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IN-VITRO STUDIES OF VITEX NEGUNDO L. AN IMPORTANT MEDICINAL PLANT

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ABSTRACT

A rapid and efficient protocol was developed for shoot induction and multiple shoot formation from apical and nodal explants of *Vitex negundo* L. an important endangered medicinal plant species. The explants were cultured on Murashige and Skoogs (MS) medium supplemented with various concentrations of auxins, cytokinins and sucrose. Highest percentage (97%) of shoot induction were observed from axillary meristem, developed 10-20 shoots when cultured in the medium containing the combination of 6-Benzyl amino purine (BAP) (0.5 mg/l) and Naphthalene acetic acid (NAA) (0.2mg/l) supplemented with 3% Sucrose within 25 days.

Key words: *In-vitro* studies, Benzyl amino purine, Naphthalene acetic acid, *Vitex negundo* L.

INTRODUCTION

Verbenaceae is a large family of herbs, shrubs and trees comprising of about 75 genera and nearly 2500 species (Nasir and Ali 1974., Sastri 1950). *V. negundo* L. is distributed in East Asia, South West China, throughout the greater part of India at warmer zones and ascending to an altitude of about 1500m in outer, western Himalayas. It is also cultivated in Pakistan. (Usman Ghani Khan, 2007., Khare, 2007., Cook, 1903). *V. negundo* L. is a large woody aromatic and multipurpose medicinal shrub belonging to the family verbinaceae (Wealth of India 1976). It is one of the common plants used in Indian system of medicine. Various parts of the plant are used in the treatment of Arthritis, joint pains and sciatica. It is also used in the treatment of chronic bronchitis, asthma and gastric troubles.

In dispersing swellings of the joints from acute rheumatism and also of the testes from suppressed gonorrhea. The methanolic root extracts of *V.negundo* significantly antagonized the *Vipera russellii* and *Naja kautha* venom induced lethal activity both in-vitro and in-vivo studies (Alam and Gomes, 2003). The stem decoction is used in the treatment of burns and scalds. The fresh berries are pounded to a pulp and are used in the form of a tincture for the relief of paralysis, pains in the limbs, weakness etc. The leaves of the plant are astringent, febrifuge, sedative tonic and vermifuge (Horowitz, 1996). The plant also shows antibacterial, antifungal, larvicidal, antihelmentic, antioxidant, and insecticidal/pesticidal activities. The plant also shows anticancerous activity against Daltons Asiatic lymphoma. It also shows gastro

protective and hepato-protective activities. Despite its economic importance the production of *V.negundo* is threatened by population growth, desertification, industrial development and attack by numerous parasites. The biotechnological approach such as plant tissue culture initiated from medicinal plants is a variable method for the large scale production of economically and medicinally important plants. The present study was undertaken to standardize a protocol for high frequency induction of multiple shoots from different explants and to regenerate plants of *V.negundo* to meet its demand in medicine and agriculture.

MATERIALS AND METHODS

Actively growing and healthy shoot material of *V.negundo* with dominant auxiliary buds were collected from an adult plant growing in the medicinal plant garden of Govt. Motilal Vigyan Mahavidyalaya Bhopal, M.P. After removing leaves, the shoots were cut into small pieces 0.5-1.0 cm each containing a single node auxiliary bud. The explants were then washed under running tap water for 30 minutes, followed by a wash with a solution of detergent for 10 min. followed by washing with surface sterilizing agent mercuric chloride (0.1% HgCl_2) solution for 3-6 min. In sterilized autoclaved beakers and finally washed three times with autoclaved water. Since the use of sodium hypo chloride and bromine water did not prevent contamination. Mercuric chloride was used as sterilizing agent throughout the experiment. The explants were then inoculated in basal medium consisting of Murashige and Skoogs salts, vitamins 30g/L. Sucrose 30g/L. Agar (qualigens India) supplemented with various growth hormones. After adjusting the PH (5.4-5.9) the medium was autoclaved at 121°C for 15-20 minutes at the pressure of 1.06 kgcm^{-2} . The

cultures were then incubated at $25 \pm 3^\circ\text{C}$ under 14/10 hours (light/dark) period with light supplied by white fluorescent tubes at 3500 lux. After 20 days of inoculation, the explants were transferred to a fresh medium. And after 40 days of inoculation data were recorded on shoot induction and number of shoot formation per explant. For multiplication of cultures in-vitro raised shoots were taken in a sterilized Petri dish and were cut into small pieces containing a single node along with dormant auxiliary buds. Then the explants were transferred to culture tubes containing MS medium supplemented with BAP (2mg/L.) and NAA (0.5mg/L.). For the induction of multiple shoots, subsequently subcultures were raised after 20 days interval to study the effect of culture passages on the explants response for shoot induction and multiple shoot formation. All the treatments were repeated at least three times with 10 replicates and data were subjected to statistical analysis.

