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Rapid induction of callus and clonal micropropagation by plant growth regulators (PGR^s) on important medicinal plant *Vitex negundo* (L.)

Shrikant B. Bhosale[1*], Subhash B. Pawar[2], Shrikant B. Mane[3], D. S. Jadhav[1] and Vikas B. Kalyankar[4]

[1]Department of Botany, Shikshan Maharshi Dnyandeo Mohekar College Kallamb, Dist. Osmanabad, (M.S.) India.
[2]Sant Ramdas College, Ghansawangi, Dist Jalna. (M. S.) India.
[3]Department of Botany, Dr Babasaheb Ambedkar Marathwada University, Aurangabad, (M.S. 431007) India.
[4]Toshniwal A. C. S College, Sengaon Dist. Hingoli, (M.S.) India.
*Corresponding Author

Abstract

In the present research, efforts have been made to enlarge appropriate experimental protocol for regeneration of rapid proliferation and conservation. Present work was done in the laboratory. *Vitex negundo* (L.) (Verbenaceae) originate to tropical Eastern and Southern Africa also called as chest tree or monks pepper in Hindi Nirgundi. It is aromatic plant extensively used in folk remedy, predominantly in South and Southeast Asia such as cough remedy, skin diseases, liver disorders and rheumatic pain as well as control mosquitoes. It is good potential bio-control manager and quality as well as quantity of the bioactive secondary metabolites. Callus creation was productively by node and apical shoot tip of investigational plant. 2.0 KIN and 0.2 IAA generate callus (yellowish green) and multiplication (Callogenesis and Rhizogenesis). Apical shoot tip produce yellowish dark green in PGR^s as like 1.8 KIN and 0.3 IAA. Near about 76 shoot were rising up by the 2.0 KIN and 0.3 IAA which

Keywords: Callus, Micropropagation, Growth Hormones, *Vitex negundo* (L.), PGR^s

Introduction

Vitex negundo **(**L.) perennial shrub belongs to the family Varbanaceae is aromatic, woody, small shrub, tri or penta-foliate leaves and purple colored flower in branched tomentose cymes. Its habitat in humid, water courses, wasteland and open mixed forest occur in Afghanistan and Asian countries (Vishwanathan A. S and Basavaraju, R). Near about 14 species were occurred in India out of 250 species of the genus *V. negundo* **(**L.). *V. negundo* **(**L.) commonly called as Chast tree, Nirgundi (Hindi) and Monks pepper.

Bark of *V. negundo* **(**L.) is thin, yellowish gray, leaflets lanceolate, terminal leaflets 5-10 x 1.6-2.3 cm lateral one smaller all nearly glabrous (Tandon Vishal R., 2005). *V. negundo* **(**L.) is propagated by seed and root suckers. Conventional propagation practices of *V. negundo* **(**L.) such as vegetative cutting but unfortunately slow and need huge number of stem segment, leading to large destruction of available genetic stock (Sharma *et al.*, 1991). *In vitro* technique for regeneration of plant applied for huge number of

prologues production. Different parts of *V. negundo* **(**L.) were used in treatment of bronchitis, asthma and gastric troubles (Vadawale A. V *et al.*, 2005). Leaves also reported that insecticidal properties and leaves extract use against cancer, *Echrlich ascites* tumor cells as well as soil reclamation and erosion. Properties of leave were use as snake neutralizing activity (Alam and Gomes, 2003). *V. negundo* **(**L.) is the rich source of active compounds such as betulinic acid and ursolic acid (Chandramu *et al*., 2003).

Leaves are aromatic bitter, acrid, astringent, anodyne, anti-inflammatory, anti- pyretic anti-helminthe and varmifuse. Chemically leaves contain alkaloids, flavonoides such as flavones, luteoline-7-glycoside, casticin, iridoid glycosides, essential oil and additional components like vitamin C, carotene, glycol-nonital, benzoic acid, β-sitosterol and C-glycoside. Flower are cool, astringent, carminative, hepatoprotective, digestive, fabrifuse and very valuable in haemorrhages and cardiac disorder. *Vitex* fruit is nervine, cephalic, aphrodisiac, emmenagogue and varmifuse. Its seed have hydrocarbon, β-sitosterol, benzoic acid and phathalic acid (Husain Akhtar *et al.*, 1992). It is use in remedy of headaches and colds, alleviation of fever, pain relief, sedation, anti-inflammatory treatment. *Vitex* Plant body or an extract of simple leaf was use an external medicine to be applied to the scalp as hair tonic (Chuyaku Jiten). Leaf is used for therapeutic reason but root, flower and fruit also have the remedial purpose (Hasan, 1982). Juice of fresh leaves removes fetid discharges and worms from ulcers. Stem and bark contain flavonoid glycosides as well as swellings of joints in rheumatic attacks. Flower oil is applied to sinuses and scrofulous sores. Root juice is tonic, expectorant and diuretic (Ghani, 1998).Tissue culture has deeply improved the scope and potentiality of mass propagation by exploiting the regenerative performance in a broad range of preferred horticultural and agricultural flora together with the medicinal ones (Roy et al. 1994, Thiruvengadam and Jayabalan, 2000, Islam *et al.*, 2001 and Jawahar *et al*., 2008).

