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IN VITRO CALLOGENESIS AND MICROPROPAGATION OF MEDICINAL PLANT – *Vitex negundo* L.

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Abstract

The woody, aromatic and medicinal shrub *Vitex negundo* was used for rapid and large-scale propagation by in vitro culture. Callusing was observed in leaf, axillary bud, node, and internode explants when supplemented with different growth regulator in different concentrations. Leaf explants showed better callusing when supplemented with 2, 4-D + NAA of (1 + 1 mg/l), BAP + NAA + Kin (1.0 + 0.5 + 0.5 mg/l), 2, 4-D (1.0mg/l). Petiole showed better respond for callusing in combination with 2, 4-D + BAP (2.0 + 1.0 mg/l). Shooting was observed in from leaf, axillary bud, nodal explants when supplemented with growth regulators in different combinations at different concentrations such as BAP (2.0 mg/l), BAP + NAA + Kin (1.0 + 0.5 + 0.5 mg/l), BAP + NAA (2.0 + 1.0 mg/l). Both shooting and rooting was obtained in MS media containing the growth regulators in combination of IBA + BAP at a concentration (0.5 + 1.5 mg/l) and IAA + IBA (1.0 + 1.0 mg /l) respectively. In vitro propagated plants were transferred to soil with a survival rate of 99 % after 45-50 days.

Keywords: *Vitex negundo,* Murashige and Skoog, Micropropagation, Growth Regulators

#Research Article

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Introduction

Micropropagation is an effective approach to conserve germplasm. Further, genetic improvement is another approach to augment drug yielding capacity of the plant. In-vitro propagation has proven as a potential technology for mass scale production of medicinal plant species (Martin, 2002; Azad *et al*, 2005; Faisal *et al*, 2003; Hassan and Roy, 2005). *Vitex negundo* L. (Family- Verbanaceae) commonly known as five-leaved Chaste or Monk's Pepper (Chopra *et al*,1956), is woody, medicinal and aromatic deciduous shrub sometimes small tree, restricted to tropical and sub-tropical regions, although a few species are also found in temperate zones (K. Padmalatha *et al*. 2009). The phytochemical components of medicinal plants often active individually, additively or synergistically in improvement of health. After having analyzed the various chemical components present in different parts of *Vitex negundo* it is imperative that focus shifts to the medicinal applications of the plant. Myriad medicinal properties have been ascribed to *Vitex negundo* L and the plant has also been extensively used in treatment of bronchitis, asthama and gastric troubles a plethora of ailments, anticancerous, dyspepsia, colic, rheumatism, worms, boils, antitumoric, antimicrobial, anti inflamatory agent. Therefore, Plant tissue culture plays a important role in search for alternative to production of desirable medicinal compounds.

Materials and Methods

Plant Material

In the present investigation the explant such as leaves, nodes, internodes, petiole, axillary bud selected from healthy and disease free plant of *Vitex negundo* L. were collected from the botanical garden of the Sant Gadge Baba Amravati University campus.

Excision and Surface Sterilization of Explants

All aseptic operations were performed in laminar airflow cabinet in order to avoid contamination. The explants were subjected to washing thoroughly with running tap water after that thoroughly with double distilled water 3-4 times, then treated with 1% Bavistin solution and washed with double distilled water. Explants were surface sterilized with 0.1% mercuric chloride (HgCl2) for 2-3 minutes followed by rinsing with sterile double distilled water for 3-4 times, to remove the traces of surface sterilants. The explants were soaked by placing them on sterile tissue paper and edges of the explants were cut with sterile scalpel near to the blower.

Culture Medium and Conditions

The explants were inoculated on MS (Murashige and Skoog, 1962) medium with different concentrations and combinations of 6-Benzylaminopurine (BAP), 2,4 – Dichlorophenoxyacetic acid (2,4-D), 6-Furfuryl-aminopurine (KIN), Indole- 3- acetic acid(IAA) Indole - 3-butyric acid (IBA), α - naphthalene acetic acid (NAA). All the plant growth regulators were procured from Himedia (Mumbai, India). The MS media (34.8 gm/lit) and CaCl2 (440 mg/lit) was dissolve in 1000 ml of sterile double distilled water and subjected to adjust the pH 5.8. Thereafter, agar powder (8 gm/lit) was added and

dissolved in the prepared media solution. After dissolution of agar, 20 ml media was poured into sterile tissue culture air tight tubes and bottles (Borosil), sterilized by autoclaving at 15 lb pressure, at 121^0C temperature for 15 min. Contamination was checked for 2 days and then inoculated by proper explants. Observations were recorded time to time and all the culture tubes and bottles were kept under observation. Moreover, after consumption of the nutrients by growing explants after some period of time, the explants were subjected to sub culturing. The photoperiod was adjusted as 16 hours light and 8 hours dark, as per the requirement. The culture was kept at 25 ± 2C temperature and 70% humidity (Gottlieb Hberlandt 1854-1945).

