STUDY OF IN-VITRO INDUCTION OF VITEX NEGUNDO L.

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Abstract: Nirgundi is an important medicinal plant widely used as an active ingredient in many medicines. It is an attempt to conserve the plant through micropropogation of nodal segment. Experiment was conducted on shoot initiation with combination of growth hormone finally tried on multiplication.

Introduction: *Vitex negundo* L. commonly known as Nirgundi, Tarvan, Sephali and Sambhalu, is an important woody, agro-forestry tree (200 to 300 cm high) in east Asia, south west China and tropical Africa (Kapur et al. 1994). *V. negundo* (L.) perennial shrub belongs to the family verbenaceae. It is aromatic, woody, small shrub, tri or penta-foliate leaves and purple colored flower in branched tomentose cymes. Its habitat in humid, water courses, waste land and open mixed forest occur in Afghanistan and Asian countries. Near about 14 species were occurred in India out of 250 species of the genus *V. Negundo* **(L.).** The leaves of *Vitex negundo* grow opposite on the stem. The leaf is compound, digitate with 3 or 5 leaflets and a 5-6 cm long petiole. The 2 lower leaflets are sessile and the others have a 1.5-2 cm long petiolule. The leaflet of *Vitex negundo* is 5-15 cm long and 2 – 3 cm wide. The shape of the blade is lanceolate, the apex is acuminate, the base is rounded and the margins are entire. The venation of the leaflets is reticulate with a prominent midrib. The flowers of *Vitex negundo* are grouped in terminal panicles. The calyx-tube is campanulate, 5-lobed and 3mm long and 2mm wide. The calyx is pale green and tomentose. The corolla is pale – purple coloured and 5 mm long. The fruit of *Vitex negundo* is a globose drupe. It is dark – purple blackish – coloured when ripe. The bark of *V.negundo* is grey coloured and rough.

It is easily available in every part of India (Andhra Pradesh, Arunachal Pradesh, Assam, Bihar, Delhi, Goa, Gujarat, Haryana, Himachal Pradesh, Jammu-Kashmir, Jharkhand, Karnataka Kerala, Madhya Pradesh Manipur, Meghalaya, Uttar Pradesh, Tamil Nadu, West Bengal and Andaman and Nikobar Islands) as well as Afghanistan, Bangladesh, Bhutan, Cambodia, China, Indonesia, Japan, Kenya, Korea, Madagascar, Malaysia, Mozambique, Myanmar, Nepal, Pakistan, Philippines, Sri Lanka, Taiwan, Tanzania, Thailand and Vietnam.(Usha et al.,2007). It contains many polyphenolic compounds, terpenoids, glycosidic iridoids and alkaloids. Whole plant is used in asthma, bronchitis, inflammations, eye diseases, leucoderma, spleen enlargement, painful teething and promotes the growth of hair. Fruits and seeds are used in cutaneous diseases and leprosy. Flowers are prescribed in cholera, diarrhoea, fever and liver complaints. The leaves are useful in rheumatism, arthritis, catarrhal fever, cephalagia, sprains, orchitis, syphilis, inflammations and ulcers (Deogade et al., 2016). It possess many therapeutic actions viz, anti inflammatory, antibacterial, moderate CNS depressant, antifertility, antispasmodic, analgesic, hepato-protective, estrogenic, anticonvulsant,diuretic, antimicrobial, antipsychotic, antidepressant, antihistamine releasing activity, mosquito repellent activity, anti-feedant, anti-flarial, and anti-androgenic. The objective of present work is *In vitro* Induction of *Vitex negundo*.

Materials and Methods:

Plant Sample Collection: Nodal explants were collected from mature plants, near Parasiya. Chhindwara (India). Nodel segment cut 1.5- 2.5 cm. Explants were washed under running tap water with Tween-20 (two dropes per 100ml water) and sterilized with 0.1% (w/v) mercuric chloride for 3 to 5 minute and then given a dip in 70% ethanol. Nodal explants were rinsed with sterile distilled water four to five times to remove traces of mercuric chloride (**Groach *et al.*, 2014**) **(Fig .1).**

Inoculation of Explants: Nodal explants was inoculated on autoclaved MS medium having sucrose (3%), agar (0.6%) and supplemented with BAP and NAA. The pH of the medium was adjusted to 6.5 ± 2 before autoclaving. Media sterilized by autoclaving at 15 p.s.i autoclaved at 121°C at 15 lb pressure for 15 minutes. The cultures were maintained at 25± 2°C under the light intensity of 2500 lux provided by cool white fluorescent lamps. Nodal explants were placed in pre sterilized clear culture tube with the help of sterile forceps and scalper.

Shoot Multiplication: For shoot multiplication, the MS media was supplemented with the combination of BAP + NAA (0.5 mg/l) + (0.5, 1.0, 1.5, 2.0, 2.5 mg/l) was used. Sub-culturing was done at every 15 days interval with the same combination and recorded the result as bud no., leaf no. shoot length and No. of shoot.

