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Direct Shoot Organogenesis from Nodal and Leaf Explants of *Munronia pinnata* (Wall.) Theob: a Valuable Medicinal Plant

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ABSTRACT. *An efficient micropropagation protocol was developed for direct plant regeneration from nodal and leaf explants of Munronia pinnata (Wall.) Theob. Nodal explants were cultured on MS medium supplemented with different concentrations (0, 0.5, 1, 2, 5 mgl^{-1}) of benzylaminopurine (BAP). Adventitious shoot initiation was most successful in 2 mgl^{-1} BAP where 96% of explants induced approximately three shoots per node with an average length of 1.4 cm. An average of six shoots per explant was produced in MS medium supplemented with 2 mgl^{-1} BAP after single subculture. Leaf explants were cultured on MS medium supplemented with BAP (0, 1, 2 mgl^{-1}) alone, either with combinations of Indole-3-acetic acid (IAA, 0.4, 0.8, 4 mgl^{-1}) or indole-3-butyric acid (IBA, 0.1, 0.5, 2, 5 mgl^{-1}). Seventy percent of explants initiated an average of nine shoots on MS medium supplemented with 1 mgl^{-1} BAP and 2 mgl^{-1} IBA. An average of four roots were developed in ninety percent of microcuttings on MS medium supplemented with 1 mgl^{-1} IBA. Upon acclimatization and soil establishment, survival rate was eighty percent after eight weeks in the greenhouse. Qualitative chemical similarity of tissue culture derived plants and wild collected plants were confirmed using thin layer chromatographic (TLC) profiling.*

INTRODUCTION

Munronia pinnata (Wall.) Theob., which belongs to family *Meliaceae*, is a small hardy perennial shrub with a 5 – 15 cm stem, pinnate leaves and a long woody root system. The entire plant is used in *ayurvedic* systems of medicine, especially to treat malarial fever, fever, dysentery and purification of blood (Jayaweera, 1982). It is grown in diverse ecosystems including rocky sites in the dry, dry-mixed to wet evergreen forests and some savannah lands in Sri Lanka from where *Munronia pinnata* propagules required for traditional systems of medicine are usually collected. The collection of this species from the wilderness was not a problem in the past and plant materials collected were within limits, allowing the regeneration of plants to sustain the demand. However, this species is still being collected from the same sources to achieve an annual demand of 10,000 kg of dried material, which is about 30,000 plants (Summithrarachi, 1996). Due to the large scale and

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unrestricted over exploitation of this natural resource to meet its ever increasing demand, the high price (1 Kg of dried products cost approximately US $ 20 - 35), coupled with habitat destruction and insufficient natural regeneration, the wild stock of this rare but medicinally important plant species has been markedly depleted. Therefore, *M. pinnata* has been included in the list of the most important 50 medicinal plants in Sri Lanka and at present, this species is under threat of extinction (SLSUMPP, 1999). Therefore, the development of rapid, large-scale propagation system of *M. pinnata* is a necessity in commercial cultivation to prevent it from being extinct. Conventional propagation of *M. pinnata* through seeds is too slow to meet the demand due to reduced span of viability and low germination (Arambewela *et al*., 2002). Therefore, micropropagation is an effective alternative for meeting the need for clones of *M. pinnata* plants within a reasonable time frame without affecting the wild population.

Micropropagation of *M. pinnata* through indirect organogenesis from callus developed on leaf and nodal explants has been reported (Silva and Hirimburegama, 2001; Sugathadasa, 2001; Fernando, 2003; Senarath, *et al*., 2007). However, shoot regeneration via an intermediate callus phase has induced somaclonal variations, making this method less desirable for large-scale clonal multiplication (Thrope *et al*., 1991; Salvi *et al*., 2001; Martin, 2003; Kongbangkerd *et al*., 2005). Shoot regeneration directly from leaf explant avoids this problem and provides a very reliable method for clonal propagation (Krul and Myerson, 1980; Bhojwani and Razdan, 1996). Propagation through axilary bud multiplication is an easy and safe method for obtaining true-to-type plants within a shorter period of time (George, 1993; Martin, 2002; Salvi *et al*., 2002). In addition, comparison of chemical composition of micropropagated and wild grown plants is important before introducing them into large scale cultivation, which has not been considered in previous studies on *M. pinanta*. Therefore, the objectives of the present study were to obtain direct organogenesis from leaf and nodal explants of *M. pinnata* and also to compare chemical composition of *in vitro* grown plants and mother plants.

