

Mass production of *Plectranthus zeylanicus*- A valuable medicinal and aromatic plant with a future value

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ABSTRACT

Plectranthus zeylanicus is a medicinal and an aromatic herb native to Sri Lanka of family Lamiaceae. It is known to contain strong aromatic chemicals as other members of the *Plectranthus* genus. Currently, because of the significance of aromatic chemicals and essential oils in perfumery industry, flavoring industry, pharmaceuticals and various other fields demands the increased production of them in lesser time and cost. The present work was aimed to develop a protocol for mass scale propagation of *Plectranthus zeylanicus* through tissue culture techniques. Shoot tips and nodal segments were selected as explants, collected from well-maintained shade house grown one-month old mother plants. Murashige and Skoog (MS) medium was used throughout the experiment. For shoot induction and proliferation, MS medium supplemented with combination of 1-Naphthalene acetic acid (NAA) and different levels of N6-benzylaminopurine (BAP) were used. In vitro rooting was achieved to 50% strength MS basal medium containing different levels of Indole Acetic Acid (IAA) and NAA. Such in vitro produced plants were acclimatized and survival percentages were obtained. The explants from nodal segments gave better results in shoot initiation compared to those from the shoot tips on all the media combinations tested. The highest rate of shoot induction (97.8%) and highest number of shoots per explant (8.9) were obtained in MS medium supplemented with 2.0 mg/l BAP from nodal explants and was significantly different at 5% significant level. The highest rate of shoot proliferation (85%) and number of shoots per explant (8.8) was obtained on media supplemented with 2.0 mg/L BAP + 0.1 mg/L NAA after six weeks of nodal culture and shoot tip cultures supplemented with 2.0 mg/L BAP + 0.1 mg/L NAA resulted highest number of shoots per explant (5.5) with 78% shoot proliferation, after 6th week of culture initiation. The shoot proliferation was more effective in nodal segments than from shoot tip cultures resulting from observations and analysis. Therefore 6th week is the best period to get optimum number of shoots from nodal segments. It was observed that with 0.5mg/L IAA with 0mg/L NAA resulted highest number of roots/explant and the longest roots were recorded with the control treatment. Therefore, it is possible to deduce that the current protocol is promising for in vitro mass propagation of *Plectranthus zeylanicus*, a valuable medicinal plant with promising future in aromatic and pharmaceutical industry.

Keywords: micropropagation, *Plectranthus zeylanicus*, plant growth regulator, rapid multiplication.

INTRODUCTION

Plectranthus zeylanicus, a valuable medicinal plant native to Sri Lanka, belongs to family Lamiaceae. This medicinal herb commonly called Iriweriya in Sinhala or Pathacur in Hindi, and Karpuravalli or Malyalam-Kannikkaurkka in Sanskrit (Sivarajan and Balachandran, 1994). This herb contributes to the immense medicinal importance. This species is used in folk (ayurvedic) medicine for the preparation of a drug, which is a carminative, tonic and cures dyspepsia, indigestion, dysentery, vomiting, dermatitis, ulcers and bleeding disorders. (Sivarajan and Balachandran, 1994). Betty and Thoppil (2004) have discovered the effectiveness of the extract of *Plectranthus*

zeylanicus as a scavenger of free radicals and its role as a natural antioxidant.

It is discovered that species of *Plectranthus* genus contain geraniol, geranyl acetate like strongly aromatic essential oils (Muthukumarana and Dharmadasa, 2014). These strongly aromatic essential oil is important for many industries and demand for those industries is increasing day by day. Rahman and Fakir (2015) have stated that many wild and semi-wild plant species, medicinal and aromatic plants have been used over the millennia for human welfare in the promotion of health and as drugs and fragrance materials. Same authors have mentioned that countries like Sri Lanka, India

and China have officially recognized the use of traditional medicines in health care delivery systems.

Plectranthus zeylanicus is normally propagated by vegetative cuttings but it is time consuming and provides a limited number of propagules. Thus, use of traditional propagation methods are not sufficient to meet the industry demand for the extraction of volatile materials from this aromatic herb. Commercial plantation of this aromatic plant species have not been attempted yet due to non-availability of quality planting materials.

While this aromatic plant has potential for commercialization to take advantage of its medicinal properties and essential oils there has to be a reliable source of this plant material before it can be considered for product development. In this regard, a simple and effective protocol of micro propagation through tissue culture would be useful for future cultivation of this plant to make it more readily available.

