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In Vitro Micropropagation of a Valuable Medicinal Plant, *Plectranthus amboinicus*

Zuraida Ab Rahman[1], Erny Sabrina Mohd Noor[2], Mohd Shukri Mat Ali[2], Razali Mirad[2], Ayu Nazreena Othman[1]

[1]Biotechnology Research Centre, MARDI Headquarters, MARDI HQ, Persiaran MARDI-UPM, Serdang, Malaysia
[2]Strategic Resource Centre, MARDI HQ, Persiaran MARDI-UPM, Serdang, Malaysia
Email: azuraida@mardi.gov.my

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Abstract

The effect of the plant growth regulators benzyl amino purine (BAP), 1-naphthaleneacetic acid (NAA) and kinetin (KIN) on *in vitro* shoot induction and proliferation of *Plectranthus amboinicus* was examined. Explants obtained from lateral shoots and apical shoots of *P. amboinicus* were inoculated on Murashige and Skoog (MS) culture medium supplemented with different concentrations of BAP, NAA and KIN. When the effect of each growth regulator was considered singly, the highest rate of shoot induction (80% of explants producing shoots) and highest number of shoots produced (2.4 shoots per explant) were obtained from lateral shoot explants cultured on MS media supplemented with 3.0 mg/L BAP within 6 - 7 weeks. Better results were obtained using MS medium supplemented with 1 mg/L BAP + 5 mg/L NAA. Shoot proliferation rose to 85%, while 5.7 shoots per explants were recorded. Among the different media tested for rooting, MS medium supplemented with 1.0 mg/L IBA was the most effective for root induction. The quality of the roots obtained was better than that obtained using MS media supplemented with NAA or IAA.

Keywords

***Plectranthus amboinicus*, Micropropagation, Medicinal Plant, Plant Growth Regulator**

1. Introduction

Plectranthus amboinicus (Lour.) Spreng, a plant native to south and east Africa, belongs to family Lumiaceae. The plant is known by different names in various languages, such as country borage, Indian borage, Patta ajavayan or Pathacur in Hindi, and Karpuravalli or Malyalam-Kannikkaurkka in Sanskrit [1]. The plant is used in

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Chinese folk medicine for the treatment of cough, sore throat, fever, mumps, and mosquito bite [2] [3]. *P. amboinicus* is also used to treat collagen-induced arthritis in rats [4]. In India, the plant is traditionally used as a carminative, digestive, expectorant, anthelmintic, diuretic and liver tonic. The leaves are also useful to treat dyspepsia, flatulence, cholera especially in children, epilepsy, chronic asthama, hicough, bronchitis, renal and vesicle calculi, hepatopathy, and malarial fever [1]. In ethnomedicine, species of the genus Plectranthus are cited as antimicrobial agents used to treat several infections [5] [6]. In Brazil, *P. amboinicus* is used for the treatment of inflammation as well as fungal and bacterial infections [7] [8]. Although *P. amboinicus* thrives in the Malaysian climate, it is not commonly encountered in the country. While this plant has potential for commercialization to take advantage of its medicinal properties, there has to be a reliable source of this plant material before it can be considered for product development. In this regard, a simple method of micropropagation through tissue culture would be useful for future cultivation of this plant to make it more readily available.

2. Materials and Methods

2.1. Plant Materials and Culture Initiation

Healthy *Plectranthus amboinicus* plants (about one month-old) were maintained in a net house for two weeks prior to explants excision and establishment *in vitro*. Shoots and stem segments of plants collected were washed in running tap water for 1 h. Three centimetre pieces of stem carrying vegetative buds were prepared from either lateral shoots or apical shoots and washed with detergent (Teepol) solution for 30 min, followed by rinsing in distilled water. The explants were transferred to a laminar air flow chamber where they were surface sterilized with 10% - 20% Clorox® containing drops of Tween-20 for 5 - 20 min on a rotary shaker. After rinses with sterile water, the explants (apical shoots and lateral shoots) were cut into 2 - 3 cm, and then cultured on Murashige and Skoog (MS) medium supplemented with the plant growth regulators benzyl amino purine (BAP), 1-naphthaleneacetic acid (NAA) and kinetin (KIN) at concentrations of 0, 0.5, 1.0, 3.0 or 5.0 mg/L. The basal MS medium that also contained 30 g/L sucrose was adjusted to pH 5.7 to 5.8 before adding 3 g/L gelrite agar for gelling. Sterilization of the culture medium was performed by autoclaving at 12˚C for 20 min. 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 h photoperiod provided by cool-white fluorescent lamps (1000 - 2000 lux). The cultures were checked regularly for contamination and observations were recorded at weekly intervals. Twenty replicated flasks were used in each treatment to compare the effects of the growth regulators. Results were expressed as percent shoot induction, the number of shoots per culture and also the length of the shoots after 45 days of culture.