MATERIALS AND METHODS

Plant material and surface sterilization

Munronia pinnata plants (3 leaflet type) obtained from the Ayurvedic plant nursery, Haldummulla were grown in polythene bags (14 x 20 cm) containing compost and sand (3:1 ratio) at 28 ± 3 °C under 12 h photoperiod in a plant house at the Department of Crop Science, University of Peradeniya, Sri Lanka. The explants were washed under running tap water for 45 min and then soaked in a 1% (v/v) Tween Twenty (Sigma), for 10 min. After washing under running tap water for 15 min, their surfaces were sterilized with 0.1% (w/v) $HgCl_2$ for 3 min and finally rinsed 4–5 times with sterile distilled water.

Culture media and culture conditions

A culture medium containing MS (Murashige and Skoog, 1962) salt supplemented with macro-elements, micro-elements, 3% (w/v) sucrose, and 0.2% (w/v) phytogel (Sigma) was used in all experiments. The pH of the medium was adjusted to 5.8 ± 0.1 by 1N NaOH or IN HCl prior to being autoclaved at 120 °C for 20 minutes at 15 psi. If otherwise not stated, all

of the cultures were incubated under 1000-1300 $\mu molm^{-2}s^{-1}$ light provided by cool white fluorescent lights for a photoperiod of 16 h at 25 ± 2 °C. Tissues were sub cultured on fresh media once in every 8 weeks.

Shoot initiation and multiplication from nodal explants

For shoot induction, single nodes were cultured on MS medium supplemented with five different concentrations of BAP (0, 0.5, 1, 2 and 5 mgl^{-1}). Eight weeks after establishment, multiplied primary shoots were sectioned into single nodes again and sub cultured onto the fresh media with same amounts of BAP except the medium containing 5 mgl^{-1} BAP since it negatively affected growth and multiplication. The frequency of shoot induction, the number of shoots per explant and the shoot length were recorded at the end of each subculture period (8 weeks).

Shoot initiation and multiplication from leaf explants

The leaf explants of $1cm^2$ size were cultured in a MS medium supplemented with BAP (0, 1, 2 mgl^{-1}), indole-3-acetic acid (IAA: 0, 0.4, 0.8, 4 mgl^{-1}), indole-3-butyric acid (IBA: 0, 0.1, 0.5, 2.0, 5.0 mgl^{-1}) at different concentrations, either individually or in combinations. All cultures were incubated under complete dark conditions for eight weeks. At the end of eight weeks, explants were sub cultured onto media with the same composition and shoots emerged after two weeks of first sub culturing. Then, 4 weeks after the first sub culture, all the cultures were transferred to 1000 $\mu molm^{-2}s^{-1}$ light intensity. The frequency at which explants produced shoots and the number of shoots per explant were recorded after sixteen weeks of culture establishment. Once plantlet regeneration was achieved, individual shoots were subcultured in culture tubes (1.5 cm x 5.0 cm) containing 10 ml of MS medium supplemented with different concentrations of BAP (0, 0.5, 1.0, 2.0 mgl^{-1}) for further multiplication. Number of shoots produced per single explant was recorded at the end of each subculture period (8 weeks).

Rooting of regenerated shoots

Microshoots of 1.5-2.0 cm in length with 3-4 pairs of leaves were excised individually and transferred either to a MS medium without sugar, with 3% sugar, 4% sgar or MS medium with 3% sugar and 1 mgl^{-1} NAA or different concentrations of IBA (0.1, 0.5, 1.0, 2.0 mgl^{-1}). The percentage of rooting and the number of roots per shoot after eight weeks of transfer onto the rooting medium was recorded.

Acclimatization

Plantlets with five or more leaves and three to four roots were removed from the culture medium, washed gently under running tap water and transferred to plastic pots containing sterile compost and sand (1:3) under diffuse light (16:8 h photoperiod, 3000 $\mu molm^{-2}s^{-1}$) conditions. Potted plantlets were covered with a transparent polythene membrane to ensure high humidity and watered every three days for a week. Polythene membranes were opened gradually in order to acclimatize plants to field conditions. After four weeks, these plants were transferred to pots containing normal compost and sand (1:3) medium and maintained in a greenhouse under normal day length conditions.

