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In Vitro Regeneration of *Vitex negundo* L., a Woody Valuable Medicinal Plant through High Frequency Axillary Shoot Proliferation

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Abstract

An efficient protocol was established for rapid and large scale propagation of woody aromatic medicinal plant *Vitex negundo* L. by *in vitro* shoot multiplication from shoot tips and nodal segments of mature plant. Of the four different growth regulators BA, Kn, GA_3, NAA and coconut water, MS fortified with BA 1.0 mg/l was found to be the most effective for inducing multiple shoots from nodal explants. The percentage (96%) of shoot multiplication per node (21.83) was highest up to second subculture passages, after which there was a gradual decline in shoot development. Best rooting was induced (93%) in excised shoots on half strength MS medium supplemented with an optimal combination of NAA (0.3 mg/l). Soil, compost and sand (1:1:1) mixture was the most suitable planting substrate for hardening. The survival rate was 80% and the regenerated plants were successfully transferred to the soil.

Key words: *Vitex negundo,* Medicinal plant, Shoot proliferation, Micropropagation, Regeneration

Introduction

Vitex negundo is commonly known as 'Nishinda' belongs to the family Verbenaceae, a woody aromatic and medicinal shrub (Sahoo and Chand, 1998), or a small slender tree with 3-5 foliate compounds odorous leaves and bluish-purple flowers in pedunculate-branched tomentose cymes, grown naturally in all districts of Bangladesh (Ghani, 2003). Its leaves contain a pale greenish yellow essential oil (Basu and Singh, 1944).

An alkaloid, nishindine (Basu and Singh, 1947) and a glucoside (Ghose and Krishna, 1936), hentriacontane, sterols, beta-sitosterol, beta-sitosterol acetate, sigmasterol ascorbic acid, p-hydroxybenzoic acid, carotene and amino acids have also been iso-

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lated from this plant (Mukherjee and Shan, 1981; Gupta and Mukat, 1976). Stem bark contains flavonoid glycosides of wogonin, aurosin, vitexin, myrecetin, luteolin, leucodelphinidin, leucocyanidin rhammoside, beta-sitosterol, vanillic acid and p-hydroxy benjoic acid (Kodandu and Venkata, 1977).

Leaves are antiparasitic and used as alternative vermifuge and anodyne. They are also very effective to reduce inflammatory swellings of joints in rheumatic attacks, relieve catarrh and headache. Juice of fresh leaves removes foetid discharges and worm from ulcers. Flowers are astringent and cooling, oil from flowers is applied to sinuses and scrofulous sores. Fruits are nervine stimulant, emmenagogue and vermifuge. The root is used as tonic, febrifuge, expectorant and diuretic. It regulates hormones, increases breast-milk production and possesses progesterogenic properties (Chevallier, 1996).

Bangladesh has a very rich flora of medicinal plants. But due to heavy demand of these plants in Pharmaceutical industries, their supplies are getting short and as a consequence of over exploitation, many important medicinal plants are put to endangered condition. In Bangladesh, no concerted effort has yet been made for careful cultivation of these plants so as to replenish the exhausting supply and to conserve the threatened species (Hossain *et. al*., 2000). Though the plant has immense medicinal value it is gradually declining from the nature due to over exploitation and environmental pollution. That is why there is an urgent need of replenishment of the short supply and conservation of this plant genetic resource. Micropropagation is an effective approach to conserve such germplasm. Further, genetic improvement is another approach to augment drug-yielding capacity of the plant. Therefore it is important to develop an efficient micropropagation technique for *Vitex negundo* to rapidly disseminate superior clones once they are identified. Tissue culture technique can play an important role in the clonal propagation of elite clones and germplasm conservation of this medicinal shrub. In recent years, numerous studies on in vitro propagation of different plant species have shown that this technique may be a solution for rapid propagation of selected plant species (Bonga and Durjan, 1987; Chalupa, 1987; McCown, 1987; Boulay, 1987). The present communication deals with the development of a protocol for *in vitro* asexual multiplication in *Vitex negundo.*