2.2. Micropropagation, Rooting and Transplantation of Plantlets

Separate experiments were conducted for shoot multiplication. Cut segments of explants were cultured on basal MS medium supplemented with either BAP (0, 0.5, 1.0, 3.0 and 5.0 g/L), NAA 0, 0.5, 1.0, 3.0 and 5.0 g/L or combinations of both growth regulators. Ten replicated flasks were used in each treatment and observations were recorded at weekly intervals. The results were expressed as percent explants showing shoot proliferation, as the number of shoots per culture and the length of the shoots after 45 days of culture. Shoots (>2.0 cm long) from the best treatment were individually placed inside the flasks for root initiation. The explants were cultured on MS medium supplemented with IBA, IAA or NAA at concentrations of 0, 0.5, 1.0, or 2.0 mg/L. 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 organic soil mixed with garden soil (1:1) and placed in the net 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.

3. Results and Discussion

3.1. Explants Establishment and Shoot Initiation

A simple and effective protocol was developed for the *in vitro* micropropagation of *Plectranthus amboinicus*. Two different types of explants (apical shoots and lateral shoots) were cultured on MS media containing differ-

ent concentrations of BAP, NAA, or KIN to evaluate their effects on shoot initiation. Explants grown on plant growth regulator-containing media showed varying success in shoot initiation depending on the type of explant and the growth regulators added. The response of explants cultured in MS media supplemented with BAP, NAA, and KIN are shown in **Table 1**. The explants from lateral shoots gave better results in shoot initiation compared

Table 1. Effect of plant growth regulators on shoot initiation from different explants after 40 days of culture.

Explant segment	Growth regulator concentration (mg/L)	Shoot induction (%)	Average number of shoots/explant
Apical shoots	BAP		
	0	20	0.3 ± 0.04
	0.5	30	0.5 ± 0.03
	1.0	20	0.5 ± 0.01
	3.0	30	0.6 ± 0.05
	5.0	35	0.6 ± 0.09
	NAA		
	0.5	30	0.3 ± 0.02
	1.0	30	0.4 ± 0.01
	3.0	25	0.4 ± 0.04
	5.0	40	0.7 ± 0.06
	KIN		
	0.5	30	0.5 ± 0.04
	1.0	35	0.5 ± 0.07
	3.0	30	0.5 ± 0.02
	5.0	30	0.4 ± 0.02
Lateral shoots	BAP		
	0	40	0.5 ± 0.03
	0.5	60	1.2 ± 0.21
	1.0	80	1.9 ± 0.31
	3.0	80	2.4 ± 0.43
	5.0	75	0.9 ± 0.09
	NAA		
	0.5	45	0.4 ± 0.03
	1.0	55	0.5 ± 0.01
	3.0	70	1.0 ± 0.11
	5.0	60	1.0 ± 0.21
	KIN		
	0.5	30	0.4 ± 0.04
	1.0	35	0.4 ± 0.03
	3.0	45	0.5 ± 0.01
	5.0	40	0.8 ± 0.07

to those from the shoot apices on all the media tested. Generally, the addition of BAP resulted in significantly higher shoot initiation and the number of shoots at the initiation stage, as compared with other growth regulators. The highest rate of shoot induction (80%) (**Figure 1(b)**) and highest number of shoots per explant (2.4) were obtained in MS medium supplemented with 3.0 mg/l BAP, followed by 1.0 mg/l BAP (80%, 1.9). The lowest rate of shoot initiation was recorded in media with no plant regulator added. Similar results were reported by Sudharson *et al.* [9] 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.* [10], BAP concentrations of up to 2.0 mgl/L were effective in inducing shoots of Musa (Grand naine). Furthermore, Yohannes and Firew [11] 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.