Thin Layer Chromatography (TLC) profiling of tissue cultured and wild grown plants

For TLC profiling, material was harvested from mature, naturally grown *M. pinnata* plants (flowering and fruit setting) and acclimatized micropropagated plants. Four grams of plant material (leaves (2.0 g), stems (1.0 g), roots (1.0 g)) was frozen in liquid nitrogen and grounded with a mortar and pestle to a fine homogenous powder. The powdered samples were shaken on an orbital shaker (Stuart Scientific-SO1) with Hexane (2 x 2 ml) for one hour. The extract was filtered through a Whatman No 1 filter paper and evaporated to dryness on a rotovapor (< 40 °C). The residue was sequentially re-extracted with hexane, dichloromethane and ethyl acetate. The extracts were re-dissolved in 1 ml of same solvent and separated by TLC on silica gel (GF254) plates using 2 different solvent systems. The solvent system used for hexane and dichloromethane extracts consisted of 95% hexane and 5% ethyl acetate while Ethylacetate: Methanol (95: 5) was used as the solvent for ethyl acetate extracts. The tank was saturated with the solvent before inserting the silica gel plate with samples. The TLC chromatograms were observed under UV light (254 nm wavelength and 366 nm wavelength) and photographed using a digital camera (Sony 7.2 M.P).

Statistical analysis

The experimental design was fully randomized (CRD). All the experiments were conducted with a minimum of 20 replicates per treatment. The experiments were repeated three times. The cultures were observed periodically and morphological changes were recorded at regular intervals. The results were analyzed using Statistical Analysis Software (SAS) version 9.1 (SAS institute, SAS Campus Drive, Cary, NC 27513, USA). Data on length was analyzed by analysis of variance (ANOVA). The significance of differences among means was tested using Least Significant Difference (LSD) at $\alpha = 0.05$. Data on number of shoots and roots was analyzed by the Chi-square method.

RESULTS

Shoot initiation and multiplication from nodal explants

Under the conditions employed, swelling of the dormant axillary bud took place within seven days, and then differentiation into multiple shoots occurred after four weeks. Explants cultured on the medium without growth regulators (control) did not multiply while the medium containing 2 mgl^{-1} BAP tested, produced an average of 3.00 ± 0.32 shoots per explant in 96% of cultures. Upon lowering the concentration of BAP, a reduction in the number of shoots per culture was recorded. Similarly, at a higher concentration (5.00 mgl^{-1}) the number as well as the percentage of shoot formation was drastically reduced (Table 1).

Nodal explant with axillary buds appeared like a clump without any callus and a number of buds of size ranging from 0.3 - 1.45 cm could be carefully separated after eight weeks. These shoots were transferred onto same medium except 5 mg ml^{-1} BAP concentration for further multiplication. The excision of node segments and their subculture on MS medium supplemented with the same growth regulators enhanced the rate of shoot multiplication (Figure 1A) and the length of shoots (Figure 1B). Of the four levels of BAP tested, 2.00

mgl^{-1} showed to be most successful, as in this concentration an average of 6.32 ± 0.24 shoots with the average length of 2.73 ± 0.08 cm were developed at the end of the first subculture or 16 weeks after establishment of the explants.

Table 1. Effect of BAP concentration on shoot induction from nodal explants of *M. pinnata*

BAP concentration (mgl^{-1})	Shoot formation (%)	Number of shoots per explant	Shoot length (cm)
0	36	0.36 ± 0.10	0.78 ± 0.08^{bd}
0.5	48	0.52 ± 0.12	1.34 ± 0.14^{bc}
1	56	1.20 ± 0.28	1.38 ± 0.16^{bc}
2	96	3.00 ± 0.32	1.45 ± 0.10^{a}
5	28	0.72 ± 0.25	0.41 ± 0.04^{bd}

