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Effect of NAA and BAP on *In-Vitro* Micropropagation of *Vitex negundo* L. - A Significant Medicinal Plant

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ABSTRACT

Vitex negundo has been used worldwide in pharmaceutical industry. Its natural vegetative propagation rate is unable to meet growing demands of pharmaceutical industries. The present study is aimed to find a highly effective and a rapid method for the Micropropagation of this medicinal plant to meet the demand. The explants of *V. negundo* were cultured on Murashige and Skoogs (MS) medium supplemented with different concentrations of auxins, cytokinins and sucrose. Among different combinations used, Murashige and Skoog medium with 0.5 (mg L^{-1}) 6-benzylaminopurine + 0.5 (mg L^{-1}) α-naphthalene acetic acid, containing 0.8% agar and 25% sugarcane juice exhibited highest shoot induction (80%) with highest number of shoots per explant (3 - 4) supplemented with 3% Sucrose and 0.8% (w/v) agar within 20 days.

1. Introduction

Plant tissue culture is one of the most important tool for large scale production of active constituents which include secondary metabolites and some important engineered molecules of biological importance. Novel methods like gene editing and abiotic stress can also improve the technique [1]. Plant tissue culture also serves as a useful tool for conservation and propagation of germplasm of the endangered and threatened plants. Micropropagation protocols have already been developed by various researchers for a huge number of medicinally important plant species [2-7]. The genetic fidelity of three successive regenerations of *Nepenthes khasiana* by *in-vitro* propagation was assessed by using three different but single primer amplification reaction (SPAR) methods [8]. *Vitex negundo* commonly called Five - Leaved Chaste Tree and Nirgundi in Sanskrit is an aromatic shrub of around 2.5 feet height with beautiful bluish purple flowers. *V. negundo* belongs to Verbenaceae, a large family comprising of herbs, shrubs and trees of about 75 genera and 2500 species. *V. negundo* finds its origin in tropical Eastern and Southern Africa and is also called as chest tree or monks pepper in Hindi. *V. negundo* is an aromatic plant used in folk medicine, especially in South Asia and Southeast Asia for Skin diseases, cough remedy, rheumatic pain and liver disorders as well as repelling mosquitoes. It possess a great potential of biologically active secondary metabolites [9]. *V. negundo* L. is also found in South West China, East Asia, throughout the greater part of India at warmer zones and ascending to an altitude of about 1500m in outer, western Himalayas. *V. negundo* L. is a large aromatic, woody and multipurpose medicinally important shrub (Wealth of India 1976**)** [10]. Seeds and leaves are bitter and leaves are used in fever, inflammation, vermifuge, headache and cough. Decoction of nirgundi is used in steam bath against arthritis or joint pains [11, 13], used as tonic and its flowers are used in fever, diarrhea and liver complaints [12]. Its Leaves and seeds possess anti-inflammatory, anti-arthritic antianalgesic, activities [14,15]. Significant anti-fungal [16] and antibacterial activity against few bacterial strains [17]. Alcoholic seed extract possess hepatoprotective activity against CCl_4 [18], leaves possess anti-tubercular drugs also thioacetamide induced hepatotoxicity [19,20]. Despite economic importance of *V. negundi,* its production is at great threat due to desertification, population growth, attack by numerous pesticides and industrial development. 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 has been undertaken to standardize a protocol with high frequency induction of multiple shoots from different explants and to regenerate plants of *V.negundo* to meet its demand in agriculture and medicine [21].

2. 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 from the material, the shoots were then cut into small pieces 0.5- 1.0 cm each containing a single node auxiliary bud. Then These Small pieces of explants were washed under tap water, followed by a wash with a solution of detergent for 10 min, then 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. Sterilizing agent used throughout the experiment was Mercuric chloride. Then the explants were 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 pH (5.4-5.9), the medium was left for autoclaving at 121^{o}C for about 20 minutes at the pressure of 1.06 kgcm^{-2}. The cultures were then incubated at 25+3^{o}C under 14/10 hours (light/dark) period with light supplied by usual white fluorescent

tubes at 3500 lux. After 20 days of inoculation, these explants were then transferred to a fresh medium. And after 40 days of inoculation data were recorded on shoot induction and also number of shoot formation per explant. For multiplication of cultures in-vitro raised shoots were taken in a sterilized Petri dish and were again cut into small pieces containing a single node along with dormant auxiliary buds. Then these explants were again transferred to culture tubes containing MS medium which were supplemented with (1) BAP alone and (2) NAA along with BAP at various concentrations. For the induction of multiple shoots, subsequently subcultures were raised after 20 days interval in order to study the effect of different 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 the data was subjected to statistical analysis. [22]

3. Results and discussion

The earlier research on *Vitex negundo* reported plant regeneration through axillary's nodes, inter nodes and leaves on media. In the present experiment the explants of *V. negundo* were cultured on freshly prepared MS media supplemented with sucrose (30gm/L.) along with low to high concentrations of Auxin and Cytokinin that were found suitable for axillary and apical explants for rapid and large scale multiplication. The explants of *V. negundo* were cultured on freshly prepared MS media supplemented with sucrose (30gm/L.) along with optimal concentrations of BAP (0.5mg/L.) and NAA (0.5mg/L) which was found to be most effective in the induction of shoots compared to other concentrations. In-vitro raised shoots (20 days old) were sub cultured on MS media supplemented with BAP and NAA at various concentrations. The highest response of nodal explants (85%) with a maximum average number of shoots (19-20) per explant was observed on MS media supplemented with BAP (1.0 mg/L.) and NAA (1.0 mg/L).