Shooting and Rooting Acclimatization and Field Transfer

After shoot and root formation the healthy plantlets were selected. These are then transferred to pots, containing mixture of soil sterilized in green house. After acclimatization 20- 30 days the plantlets were potted in earthen pots with garden soil.
For shoot and root response the average shoot and root length per explants was evaluated after 3 days of culture. Observations were recorded for the initiation of callus induction and average number of shoots after 30 days of inoculation and observation was made to counter check contamination and progress in plant development.

Result and Discussion

Callus induction

Among the various explants used, leaf explants exhibited maximum i.e. 5.0 cm callus induction. However, other explants also respond for callus formation like petiole and nodal explants in moderate 1.0 and 2.0 cm respectively. The amount of callus formed was found to be different in various combinations of growth regulators under investigation. Moreover, it also differs in various concentrations of the same growth regulator (1.0, 2.0, 5.0).

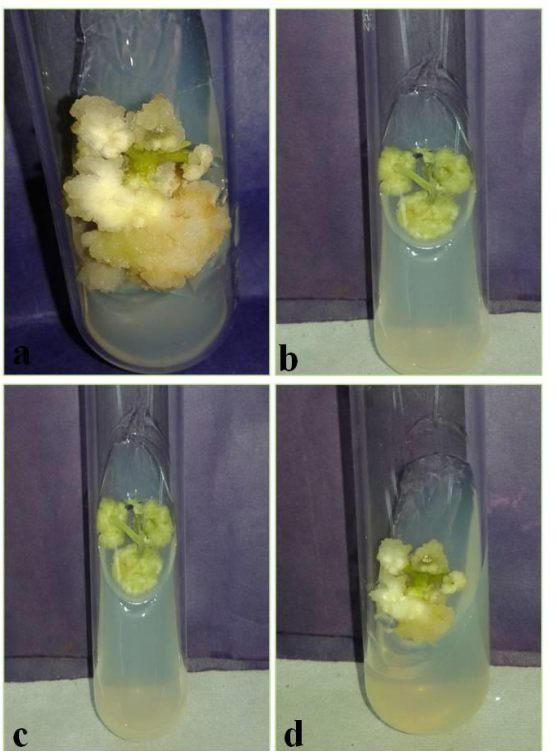

Plate: 1 Micrograph (a, b) showing callus induction from leaf explants of *V negundo* in BAP (2.0 mg/l), 2,4-D + NAA (1.0 + 1. 0 mg/l) BAP + NAA + kin (1.0 + 0.5 + 0.5 mg/l). and for petiole explants **(c, d)** shows 2,4-D + BAP (2.0 + 1.0 mg/l) 2,4-D + NAA(2.0 + 1.0 mg/l.)

Table:1. Response of Different Combinations and Concentrations of Growth Regulators on Callus Formation From Leaf, Nodal, Petiole, Intermodal, Axillary Bud Explants of *Vitex negundo* L.

Growth Regulators	Concn. (mg/l)	Explants	Callogenesis	Time	C/T
BAP	2.0	Leaf	+++	12-15	W/F
BAP + NAA + KIN	1.0 + 0.5 + 0.5	Leaf	++	10-12	G/F
2,4-D + NAA	1.0 + 1.0	Leaf	+++	7-10	G/H
2,4-D	1.0	Leaf	+	20-22	W/F
KIN	1.5	Petiole	-	-	-
2,4-D + BAP	2.0 + 1.0	Petiole	++	15-16	G/H
2,4-D + NAA	2.0 + 1.0	Petiole	+++	12-15	W/F
IBA + BAP	0.5 + 1.5	Node	+++	12-15	B/F
BAP + NAA + Kin	2.0 + 0.5 + 0.5	Axillary Bud	+++	11-12	W/F
BAP + 2,4-D	2.0 + 1.0	Node	+++	15-18	W/F
BAP	2.5,2.0	Node	+++	12-15	B/F
IAA+IBA	1.0+1.0	Internodal	+++	15-18	B/H

Concn. – Concentration, C/T – Colour Texture, SL – Shoot Length, RL – Root length, No callus (-), No shooting (-) No rooting (-), Poor callus Formation (+), Good Response(++), Better Response(+++).

Table 2: Response of Different Combinations and Concentrations of Growth Regulators on Shooting and Rooting Formation from Leaf, Axillary Bud, Nodal, Petiole and Intermodal Explants of *Vitex negundo* L.

