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IN VITRO SELECTION AND PLANT REGENERATION OF *PLECTRANTHUS AMBONICUS* LOUR., AN IMPORTANT MEDICINAL PLANT

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ABSTRACT

An efficient plant regeneration protocol was developed for mass propagation of *Plectranthus amboinicus* from apical and lateral shoot explants on Murashige and Skoog's (MS) medium supplemented with 3.0 mg/l 6-benzylaminopurine (BAP), 5.0mg/l 1-napthalene acetic acid (NAA) and 3% (w/v) sucrose within 4 weeks of culture. The maximum numbers of shoot buds were obtained on MS medium supplemented with 3.0 mg/L BAP from lateral shoots within 6-7 weeks of sub culture. Inclusion of 1.0 mg/l gibberellic acid into the medium enhances the rate of shoot elongation and multiplication. Multiplication was higher when the cultures were incubated under continuous light rather than 16h photoperiod. MS medium supplemented with 1.0 mg/l BAP in combination with 5.0 mg/l NAA and 1.0 mg/l gibberlic acid showed best metabolic response with an average of 7.5 shoots per explants developed on 88% of cultures in 5-6 weeks. Repeated subculture on regeneration medium induces higher rate of shoot regeneration. Rooting was readily achieved upon transferring the micro shoots on to MS basal semi-solid medium supplemented with 0.5 mg/L IBA with number of roots per shoots (10-12.5) and root length (4.1 ± 0.2) cm after 4-5weeks. Micropropagated plantlets were acclimatized and successfully grown in poly trays containing sand, vermiculite and soil (1:1:2) and covered with transparent plastic bags to prevent loss of humidity and acclimatized for a period of 3 weeks. About 98% micropropagated plantlets were hardened successfully. This study will help for propagation of quality planting material of *P. amboinicus* for commercialization required for therapeutic purposes.

KEYWORDS: *Plectranthus amboinicus*, Micropropagation, Medicinal Plant, Plant Growth Regulator.

INTRODUCTION

Plectranthus ambonicus Lour., originally classified under the genus *Coleus* but was moved to the *Plectranthus* genus (Arumugam et al. 2016), under Family- Lamiaceae, syn. *Coleus amboinicus* Lour. The origin of *P. amboinicus* is unknown, but it may be native to Africa and possibly India (Wagner and Lorence 2014). Spreng is commonly known as Indian/ country borage and 'Pathorchur' in Hindi and Bengali (Kumar et al. 2007). It is known as karpooravalli in Tamil, because of its flavour of camphor. In Odia it is called as "Runkuna". *Plectranthus amboinicus* is a large succulent herb, fleshy and highly aromatic, much branched, possessing short soft erect hairs, with distinctive smelling leaves. The leaves of the plant are bitter, acrid and are being widely used traditionally for various purposes. *Plectranthus amboinicus* (Lour) Spreng has been traditionally used to cure various illnesses such as chronic cough, bronchitis, asthma, diarrhea and epilepsy (Khare et al. 2011). In vivo studies using animal model showed that the plant has analgesic and anti-inflammatory activity (Chiu et al. 2012), anti-inflammatory and antitumour activity against Sarcoma-180 and Ehrlich ascites carcinoma (Gurgel et al. 2009), anti rheumatoid arthritis (Chang et al. 2010), antimicrobial activity against *Staphylococcus aureus* (Oliveira et al. 2013). In vitro studies also reported that the plant extract showed antioxidant and antibacterial activities (Bhatt et al. 2012), antidandruff (Selvakumar et al. 2012) and antifungal activity in food system (Murthy et al. 2009). There are other Indian traditional medicinal uses of this plant such as for skin ulcerations, scorpion bite, skin allergy, wounds, diarrhoea, and the leaves are being used as a hepatoprotective, to promote liver health. The leaves of the green type of country borage are often eaten raw with bread and butter. Despite many studies have already explored wide range of bioactivities of the plant, there is still however lack of study reporting how to grow the plant in *in vitro* condition. Micropropagation insures a good regular supply of medicinal plants, using minimum space and time (Prakash et al. 2007). There are various advantages of *in vitro* micro propagation of medicinal plant (Sidhu *2011)*. Before it can be considered for product development, a simple method of micro propagation through tissue culture would be useful for future cultivation of *Plectranthus amboinicus* to make it more readily available.

MATERIALS AND METHODS

Plant material and explants source

Actively growing, healthy and disease free young shoots of *Plectranthus amboinicus* were collected from green house grown plants and washed with 2% (v/v) detergent Teepol (Qualigen, India) and rinsed several times with running tap water. The explants were surface sterilized in 0.1% (w/v) aqueous mercuric chloride solution for 3-4min followed by four washing with sterile distilled water. *P. amboinicus* was extremely sensitive to surface sterilizing agent as well was water, therefore, the surface sterilization procedure was optimized and this helped in preventing blackening of tissues and establishment of clean cultures. The apical and lateral shoots (~ 2.0 mm) were isolated and used as explants.