Table-1 EFFECT OF GROWTH REGULATORS ON INITIATION OF SHOOTS

S.no	Medium + Growth hormones mg/l	%age of explants shoot induction	No. of shoots	Shoot length in cm.	Callussing
1	MS + 0.5BAP	70%	1- 3	2-3	-
2	MS + 1.0BAP	70%	1-2	2 -3	-
3	MS + 0.5BAP + 0.5 NAA	80%	3- 4	2-3	-
4	MS + 1.0BAP + 0.5NAA	60%	2-3	1-2	++

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

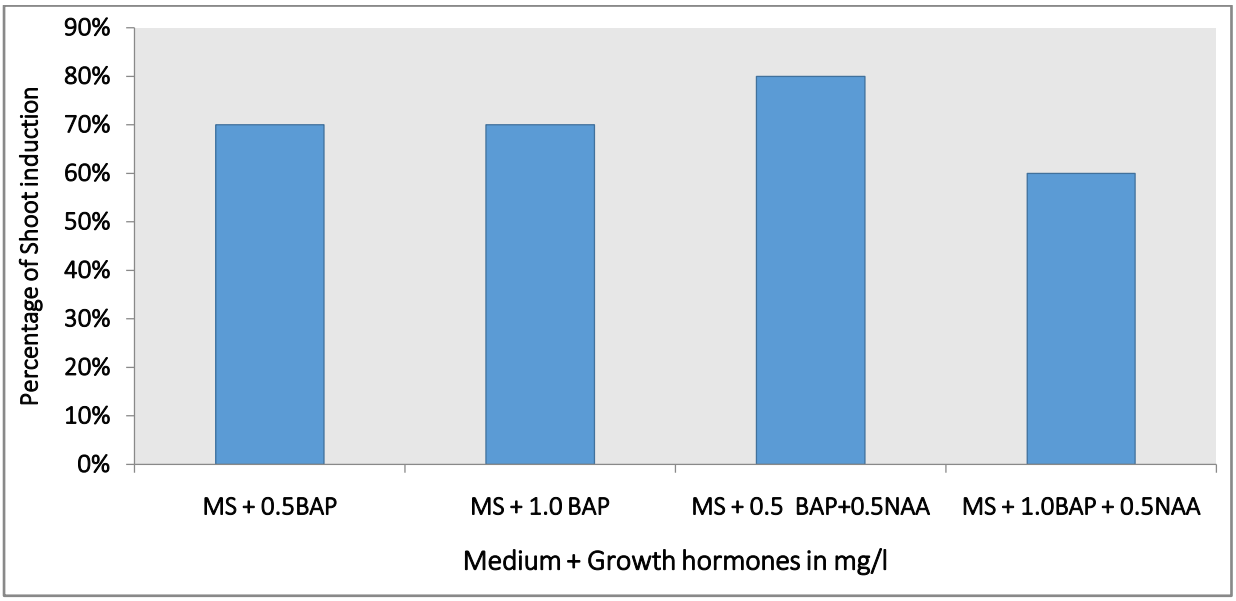
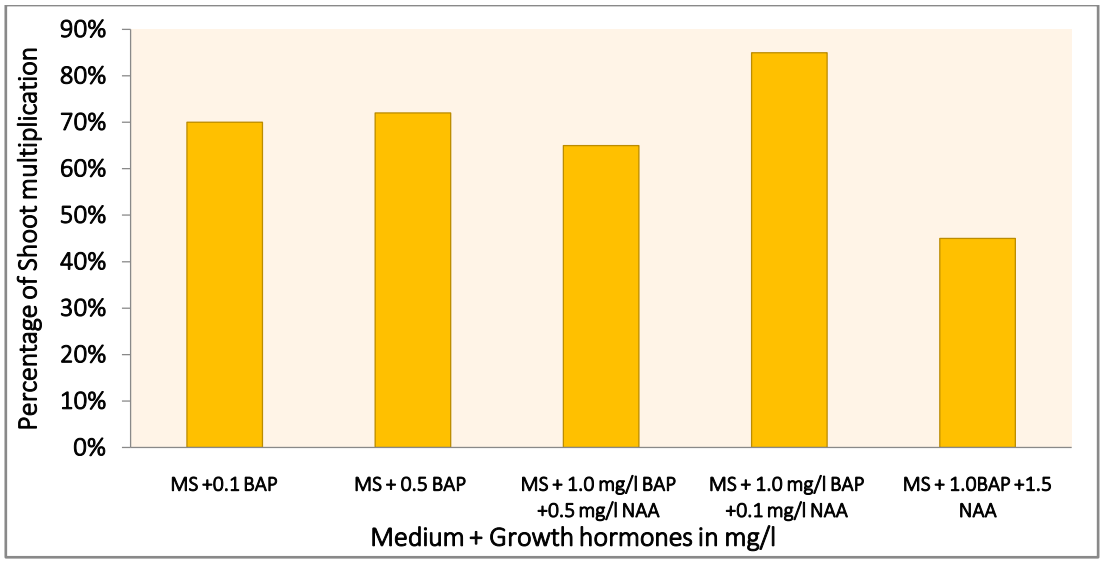