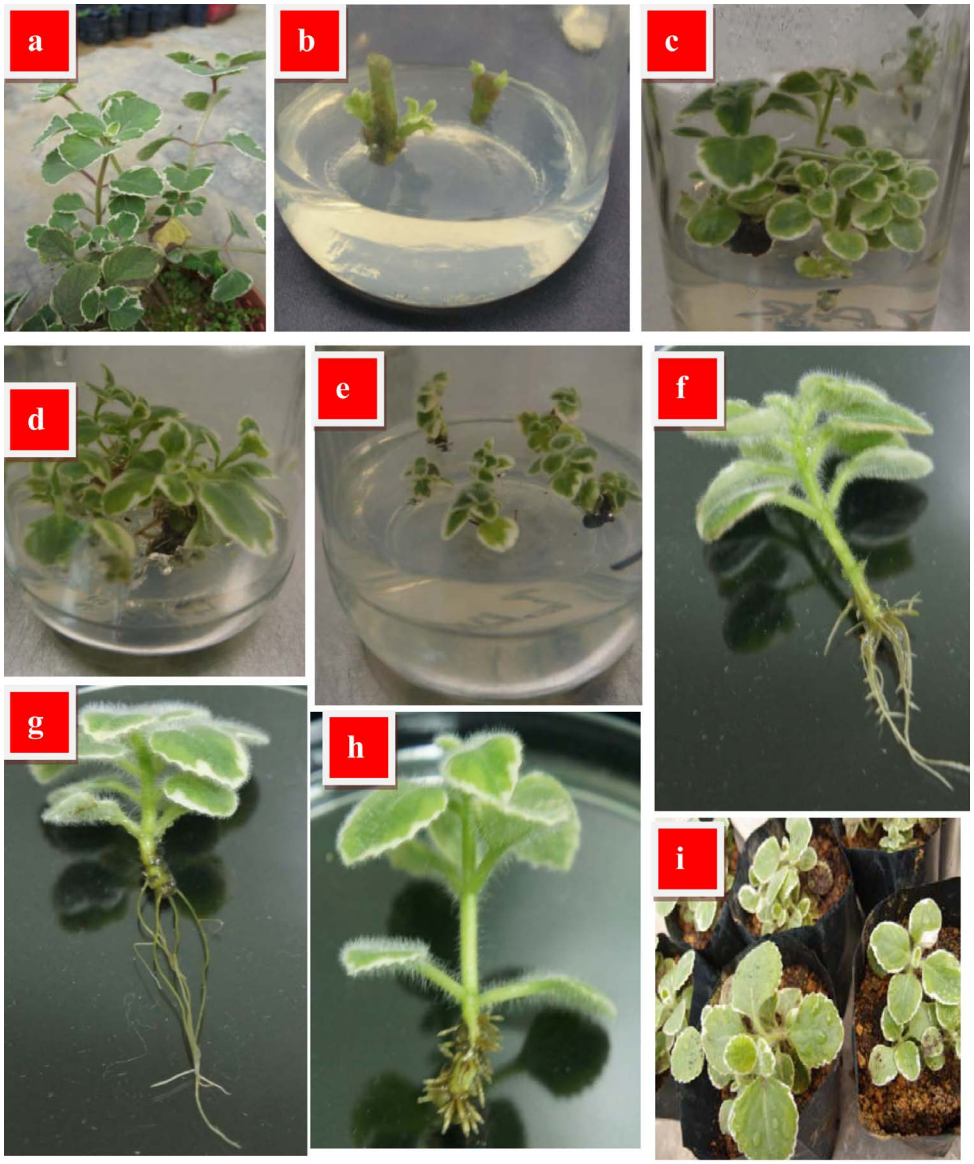

Figure 1. Direct plant regeneration of *in vitro* cultured *Plectranthus amboinicus.* Sources of mother plant planted in polybag and placed in net house (a). Inanition of side shoots from t of shoots from stem on medium with 3.0 mg/L BAP (b); Prolifaration and development of shoots (c) (d); Shoot stunted cultured on 5 mg/L BAP (e); Root elongation on basal medium with 0.5 mg/L IBA (f); MS withour plang growth regulator (g) and MS with 0.5 mg/L NAA (h); Plantlets transferred into polibag after one month (i).

3.2. Shoot Multiplication

Different concentrations of BAP or NAA added to MS medium singly or in combination 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 (5.7) was obtained on media supplemented with 1 mg/L BAP + 5 mg/L NAA after six weeks of culture (**Table 2**) (**Figure 1(c)**, **Figure 1(d)**). On the other hand, no shoots were recorded in MS medium that contained, 0.5 mg/L BAP, 0.5 mg/L NAA, 0.5 mg/L BAP + 0.5 mg/L NAA, or that contained no plant growth regulators. This result indicated 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 has been highlighted in various studies [12]-[15]. 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. The percentage of shoot regeneration in Artimisia was highest with a combination of BAP and NAA, both at the concentration of 0.5 mg/L [16]. Brand and Lineberger [17] also recorded explants of Liquidambar styraciflua L. forming adventitious shoots when cultured on medium supplemented with 2.5 mg/L BAP + 0.1 mg/L NAA. Rao *et al.* [18] reported that treatment of BAP (1.0 mg/L) and NAA (0.2 mg/L) on mulberry resulted in apical shoot explant proliferation and regeneration. The greatest mean shoot length (3.7 cm) was obtained on the medium containing 1.0 mg/L NAA although the number of shoots was showed in this treatment was lower. Reduced concentrations of BAP or NAA (0.5 - 1.0 mg/L) tended to increase shoot length. In addition, the regenerated shoots exhibited abnormal morphology at high concentrations of BAP and the explants produced dense clumps of non-elongating shoots (**Figure 1(e)**).