Materials and Methods

Source of plant material

Vitex negundo **(**L.) is herbaceous plant. Authenticated plants of *Vitex negundo* **(**L.) were collected from garden of S.M.D.M. College, Kallamb and also in the Department of Botany were used as the source of explants. Excised shoot tips, stem segments, node and internodes were used as explants.

Culture Media

MS medium (Murashige and Skoog, 1962) variously supplemented with IAA and KIN was used for multiple shooting from apical shoots and nodal explants of *Vitex negundo* **(**L.) for rooting, half strength MS medium supplemented with various concentrations of auxins IAA and KIN were used.

Culture Conditions

MS medium containing 3% sucrose was gelled with 3 gm/L solidified agent Agar-Agar and the pH was adjusted to 5.8 after addition of the growth regulators. The media were sterilized by autoclave under 15 psi and 121°C. After inoculation, culture vessels were transferred to tissue culture room. The explants were incubated in a culture room where the temperature was maintained at 25-26°C, humidity at 85% and photoperiod of 16h light and 8h dark. Data was measured after 30 days for five replicates for shoot multiplication and shoot length Mean (μ) values with the standard error (SE).

Growth regulators

Auxins and cytokinins were the two major phytohormones used in different concentration and combination in various media for induction of callogenesis, caulogenesis and rhizogenesis.

Auxins: **-** Powder of Auxins were dissolved in 1N NaOH and made up the volume with sterilized distilled water and then used or stored in freezer as stock for further use which was indole acetic acid (IAA).

Cytokinins: - The cytokinins were dissolved in 1N NaOH and then used or stored as stock for further use which was Kinetin (KIN).

Data Collection

The day of initial callus formation, the morphology and color of the callus were recorded. At the end the observation period, percentage of the explants forming callus as well as the degree of callus formation was measured.

Results and Discussion

Node and apical shoot tip explants of *Vitex negundo* **(**L.) grown on growth hormones free medium (MS) found no effect on callus and multiplication of plant. Tissue culture medium (MS) with different concentration of KIN (1.0, 1.2, 1.4, 1.6, 1.8 and 2.0) and IAA (0.2) mg/L but both PGR^s combination shows better multiplication percentage.

The present research, explants of *Vitex negundo* **(**L.) (node and apical shoot tip) was essential for the regeneration of plant. Out of six different concentrations combination of KIN 2.0 and IAA 0.2 shows very profuse callus formation with yellowish green colored which was best for better propagation 1.8 mg/L KIN and 0.2 mg/L IAA gives profuse callus formation with yellowish green color in nature. Growth hormones concentration of KIN and IAA also shows effective on apical shoot tip 1.8 mg/L KIN and 0.3 mg/L IAA shows very profuse callus formation and colored appear to callus was yellowish dark green.

Node and apical shoot tip were inoculated on (MS) medium supplement 3 % sucrose, 2.5 % Agar-Agar and different PGR^s combination as shown in table 1. Present investigation observation proves that highest number of shoot percentage was recorded in their sub-culturing. Combination of IAA was recorded achievement of rooting to experimental plant *in vitro* rhizogenesis (0.2 mg/L for node and 0.3 mg/L for apical shoot tip). Thick long root with root hairs were shown by combination of KIN 1.8 mg/L and IAA 0.2 mg/L on nodal explants. As well as KIN 2.0 mg/L and IAA 0.3 mg/L shows thick long root with root hairs on apical shoot tip of *Vitex negundo* **(**L.) (Table 2). *In vitro* regeneration plants were hardened in poly-house soil and lastly in soil *in vivo* trails which was shown 66 % viability in natural condition.

Table 1. Effect of PGR^S on node and apical shoot tip of *Vitex negundo* (L.) and callus color with callus formation frequency.

Explant	Conc. of growth regulator (mg/L)		Frequency of Callus formation	Color of callus	Shoot length (Mean± SE)
	KIN	IAA			
Node	1.0	0.2	+	Whitish	1.70± 0.130
	1.2	0.2	+++	Yellowish	18± 1.632
	1.4	0.2	+++	Whitish	2.14± 0.140
	1.6	0.2	+++	Yellowish	9 ± 0.881
	1.8	0.2	++++	Yellowish green	1.70± 0.130
	2.0	0.2	+++++	Yellowish green	5 ± 0.577
Apical shoot tip	1.0	0.3	+	Whitish	2.00 ± 0.18
	1.2	0.3	+++	Whitish	2.00 ± 0.81
	1.4	0.3	+++	Creamish	8.37 ± 2.06
	1.6	0.3	+++	Yellowish light green	2.70 ± 1.33
	1.8	0.3	+++++	Yellowish dark green	7.20 ± 2.14
	2.0	0.3	++++	Yellowish green	2.52± 0.122

+ indicates the frequency of callus induction in
+ : very weak ; +++: Moderate; ++++: Profuse; +++++: Very profuse
After 25 days, mean ± SE of 5 replicates

Table 2. Effect of PGR's on root morphology of *Vitex negundo* (L.).