Culture medium and condition

The meristem (apical and lateral) was placed on semi-solid basal MS (Murashig and skoog 1962) medium, supplemented with various concentration of BAP (0,0.5,1,2,3,3.5mg/l), 3% (w/v) sucrose and gelled with 0.7% (w/v) agar, for shoot bud induction. Ascorbic acid also used to reduce the amount of phenolic secretion of the explants in the initial media. The pH of the media was adjusted to 5.8 using 0.1N NaOH or 0.1N HCl before autoclaving. The cultures were maintained at 25±2ºC under 16h photoperiod light from cool, white fluorescent lamps. The obtained shoot buds were transferred after 5-6 weeks of inoculation to MS medium containing various concentrations BAP (0.5, 1, 2, 3mg/l), NAA (1, 3, 5 mg/l) and GA_3 (1mg/l) in combinations for shoot proliferation and multiplication. The cultures were maintained by regular subcultures at 4-week intervals on fresh medium with the same compositions.

Induction of rooting and acclimatization

Elongated shoots were transferred to rooting medium, which consisted of MS supplemented with different concentrations of IBA (0, 0.5, 1 mg/l) and NAA (0.5, 1, 2mg/l). All the cultures were incubated in culture room at 25 ± 2°C, light intensity (3000lux) with a photoperiod of 16 hours with 60-70% relative humidity. The cultures were monitored and the data were recorded at every seven day interval. After 15-20 days of culture, the sufficient rooted plantlets were dipped in bavistin solution for approx 2-3 minutes and planted carefully in the poly bags containing soil mixtures (organic soil mixed with garden soil 1:1). They were maintained at about 70% relative humidity in the greenhouse with 75% shading to produce newer leaves/roots.

Observation of cultures and presentation of results

Twenty cultures were used per treatment and each experiment was repeated at least three times. The data pertaining to mean percentage of cultures showing response, number of shoots/culture and mean percentage of rooting were statistically analysed by the Post-Hoc Multiple Comparison test (Selvakumar et al. 2012). Between the treatments, the average figures followed by the same letters were not significantly different at $P < 0.05$ level.

RESULT AND DISCUSSION

Explants Establishment and Shoot bud Initiation

Meristem proliferation was initiated from apical and axillary explants of *Plectranthus amboinicus* within 8-10 days of inoculation onto MS basal medium supplemented with different concentrations of the cyokinins. The maximum shoot proliferation was observed in axillary meristems cultured on MS medium supplemented with 0.5 – 3.5 mg/l BAP within 4 weeks of culture under 16h photoperiod**.** The explants from nodal segments gave better results in shoot bud initiation compared to shoot tip (Figure.1-B,&C). The highest rate of shoot induction 83% with average 4.6 shoots per explant were obtained in MS medium supplemented with 3.0 mg/l BAP, followed by 2.0 mg/l BAP (80%, 1.9 shoots per explant). Similar results were reported by Sudharson et al. (2014) in their studies on the medicinal plant *Hybanthus enneaspermus*. They found that supplementation with 2.0 mg/L BAP gave better results than when the growth regulator was added in higher or lower concentrations. According to Dharaneeswara et al. (2014) BAP concentrations upto 2.0 mgl/l was effective in inducing shoots of Musa (Grand naine). Furthermore, 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.

Table 1: Effect of different concentrations of BAP on shoot bud initiation from different explants after 4 weeks of culture (*means of 10 replicates per treatment; repeated thrice).

Explants segment	Growth regulator BAP concentration (mg/l)	Shoot induction %*	Average no of shoot buds per explant (mean±SD)*
Node	0	20	0.2 ± 0.02
	0.5	60	1.2 ± 0.02
	1	75	1.6 ± 0.34
	2	80	1.9 ± 0.32
	3	**83**	**4.6 ± 0.4**
	3.5	78	1.6 ± 0.4
Shoot tip	0	0	0
	0.5	30	0.2 ± 0.01
	1	20	0.2 ± 0.01
	2	30	0.5 ± 0.5
	3	35	0.5 ± 0.56
	3.5	32	0.5 ± 0.06

