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# Micropropagation of *Vitex negundo* L. - A Significant Medicinal Plant

***Shamima Nasrin[1], Md. Shamim Hossain[2], Md. Ashrafuzzaman Sapon[2-3], and Monokesh Kumer Sen[2]***

[1]Scientific Officer, Plant Biotechnology Division, National Institute of Biotechnology, Ganakbari, Savar, Dhaka, Bangladesh

[2]Department of Biotechnology and Genetic Engineering,
Faculty of Applied Science and Technology, Islamic University, Kushtia-7003, Bangladesh

[3]Bioscience Division, Graduate school of Science & Technology, Shizuoka University, Shizuoka, Japan



**ABSTRACT:**

*Introduction:* *Vitex negundo* L. (Nisinda locally), belongs to the family Verbenaceae, found almost everywhere in Bangladesh is a medicinal aromatic shrub. *Materials and Methods:* An attempt was taken to its micropropagation from field-grown explants (shoot-tip) in Murashige and Skoog medium fortified with various concentrations of phytohormones. *Results:* Experimentally, the best shoot induction was observed in full strength MS medium supplemented with BAP 1.0mg/L and Kin 0.5mg/L. However, 0.5mg/L IBA in half strength MS medium was enabled to induce 80% root initiation with the highest root number and longest shoot length. Well-developed roots were successfully subjected to hardening process and acclimatized. *Conclusion:* Regenerated plantlets were same as the natural plants and showed 80.56% survival frequency with frisky and seductive appearances without any abnormalities.

**KEYWORDS:** *Vitex negundo*, micropropagation, Murashige and Skoog medium, acclimatized.

## 1 INTRODUCTION

*Vitex negundo* Linn. (Verbenaceae) is a woody, aromatic shrub found in different parts of the world including Bangladesh. It is used as a valuable medicinal plant in traditional and modern system of medicines. Leaf is used as aromatic, tonic and vermifuge [1]. Root is used in dyspepsia, colic, rheumatism, worms, boils and leprosy [1]. Fruit is nervine, cephalic and emmenagogue; dried fruit acts as a vermifuge as well as flowers are cool and astringent [1]. It has also been reported as an antitumoric [2] antimicrobial [3] antiinflamatory agent [4]. Conventional propagation method is not satisfactory to meet huge demand and may spread diseases. Therefore, biotechnological approach plays a vital role in search for alternative to production of desirable medicinal compounds from plants [5]. Therefore, keeping in the view of importance of *V. negundo*, the following studies were conducted to establish the optimum condition for *in vitro* propagation *V. negundo* plantlets using shoot-tip explants and acclimatized.

## 2 MATERIALS AND METHODS

The experiment was conducted at Plant Biotechnology Division of National Institute of Biotechnology (NIB), Bangladesh. Field grown explants were sterilized with 0.1% mercuric chloride (Merck, Germany) for 5 min and subsequently disinfected with 70% ethanol (Merck, Germany) for 30 sec. Murashige and Skoog (Duchefa, The Netherlands) media (1962) [6] supplemented with 3% (w/v) sucrose (Merck, Germany) and different concentrations of BAP, Kin, NAA, $GA_3$, (Duchefa, The Netherlands) coconut water (CW) (Locally Collected) were used for shoot and root initiation and multiplication. pH (Jenway 3520 pH Meter, Bibby Scientific Ltd., UK) was adjusted to 5.8. Autoclaved medium (ALP Co. Ltd., CL-40M, Japan) was used to inoculate sterilized explants and temperature of the growth chamber was maintained at $25\pm2^0$ C with a photoperiod of 16 hours lightand 8 hours dark (50 W, Philips Agro-Lite). 1:1 mixture of garden soil and farmyard manure (Locally Prepared) was

**Corresponding Author:**Monokesh Kumer Sen 

used for hardening and acclimatization **(Figure 1)**. The percentage of initiation, days for initiation, length and number of shoots and roots and regeneration percentage during acclimatization of plants were recorded **(Table 1 and 2)**.

### 2.1 STATISTICAL ANALYSIS

Results were expressed as mean ± SD (Standard deviation of mean) **(Table 1 and 2)**. The statistical program used was Microsoft Office Excel 2007. Photos were taken with (SANYO, Japan) camera.

## 3 RESULTS AND DISCUSSION

### 3.1 INDUCTION AND PROLIFERATION OF SHOOTS FROM SHOOT-TIP EXPLANTS

Concerted effects of BAP 1.0mg/L and Kin 0.5mg/L showed the maximum 95% induction, also the number of shoots per explants (19.33±1.25) and shoots length (6.6±0.22 cm) were maximum **(Table 1 and Figure 1)**. Synergistic effects of BAP and Kin also demonstrated in other plants by Biradar et al. (2012) [7] and Sen et al. (2013) [8]. BAP in combination with $GA_3$ gave 87% initiation and shoots number were 13.33±1.25 per explants. Concerted effects of BAP and GA3 for shoot proliferation was also reported by Haque et al. (2009) [9]. Coconut water was used as phytohormone and it showed 80% shoot inducing frequency **(Table 1)**. Addition of coconut water to the media stimulated more multiple axillary shoots growth also demonstrated by Kwapata et al. (1999) [10].

