Madras Agric. J., 93 (7-12) : 217-221 July-December 2006

https://doi.org/10.29321/MAJ.10.100754

Effect of phytohormones on the *In vitro* callus formation in *Vitex negundo* L.

A.NIRMALAKUMARI

Dept. of Millets, Centre for Plant Breeding and Genetics, Tamil Nadu Agrl. Univ., Coimbatore-3.

Abstract: Effect of basal media and their strength, growth hormone composition and concentration and different explants on callus induction, proliferation and induction of embryogenesis were examined in *Vitex negundo* L. Sixteen media composition analysis of callus induction frequency and thirty two combinations for regeneration response were examined. Four explants *viz.*, leaf, stem, shoot-tip and bark were tried. Basal medium MS was found best for callus induction and regeneration in its full strength. For callus induction MS +2,4-D, (2-2.5 mg 1^{-1}) + Kn (0.5-0.75 mg 1^{-1}) and for embryoid formation MS + BAP (1.5 mg 1^{-1}) + IAA (0.5 -0.75 mg 1^{-1}) were found efficient. Among explants, leaf explant produced highest callus induction and proliferation response followed by stem and shoot-tip while bark explants responded poorly.

Keywords : Nochi, callus induction, proliferation, embryogenic calli, growth regulators.

Introduction

Nochi *(Vitex negundo* L.) is a common hedge plant seen in farms and road sides and belongs to the family Verbanaceae. It is otherwise known as "Indian privet". Nochi grows as a shrub or small slender tree in moist areas especially on the banks of rivers through out India up to an altitude of 1500 meters.

Besides neem, the novel plant products like leaves of nochi were found to be the effective cereal grain protectants in rural storage (Anand Prakash *et al.,* 1992). Antifeedants are desirable chemicals as they offer protection without disturbing any ecosystem and hence have gained momentum in modern pest control. Antifeedants are considerably safe as they minimize pesticide residue in food and ensure safety to people and wild life. High insecticidal and repellent activities of *V. negundo* against mosquitoes and house flies have been reported (Chopra *et al.,* 1958).

Instead of taking whole plants and spending a lot of time and trouble to purify the required product, bio-technologists can take isolated cells from the plant or plant organs *viz.,* leaf, stem and shoot. Grown in tissue culture media, under right conditions, the cells will make the product in large quantities. All parts of the plant including leaves, bark, fruit, seed, stem and root are used in medicine and also in agriculture. The production of valuable products by plant cells grown in tissue culture avoiding seasonal and climatic fluctuations on available yield is now economically feasible, at least in few cases (Curtin, 1983; Stafford *et al.,* 1986).

One main objective of tissue culture studies is the development of protocols to induce callus, cell suspension cultures and to regenerate whole plants from them. The *in vitro* manipulation of many plants has been well documented. However, these is no report available on the callus induction and subsequent plant regeneration

Table 1. Effect of basal media and growth regulators on callus induction for different explants of *Vitex negundo.*

Hormonal combinations (mg l^{-1})	Callus induction (%)							
	MS				B5			
	Leaf	Stem	Shoot tip	Bark	Leaf	Stem	Shoot tip	Bark
1.0 2,4-D + 0.5 Kn	16.5	5.9	21.7	—	10.3	3.4	15.1	-
1.0 2,4-D +0.75 Kn	11.4	6.4	20.6	—	9.1	4.9	17.5	-
1.5 2,4-D + 0.5 Kn	38.1	23.8	31.0	—	27.4	18.6	22.6	-
1.5 2,4-D +0.75 Kn	44.2	29.7	30.3	—	25.0	16.4	20.9	-
2.0 2,4-D + 0.5 Kn	93.7	68.5	52.8	3.3	82.2	32.7	41.0	1.5
2.0 2,4-D + 0.75 Kn	84.6	71.0	57.9	2.1	77.5	35.0	48.4	1.3
2.5 2,4-D + 0.5 Kn	75.0	87.7	12.1	5.3	59.8	62.2	8.3	2.7
2.5 2,4-D + 0.75 Kn	60.9	83.2	11.3	6.4	47.6	58.1	7.5	3.3

of *V. negundo.* In the present work, for the first time a simple and reproducible method for the induction of callus in *Vitex negundo* has been reported. This will facilitate the establishment of specific high yielding cell lines of this plant and its micro propagation where "Vitexol" is the main secondary metabolite with insecticidal property from nochi.