Means followed by the same letter are not significantly different

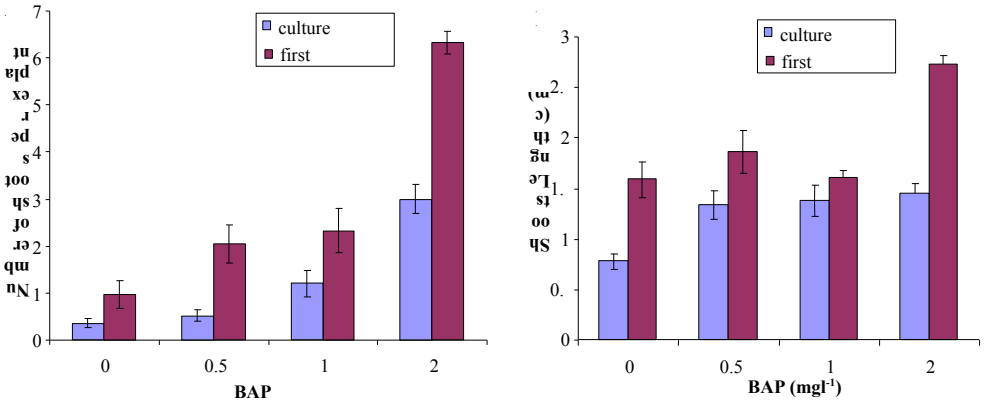

Figure 1. Effect of progressive culture of nodal explant derived *M. pinnata* shoots on MS medium supplemented with same concentration of BAP

Shoot initiation and multiplication from leaf explants

Direct shoot regeneration occurred from the cut margins of leaf explant within ten weeks of culture induction in the dark. Response of explants to shoot induction medium was vigorous and multiple shoots were generated from the cut margins. The morphogenetic responses of leaf explants to various concentrations of auxine alone (IBA) or combinations of auxins and cytokines are summarized in Table 2.

Except the leaf explants cultured on media without growth regulators (control) or IBA alone, all the other treatments induced shoot regeneration through direct organogenesis (Table 2) and the primodia always regenerated directly from leaf without forming callus. Among the various combinations of growth regulators, leaf explants cultured on MS medium supplemented with 1.00 mgl^{-1} BAP and 2 mgl^{-1} IBA exhibited the highest shoot regeneration

frequency (70%) with highest number of shoots (8.55 ± 1.47 per explants). Upon increasing

Table 2. Shoot initiation from leaf explants of *M. pinnata* on MS medium supplemented with different growth regulators

Growth regulator (mgl^{-1})			Percentage response	Number of shoots per explants
BAP	IAA	IBA		
Growth regulator free			0	0
1	0.4		30	2.05 ± 0.79
1	0.8		45	3.60 ± 1.04
1	4.0		20	0.35 ± 0.18
2	0.4		50	3.65 ± 0.97
2	0.8		30	0.8 0 ± 0.30
0		0.1	0	0
0		0.5	0	0
0		2.0	0	0
0		5.0	0	0
1		0.0	30	1.25 ± 0.57
1		0.1	25	1.35 ± 0.55
1		0.5	40	2.10 ± 0.72
1		2.0	70	8.55 ± 1.47
1		5.0	30	4.50 ± 1.57
2		0.0	40	3.25 ± 1.11
2		0.1	10	0.30 ± 0.25
2		0.5	30	2.25 ± 0.89
2		2.0	25	1.80 ± 0.86
2		5.0	50	4.40 ± 1.00

Values represent means ± standard error of three independent experiments, each with 25 replicates. Observations were made after 16 weeks of culture

Figure 2. *In vitro* propagation of *M. pinnata*.

and decreasing the concentration of IBA, a gradual decrease in regeneration frequency and the number of shoots per explant were recorded (Table 2).

Leaf explants with regenerated shoots appeared like a clump (Figures 2A, 2B). The shoots were separated from the explants and prolific shoot formation from single shoot was subsequently achieved on MS medium consisting of 0, 0.5, 1 and 2 mgl^{-1} BAP concentrations (Table 3). Highest shoot multiplication was observed in the medium containing 2.00 mgl^{-1}BAP and this was 13.33 ± 0.32 shoots from a single shoot within 8 weeks.

