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Mass Propagation of *Vitex negundo* L., *In vitro*

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Abstract

Shoot proliferation was obtained from shoot tips and nodal explants of *Vitex negundo* L. on MS medium supplemented with either BAP or KIN (0.1 - 2.0 mg/L) alone or in combination with NAA (0.1 mg/L). The concentrations of cytokinins combined with NAA produced multiple shoots from shoot tips and nodal explants. The highest mean percentage (84.3 ± 8.0) of shoot multiplication's were observed on nodal explants in the presence of BAP (1.5 mg/L) and NAA (0.1 mg/L) followed by shoot tips (65.0 ± 5.0). The regenerated shootlets were rooted on MS basal medium IAA, IBA, NAA (0.1 - 1.5 mg/L). The maximum number of roots (51.0 ± 2. 6) was achieved on the medium containing IBA (1.0 mg/L) followed by other auxins (NAA, IAA). The regenerated plants were successfully transferred to a mixture of vermiculate and soil. About 95% of the plantlets survived when transferred to the field.

Introduction

Vitex negundo L. (Family Verbinaceae) is an aromatic shrub which many parts are used in medicine. The plant has considerable medicinal importance such as treatment of febrile, catarrhal, rheumatic affections and in the ayurvedic system of medicine (Nadkarni, 1954). The extract of leaves showed anticancer activity against *echrlich ascites* tumor cells (Ambasta, 1986). The leaves were reported to posses insecticidal properties (Chadha, 1976). Apart from it's diverse medicinal uses the plant is also known for reclaiming degraded soil and to prevent soil erosion.

Natural regeneration and conventional propagation of *V. negundo* through vegetative cuttings is slow and a large number of cuttings do not survive during transport and transplanting plantation. It can also be propagated through seeds or root suckeres, but these methods are not very efficient in producing a sufficient number of planting stocks as the germination frequency of the seeds is poor and production of root suckered is strictly age dependent. Micropropagation techniques appear to be most suitable for producing large quantities of genetically identical propagules from elite source (Evans 1990). With recent spurt in demands for herbal based medicines, tonics, cosmetics and plants with insecticidal and pesticidal properties. Tissue culture techniques have been reported for conservation and multiplication of several medicinal plants in *Acmella oppositifolia* (Garciglia et al., 1996), *Psoralia corylifolia* (Saxena et al., 1998), *Plumbago zeylanica* (Sahoo and Debuta, 1998), *Houttynia cordata* (Handique and Bora, 1999). There are only very few reports on *in vitro* studies of *Vitex negundo*. They reported that the micropropagation of *Vitex negundo* was reported from nodal explants by (Sahoo and Chand, 1998). Maximum number of shoots was developed on MS medium containing BA (2.0 mg/L) and GA_3 (0.4 mg/L). There is an urgent need to rapidly multiply the elite stocks for large scale plantations. Therefore, the present investigation was undertaken to establish protocols for regenerating large number of plant lets from the shoots and nodal segments.

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Materials and Methods

The shoot tips and nodal segments were collected from mature *V. negundo* plants were washed in followed by 1% Teepol for about 10 min. and then washed under tap water thoroughly before presterilization. Then explants were rinsed with 80% ethanol (30 sec.) and surface sterilized for 3 min. with 0.1% w/v mercuric chloride ($HgCl_2$) and finally washed (3 times) with sterilized distilled water.

The shoot tip explants were cut into (0.3 - 0.5 cm) and nodal segments (1.0 - 1.5 cm) and placed on MS medium (Murashige and Skoog, 1962) containing 3% sucrose, auxin NAA and cytokinins (BAP or KIN) at various concentrations and combinations for shoot proliferation. The pH of all media was adjusted to 5.8 prior to gelling with 0.8% w/v agar-agar and steam sterilized for 15 min. at 121℃ under 1.1 kg /cm^2 pressure. The cultures were grown at 26 ± 1℃ under 16h photoperiod with light intensity of 80μE $m^{-2}s^{-1}$.