***Table 1. Effect of shoot induction media on shoot-tip of V. negundo***

| Treatments | Composition | Shoot inducing frequency (%) | Number of shoot per explant | Shoot length (cm) |
|---|---|---|---|---|
| $S_1$ | MS+BAP (0.5mg/L+5% coconut water | 70 | 2.33±0.47 | 2.4±0.08 |
| $S_2$ | MS+BAP (1.0mg/L)+5% coconut water | 80 | 6.0±0.0 | 3.47±0.11 |
| $S_3$ | MS+BAP (2.0mg/L) | 65 | 4.33±0.47 | 2.13±0.17 |
| $S_4$ | MS+BAP (1.0mg/L)+ NAA (0.5mg/L) | 85 | 6.67±0.47 | 4.07±0.12 |
| $S_5$ | MS+BAP (0.5 mg/L)+ NAA (1.0 mg/L) | 87 | 7.33±0.47 | 5.25±0.02 |
| $S_6$ | MS+BAP (0.5mg/L) + Kin (0.2mg/L) | 90 | 11.67±1.25 | 5.74±0.13 |
| $S_7$ | MS+BAP (1.0mg/L)+ Kin (0.5mg/L) | 95 | 19.33±1.25 | 6.6±0.22 |
| $S_8$ | MS+BAP (1.0mg/L)+ Kin (0.5 mg/L)+$GA_3$ (0.5mg/L) | 92 | 17.0±0.81 | 6.2±0.08 |
| $S_9$ | MS+BAP (1.0mg/L)+ Kin (0.5mg/L)+NAA (0.5mg/L) | 90 | 14.33±1.25 | 5.77±0.08 |
| $S_{10}$ | MS+BAP (1.0mg/L)+ $GA_3$ (0.5mg/L) | 87 | 13.33±1.25 | 4.88±0.08 |
| $S_{11}$ | MS+BAP (0.5mg/L)+ NAA (1.0mg/L) | 89 | 12.0±1.62 | 3.77±0.09 |

### 3.2 GENESIS AND PROLIFERATION OF ROOTS FROM SHOOT-TIP DERIVED SHOOTS

In **Table 2**, IBA (0.5mg/L) in half strength MS medium showed the highest root inducing frequency (80%). Number of root per microculturing was also the maximum (15.0±0.82) and the highest root length was recorded (6.77±0.58 cm). Similar result also illustrated by (Lalitha et al., 2013) [11]. The highest root number (9.0±0.82) was found when half strength MS medium was fortified with 1.0mg/L NAA and it took 24-26 days to initiate root. The lowest root length was 1.53±0.41 cm when only half strength MS medium was used without auxins supplementation. Rooting of elongated shoots were successfully achieved (90%) in half strength MS with 1.0mg/L NAA [12].IBA was found more effective rooting hormone comprising 80% rooting efficiency compare with NAA **(Figure 1)**. Velayutham et al. (2006) [13] also documented nearly similar result.

*Table 2. Effect of root induction media on shoots of V. negundo*

| Treatments | Composition | Root inducing frequency (%) | Days taken to root initiation | Number of roots per micro culturing | Root length (cm) |
|---|---|---|---|---|---|
| $R_1$ | ½MS | 50 | 28-30 | 2.33±0.47 | 1.53±0.41 |
| $R_2$ | ½MS+NAA (0.2mg/L) | 60 | 26-28 | 3.0±0.0 | 2.6±0.43 |
| $R_3$ | ½MS+NAA (0.5mg/L) | 70 | 24-26 | 7.33±0.94 | 5.0±0.82 |
| $R_4$ | ½MS+NAA (1.0mg/L) | 60 | 24-26 | 9.0±0.82 | 5.56±0.47 |
| $R_5$ | ½MS+IBA (0.2mg/L) | 70 | 22-24 | 11.33±0.94 | 5.67±0.42 |
| $R_6$ | ½MS+IBA (0.5mg/L) | 80 | 22-24 | 15.0±0.82 | 6.77±0.58 |
| $R_7$ | ½MS+IBA (1.0mg/L) | 70 | 23-25 | 13.33±0.47 | 5.67±0.47 |

*Figure 1. Micropropagation of V. negundo from shoot-tip*

**A.** Shoot initiation and proliferation in MS+BAP (1.0mg/L) + Kin (0.5mg/L) after 15 days of culture.

**B.** Shoot proliferation on the same medium after 32 days of subculture.

**C.** Rooted shoots on ½MS+IBA (0.5mg/L) after 30 days of culture.

**D.** Hardened plants of *V. negundo* in potted soil after 20 days of transplantation.

### 3.3 Acclimatization and Hardening

Plastic pots containing garden soil and farmyard manure (1:1) were used for hardening. The pots were covered with porous polyethylene bags and removed after 2 weeks and transferred to normal room temperature. Almost 80.56% plants survived as shown in **Figure 1**.

## 4 Conclusion

The findings from this experiment pointed to the possibility of consistent mass production of *V. negundo* L. from shoot-tip as a reliable planting material.

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