MATERIALS AND METHODS

Plant Materials

Mother plants were maintained in shade house conditions, Department of Crop Science, Faculty of Agriculture, University of Ruhuna, Mapalana, Kamburupitiya, Sri Lanka. Watering was done regularly and all other agronomic practices were followed as required. Shoot tips and nodal segments were used as explants. Mother plants were sprayed with the fungicide solution (Daconil 1ml/L) prior to the collection of explants and after 1 hour, explants were collected from freshly emerged sprouts. The collected explants were washed with liquid detergent (2-3 drops of Teepol). Then the explants were kept under running tap water for 1 hour followed by dipping in a fungicide solution (Daconil-concentration 1ml/L) for 30 minutes. The explants were transferred to a laminar air flow chamber where they were surface sterilized with 10% Clorox® containing drops of Tween-20 for 15 minutes. After rinses with sterile distilled water for 3 times, the explants (shoot tips and nodal segments) were cut into 2 - 3 cm.

Shoot induction and proliferation

Basal MS medium with 30 g/l sucrose and gelled with 0.8% (w/v) agar and the pH was adjusted to 5.8 and sterilized in an autoclave under 15 psi and 121°C. The medium was supplemented with combinations of BAP (0.5, 1.0, 1.5 and 2.0 mg/L) and NAA (0.1mg/L). Explants were inoculated on to 40 mL of medium contained in 150 ml flasks. The cultures were incubated in a plant growth room at a temperature of 25°C ± 1°C with a 16/8 h photoperiod provided by cool-white fluorescent lamps (1000 -2000 lux) and were checked regularly for contamination. Percentage of shoot induction, number of shoots per explant were obtained after 12 days and shoot proliferation rate was recorded at weekly intervals for ten weeks. Twenty replicated flasks were used in each treatment to compare the effects of the growth regulators in Complete Randomized Design (CRD) and data were analyzed using Minitab 17 statistical software using ANOVA and means were separated using Duncan's Multiple Range Test (DMRT).

***In vitro* rooting and acclimatization of Plantlets**

Shoots (>2.0 cm long) from the best treatment were individually placed inside the flasks for root initiation. The explants were cultured on 50% MS basal medium with glucose (15g/L) and gelled with 0.8% (w/v) agar supplemented with IAA and NAA with different combination levels as 0.25 mg/L IAA and NAA, 0.5 mg/L IAA and NAA, 0.5 mg/L IAA with 0mg/L NAA and 0 mg/L IAA with 0.25, 0.5 & 1.0 mg/L NAA. Twenty replicated flasks were used in each treatment. The number of shoots that produced roots, as well as the number and length of the induced roots were recorded after 30 days. Complete plantlets produced *in vitro* were removed from the culture medium and the roots were washed to remove the agar. The plantlets were then transferred into pots containing top soil: compost: sand in 1:1:1/2 ratio and placed in the shade house under controlled conditions with 75% shading and temperature at 28°C-32°C. To maintain humidity, the plants were watered periodically twice a day. Observations were recorded on the percent survival of rooted and acclimatized plants.

Table 1: Effect of plant growth regulators on shoot induction from different explants after 12 days of culture.

| Explant segment | Growth regulator concentration (mg/L) | | Shoot induction (%) | Average number of shoots/explant |
|-----------------|---------------------------------------|-----|---------------------|----------------------------------|
| | BAP | NAA | | |
| Shoot tips | 0.5 | 0.1 | 70.4 | 4.0 ± 0.02 ^a |
| | 1.0 | 0.1 | 76.2 | 4.3 ± 0.01 ^a |
| | 1.5 | 0.1 | 80.8 | 4.5 ± 0.04 ^a |
| | 2.0 | 0.1 | 85.2 | 5.2 ± 0.06 ^a |
| Nodal segments | 0.5 | 0.1 | 66.8 | 3.3 ± 0.04 ^c |
| | 1.0 | 0.1 | 82.4 | 4.9 ± 0.07 ^b |
| | 1.5 | 0.1 | 85.2 | 5.7 ± 0.02 ^b |
| | 2.0 | 0.1 | 97.8 | 8.9 ± 0.02 ^a |

Means with same letters are not significantly different at P>0.05 level

Table 2: Effect of different concentrations of IAA and NAA on the rate of explant rooting, number of roots per plant and average root length.

| IAA | NAA | No. of Roots/Explant | Average Root Length (cm) |
|------|------|--------------------------|--------------------------|
| 0 | 0 | 7.8 ± 0.39 ^e | 3.1 ± 0.9 ^a |
| 0.25 | 0.25 | 12.5 ± 0.91 ^b | 2.1 ± 0.4 ^b |
| 0.5 | 0.5 | 13.9 ± 0.72 ^a | 2.0 ± 0.3 ^c |
| 0.5 | 0 | 14.2 ± 1.09 ^a | 2.4 ± 0.1 ^c |
| 0 | 0.5 | 11.6 ± 0.34 ^c | 1.5 ± 0.4 ^d |
| 0 | 0.25 | 8.7 ± 0.51 ^d | 0.6 ± 0.04 ^e |
| 0 | 1 | 7.5 ± 0.44 ^e | 0.4 ± 0.03 ^f |