Well developed shootlets were cultured on MS medium supplemented with different concentrations of IAA (0.1-2.0 mg/L), IBA (0.1-2.0 mg/L) or NAA (0.1-2.0 mg/L) for root induction. Rooted plants were maintained under the same incubation conditions as described above for shoot induction. Subsequently the rooted plants were removed from cultures and washed with running tap water and transplanted in to plastic cups containing vermiculite and soil and kept in the culture room for (3 weeks). Later the plants were hardened in the field. The experiment consisted of 25 explants for each experiment was repeated thrice and the data was statistically analysed by determining mean ± SE.

Results

Surface sterilization of shoot tips and nodal explants with 0.1% $HgCl_2$ produced 90% free of contamination. Both shoot tips and nodal explants when cultured on MS medium supplemented with BAP and KIN started to proliferate new shoots after two weeks of culture. The frequency as well as number of shoots per culture was obtained from both explants in the presence of BAP (1.5 mg/L) and Kin (1. 5 mg/L) summarized (Table 1). The development of shoot buds exhibited different morphology and when cultured in different concentration of BAP along with NAA. The maximum percentage of shoot buds developed from nodal (84.3 ± 8.0) and shoot tip (65.0 ± 5.0) explants were observed on medium with NAA (0.1 mg/L) and BAP (1.5 mg/L) summarized (Table 2). Nodal explants produced more number of shoots than shoot tip explants. Well developed shoots were transferred to MS medium supplemented with IAA, IBA, and NAA (0.1-2.0 mg/L) with different concentrations for root induction's (Table 3). Among the three auxins used, IBA was effective for root induction. The maximum number of roots (5 - 7) were produced from the shoots when they were cultured in the medium with 1.0 mg/L IBA. Well developed shoots with roots were transferred to pots containing sterile vermiculite soil and established in green house conditions. Almost 95% of the plantlets survived when transferred to the field.

Table 1. Effect of BAP and KIN on shoot bud proliferation from shoot tips and nodal region explants of *Vitex negundo* L.

Growth regulators (mg/L)	Percentage of shoots /explants (mean ± SE)		Number of shoots / culture		Shoot length/ explants (cm)		Number of nodes/ explant	
BAP	STE	NRE	STE	NRE	STE	NRE	STE	NRE
0.1	25.3±4.5	41.3±3.5	21.6±2.0	24.6±3.7	2.6±0.30	3.0±0.16	1.7±0.3	2.0±0.2
0.5	37.6±2.5	57.3±4.7	29.3±3.5	32.6±3.5	3.1±0.30	3.9±0.45	2.0±0.2	2.3±0.3
1.0	52.0±3.0	58.3±6.1	28.3±3.0	31.0±4.0	4.2±0.25	4.4±0.30	2.4±0.4	2.5±0.4
1.5	59.6±4.1	79.3±4.5	32.6±5.5	46.3±3.0	4.3±0.41	5.3±0.52	3.0±0.5	3.3±0.6
2.0	16.0±4.5	20.3±3.0	16.0±3.6	18.0±2.6	2.1±0.32	2.7±0.15	1.6±0.2	1.7±0.2
KIN								
0.1	16.6±3.5	24.0±5.5	17.6±1.5	19.6±1.5	25.3±4.5	41.3±3.5	1.4±0.15	1.7±0.25
0.5	21.3±1.5	24.3±3.0	23.3±2.5	25.0±2.0	37.6±2.5	57.3±4.7	1.8±0.36	1.9±0.26
1.0	52.0±3.0	58.3±6.1	20.0±3.0	24.3±3.0	52.0±3.0	58.3±6.1	2.1±0.20	2.2±0.25
1.5	59.6±4.1	79.3±4.5	25.6±4.0	79.3±3.0	59.6±4.1	79.3±4.5	2.4±0.25	2.5±0.30
2.0	16.0±4.5	20.3±3.0	13.3±3.5	17.0±3.6	16.0±4.5	20.3±3.0	1.1±0.15	1.3±0.20

The experiment consisted of 25 explants and the experiment was repeated thrice and the data's were statistically analysed determining mean SE.