Materials and Methods

Four different explants *viz.,* shoot tip, leaf, stem and bark were collected from twelve years old nochi tree (Botanic Garden, TNAU, Coimbatore). The explants were washed with Tween - 20 and rinsed thoroughly in distilled water. They were surface sterilized with 70% ethanol for 30 seconds and 0.1% (w/v) mercuric chloride for 7 min. They were rinsed 5-6 times in sterile distilled water. The shoot tips were taken with terminal bud and 2 unfolded leaves. The leaf segment was 1-1.5 sq. cm with mid-rib, veins and margin. The internodal stem bits of 1 cm were taken as stem explants and peeled afresh 1 cm length barks were taken for culturing on various media. They were kept in dark for callus induction. The primary calli were separated from initial explants after 25 days and sub- cultured every 22 days. The pH of all media was adjusted to 5.7 and solidified with 0.8% (w/v) agar. Cultures after sub-culturing were maintained at 25 + 2°C under a 16h photoperiod from cool white fluorescent tube lights (2000 lux).

The two different basal media used for callus initiation were Murashige and Skoog (MS) and Gamborg (B5) containing 3% sucrose and different combinations of growth regulators.

The auxins, 2, 4-D, (1, 1.5, 2.0, 2.5 mg l^{-1}), IAA (0.5, 0.75 mg l^{-1}), NAA (0.5, 0.75 mg l^{-1}) and cytokinins, BAP (1,1.5. 2, 2.5 mg l^{-1}) and Kn (0.5, 0.75 mg l^{-1}) were

used. For callus induction and proliferation 2,4-D and Kn and in the regeneration medium BAP and IAA or NAA combinations were used. The different basal media and growth regulator formulations used were arrived at after carrying out extensive preliminary experiments.

Results and Discussion

The MS basal medium supplemented with various growth hormones was found suitable for callus induction. The callus induction frequency was high in all the cases in MS basal than in B5 basal medium (Table 1). The two media differ significantly with respect to nitrogen and phosphorus. The level of phosphate and nitrate ions are high in MS than B5. So, higher amount of those ions are needed for increased callus induction.

Explants significantly accounted for the variation in the callus induction frequency. The leaf explant produced the highest callus induction frequency (93.7%) followed by stem explants (87.7%). The bark explant produced the least callus induction frequency (2.1%).

Plant growth regulator combinations significantly influenced the callus induction frequency and the proportion of embryogenic calli (Table 1).

After one week, the first visible callus appeared on the mid-rib, veins and margins of leaf explant. After nine days, compact white granular calli appeared on the cut ends of the stem explants. From the shoot-tip, butter white calli appeared on the entire surface after 12 days of inoculation. The bark explant produced dull white calli on the upper surface near the nodal bulging after 15-17 days.

The media combination for leaf callus induction was MS + 2, 4-D (2.0 mg l^{-1}) + Kn (0.5 mg l^{-1}). It was different for stem explants (MS + 2, 4-D (2.5 mg l^{-1}) + Kn (0.5 mg l^{-1}). The shoot-tip produced more calli with 2, 4-D (2.0 mg l^{-1}) and Kn (0.75 mg l^{-1}). The bark showed relatively good callus induction frequency in MS medium with highest 2,4-D (2.5 mg l^{-1}) and Kn (0.75 mg l^{-1}) concentrations in this study.