3.3. Rooting and Acclimatization

Roots were produced in all media, including the medium that was free of growth regulators (**Table 3**). However, supplementation with IBA, IAA, and NAA affected rooting in *Plectranthus amboinicus* shoots differently.

Table 2. Effect of BAP and NAA concentrations on shoot proliferation, number of shoots/explants and average length of shoots.

BAP (mg/L)	NAA (mg/L)	% of explants showing shoot proliferation	Average number of shoots per explant (mean ± SD)	Average length of shoot (cm)
0	0	0	1.0 ± 0.01	2.6
0.5	0	0	1.0 ± 0.01	2.5
1.0	0	5	1.0 ± 0.01	1.9
3.0	0	30	1.5 ± 0.12	2.2
5.0	0	30	2.5 ± 0.02	1.5
0	0.5	0	1.0 ± 0.01	3.1
0	1.0	5	1.2 ± 0.51	3.7
0	3.0	50	3.5 ± 0.11	1.5
0	5.0	60	3.7 ± 0.42	1.2
0.5	0.5	0	1.0 ± 0.01	2.7
0.5	1.0	20	2.1 ± 0.02	2.8
0.5	3.0	40	3.1 ± 0.45	1.7
0.5	5.0	40	3.4 ± 0.13	1.4
1.0	0.5	10	1.3 ± 0.07	1.6
1.0	1.0	40	3.0 ± 0.12	2.0
1.0	3.0	55	3.1 ± 0.05	2.4
1.0	5.0	85	5.7 ± 0.32	2.3

Table 3. Effect of different concentrations of IBA, IAA and NAA on the rate of explant rooting, number of roots per plant and average root length.

BAP (mg/L)	IAA (mg/L)	NAA (mg/L)	% of explants rooted	No. of roots/explant	Average root length (cm)
0	0	0	100	9.7 ± 0.39	3.1 ± 0.9
0.5	0	0	100	12.5 ± 0.91	2.1 ± 0.4
1.0	0	0	100	10.8 ± 0.72	2.0 ± 0.3
0	0.5	0	80	8.3 ± 1.09	1.4 ± 0.1
0	1.0	0	85	9.5 ± 0.34	1.5 ± 0.4
0	0	0.5	75	8.6 ± 0.51	0.6 ± 0.04
0	0	1.0	75	7.8 ± 0.44	0.4 ± 0.03

Among the growth regulators, IBA had the largest effect on root formation (**Figure 1(f)**). The percentage of rooted explants (100%) and the number of roots (10 - 12.5 roots/explant) produced were highest in media containing IBA. However, IBA-induced roots (2.0 - 2.1 cm) were shorter than those obtained without using growth regulators (**Figure 1(g)**). Treatment with NAA showed all the root stunted with shoot roots produced (**Figure 1(h)**). These observations were similar to the findings on Gentiana lutea by Mariya *et al.* [19] 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. Arun *et al.* [20] 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. They also observed that the medium supplemented with 1.0 mg/L IBA alone induced rooting only in 66% of explants and only 6.5 roots/explant were obtained. In agreement with the results of the present study, the lower response of NAA in promoting root formation of *in vitro* grown banana explants was also reported by Arun *et al.* [20]. They found that the culture medium containing 1.0 mg/L NAA was less efficient than IBA in the promotion of rooting, with the former inducing only 1.64 roots/explant. As in the present study, Ravanfar *et al.* [21] found that maximum root length (2.46 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 soil mixtures (organic soil mixed with garden soil 1:1). They were maintained at about 70% relative humidity in the greenhouse with 75% shading (**Figure 1(i)**). A survival rate 98% was achieved after 6 weeks.

4. Conclusion

In this study on the *in vitro* micropropagation of the medicinal plant *Plectranthus amboinicus*, MS culture medium containing 3.0 mg/L BAP gave the best rate of shoot initiation using explants derived from lateral shoots. The highest rate of shoot proliferation was obtained using BAP at 1.0 mg/L in combination with NAA at 5.0 mg/L. BAP at 0.5 - 1.0 mg/L was most suited for root initiation. The plantlets obtained survived and grew normally in the greenhouse. This procedure was recommended for rapid *in vitro* shoot micropropagation of *P. amboinicus*.

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