Means with same letters are not significantly different at P>0.05 level

RESULTS AND DISCUSSION

Explants establishment and shoot initiation

A simple and effective protocol was developed for the *in vitro* micropropagation of *Plectranthus zeylanicus*. Two different types of explants (shoot tips and nodal segments) were cultured on MS media containing different concentrations of BAP and NAA to evaluate their effects on shoot initiation. Explants showed varying success in shoot initiation depending on the type of explants and the growth regulators added. The response of explants cultured in MS media supplemented with BAP and NAA are shown in Table 1.

The explants from nodal segments gave better results in shoot initiation compared to those from

the shoot tips on all the media combinations tested. Generally, the increased level of BAP resulted in significantly higher shoot initiation and the number of shoots at the initiation stage, with constant level of NAA. Although bud break was dependent on BAP supply, the synergistic combination of BAP and NAA induced the optimum frequency of shoot formation as well as shoot number. The highest rate of shoot induction (97.8%) and highest number of shoots per explant (8.9) were obtained in MS medium supplemented with 2.0 mg/l BAP + 0.1 mg/l NAA which was significantly different from all other hormonal combinations and type of explant used at 5% significant level (Table 1). Similar results were reported by Sudharson *et al.* (2014) in their

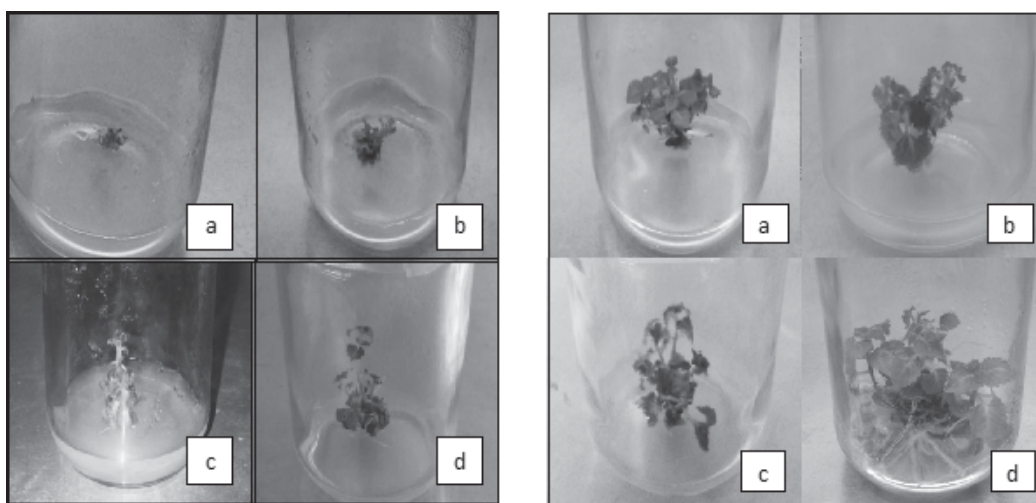


Plate 1: Effect of different treatments for shoot tip proliferation from shoot tip explants (Left) and nodal segments (Right); (a) BAP 0.5mg/L + NAA 0.5mg/L NAA, (b) BAP 1mg/L + NAA 0.5mg/L NAA, (c) BAP 1.5mg/L + NAA 0.5mg/L NAA, (d) BAP 2mg/L + NAA 0.5mg/L NAA.

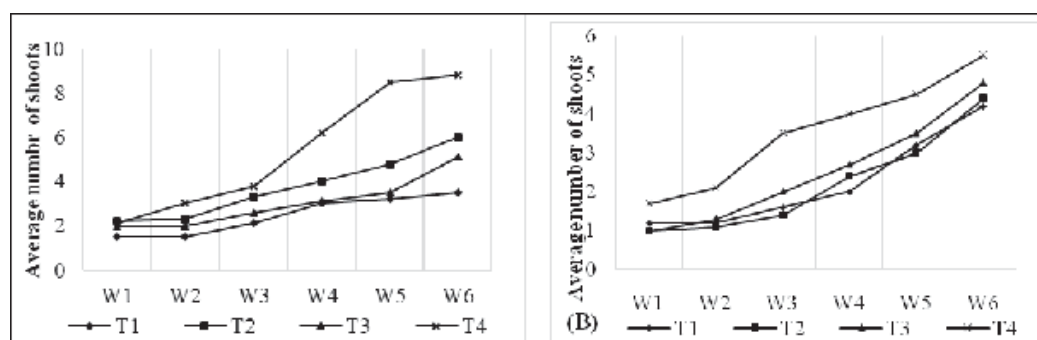


Fig. 1: Plantlet proliferation rate of *Plectranthus zeylanicus* from nodal segments (A) and from shoot tips (B).

