* was also recorded earlier by several workers (Azad et al. 1994, Uddin et al. 1998).

Regenerated shoots were excised and cultured on half strength of MS supplemented with three concentrations (0.5, 1.0 and 1.5 mg/l) of IBA for root induction. Rooting was noticed in all the concentrations used, however, maximum number of shoots rooted in 0.5 mg/l IBA (90%) followed by 1.5 mg/l (70%) and 1.0 mg/l (65%). Induction of root under 0.5 mg/l IBA is illustrated in Fig. 2C. Number of roots (7.0) per cutting and length of root (2.85 cm) were

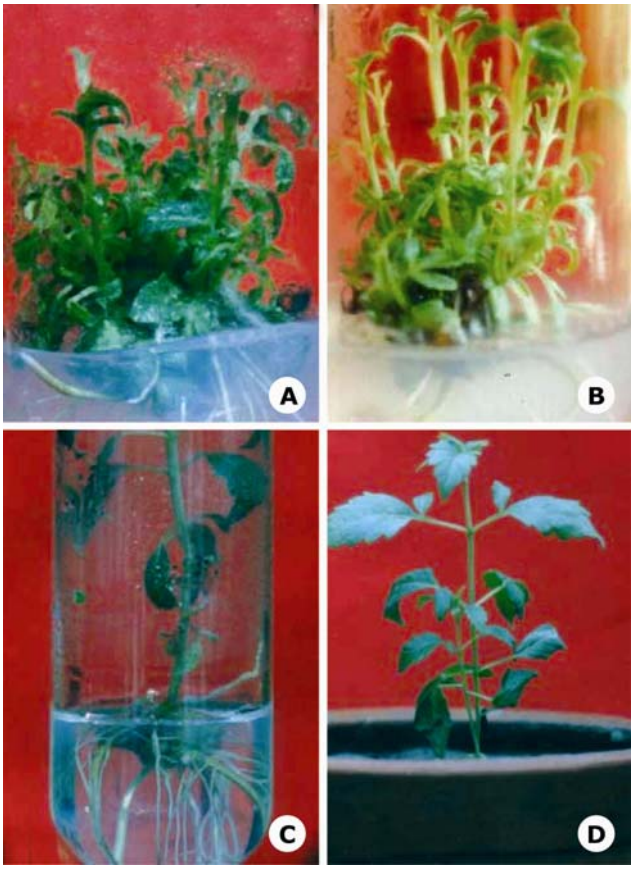

Fig. 2. Multiple shoots developed from shoot tip cultured onto MS containing 2.0 mg/l BA (A) and 0.5 2iP and 0.1 NAA mg/l (B). (C) Rooting of excised shoot on half MS fortified with 0. 5 mg/l IBA. (D) A plant in pot.

recorded highest in culture containing 0.5 mg/l IBA (Fig. 1). Several authors reported that IBA is an effective auxin in the induction of roots in different ornamental, medicinal and fruit plants like chrysanthemum (Hoque et al. 1998), carnation (Shibli et al. 1999), neem (Sarker et al. 1997), apple (Caboni and Tonelli 1999). The *in vitro* raised healthy plantlets were transferred to earthen pots, gradually acclimatized (Fig. 2D) and finally transferred to the field.

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