(A) Shoot growth from leaf explant after 12 weeks on MS medium with 1 mgl^{-1} BAP and 2 mgl^{-1} IBA (B) Shoots regenerated from leaf explants after 16 weeks on MS medium with 1 mgl^{-1} BAP and 2 mgl^{-1} IBA (C) Rooting of plantlets on MS medium with 1 mgl^{-1} IBA (D) Rooting of plantlets on MS medium with 1 mgl^{-1} NAA (E) Micropropagated plants 8 weeks after transfer to soil

Table 3. Effect of BAP on multiplication of leaf explant derived shoots

BAP (mgl^{-1})	Number of shoots per explant ± SE
0	7.07 ± 0.23
0.5	8.47 ± 0.39
1	11.67 ± 0.40
2	13.33 ± 0.32

In vitro* rooting of *M. pinnata

Root formation from the basal cut end of the shoots was observed two weeks after transferring to the rooting media and the frequency of rooting increased gradually over time and reached a maximum after eight weeks of culture. Shoots cultured in a medium without sugar and auxins did not produce roots. In the absence of auxins, only 40% of shoots produced roots in the medium containing 3% sugar, while 60% in the medium containing 4% sugar. A similar low response in the absence of growth regulators was also reported in previous studies on *M. pinnata* (Sugathadasa, 2001; Fernando, 2003; Yapabandara *et al.,* 2003). The rooting of microcuttings was significantly affected by the concentration of IBA. The highest percentage of root induction (90%) together with the highest root number (4.2 ± 0.66) was achieved in MS medium fortified with 1 mgl^{-1} IBA and 3% sugar (Figure 2C). Upon increasing and decreasing the concentration of IBA, number of roots per culture as well as the percent response were drastically reduced (Table 4). Even though 65% of cuttings produced average of 4.0 ± 0.91 roots in medium fortified with 1 mgl/ NAA, a callus formed at the base of every single cutting (Figure 2D). Roots developed were short and thicker than other treatments (Figure 2D).

Acclimatization

Plantlets with 6-8 fully expanded leaves and well-developed roots were subsequently transplanted to small pots filled with sterilized sand and acclimatized in a mist chamber for 2 weeks at high relative humidity (75%). These plants were then transferred to the greenhouse. About 80% of the regenerated plants survived after eight weeks following transfer from sand

to natural soil and no detectable variation in morphology or growth characteristics was observed (Figure 2E).

Table 4. Rooting of *in vitro* derived *M. pinnata* plantlets on MS medium supplemented with different percentages of sugars and auxin concentrations

Growth regulators (mgl^{-1})		Sugar (%)	Percentage Response	Number of roots per shoot	Percentage of callus
IBA	NAA				
		0	0	0	0
		4	60	3.95 ± 1.18	0
		3	40	1.65 ± 0.61	0
	1	3	65	4.00 ± 0.91	100
0.1		3	50	2.90 ± 1.06	0
0.5		3	70	2.00 ± 0.42	0
1.0		3	90	4.20 ± 0.66	0
2.		3	45	1.40 ± 0.47	0

TLC profiling of tissue cultured and wild collected plants

The TLC chromatogram pattern in the cloned plantlets were identical to that of the mother plants, under both 253 nm and 366 nm wavelength for all extracts *i.e.* hexane (Figure 3A), dichloromethane (Figure 3B) and ethylacetate (Figure 3C).

DISCUSSION

Successful application of micropropagation techniques involves the regeneration of plants from cultured tissues at high frequencies without genotypic changes. Axillary bud multiplication and adventitious shoot formation directly from explants are unquestionably better approaches than the callus method for clonal propagation of the plant species since propagation through calli often produced cytologically abnormal plants (Cassells *et al.*, 1980). Therefore, in the present study, direct shoot organogenesis of leaf and shoot explants of *Munronia pinata* was investigated.

Using tissue culture, the rate of shoot multiplication of axillary buds can be substantially enhanced by growing shoots in a nutrient medium containing a suitable cytokinin, with or without an auxin. In the present study, 2.00 mgl^{-1} BAP proved to be more effective in axillary bud multiplication of *M. pinnata*. Among the different levels of BAP tested, 2.00 mgl^{-1} BAP produced the maximum number of shoots with highest average length. Increasing BAP concentration to 5.00 mgl^{-1} negatively modified the frequency of shoot induction and growth. A similar result was reported in *in vitro* multiplication of *Rotula aquatica* (Martin, 2003).