Table-2 EFFECT OF GROWTH REGULATORS ON SHOOTS MULTIPLICATION.

S.no	Medium + Growth hormones mg/l	% age of shoot multiplication	No. of shoots per culture	Average shoot length in cm.	Callussing
1.	MS +0.1 BAP	70%	10-12	3-4	-
2	MS + 0.5 BAP	72%	15	1-3	-
3	MS + 1.0 mg/l BAP +0.5 mg/l NAA	65 %	9-11	1-2	+
4	MS + 1.0 mg/l BAP + 1.0 mg/l NAA	85 %	19-20	4-5	++
4	MS + 1.0BAP +1.5 NAA	45%	8-9	1-2	+

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



For initiation of shoots the explants shows response in every medium tried for experiment the bud break and the initiation of shoots was reported maximum in the medium containing optimal concentrations of BAP (0.5mg/L.) and NAA (0.5mg/L) 3-4 shoots were initiated which were 2–3 cm long within one week. The addition of NAA also promotes the percentage of shoots initiation. At high concentrations of Cytokinin and low Auxin, produces very little callus at the lower end of shoots. The initiated shoots were transferred in the fresh media containing BAP alone and with combination of Auxin. The higher multiplication of shoots was reported in the medium containing 1.0 mg/l BAP with combination of 1.0 mg/l NAA. Both concentration and combination of growth hormones show multiplication within two to three weeks of incubation. Addition of NAA enhance the shoot production from the nodes and emergence of shoot buds at the base of internodes which later differentiated into shoots. Proliferation of shoot bud and elongation growth of shoots was comparatively higher in the MS media containing BAP and NAA. Increase in length of shoot was observed faster in the medium about 19-20 shoots of 4 to 5 cm length has been achieved within 15-20 days of culture which increases with the age of culture. The multiplication pattern which we observe in *Vitex negundo* was that the shoots multiplied at the axillary nodes of the explant with the formation of normal callusing. Somewhat similar type of results have been observed and reported by various researchers which have worked on *V. negundo* and also on various other medicinal plants through micropropagation which are sufficient to support our research and help commercial producers to a great extent [22-27].

Fig. A: Shoot initiation of *Vitex negundo*. from Apical & Axillary Meristem

Fig. B: Shoot multiplication and Elongation of *Vitex negundo*.

Research is said to be more successful if it is cost effective when number of shoots per sub-culture and quantity of media per sub-culture was standardized from the economical point of view. The system demonstrated a continuous supply of shoots up to ten cycles without any decline in shoot number in subsequent sub-cultures. Subcultures were performed frequently (3-weeks), as the delayed sub-cultures (more than 4-weeks) in the medium were found to cause vitrification of shoots. We reported high level of regeneration in shoot buds from axillary and apical explants

with continues proliferation as well as elongation of shoot buds. Shoot buds and the induction on MS media supplemented with 1.0mg/l BAP with 1.0mg/ l NAA. Hence we suggest commercial producers that they may use this method for commercial production of such a medicinally important plant.

4. Conclusion

The main objective of our study was to explore the possibility of raising an effective shoot number for micropropagation protocol. *Vitex negundo* holds great position as a medicinal plant especially in its availability and indeed there is no surprise when this plant is referred to *'sarvaroganivarini'* (remedy for all diseases) in Indian traditional circles. Although *Vitex Negundo* propagates vegetatively, but its propagation rate is unable to meet growing demands of pharmaceutical industries. Thus commercially viable protocols have been established for mass propagation of *Vitex negundo*. The procedure described here will go a long way to meeting on one hand the never increasing demand of the pharmaceutical industries and on the other hand save the species from the extinction. Present study also reported high level regeneration of shoot buds from axillary and apical explants with continues proliferation as well as elongation of shoot buds. Shoot buds and the induction on MS media supplemented with 1.0 mg/l BAP with 1.0 mg/ l NAA.

Conflicts of interest

The authors declare no conflicts of interest.

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