RESULTS AND DISCUSSION

In the present study both apical and explants were used. But the nodal explants were found to be more effective for shoot induction and multiple shoot formation when culture on MS medium supplemented with various phytohormones as compared to other explants. The nodal explants of *V.negundo* L. were cultured on MS medium supplemented with various concentrations of BAP or KN individually or in combination with NAA or IAA resulted in induction of healthy shoots. When the explants were cultured on MS medium supplemented with cytokinins alone lesser number of shoots were induced in comparison to the MS medium supplemented with combination treatment of auxin and cytokinin.

Table 1. Effect of different concentration of BAP on shoot induction from axillary and apical nodes of *V. negundo* L. in MS medium

Growth regulator	Percentage explants shoot induction	Number of shoots produced/explants \pm SE	Mean length of shoots in (cms) \pm SE
BAP (0.5 mg/l)	80%	2.00 \pm 0.15	0.75 \pm 0.06
BAP (1.0mg/l)	57%	1.47 \pm 0.12	1.38 \pm 0.06
BAP (1.5 mg/l)	50%	1.15 \pm 0.11	1.9 \pm 0.11
BAP (2.0 mg/l)	45%	1.4 \pm 0.12	1.7 \pm 0.03

Each value represents mean \pm SE calculated from three separate experiments each with 10 replicates per treatment.

Table 2. Effect of different concentration of BAP and NAA on shoot multiplication of *V. negundo* L. in MS medium.

Growth regulators	Percentage explants showing shoot multiplication	Number of shoots produced/explant \pm SE	Mean length of shoots in (cms) \pm SE
BAP (1 mg/l) + NAA (0.1mg/l)	43%	1.38 \pm 0.13	1.85 \pm 0.02
BAP (1 mg/l) + NAA(0.5mg/l)	50%	3.4 \pm 0.14	2.2 \pm 0.06
BAP (2 mg/l) + NAA (0.5mg/l)	46%	4.2 \pm 0.10	1.3 \pm 0.06
BAP (3 mg/l) + NAA(0.5mg/l)	56%	6.00 \pm 0.16	2.01 \pm 0.01
BAP (0.5 mg/l) + NAA (1.0mg/l)	76%	6.80 \pm 0.20	1.67 \pm 0.08
BAP (0.5 mg/l) + NAA (2.0mg/l)	90%	9.40 \pm 0.11	1.8 \pm 0.04

Each value represents mean \pm SE calculated from three separate experiments each with 10 replicates per treatment.

The explants were cultured on MS medium supplemented with sucrose (30gm/L.) along with optimal concentrations of BAP (0.5mg/L.) and NAA (2mg/L.) which was found to be the most effective in the induction of shoots compared to other concentrations. In-vitro raised shoots (20-30days old) were sub cultured on MS medium supplemented with BAP (0.5mg/L.) and NAA (2mg/L.). The highest response of nodal

explants (90%) with a maximum average number of shoots (3.40 \pm 0.11) per explant was observed. There have been several reports of micro propagation with nodal segment and shoot tips of tropical medicinal plants in the juvenile phase of development (Kukreja et al., 1988). Here the protocol is described for rapid and large scale propagation of the woody aromatic and medicinal shrub *V.negundo* by in-vitro