Axillary bud multiplication stops at some point in a single cycle adding a limit to number of plants that can be obtained. At this stage, planting of shoots on a fresh medium with same hormone composition facilitates repeating the multiplication cycle. In the present study, planting of shoot on fresh medium supplemented with the same growth regulators enhanced

both rate of shoots multiplication and the average length of shoots. During subculture, basal axillary buds also underwent initiation. In a previous study on *M. pinnata*, Yapabandara *et al.*, (2003) achieved about fifteen shoots per explant with an average length of 2.00 cm. However, they used shoot tips as the explant which is one per mother plant. In the present study, about 15-20 nodes were obtained from one mother plant showing the new method is very effective in large-scale propagation.

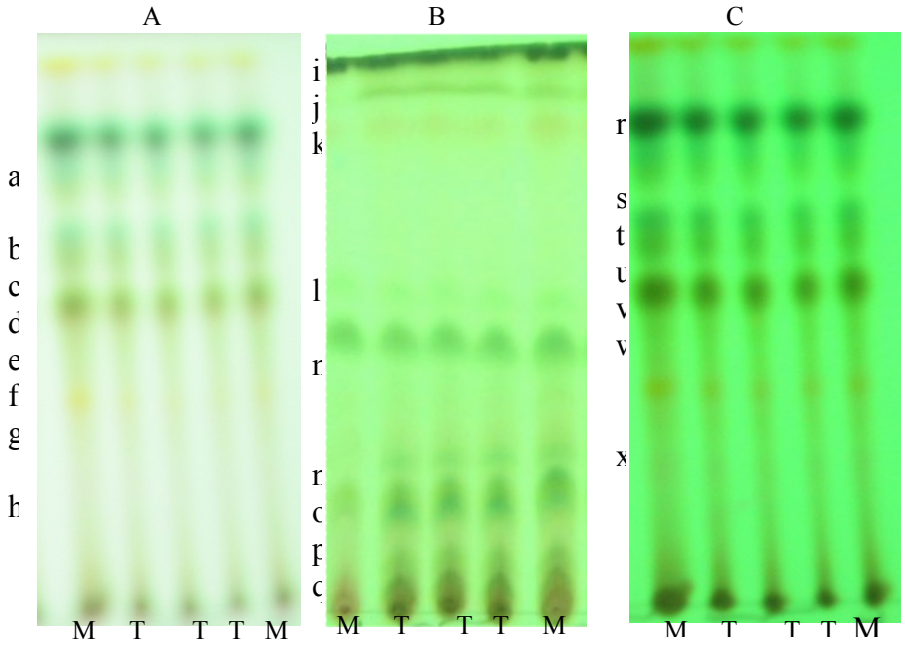

Figure 3. TLC chromatograms of tissue cultured plants (eight weeks after soil establishment) and mother plants obtained at 254nm wavelength (A) Hexane extracts (B) Dichloromethane extracts and (C) Ethylacetate extracts

M: mother plants, T: tissue cultured, a-x: different compounds

Adventitious bud formation is the process where shoot buds arise directly from a plant organ or piece of tissue without an intermediate callus phase. In tissue culture, under the influence of an appropriate combination of growth regulators, adventitious buds can be induced on explants of the species normally not propagated vegetatively (Cassells *et al.,* 1980). The effect of BAP and IBA on multiple shoot bud differentiation has been demonstrated in a number of medicinal plant species using a variety of explants (Azad *et al.,* 2005; Feyissa *et al.,* 2005). In the present study, 1.00 mgl^{-1} BAP and 2.00 mgl^{-1} IBA proved to be more effective than other concentrations and other combinations of phytohormones tested. Application of auxin, IBA alone did not give any response. The synergetic effect of BAP in combination with an auxin in shoot induction has been demonstrated in medicinal plants such as *Bupleurum fruticosum* (Fraternale *et al*., 2002) and Turmeric (Salvi *et al*., 2002). In accordance with these reports, the present study also illustrates the positive effect of

applying a low concentration of auxin in combination with a cytokinin than application of auxin alone.