NRE Nodal region of explants

STE Shoot tip explants

Table 2. Effect of BAP and in combination with NAA) 0. 1mg/L on shoot bud multiplication response of shoot tip and nodal explants of *Vitex negundo* L.

Growth regulators (mg/L)	% of shoot proliferation (mean±SE)	
	Shoot tip	Nodal
BAP + NAA		
0.1±0.1	29.6±3.0	36.6±3.5
0.5±0.1	43.6±3.5	52.0±3.0
1.0±0.1	49.6±4.5	54.6±3.0
1.5±0.1	65.0±5.0	84.3±8.0
2.0±0.1	20.3±5.0	28.3±3.0

The experiment consisted of 25 explants and the experiment was repeated thrice and the datas were statistically analysed determining mean±SE.

Table 3. Root development of shoot on MS medium supplemented with different concentration of IAA, NAA, IBA from *Vitex negundo* L.

Auxin Concentration (mg/L)	% of Rooting (mean±SE)	No. of roots / shoots
IAA		
0.1	16.6±4.5	3.0±1.0
0.5	23.0±4.0	3.6±1.5
1.0	33.2±3.5	1.0±1.0
1.5	24.0±2.6	4.0±2.0
NAA		
0.1	11.3±2.0	2.0±1.0
0.5	13.6±1.5	2.6±1.5
1.0	17.0±2.0	3.0±1.0
1.5	-----	-----
IBA		
0.1	18.6±3.5	3.0±1.0
0.5	37.5±2.6	4.0±1.0
1.0	51.0±2.6	2.0±2.0
1.5	42.6±2.5	4.6±1.5

The experiment consisted of 25 explants and the experiment was repeated thrice and the datas were statistically analysed determining mean±SE.

Discussion

The primary establishment of *in vitro* cultures from field grown plants was difficult because of profuse surface contamination attached to explants. To overcome contamination problem surface sterilization of explants was done with $HgCl_2$ treatment. BAP was most effective than KIN. Maximum of 32 shoots from shoot tip and 49 shoots from nodal explant were obtained on BAP (1.5 mg/L). 25 shoots from shoot tip and 27 shoots from nodal explants were obtained on KN (1.5 mg/L). Similar results were reported in Eucalypt species (Das and Mitra, 1990; Tibok et al., 1995; Ito et al., 1996).

In the present study it was observed that nodal bud explants showed (79.3 %) than shoot tip explants (59.6 %) of response on medium containing BAP (1.5 mg/L). Among the various concentration and combination of BAP and NAA tested, nodal explants showed maximum number of multiple shoots (84.3 ± 8.0) on the medium containing BAP (1.5 mg/L) and NAA (0.1 mg/L) than individual concentration of BAP showed maximum response than BAP. The similar results were reported in *Rouvolfia micrantha* indicating that combinations of BAP and NAA (Sudha and Seeni, 1996). In contrast to the present study Sahoo and Chand, 1998 reported that BAP (2.0 mg/L) and GA_3 (0.4 mg/L) was optimal and superior for *in vitro* multiplication of shoots from nodal segments. In the present experiment IBA was found to be best auxin for root induction. Similar results were reported in *Cephaelis ipecacuanha* (Jha and Jha, 1989), *Plantago ovata* (Wakhlu and Barna, 1989), *Rheum emodi* (Lal and Ahuja, 1989) and other plant species.

The clonal micropropagation method for *V. negundo* developed in this study appeared to be very efficient in the rate of shoot multiplication, the shoots were rooted rapidly. After a simple brief hardening, micropropagated plantlets of *V. negundo* showed had 95 % survival when transferred to the soil. The present procedure would provide an effective strategy for the conservation and large scale propagation of this important aromatic medicinal shrubs.