Materials and Methods

Shoot tips and nodal explants of *Vitex negundo* L. collected from the garden of medicinal plants in the premises of BCSIR Laboratories, Dhaka-1205, were used for this experiment. The explants were washed thoroughly under running tap water, presoaked in liquid detergent for about 30 min, wiped with cotton and dipped in 70% (v/v) ethanol for 1 min. They were then surface

sterilized with 0.1% (w/v) mercuric chloride for 5 min, followed by five rinses with sterile distilled water inside a laminar air flow cabinet. The surface sterilized explants were sized to 1.0-1.5 cm in length containing a single node with an axillary bud or a shoot tip with an apical bud. The explants were placed vertically on the culture medium.

For shoot induction agar-gelled MS (Murashige and Skoog, 1962) basal media supplemented with BA, Kn, GA_3, coconut water (CW) at varying concentrations were prepared. The new shoots induced from the *in vitro* cultures were further used as an explants for adventitious shoot regeneration. For *in vitro* rooting, individual shoots (3-5 cm) were cut from the proliferated shoot cultures and implanted on half strength MS with different concentrations and combinations of NAA and IBA. All media were supplemented with 30 g/l sucrose and gelled with 7 g/l agar (Difco). Prepared media were dispensed into 150 x15 mm culture tubes or 250 ml conical flasks according to experiment. The pH of the media was adjusted to 5.8 before autoclaving at $121^{O}C$ for 20 min. The cultures were incubated for 16 h photoperiod at $24 \pm 2^{O}C$ under a fluorescent light.

Visual observation of culture was made every week. Data on shoot induction and proliferation and root induction were recorded after three weeks of inoculation and used for calculation. For each treatment 15 explants were used and all the treatments were repeated thrice. Data were analyzed statistically according to Mian and Mian (1984). The healthy plantlets were taken out from the culture tubes, washed to make free from agar gel with running tap water and transplanted to plastic pots containing soil, sand and compost (1:1:1) for hardening. The plantlets were kept in a poly chamber at 80% relative humidity, $32 \pm 2^{O}C$ under 12 h photoperiod for acclimation. Established plantlets were transplanted in earthen pots under natural conditions and the survival rate was recorded.

Results and Discussion

Shoot tips and nodal explants of *Vitex negundo* were cultured on MS media supplemented with various concentration of BA alone, BA with NAA or 5% CW and Kn with GA_3 for shoot induction. The explants were found to be swollen and they produced two to five shoots within three-four weeks after inoculation (Fig 1b). The highest percentage of shooting response of the inoculated explants was 96% with 21.83 shoots per culture when the explants were cultured in MS medium with 1.0 mg/l BA, which was followed by the media supplemented with 1.0 mg/l BA + 5% CW, 1.5 mg/l BA, 2.0 mg/l BA, 0.4 mg/l BA + 0.1 mg/l NAA, 0.1 mg/l BA and 0.2 mg/l Kn + GA_3 (Table I, Fig 1c, d, e). Both the explants responded in the same medium but the highest number of micro shoots were found to be induced in nodal explants. Usha *et al.* (2007) reported that maximum shoot proliferation (6.3) of

Table I: Effect of cytokinin(s) on shoot regeneration from nodal explants of *Vitex negundo*

Supplements					Shoot sprouting frequency (%)	Number of shoots per explant Mean ± SE	Shoot length (cm) Mean ± SE
Growth regulators/ (mg/l)				CW			
BA	Kn	GA_3	NAA	(%)			
0.1	0	0	0	0	53	2.83 ± 1.28	5.33 ± 0.13
0.5	0	0	0	0	80	3.78 ± 1.47	3.44 ± 2.47
1	0	0	0	0	96	21.83 ± 1.28	3.75 ± 0.22
1.5	0	0	0	0	87	6.76 ± 6.39	3.75 ± 1.41
2	0	0	0	0	87	6.00 ± 4.44	3.28 ± 0.39
1	0	0	0	5	93	7.40 ± 7.08	4.33 ± 1.13
0	0.2	0.1	0	0	60	2.43 ± 0.2	2.32 ± 0.18
0.4	0	0	0.1	0	67	3.12 ± 0.26	3.56 ± 0.03