Leaf explant with regenerated shoots appeared like a clump. The shoots were separated from the explants and prolific shoot formation from single shoot was subsequently achieved on MS medium which consisted of different BAP concentrations (Table 3). A concentration of 2.00 mgl^{-1} BAP was found to be the most effective for shoot multiplication, in which 13.33 ± 0.32 shoots developed from a single shoot. This result is in accordance with the results obtained with the nodal explants, exemplifying the positive effect of 2 mgl^{-1} BAP on shoot multiplication efficacy of *M. pinnata*. However, number of shoots recorded per explant was significantly higher from shoots that originated from leaf segments (13.33 ± 0.32) compared to shoots derived from nodal explants (6.32 ± 0.24). Shoots that originated from leaf explants were more responsive than those of nodal explants and this differential morphogenetic response could be due to differences between the physiological states of the buds on different regions of the plants. As described by Lisowska and Wysonkinska (2000), this difference between plantlets obtained from different explant types could also be a reflection of probable differences of endogenous growth regulator levels in the explant source or different tissue sensitivities to plant growth regulators. However, the observed shoot forming ability of both the *in vitro* derived explant types greatly enhances the mass multiplication potential of the culture systems. This propagation regime has the capacity for producing more than 100 plants from 1cm^2 leaf explant within 24 weeks, making it highly attractive for implementation.

To optimize the rooting of microcuttings, two different auxins, IBA and NAA were tested at various concentrations. Rooting response was very low in the absence of auxin and a similar low response in the absence of growth regulators was also reported in previous studies on *M. pinnata* (Sugathadasa, 2001; Fernando, 2003; Yapabandara *et al*., 2003). In general, IBA is known to promote rooting in a wide range of plant species. In the present case, rooting of *M. pinnata* was significantly affected by the concentration of IBA in the medium. The best rooting was achieved in MS medium fortified with 1 mgl^{-1} IBA. Percentage response and number of roots per plant achieved in present study are much higher than previous studies on *M. pinnata* where Senarath *et al.,* (2007) reported 75% rooting with maximum length of 0.96 mm (in ½ MS + 0.2 mgl^{-1} IBA) and Yapabandara *et al.,* (2003) observed 60% rooting (on MS + 0.2 mgl^{-1} IBA). In the 1 mgl^{-1} NAA medium, a callus was formed at the base of the microcutting, retarding root formation and the subsequent growth of roots. Similarly, callus formation in NAA supplemented media has been observed during *in vitro* rooting of medicinally important *Holostemma ada-kodian*, (Martin, 2002) and *Charybdis numidica* (Kongbangkerd *et al.,* 2005).

Although micropropagation is a feasible solution to provide large number of plants in shorter period for cultivation and conservation purposes, *in vitro* culture procedures such as application of phytohormones and *in vitro* culture conditions may affect biochemical pathways as well as production and storage of chemical substances in certain plant species. In general, *ayurvedic* doctors and traditional practitioners prefer plants propagated with natural means than *in vitro* originated material. Therefore, after optimizing all propagation steps, the qualitative chemical composition of tissue cultured plants and mother plants were compared. TLC fingerprint profiles of tissue culture derived plants and mother plants were identical, exemplifying their qualitative similarity in chemical composition. Similar results

were also obtained in *Rauvolfia serpentina* (Sugathadasa, 2001), *Lonicera tatarica* (Palacios *et al,* 2002) *Harpagopphytum procumbers* and *H. Zeyheri* (Levieille and Wilson, 2002) *Charybidis numidica* (Kongbangkerd *et al.,* 2005) and *Celastrus paniculatus* (Martin *et al.,* 2006).

CONCLUSIONS

This study developed efficient methods for adventitious shoot regeneration from leaf and nodal explants of *M. pinnata.* Approximately 114 shoots could be obtained from one leaf explant (10 x 10 mm) in 24 weeks, while one node produced 18 shoots in 16 weeks. About 90% of multiplied shoots could be rooted on MS medium supplemented with 3% sugar and 1 mg/L IBA. Eighty percent of rooted plantlets survived under greenhouse conditions after eight weeks following soil transfer. The TLC chromatogram pattern in micropropagated plants was identical to those of mother plants.

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