studies on the medicinal plant *Hybanthus enneaspermus*, that supplementation of MS medium with 2.0 mg/L BAP give better results than when the growth regulator was added in higher or lower concentrations. According to Dharaneeswara *et al.* (2014), BAP concentrations of up to 2.0 mg/L were effective in inducing shoots of *Grand naine*.

Shoot proliferation and *in vitro* rooting

Different concentrations of BAP or NAA added to MS medium in combination has affected shoot proliferation rate, the number of shoots produced and the average length of the shoots. The highest rate of shoot proliferation (85%) and number of shoots per explant (8.8) was obtained on media supplemented with 2.0 mg/L BAP + 0.1 mg/L NAA

after six weeks of nodal culture (Figure 1). Same way, shoot tip cultures supplemented with 2.0 mg/L BAP + 0.1 mg/L NAA resulted in highest number of shoots per explant (5.5) with 78% shoot proliferation, after 6th week of culture initiation (Figure1). But, the shoot proliferation was more effective in nodal segments than from shoot tip cultures (Plate1- Right). Therefore 6thweek is the best period to get optimum number of shoots from nodal segments. Yohannes and Firew(2014) reported that the cotyledonary node explants of Yeheb (*Cordeauxia edulis*) cultured on MS medium supplemented with 2.0 mg/L BAP resulted in the highest rate of shoot initiation (89 %) and the highest number of shoots per culture after nine weeks. Consistent with this result, Daneshvar *et al.* (2013) reported that 2.5 mg/L BAP + 0.15 mg/

L NAA in MS medium produced the highest number of *Aloe vera* plantlets (up to 28.47 plantlets per explants). Amiri *et al.* (2011) reported that the maximum shoot regeneration and maximum number of regenerated shoots in *Datura stramonium* were obtained in the treatment containing 2 mg/L BAP + 1 mg/L NAA.

Rooting and Acclimatization

Roots were produced in all media. However, supplementation with IBA and NAA affected rooting in *Plectranthus zeylanicus* shoots differently (Table 2). Visual observations during the study revealed that roots of micro-shoots grown on medium containing high NAA levels were morphologically different from roots of other shoots supplemented with high IBA with or without NAA. These roots appeared thicker and short. Among the tested treatment combinations 0.5mg/L IAA with 0.5mg/L NAA and 0.5mg/L IAA with 0 mg/L NAA showed no significant difference resulting the highest number of roots per explant ($P>0.05$).

But, with 0.5 mg/L NAA, callus production was high and it is not a desirable observation during rooting stage. Therefore, 0.5mg/L IAA without NAA is the suitable hormone level for rooting of *Plectranthus zeylanicus*. According to the findings of Rahaman *et al.* (2015) the percentage of rooted explants (100%) and the number of roots (10 - 12.5 roots/explant) produced were highest in media containing IAA 1.0mg/L with 0.5mg/L NAA. The lower response of NAA in promoting root formation of *in vitro* grown banana explants was also reported by Arun *et al.* (2012). They found that the culture medium containing 1.0 mg/L NAA was less efficient than IAA in the promotion of rooting, with the former inducing only 1.64 roots/explant. As in the present study, Ravanfar *et al.* (2009) and Rahaman *et al.* (2015) recorded that maximum root length (3.1 cm) was attained on growth regulator free MS medium.

The acclimatization of rooted plants in *ex vitro* conditions was carried out with the plants bearing well developed roots transferred to small pots containing potting mixtures (top soil: compost: sand in 1:1:1/2). They were maintained at about 70%

relative humidity in the greenhouse with 75% shading. A survival rate 100% was achieved after 6 weeks.

CONCLUSION

This study provides an efficient *in vitro* propagation method for *Plectranthus zeylanicus* using a simple and efficient protocol for producing true to type plants in a relatively short period and with high multiplication rate using nodal tips and shoot tips. Hence, this protocol, culturing of nodal segments in MS medium supplemented with 2.0 mg/l BAP + 0.1 mg/l NAA for shoot induction and proliferation and for rooting ½ MS supplemented with 0.5 mg/l IAA is found to be optimal for *in vitro* mass production of the plant *Plectranthus zeylanicus*. to solve the commercialization and conservation bottle necks of this economically valuable aromatic and medicinal plant.

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