Vitex negundo occurred when the explants were cultured on MS medium supplemented with 2.0 mg/l BA and 0.5 mg/l NAA than BA alone after 4 weeks. But Sahoo and Chand (1998) reported that the highest number of shoots per node were six to eight during the first three culture passages, after which there was a gradual decline in shoot development. In the present investigation the highest numbers of multiple shoots were 21.83 when cultured on MS medium supplemented with 1.0 mg/l BA alone. It was also found that the multiple shoot number was declined after three sub cultures. Though addition of CW to the medium increased the number of shoots as reported by Roy *et al.* (1998) and Hassan and Roy (2005) in *Elaeocarpus robustus* and *Gloriosa superba*, respectively, in the present investigation, addition of 5% CW in the nutrient medium was not found to be effective for further shoot multiplication and growth.

About 93% rooting was observed in regenerated shoots of *Vitex negundo* when the excised shoots were cultured individually on half-strength MS medium supplemented with 0.3 mg/l NAA (Table II). Roots initiated within second weeks of culture (Fig 1e). Use of auxins singly or in combination for rooting was also reported by different researchers (Sahoo and Chand, 1998; Usha *et. al*., 2007; Vadawale *et. al*., 2006; Hassan and Roy, 2005; Rahman *et al.*, 2006; Baksha *et al*., 2007).

After four weeks the rooted shoots were transferred to pots. None of the plantlets survived when directly transferred from rooting medium to the pot under natural conditions. About 80 percent of the transplanted plants of *Vitex negundo* survived if the plants in the rooting culture tubes were kept in normal room temperature for seven days before