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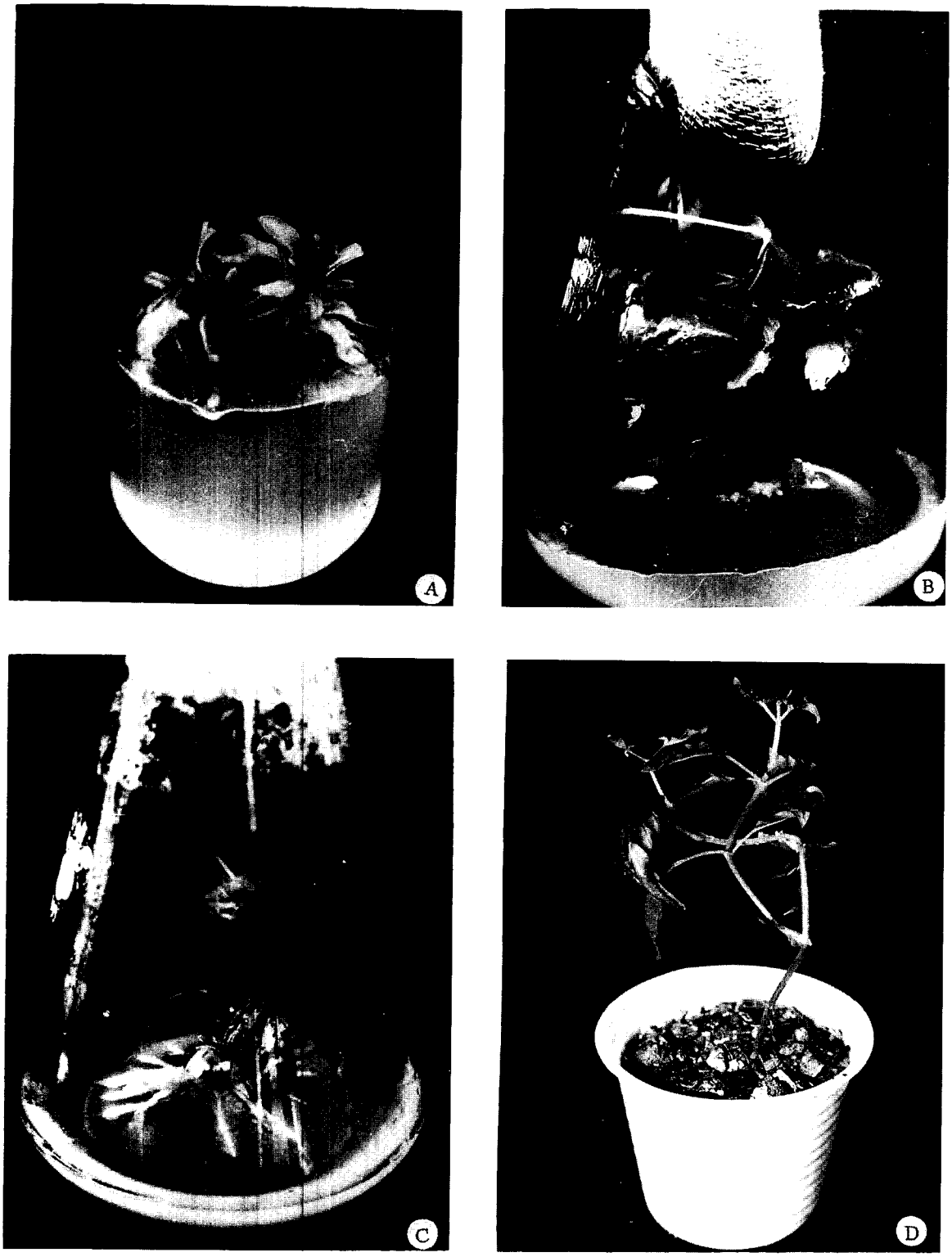

Plate 1. *In vitro* propagation of *Vitex negundo* L.
Figure A. Intiation of shoots on MS medium with BAP 1.5 mg/L
Figure B. Shoot multiplication with BAP 1.5 mg/L plus NAA 0.1 mg/L
Figure C. Rooting of the regenerated shoots
Figure D. Established plantlet

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