Conc. of PGR's (mg/l)		Mean no of root	Root morphology
KIN	IAA		
1.0	0.2	5.30±1.10	Thin shot
1.2	0.2	3.90±1.00	Fragile
1.4	0.2	7.31±0.65	Long Fragile
1.6	0.2	3.05±0.52	Sturdy and callus base
1.8	0.2	7.35±1.08	Thick long and root hairs
2.0	0.2	2.72±0.68	Long thin and Fragile
1.0	0.3	7.40±1.16	Thick shot
1.2	0.3	10.52±1.55	Short Fragile
1.4	0.3	4.83±0.79	Long Fragile and callus base
1.6	0.3	9.21±0.88	Thick short and callus base
1.8	0.3	4.28±0.96	Thin long and root hairs
2.0	0.3	3.40±2.85	Thick long and root hairs

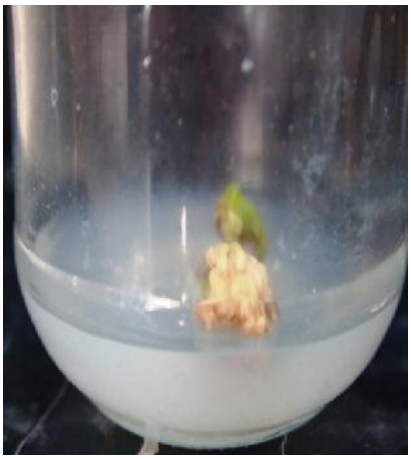

Photo. 1. Callus formation

Photo .2 Multiple shoots proliferation

Effective method has been developed for rapid callus induction *in vitro* culture of *Vitex negundo* (L.). Result represents of complete callus, multiplication, shoot and root regeneration of *Vitex negundo* (L.). Callus production was successfully by node and apical shoot tip of experimental plant. Mature and full developed callus is very good source for induction shoot and root multiplication. The key of this experiment was *in vitro*, callus and multiplication with the help of PGR^s (Plant Growth Regulators). 2.0 KIN and 0.2 IAA gives very healthy callus (yellowish green) for better multiplication (Callogenesis and Rhizogenesis) in nodal PGR^s induction. Apical shoot tip shoes yellowish dark green in PGR^s concentrations such as 1.8 KIN and 0.3 IAA. Especially 76 shoot were shown by the 2.0 KIN and 0.3 IAA which was very profuse. Thick short and callus base root shoes by 1.6 KIN and 1.8 KIN with root hairs (Table 3; Fig.1). The findings of the investigation could be helpful in understanding the details capacity of PGR^s and its ability to callus production and multiplication of *Vitex negundo* (L.). Node and apical shoot tip would be rapid callus production and multiplication of this valuable medicinal plant *Vitex negundo* (L.).

Table 3. Effect of PGR's on shoot induction of *Vitex negundo* (L.).

Concentrations of PGR's (mg/L)		No of shoot induced
KIN	IAA	
1.0	0.2	22
1.2	0.2	28
1.4	0.2	30
1.6	0.2	29
1.8	0.2	45
2.0	0.2	65
1.0	0.3	18
1.2	0.3	31
1.4	0.3	35
1.6	0.3	42
1.8	0.3	72
2.0	0.3	76

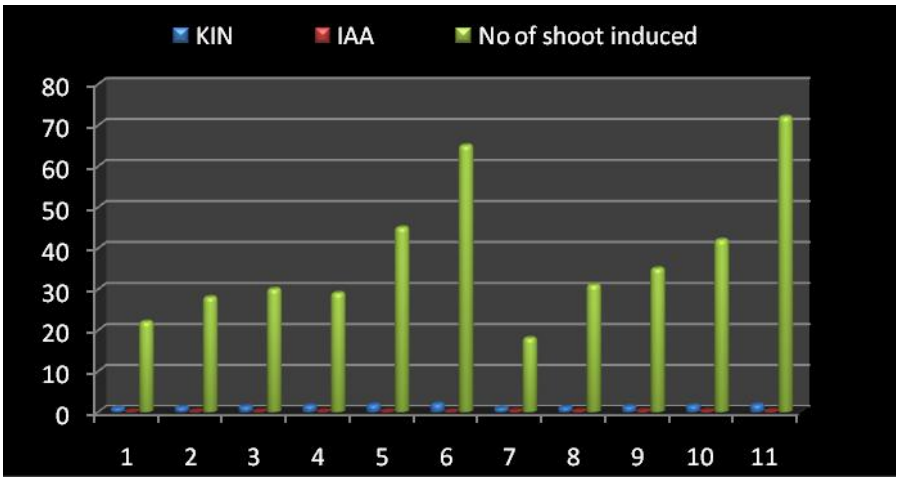

Fig 1. Graphical representation of shoot induction

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