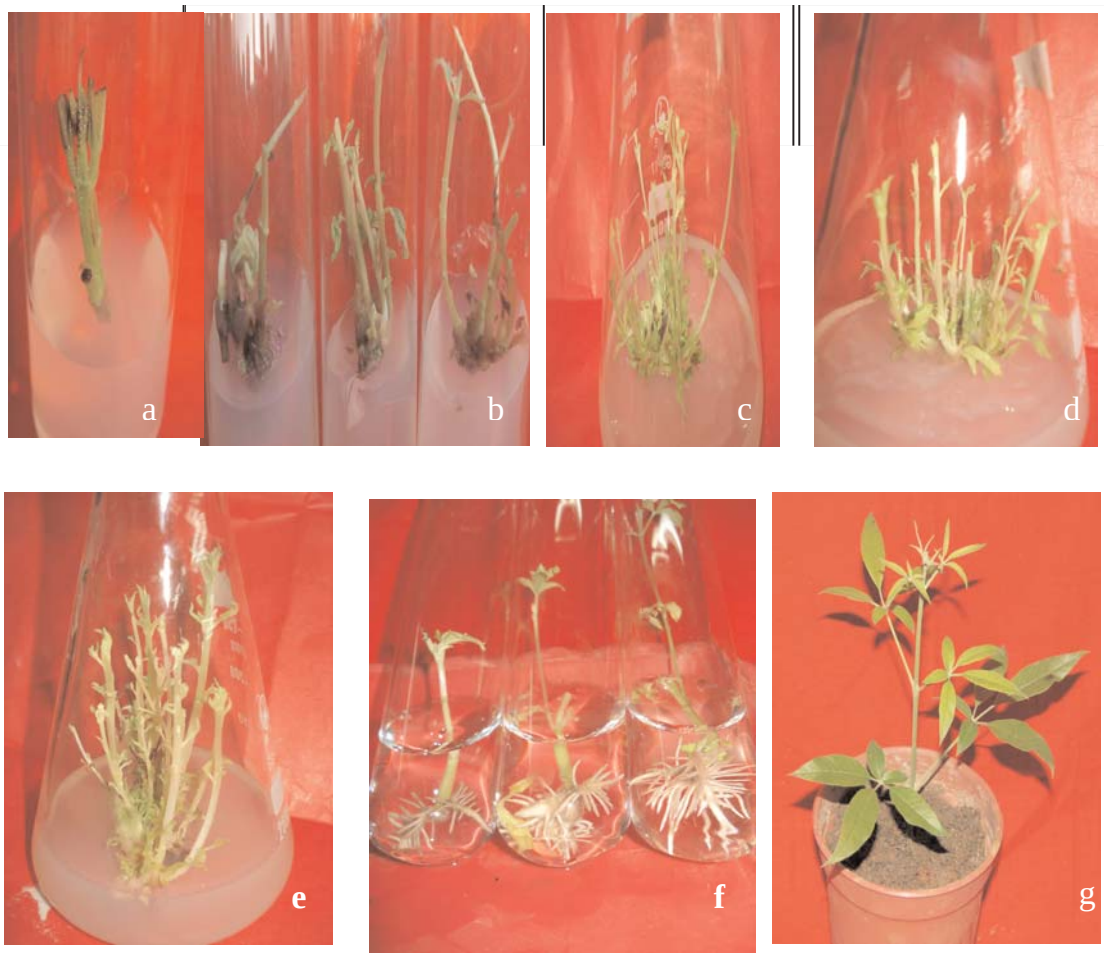

Fig 1. Regeneration of plantlets *in vitro* from nodal explants of *Vitex negundo*

(a) Inoculation of explants on regeneration medium at first day.

(b Shoot regeneration from nodal explants after three weeks. (c) Shoot regeneration from nodal explants on MS containing 1.0 mg/lBA + 5% CW after three weeks.

(d) Shoot regeneration from nodal explants on MS containing 1.0 mg/l BA after three weeks.

(e) Shoot regeneration from shoot tips on MS containing 1.0 mg/l BA after three weeks

(f) Adventitious root formation on regenerated shoots on half MS containing 0.3 mg/l NAA after two weeks.

(g) Establishment of *in vitro* grown *Vitex negundo* plantlets in outside pot after two months.

Table II: Effect of auxin(s) on root induction in regenerated shoots of *Vitex negundo* on half strength MS medium

Auxin(s)(mg/l)		Shoots rooted (%) (± SE)	Days required for root induction (± SE)
IBA	NAA		
0	0.1	77.6 ± 1.18	17.4 ± 0.82
0	0.2	87.2 ± 1.32	18.2 ± 0.96
0	0.3	93.2 ± 1.77	15.2 ± 1.9
0	0.4	74.2 ± 0.28	16.4 ± 0.46
0	0.5	81.6 ± 0.46	20.6 ± 0.46
0	1	86.4 ± 0.46	21.4 ± 0.46
0.1	0	85.0 ± 0.80	15.6 ± 0.46
0.2	0	80.0 ± 0.25	18.2 ± 1.32
0.5	0	71.2 ± 0.61	20.2 ± 0.43
1	0	68.0 ± 0.45	21.8 ± 0.25

transplantation in pots and reared for three weeks. The plantlets were reared under semi-controlled temperature (30±2^{O}C) and light (2000 lux) in a chamber with 80 percent humidity. During this period of acclimation shoots elongated, leaves expanded and turned deep green looking good and healthier (Fig 1f).

References

Baksha, R. Jahan, M.A.A. Khatun, R. Munshi, J.L.(2007) In vitro rapid clonal propagation of *Rauvolfia serpentina* (Linn.) Benth. *Bangladesh J. Sc. Ind. Res.* **42(1):** 37-44.

Basu, U.K. Singh, N.B. (1944) *Indian Journal of Pharmacy*. **6:** 71-73.

Basu, U.K. Singh N.B. (1947) *Quarterly Journal of Pharmacy and Pharmacology* **20:** 1367.