Result and Discussion: In vitro propagation technique is a powerful tool for plant germplasm conservation hence tissue culture is the only rapid process for the mass propagation of plants. The ability to generate plants directly for explants is fundamental to clonal multiplication of elite germplasm via micropropagtion.

Successful surface sterilization is one of the most critical steps of in vitro culture. Nodal explant were used as the explant for initiation of in vitro culture. To overcome contamination problem, surface sterilization of explants was done with 0.1% aqueous solution of Mercuric chloride ($HgCl_2$). The results showed that BAP alone at higher concentration or in combination with NAA was more effective for shoot formation as compared to other lower concentrations (Table 1). The highest degree of shoot initiation was observed on MS medium supplemented with 1.5 mg/l BAP + 0.5 mg/l NAA (Fig.1).

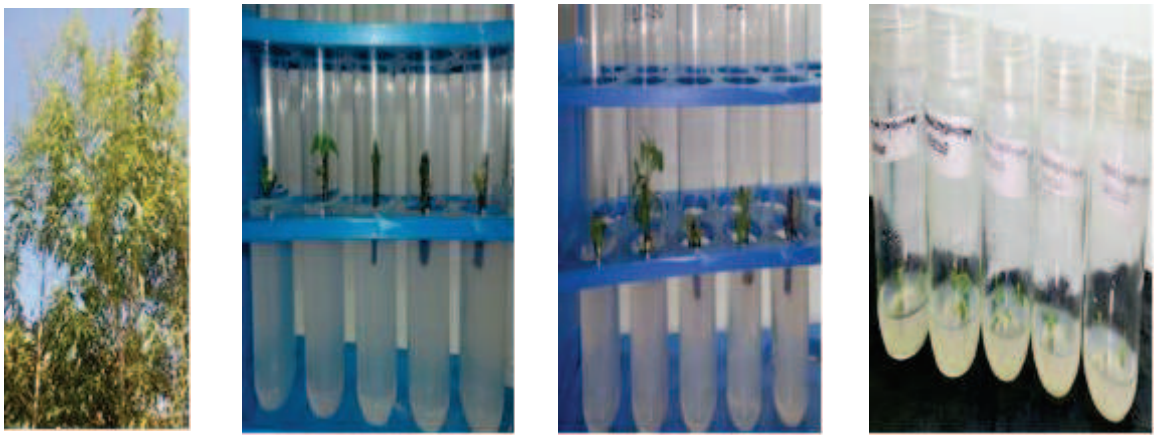

Fig. 1: Micropropogation of *Vitex Negundo* by Using Nodal Segment

A) Effect of plant growth regulator on MS media for shoot initiation in *Vitex negundo*. T1=MS+BAP+NAA(0.5+0.5mg/l),T2=MS+BAP+NAA(1.0+0.5mg/l),T3=MS+BAP+NAA(1.5+0.5mg/l),T4=MS +BAP+NAA(2.0+0.5 mg/l) and T5= MS+BAP+NAA(2.5+0.5 mg/l).
B) Effect of plant growth regulator on MS media for shoot multiplication in Vitex *negundo*. T1=MS+BAP+NAA(0.5+0.5mg/l),T2=MS+BAP+NAA(1.0+0.5mg/l),T3=MS+BAP+NAA(1.5+0.5mg/l),T4=MS +BAP+NAA(2.0+0.5 mg/l) and T5= MS+BAP+NAA(2.5+0.5 mg/l)

Further, decrease of this concentration not only decreased the percent bud break, but also decreased the number of shoots produced. Lal et al. (2010), Verma et al. (2011) also noted the synergistic effect of BAP in combination with an auxin for efficient shoot regeneration.

NAA regulates not only vegetative growth but also organ growth, whereas BAP facilitates cell division and sprouting (Pan 2001). Surface sterilized explants were cultured on MS medium supplemented with BAP + NAA (0.5 mg/l) + (0.5, 1.0, 1.5, 2.0, 2.5 mg/l).

Table 2: Effect of plant growth regulator (BAP-0.5-2.5 mg/l + NAA- 0.5 mg/l) on MS media for Nodel explant initiation in *Vitex negundo*

Sl. No.	Treatment	Bud No.	Leaf No.	Shoot No.	Shoot Length
1	T1	1.66	1.66	0.4	0.37
2	T2	1.2	1.73	0.66	0.68
3	T3	1.6	2.2	0.73	0.54
4	T4	1.26	1.46	0.6	0.3
5	T5	1.06	1.6	0.4	0.33

Among the different replicates on which BAP and NAA tested, the best response (shoot length 0.68 cm) was obtained after 22 days of incubation (**Table 1**). **Figure 1** represents the successful results for shoot proliferation from nodal explants.

Summary: *In vitro* propagation technique is a powerful tool for plant germplasm conservation hence tissue culture is the only rapid process for the mass propagation of plants. The ability to generate plants directly for explants is fundamental to clonal multiplication of elite germplasm via micropropagtion. The highest degree of shoot initiation was observed on MS medium supplemented with 1.5 mg/l BAP + 0.5 mg/l NAA.

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