Shoot Multiplication and Elongation

Combination of BA + NAA favours more effective for shoot bud proliferation. Inclusion of GA_3 1mg/l, in the culture medium enhances the rate of shootbud proliferation and multiplication. The percentage of shoot bud regeneration was maximum (88%) within 4–6 weeks of culture on MS medium fortified with 1.0 mg/l BAP + 5 mg/l NAA + 1mg/l GA_3 and 3% sucrose (Table-2). Very less shoot proliferation was observed in 0.5mg/L BAP + 1mg/L NAA + 1mg/L GA_3. The present findings showed that the addition of BAP and NAA in combination further promoted the proliferation of shoots compared to the growth regulators applied singly. The importance of plant growth regulators on shoot propagation had been highlighted in various studies. 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 Aloevera 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. In contrast, the present findings showed that the direct shoot bud regeneration was achieved on medium containing 1.0 mg/l BAP, 5.0 mg/l NAA, 1.0 mg/l Ga_3 and a maximum 7.5 shoots per culture within 4–5 weeks of first subculture (Table-2, Fig. 1 (D)). The rate of shoot bud proliferation declined on medium containing lower concentrations of NAA. The rate of shoot bud proliferation ability was maintained up to 6th subculture period on MS medium supplemented with 1.0 mg/l BA, 5.0 mg/l NAA and 1.0 mg/l Ga_3 by regular subculture at

every 2 weeks. This might be due to a balance between the endogenous and exogenous growth regulators and the ionic concentrations of nutrient salts (Rout 2002). The greatest mean shoot length (3.1 cm) was obtained on the medium containing 3.0 mg/L BAP + 1mg/L GA_3.

Table 2: Effect of BAP, NAA and GA_3 concentrations on shoot proliferation, number of shoots/explants and average length of shoots (*means of 10 replicates per treatment; repeated thrice).

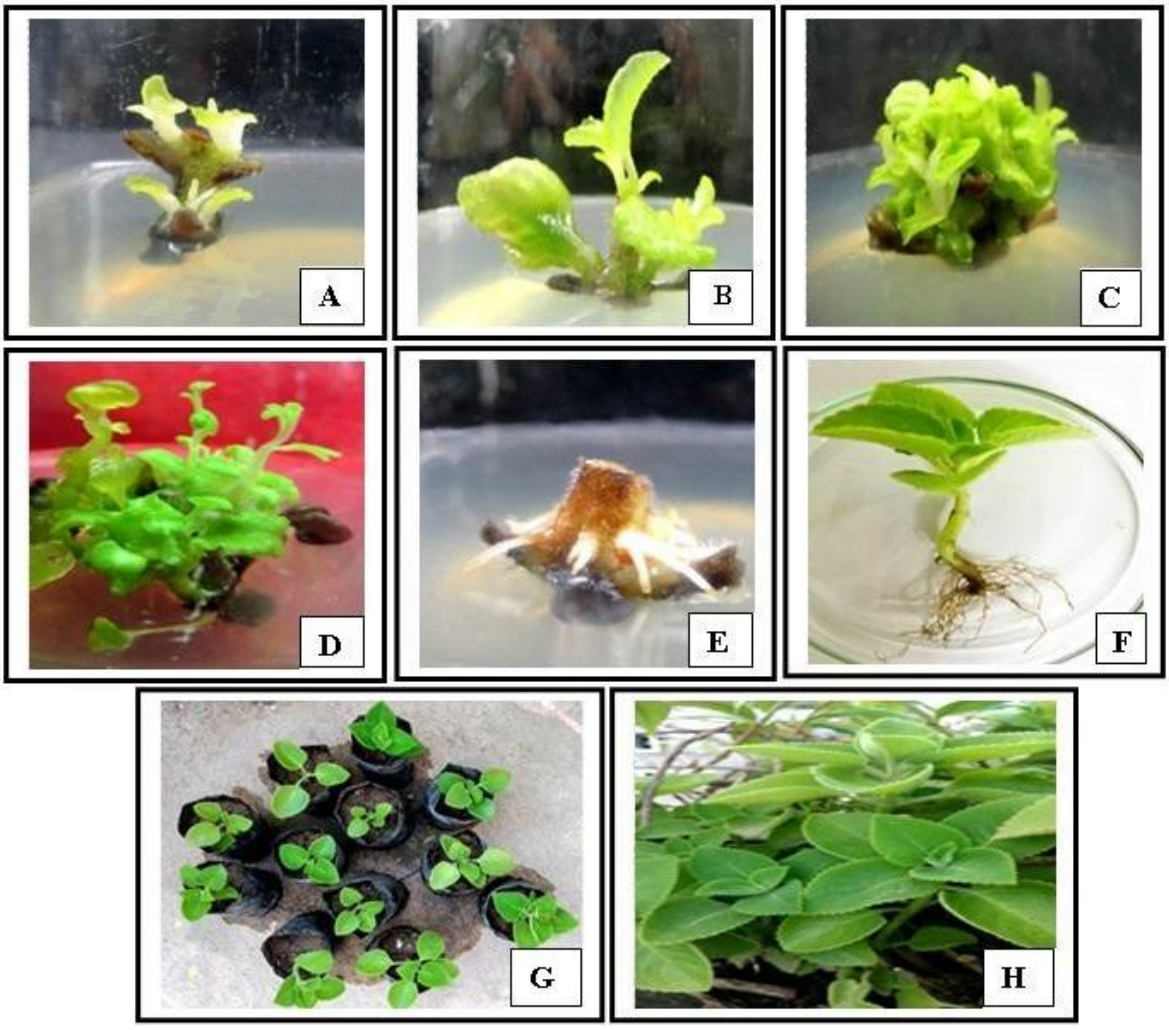
BAP (mg/l)	NAA (mg/l)	GA_3 (mg/l)	% of explants showing shoot proliferation	Average number of shoots per explant (mean ± SD)*	Average length of shoot (cm)*
0	0	0	0	1.0 ± 0.01	2.6±0.13
1	0	1	0	1.0 ± 0.01	1.5±0.03
2	0	1	55	3.8 ± 0.12	1.6±0.02
3	0	1	70	4.2 ± 0.02	3.1±0.12
0.5	1	1	10	2.2± 0.04	2.5±0.03
0.5	3	1	35	3.0 ± 0.13	1.7±0.06
0.5	5	1	38	3.0± 0.45	1.6±0.04
1	1	1	43	2.0± 0.12	2.0±0.02
1	3	1	50	2.8± 0.05	2.2±0.1
1	5	1	88	7.5 ± 0.30	2.4±0.04