Primary calli from the explants were separated and sub-cultured on fresh medium after 25 days. Basal medium MS was used with its full as well as half strength. Embryogenic calli was observed in more cases with full strength (Table 2). The embryogenic calli were milk yellow, more compact with globular embryoid like structures (Orton, 1979; Nabors *et al.,* 1983; Binh *et al.,* 1989). These embryogenic calli on regenerations medium after 13-15 days gave rise to green, compact and granular embryoids. Out of 32 regeneration media combinations used, MS + BAP (1.5 mg l^{1}) + IAA (0.5 or 0.75 mg l^{-1}) showed higher embryogenic calli formation in leaf followed by stem calli.

The callus obtained with 9 combinations of BAP and NAA was greenish in appearance along with browning and heterogenous, containing both hard and soft areas even through successive sub-cultures of the more friable areas. The callus obtained in BAP and IAA was light yellow to dull white in colour and friable. Similar results were observed by Thulaseedharan and Vaidyanathan (1990) in *Vicoa indica* calli.

Generally most of the plant species required an auxin for dedifferentiation and profuse callus induction. The differentiation and embryoid formation were noticed with high BAP and low NAA or IAA. Kinetin with 0.2 mg l^{-1} of 2, 4-D produced embryogenic calli on explants maintained in dark for 7 to 8 week (Hashim, 1990).

Table 2. Effect of the strength of MS medium and growth regulators on embryoid production in *Vitex negundo*

Hormonal combinations (mg l^{-1})	Strength of basal medium							
	½ MS				MS			
	Leaf	Stem	Shoot tip	Bark	Leaf	Stem	Shoot tip	Bark
1.0 BAP + 0.5 IAA	++	+	+	0	++	+	+	0
1.0 BAP + 0.75 IAA	++	+	+	0	++	+	+	0
1.0 BAP + 0.5 NAA	+	0	0	0	+	0	0	0
1.0 BAP + 0.75 NAA	+	0	0	0	+	0	0	0
1.5 BAP+ 0.5 IAA	++++	+++	++	+	+++	++	++	+
1.5 BAP+ 0.75 IAA	++++	+++	++	+	+++	++	++	+
1.5 BAP+ 0.5 NAA	++	+	+	0	++	++	+	0
1.5 BAP+ 0.75 NAA	++	+	+	0	++	++	+	0
2.0 BAP + 0.5 IAA	+++	++	++	+	++	+	+	+
2.0 BAP + 0.75 IAA	+++	++	++	+	++	+	+	+
2.0 BAP+ 0.5 NAA	++	++	++	+	++	+	+	0
2.0 BAP+ 0.75 NAA	++	++	++	+	++	+	+	0
2.5 BAP + 0.5 IAA	++	+	+	0	+	+	+	+
2.5 BAP+ 0.75 IAA	++	+	+	0	+	+	+	+
2.5 BAP+ 0.5 NAA	+	+	0	0	+	0	0	0
2.5 BAP + 0.75 NAA	+	+	0	0	+	0	0	0

(+) Relative embryogenic calli formed rated on a scale of + to ++++

(0) sub-cultured calli remained as such for a few days and slowly become necrotic

In the present study, somatic embryogenesis *via* callus formation following indirect embryogenesis was noticed as described by Williams and Maheswaran (1986). From this study, it was found possible to induce and proliferate large amount of calli from leaf and stem explants using high concentrations of 2, 4 -D (2-2.5 mg l^{-1}) and low Kn (0.5-0.75 mg l^{-1}). They gave rise to embryogenic calli on MS + BAP (1.5 mg l^{-1}) + IAA (0.5 mg l^{-1}) which can be further utilized in the following ways.

i) Plantlet regeneration

ii) Cell suspension cultures for production of vitexol or other secondary metabolites

iii) Production of synthetic seeds through immobilization or

iv) Directly utilized for product extraction in one stage culture.

Universal response from all explants for callus induction and proliferation is critically important, especially, in a plant like *Vitex negundo,* where all parts of the plant are economically important for producing secondary metabolites.

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