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IN VITRO MASS PROPAGATION AND GREENHOUSE ESTABLISHMENT OF *Munronia pinnata*

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Thesis submitted to the University of Sri Jayewardenapura for the award of the Degree of Master of Philosophy in Botany on Plant tissue culture

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To my father who has taken care of six of us all by himself since 1984,

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teachers whose enthusiastic encouragement made me to successful in higher education.

ABBREVIATIONS

BAP	-	6-benzylaminopurine; BA
DDH_2O	-	double-distilled water
2,4-D	-	2,4-dichlorophenoxyacetic acid
GA_3	-	giberellic acid
IBA	-	indole-3-butyric acid
IAA	-	indole-3-acetic acid
Kin	-	kinetin
MS	-	Murashige and Skoog`s (1962) medium
NAA	-	α-naphthaleneacetic acid

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K.G.P.H. CHANDRASENA

ABSTRACT

Munronia pinnata (Wall) Theob. (Sin: Binkohomba, Family Meliaceae) is a valuable medicinal plant, which is widely used in ayurvedic medicine in Sri Lanka. The status of the species and the existence of specific threats such as over exploitation and low percentage of seed germination had caused to adopt *ex situ* conservation methods via micropropagation techniques. *In vitro* culture techniques are widely used in plant conservation of a large number of species. The aim of this work was to establish a method for micropropagation and greenhouse establishment of *M. pinnata*.

Petioles, stem parts, petals and leaves were tested to select a suitable explant source for initiation of callus. Explants were cultured on MS basal media with 1.1:0.5 mg/l 2,4-D: BAP and they were maintained in complete dark at 25±1°C. Leaf disc explants showed the best result by producing the highest amount of callus (0.17±0.04 g). For the initiation of callus, combinations of 2,4-D and BAP were tested on MS basal medium and 1.1 mg/l 2,4-D and 0.3 mg/l BAP produced the highest amount of callus (0.304±0.05 g) after six weeks of incubation in complete dark at 25±1°C.

It was observed that the callus initiation varies with the maturity stage of the leaves and the first fully opened leaves produced higher amount of callus (0.06±0.01 g) than the other

tested mature leaves. Variation of the callus initiation within the leaf was also tested (midrib and lateral parts) and it has shown the ability of producing more calli along the midrib. Six-week-old calli were transferred to shoot initiation media, which contained combinations of NAA and BAP (1-5 mg/l NAA and 1-5 mg/l BAP) in MS basal medium. Among the tested treatments, 3 mg/l NAA and 3 mg/l BAP produced the highest number of shoots (32.9±2.2) with highest shoot length of 31.63±1.9 mm and with 6.12±2.2 of number of leaves in 16h photo period at 25±1°C after eight weeks of inoculation. Out of tested light intensities (1000, 2000, 3000, 4000 Lux), 3000 Lux light intensity gave the highest values for mean number of shoots 33.4 ±2.9 mean shoot length 30.52±2.7 mm and mean number of leaves (6.32±0.5).

Shoots were transferred to a series of root initiation media (0.1–0.5 mg/l IBA in full and ½ MS basal media separately, 0.1-0.5 mg/l IAA in full and ½ MS basal media separately) and ½ MS medium with 0.2 mg/l IBA produced mean root length of 9.68±1.1 mm, mean number of roots, 3.25±0.3 and percentage of root initiation was 75 in 16 h photo period at 25±1°C. The same basal medium (½ MS) supplemented with 0.2 mg/l IAA gave 15.5±2.5 mm of mean root length, 3.15±0.3 mean number of roots and 70% of root initiation in 16h photo period at 25±1°C.

For acclimatization, tissue cultured plants were introduced into the potting mixtures containing different ratios of compost and sand, and 60 % of survival rate was achieved

with the mixture of compost: sand (1:3) after maintaining pots in the growth chamber for four weeks.

Growth of *in vitro* produced plants compared with seed-raised plants of same maternal genotype over a period of nine months. Height increment was significantly different between two plant types. Seed-raised plants have shown mean height increment of 5.31±0.3 cm while it was 3.34±0.4 cm in tissue cultured plants. *In vtiro* propagated plantlets showed a higher number of leaves (12.2±1.2) while it was 9.5±0.8 in seed-raised plants. Only tissue cultured plants have shown branching (1.3 ±0.7), which is a much favorable quality when compared with the rarely branched or unbranched natural plants. Photosynthetic ability was slightly higher in tissue cultured plants (6.96 $\mu molCO_2m^{-2}s^{-1}$) than seed-raised plants (5.9 $\mu molCO_2m^{-2}s^{-1}$) after six months and values of nine month old plants revealed further improvement of photosynthesis in both plant types with time (tissue cultured plants, 7.6 $\mu molCO_2m^{-2}s^{-1}$ and seed-raised plants, 6.6 $\mu molCO_2m^{-2}s^{-1}$). Ability of water control was poor in tissue cultured plants (4.5 scm^{-1}) when compared to the seed-raised plants after six months (7.2 scm^{-1}), but became more controllable condition after nine months (tissue cultured plants 4.9, scm^{-1} and seed-raised plants, 8.0 scm^{-1}) of growth in the greenhouse. All these measures proved the possibility of propagating the plant *in vitro* with some superior qualities and would establish under natural conditions. Therefore tissue culture could be used as a technique for clonal propagation of the species and would be beneficial in supplying plants for large scale growers or companies and to use in *in situ* conservation.