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Full Length Research Paper

In-vitro Shoot Proliferation from Nodal Explant of *Vitex negundo*

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Abstract

Micro propagation of V.negundo from nodal explant with stem was done on three different MS media .It was found that media containing BAP (1mg/l) + GA3 (0.25mg/l) has maximum proliferation response in comparison to media containing only BAP (1mg/l) or NAA(0.1mg/l)+ BAP(0.4mg/l).

Key words: *Vitex negundo, Micro propagation, nodal explants, effect of growth regulators.*

Introduction

Vitex negundo is a large, woody, aromatic and medicinal shrub or sometimes a small tree distributed in several parts of India (Anonymous, 1976). It is a flowering plant of family *Lamiaceae,* represented by 236 genera and 7000 species .*Lamiaceae* ,or mint family is the 7th largest flowering plant family and includes many well known plants ,herbs, shrubs and trees of horticultural, economic and medicinal importance .Members of this family range from small plants in garden to herbs like basils *Ocimum* ,to well known shrubs *Lavender and Vitex* ,to the huge rain forest tree, *Tectona Grindis* (Davies,2007).

It is native to Europe, Asia, and possibly portions of Northern Africa (Bailey and Bailey 1976, Kriissmann, 1977).It is distributed throughout Bangladesh , India, Ceylon, Afghanistan, Philippine Islands and Tropical Africa, Madagascar and China (Bansod and Harley, 2009).

A perfect example of medicinal plant credited with innumerable medicinal qualities validated by modern science and used since ancient times is *Vitex negundo*. All parts of the plant are useful in medicines but leaves and roots are important as drugs. Leaves are antiparisitic and used as alternative vermifuge and anodyne .They are also very effective to reduce inflammatory swellings of joints in rheumatic attacks, relieve catarrh and headache. The root is used as tonic, febrifuge, expectorant and diuretic. It regulates hormones, increases breast-milk production and possesses progesterogenic properties (Chevallier, 1996). Flowers are cool, astringent, carminative, hepatoprotective, digestive, febrifuge, vermifuge and are useful in hemorrhages and cardiac disorders. Fruit is nervine, cephalic, aphrodisiac, emmenagogue and vermifuge (Hussain et al., 1992). The leaves *V. negundo* are antibacterial, antitumor, astringent, febrifuge, sedative, tonic and vermifuge (Chopra *et al.,* 1986), and anti HIV properties (Chandramu et al., 2003).

Medicinal plants are of great interest in the field of biotechnology as most of the drug industries depend, in part, on plants for the production of pharmaceutical compounds (Chand et al., 1997).

Despite its economic importance, the production of *Vitex negundo* L. is threatened by population growth, desertification, industrial development and attack by numerous parasites. Micropropagation has many advantages over conventional propagation of plants (Stushnoff & Fear, 1985) and isimportant for the regeneration following transformation (Ainsley *et al*., 2000) and cryopreservation (Channuntapipat *et al*., 2000). *In vitro* techniques are effectively utilized for germplasm conservation of rare, endangered, aromatic and medicinal plants (Arora and Bhojwani 1989, Sudha and Seeni, 1984).

The present study was undertaken to optimize a protocol for high frequency induction of multiple shoots from the nodal explants and regenerate plants of *V. negundo* to meet its demand in medicine and agriculture.

Materials & Method

Explant collection

Explanting material of *V. negundo* was collected from Nainital district of Uttarakhand state during the months of February- march. Healthy, soft nodal segments were collected and brought to laboratory.

Culture media and establishment

Explants were firstly inoculated for 8 days on MS **(Murashige & Skoog., 1962)** media with no growth hormones for establishment. After establishment the explants were inoculated to three different MS media containing different growth hormones and in different concentration shown in table 2.

Table 1. Combination of growth regulators tested for shoot proliferation from nodal explant

S. No	BAP (mg/l)	NAA(mg/l)	GA3 (mg/l)
PM1	1	-	-
PM2	0.4	0.1	-
PM3	1	-	0.25

In vitro shooting was observed and no of shoots proliferated were counted in different time intervals for different media and analysis was made to find best media for shoot proliferation.