Bonga, J.M. Durzan, D.J. (1987) Cell and Tissue culture in Forestry. (Martinus Nijhoff Publishers, Dordrecht).

Boulay, M. (1987) *In Vitro* Propagation of Tree Species *(Plant Tissue and Cell Cult.* Alan, R. Liss. Inc, New York).

Chalupa, V. (1987) Clonal propagation of broad-leaved forest trees. *In Vitro Commun. Inst. Forest. Cechosl*.**12 :** 255-257.

Chevallier, A. (1996) The Encyclopedia of Medicinal Plants. 1st Ed.(DK Publishing Inc. Newyork, USA) 55-281.

Ghani A. (2003) Medicinal Plants of Bangladesh with Chemical Constituents and Uses. 2nd ed. *(Asiatic military presssociety of Bangladesh,* Dhaka, 1000) 426.

Ghose, T. P. Krishna, S. (1936) *Journal of Indian Chemistry.* **13:** 634.

Gupta, G.S. Mukat, B. (1976) Agricultural *University Journal of Research in Science.* **25:** 63-65.

Hossain, S.S. Rahman, S. Joydhar, A. Islam, S. Hossain, M.(2000) *In vitro* propagation of thankuni (*Centella asiatica L.*). *Plant Tissue Cult.*; **10(1)**: 12-23.

Hassan A.K.M.S. Roy, S.K. (2004) Micropropagation of *Smilax zeylanica* L., a perennial climbing medicinal shrub, through axillary shoot proliferation. *Bangladesh J. Life Sci.* **16(1):** 33-39.

Hassan, A.K.M.S. Roy, S.K. (2005) Micropropagation of *Gloriosa superba* L. through high frequency shoot proliferation *Plant Tissue Cult. and Biotech.* **15(1)**: 67-74.

Jeong, P. H. Cheol, C.H. Mee, M.B. (2004) Mass production of dune plant, *Vitex rotundifolia via* micropropagation. *Journal of Plant Biotechnology.* **6:** 165-169.

Kodandu, R.U. Venkata, R.D. (1977) *Indian Journal of Pharmacy.* **39(2):** 41.

McCown, D.D. McCown, B.H. (1987) Northern American Hard Woods. In: *Cell and Tissue Cult. in Forestry* Volm. 3. Martinus Nijhoff Publishers, Dordrecht.

Mian, M.A. Mian, M.A. (1984) An Introduction to Statistics. 4th Ed. (Ideal library, Dhaka) 125-129.

Mukherjee, K.S. Shan, B. (1981) *Journal Of Indian Chemical Society.* **58(1):** 97-98.

Murashige, T. Skoog, F. (1962) A received medium for rapid growth and bioassay with tobacco tissue cult. *Physiology of Plants.* **11:** 47-53.

Rahman, S.M.R., Afroz, F., Sultana, K., Sen, P.K. Ali, M.R. (2006) Effect of growth regulators and state of medium on micropropagation of *Adhatoda vasica* (Nees.) *Khulna University Studies*, Special Issue (1st Research Cell Conference): 55-59.

Roy, S.K. Islam, M.S. Hadiuzzaman, S. (1998). Micropropagation of *Elaeocarpus robustus. Plant Cell Rep*.**17:**

Sahoo, Y. Chand, P.K. (1998) Micropropagation of *Vitex negundo* L., a woody aromatic medicinal plant shrub, through high frequency axillary shoot proliferation. *Plant Cell Rep*. **18:** 301-307.

Usha, P.K., Benjamin, S. Mohanan, K.V. Raghu, A.V. (2007) An efficient micropropagation system for *Vitex negundo* L., an important woody aromatic medicinal plant, through shoot tip culture. *Research Journal of Botany.* **2:** 102-107.

Vadawale, A.V. Barve, D.M. Dave, A.M. (2006) *In vitro* flowering and rapid propagation of *Vitex negundo* L.- a medicinal plant. *Indian Journal of Biotechnology.* **5:** 112-116.

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