culture of nodal segments from mature healthy plants. Here different concentrations of cytokinins were used as supplements to the MS medium. Among the cytokinins tested BAP was found to be most effective than other cytokinins for the induction of shoots. The bud breaking and shoot induction in cultures of nodal explants indicate the function of cytokinins (Sahoo and Chand 1998). In the present investigation bud breaking and multiple shoot induction was increased in treatments of BAP up to 0.5mg/L. However there was decline in shoot induction beyond this dosage. In each explant 4-6 axillary buds were formed within 15-20 days after inoculation. The number of shoot formation per explants was increased when the cultures were transferred to a fresh medium. The enhancing effect of MS medium supplemented with auxins and cytokinins in shoot multiplication was also studied on *Gomphrena officinalis* (Mereker et al., 1992) and on *Rauvolfia serpentina*. Similar observations were made by Sahoo and Chand (1998) in the shoot multiplication of *V.negundo*

when sub cultured on MS medium supplemented with BA (4.40um/L.) and GA₃ (1.15um/L) up to two subcultures and then there was a gradual decline. Similar results were found on shoot induction and multiple shoot formation from nodal explants of *V.negundo* in the combination treatment of BA (16.80um/L.) and IBA (2.25um/L.) supplemented with 100mg/L. silver nitrate. Noman et al., (2010) observed the high frequency bud initiation and shoot proliferation from callus by using BAP (0.3mg/L) and IAA (0.3mg/L). In the present study MS medium along with NAA and BAP has been used which has also been reported the best shoot proliferating combination in *Heracleum candicans* (Wakhlu and Sharma, 1999) *Centella asiatica* (Shashikala et al., 2005) and *Cardiospermum halicacabum* (Jawahar et al., 2008). In contrast Fraternale et al., (2002) reported that high concentration of auxin with cytokinin was stable for shoot multiplication in *Bupleurum fruticosum*.

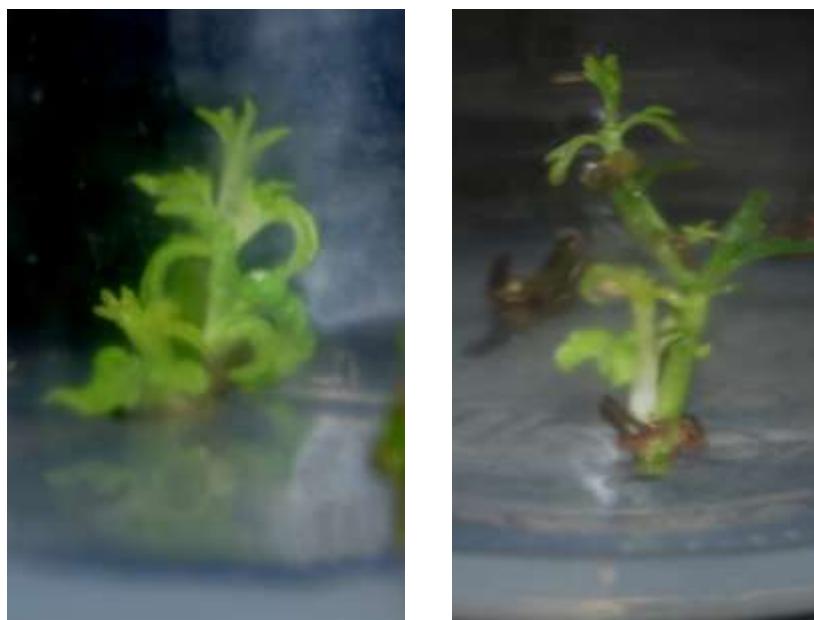


Fig.A. Shoot initiation of *Vitex negundo* L. from apical and nodal explant



Fig.B Shoot multiplication of *Vitex negundo* L.

CONCLUSION

A simple and efficient method has been developed for shoot induction and multiple shoot formation and thus increasing the production of *V. negundo*. This is suitable for conservation of germplasm of this multipurpose medicinal plant species. Despite its economic importance the production of *V. negundo* is threatened by population growth, desertification, industrial development and attack by numerous parasites. The classical conservation techniques such as crossing over, Sexual and somatic hybridization and breeding give a genetically blind mixture. Propagation through vegetative cuttings is very slow and a large number of cuttings do not survive during transportation. It can also be propagated through seeds or root suckers. Poor viability of the seeds and the production of root sucker is strictly age dependent. The biotechnological approach such as plant tissue culture initiated from medicinal plants is a variable method for the large scale production of economically and medicinally important plants. The present study was undertaken to standardize a protocol for high frequency induction of multiple shoots from different explants and to regenerate plants of *V. negundo* to meet its demand in medicine and agriculture.

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