Growth Regulators	Explant	Concn.	Shoot inducing frequency	Time	SL Mean	RL Mean
BAP	Leaf	2.5, 2.0 mg/l	100	12-15	3.79±0.08	2.98±0.40
IBA + BAP	Leaf	0.5 + 1.5 mg/l	100	10-15	6.1±00.6	2.33±2.19
BAP + NAA + KIN	Axillary bud	2.0 + 0.5+0.5 mg/l	90	15-17	6.00±0.07	3.33±3.85
BAP + 2,4-D	Node	2.0 + 1.0 mg/l	70	15-18	4.98±0.09	-
IAA + IBA	Inter node	1.0 + 1.0 mg/l	100	11-15	5.25±0.02	-
BAP + 2,4-D	Node	2.0 + 1.0 mg/l	100	15-16	5.00±1.00	-
2,4-D + BAP	Petiole	2.0 + 1.0 mg/l	80	16-17	3.33±1.22	-

Concn. – Concentration, C/T – Colour Texture, SL – Shoot Length, RL – Root length, No callus (-), No shooting (-) No rooting (-), Poor callus Formation (+), Good Response(++), Better Response(+++).

Plate 2: Micrograph (e) showing callus, shooting and rooting were observed by leaf and nodal explants in BAP (2.5, 2.0 mg/l) and IBA + BAP (0.5 + 1.5 mg/l), axillary bud explants (f) shows in BAP + NAA + KIN (2.0 + 0.5 + 0.5 mg/l). Callus and multiple shooting obtained (g) from nodal explants in combination BAP + 2,4-D (2.0 + 1.0 mg/l) and internodal explants (h) IAA + IBA (1.0 + 1.0 mg/l).

Plant tissue culture is a set of techniques designed for the growth and multiplication of cells and tissues using nutrients solutions in an aseptic and controlled environment. This technology explor's conditions that promote cell division and genetic programming In – vitro conditions. In - vitro propagation is a handy technique for rapid multiplication of endangered, threatened, horticultural and agro-economic crop plants (Satyanarayan, 2005). Sahoo Y (1998) *Vitex negundo* by in vitro culture of nodal segments from mature plants. The three different cytokinins – N6-benzyladenine (BA), kinetin, and thidiazuron – evaluated as supplements to Murashige and Skoog (MS) medium, BA at an optimal concentration of 2.0 mg/l was most effective in inducing bud break. In present investigation the induction of callus in leaf, petiole internodal and nodal explants of *Vitex negundo* L. It was achieved when media was supplemented with combination of BAP + NAA (1 + 1, 0.5 + 1, 1 + 1, 1.5 + 1.0 mg/l), 2.4-D + BAP (1 + 1, 2.0 + 1.0 mg/l) 2,4-D (1.0,1.5,2.0 mg/l) IBA + IAA (1 + 1 mg/l) produced better callus induction and found to be significant for callus induction and the callus was brownish, green, and white in colour (Table 1). However better callus induction was achieved in leaf explants when supplemented with growth regulators such as 2,4-D + NAA (1.0 + 1.0 mg/l), BAP (2.0 mg/l), 2,4-D (1.0 mg/l), and callus obtained was green/ white, hard, fragile (Photo plate 1). However good shooting was noticed in leaf, axillary bud and internodal explants in MS media supplemented with combination of growth regulators in different concentration such as BAP + NAA + Kin (1.0 + 0.5 + 0.5 mg/l), BAP + NAA + Kin (2.0 +0 .5+0 .5 mg/l) IAA + IBA (1.0 + 1. 0 g/l). The shoot were attained a length of 1.5 – 6.11 cm. Rooting was also observed but only in combination of IBA+ BAP (0.5 + 1.5 mg/lit), BAP (2.5, 2.0 mg/l), BAP + NAA + KIN (2.0 + 0.5+0.5 mg/l), IBA + BAP (0.5 + 1.5 mg/lit) in this combination

the root attained a length of 6.1 cm. (Photo plate 2) It was observed that BAP play a very significant role for the initiation of shooting growth and significant result was achieved. No shoot induction was reported when nodal segments were cultured on hormone-free MS medium. Acclimatization and hardening done after 50 days in pots containing garden soil and farmyard manure (1:1) were used for hardening in the green house as shown in (Photo plate 3 k, l)

Plate:3 Micrograph showing i, j Callus induction, shooting and rooting observed from nodal and petiole explant in BAP+2,4-D (2.0 + 1.0 mg/l), 2, 4-D + BAP (2.0 + 1.0 mg/l). k, l Acclimatized plantlets in pots.

Conclusion

Hence from overall observations and results the present investigation came to the conclusion that the *Vitex negundo* L responded better for shoot initiation, rooting and callusing. Axillary bud responded better when supplemented with different growth regulators in different combinations of BAP+ NAA +KIN (2.0 + 0.5 + 0.5 mg/l) and nodal explants responded for shooting in combination of BAP + 2,4-D (2.0 + 1.0 mg/l) and results better shoot initiation and leaf explants also responds better for shoot induction in BAP (2.0 mg/l), the growth hormone IBA (1.5mg/l) shows better response for root induction.

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