Rooting

Elongated shoots (3–4 cm long) were excised from parent culture and transfer on to basal MS medium with or without growth regulators. Initiations of root from microshoots were delayed in the medium without auxin. Inclusion of NAA or IBA (0.25–0.5 mg/l) with 2% sucrose induced rooting within 3 weeks of culture (fig- E&F). Among the growth regulators, The percentage of rooted explants (100%) and the number of roots per shoots (10-12.5) with root length (4.1 ± 0.2) cm produced were highest in media containing IBA 0.5mg/l (Table-3). These observations were similar to the findings on *Gentiana lutea* by Petrova et al. 2011, who reported high root proliferation per explant obtained on MS medium containing 1 mg/l IBA. They also stated that *in vitro* grown shoots on MS medium containing 0.5 mg/l IBA produced 3.08 roots per explant. Kumar et al. (2012) found that with the Malbhog cultivar of Banana, a combination of 1.0 mg/L IBA and 0.5 mg/L IAA produced the best rate of rooting, with 8.5 roots/ explants. The present results indicate that the percentage of rooting and number of roots per shoot were much higher than the previous report by other researchers.

Table 3: Effect of different concentrations of IBA and NAA on the rate of explant rooting, number of roots per plant and average root length (*means of 10 replicates per treatment; repeated thrice).

IBA (mg/l)	NAA (mg/l)	% of explants rooted	No.of roots/explants (mean±SD)*	Average root length (cm) (mean±SD)*
0.25	0	89	9.7 ± 0.39	2.1 ± 0.9
0.5	0	100	12.5 ± 0.91	4.1 ± 0.2
1	0	100	10.8 ± 0.72	2.0 ± 0.3
0	0.25	70	7.6 ± 0.51	0.6 ± 0.04
0	0.5	75	8.8 ± 0.44	0.4 ± 0.03
0	1.0	75	8.3 ± 1.09	0.4 ± 0.04



"Fig.1" :- *In vitro* grown *Plectranthus amboinicus*,- (A) shoot bud induction after 2weeks of culture, (B) Growth of shoot bud after 3weeks of culture (C) Growth of Multiple shoot buds (D) Elongation of shoot bud, (E) Root induction in rooting medium, (F) well developed rooted plant, (G) Acclimatization of plants in green house, (H) Hardened plant ready for sale.

Acclimatization

The acclimatization of rooted plants in *in-vivo* conditions was carried out with the plants bearing well-developed roots transferred to small polly bags containing soil mixtures (organic soil mixed with garden soil 1:1). Water was sprayed at every two days interval. Excess water will damage the plants. All the regenerated plantlets were maintained at about 70% relative humidity in the greenhouse with 75% shading (fig.1 (G)). A survival rate 98% was achieved after 6 weeks. (Fig.1 (H)). The plants grew well and attained 4-5 cm height within 2 months of transfer. The acclimatized plants exhibited normal growth and no sign of morphological variation was noticed.

CONCLUSION

A suitable micropropagation method was developed through this research work to make the plant *Plectranthus amboinicus* commercially available to fulfill growing medicinal demand as the plant was used all over world of Medicine for centuries for everything from mosquito bite to bronchitis.

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