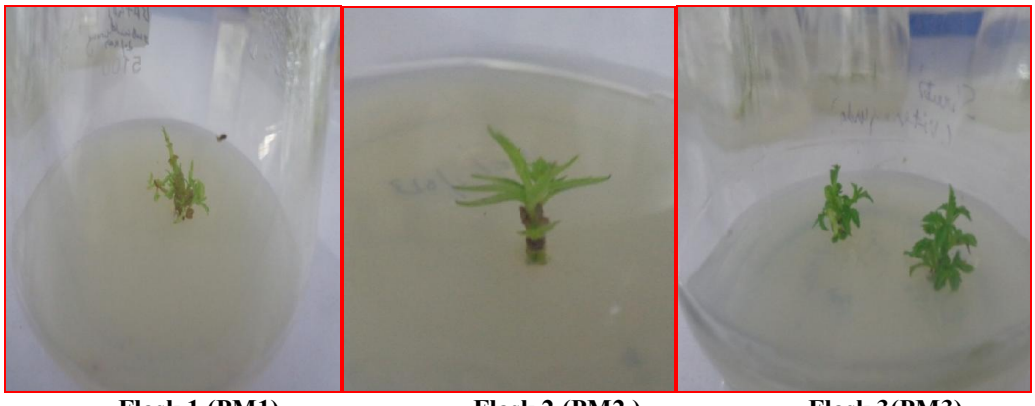
Results & discussion

Proliferation

In case of PM1 media proliferation response is good and percentage survival is 100%. In case of PM2 proliferation response is good and survival percentage is 88.8%, while in case of PM3 proliferation response as well as survival is maximum. Thus it shows that survival as well as proliferation is increased by addition of GA3 in media, while media supplemented with NAA played no significant role in proliferation.

Table 2. Explants were inoculated to three different MS media containing different growth hormones and in different concentration

Media	No of Explant	No. Of Survived	Proliferation Response	%of survival
PM1 M.S+BAP(1mg/l)	10	10	++	100%
PM2 M.S+BAP (.4mg/l+NAA(.1mg/l)	9	8	++	88.8%
PM3 M.S+BAP(1mg/l)+ GA3(.25mg/l)	9	9	+++	100%



Flask 1 (PM1) **Flask 2 (PM2)** **Flask 3(PM3)**

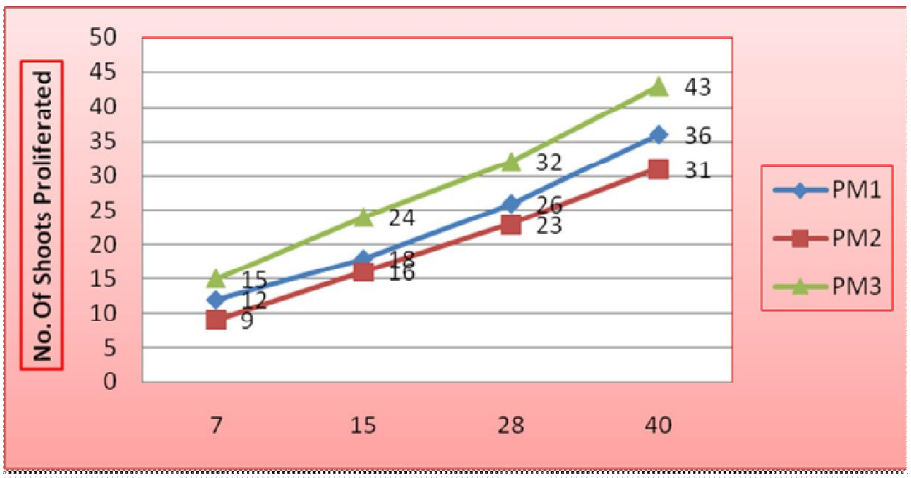


Fig. 1. Graph Showing Shoot Proliferation in Different Compositions of Media

While study of *in vitro* propagation of *V.negundo* using nodal explant it was found that MS media supplemented with BAP & GA3 gave maximum proliferation response in comparison to media supplemented with BAP & NAA+BAP. **Sahoo and Chand (1998)** in their research paper "Micropropagation of *Vitex negundo* L, a woody aromatic medicinal shrub through high frequency axiallary shoot proliferation" reported the same results. They reported that enhanced frequency of shoot proliferation and internode elongation is dependent on GA3 when used at optimal concentration along with BAP.

Conclusion

While studying *in vitro* propagation of *V .negundo* using nodal explant it was found that MS media supplemented with BAP(1mg/l) & GA3(0.25mg/l) enhances shoot proliferation and elongation in comparison to media supplemented with BAP(1mg/l) or NAA(0.1mg/l) +BAP(0.4mg/l).

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