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AN EFFICIENT MULTIPLE SHOOT INDUCTION PROTOCOL FROM NODAL AND ROOT EXPLANTS OF *ATALANTIA MONOPHYLLA* (L.) DC., A MEDICINAL PLANT.

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ABSTRACT

Atalantia monophylla (L.) DC. is a medium sized medicinal tree used in Ayurveda, Siddha and Traditional systems of medicine. It is used for the treatment of chronic rheumatism and paralysis, as an antidote for snake bites and shows antibacterial, antifungal and anti-feedant activity. The study aims to develop a protocol for multiple shoot induction from nodal and root explants. After surface sterilization the nodal portions were inoculated in MS medium supplemented with various concentrations of BAP and KIN alone or in combination. The root explants were inoculated into White's root culture medium supplemented with various concentrations of BAP. In nodal culture the best response was obtained when MS medium supplemented with 3.5mg/l BAP and 1.0 mg/l KIN (8 shoots). While in root culture the best response of 5 shoots were obtained when White's root culture medium was supplemented with 5mg/l BAP. The regenerated shoots were further subcultured into MS medium supplemented with IAA and IBA to produce individual plants. This study revealed that both nodal and root explants could be efficiently used for direct shoot regeneration of *A. monophylla*.

KEYWORDS: *Atalantia*, Rutaceae, Tissue culture, Medicinal plant

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INTRODUCTION

Atalantia monophylla (L.) DC. is a medium sized tree found in dry evergreen forests and coastal shrub forests, belonging to the family Rutaceae. It is commonly known as "Kattu-naregam" in Malayalam and "Kattu-elumichai" in Tamil. *A. monophylla* resemble *Citrus* in general aspects which are small to medium trees, spinous, bearing white fragrant flowers and small globose fruits that look like small greenish-yellow limes[1]. The plant is used in Ayurveda, Siddha and traditional systems of medicine. Various parts of *A. monophylla* have been used in folk medicine for several purposes such as the treatment of chronic rheumatism and paralysis[2]. Traditional medicinal practitioners in Villupuram district, Tamil nadu use the leaves for the treatment of rheumatoid pain and glandular swelling and the essential oil of the fruit is used for joint pain[3]. The essential oil from the leaves showed antimicrobial and strong inhibitory activities against certain pathogenic fungi[4]. Berries are used for making pickles and leaves are used as flavoring agent. Wood is hard and close-grained which is highly recommended for making furniture as it is strong and shock resistant. It is also a source of firewood for the local communities. The plant is used as a rootstock for breeding new cultivars of *Citrus*. Juice of berries is used for dyeing purpose[5]. The plant is a valuable source of coumarins, terpeniods and anti-allergic acridone alkaloids [6-7]. The natural population is gradually shrinking due to habitat destruction, collection of wood, seeds for pickling and extraction of leaves, fruits and roots for medicinal purposes by the local communities. Hence there is a need for developing an appropriate *in vitro* conservation strategy for this species. Micropropagation offers a great potential for large scale multiplication of useful plant species and subsequent exploitation[8]. Multiple shoots are normally induced from explants such as shoot tip, nodal, leaves and rarely from roots. *De novo* shoot regeneration from root explants were reported in several plant species such as *Citrus aurantifolia, Hypericum perforatum, Swertia chirata* and *Garcinia indica*[9-12]. The root and bud formation in tissue culture is dependent on a specific equilibrium between the auxins and the cytokinins, gibberellins and cytokinins ratio, which control the shoot and leaf development[13]. Root tissue has been proven to be highly regenerative explants and is relatively easy to maintain and manipulate *in vitro*[14-15]. Besides, shoots developing on root segments of the same plant have been reported to be genetically uniform and the method, an alternative way for clonal propagation[16-18]. Root explants are advantageous over other explants in terms of their easy manipulation and higher regeneration potential[19]. Tissue culture research in genus *Atalantia* is limited to callus initiation in *A. monophylla* and *A. ceylanica*[20] and somatic embryogenesis in *A. ceylanica*[21]. Micropropagation works were successfully carried out in *Atalantia* related genus such as *Citrus* and *Severina*[22-23]. Therefore in the present study an attempt was made to develop an *in vitro* conservation strategy through multiple shoot formation from both nodal and root explants for this important medicinal plant.

MATERIALS AND METHODS

Explant source

The seeds of *A. monophylla* were collected from Nagamalai hills, Madurai, Tamil Nadu. The seeds were germinated in the Botanical garden of the Madurai Kamaraj University. The explants (both nodes and roots) were taken from six month old germinated seedlings. The plant was identified at Botanical survey of India, Southern circle, Coimbatore (Fig. 1).

Surface Sterilization

The explants were washed thoroughly in running tap water for 20 minutes and placed in a detergent solution (5% Teepol) for 15 minutes. Then they were rinsed with distilled water for 10 minutes. After that the explants were taken to laminar air flow and surface sterilized with 5% sodium hypochlorite for 2 minutes and finally in 0.1% mercuric chloride solution for 3 minutes followed by thorough rinsed with sterile distilled water. The explants

were then individually inoculated on solidified MS media with various concentrations and types of growth hormones.

Multiple shoot induction

Both nodal and root explants were used for multiple shoot induction. The experiment was conducted to find out the suitable growth hormone concentrations for the maximum production of multiple shoots. The nodal explants were inoculated in MS medium and root explants were inoculated in White's root culture medium. Different concentration of plant growth regulators (PGRs) such as 6-Benzylaminopurine (BAP) and Kinetin (KIN) alone or in combination was tried out. The explants were trimmed gently with the help of the sterile surgical blade (Lister No.10) and the explants were vertically inoculated into the medium. After inoculation the percentage of shoot induction and length of the shoot was observed after 6-7weeks of culture. The cultures were subcultured at an interval of 2 weeks. All the cultures were maintained in the culture room at temperature 25±2°C and relative humidity of 80-85%. The cultures were kept under fluorescent light at an intensityLux 2000 lux provided from fluorescent lamps with 16 hours light and 8 hour dark conditions.

Rooting and hardening

The obtained shoots from both nodal and root cultures were separated and subcultured into MS medium supplemented with various concentrations of IAA and IBA for rooting. Rooted plantlets were transferred to paper cups containing sterile soil for hardening at 16/8 hr photoperiod conditions and covered with plastic bag to maintain 80-90% humidity. Subsequently, the plantlets were transferred to greenhouse and after one month they were transferred to the soil.

RESULTS AND DISCUSSION

Multiple shoot initiation from nodal explants

Multiple shoot initiation from nodal explants was carried out using MS medium supplemented with different concentrations of BAP and Kinetin (Fig. 2 a-d). Among the different concentration of media tried out for initiating shoots from nodal explants, maximum shoots were obtained in MS medium supplemented with 3.5 mg/l BAP and 1.0 mg/l KIN. 83% of the inoculated shoots responded and maximum of 8 shoots were obtained (Table 1, Fig. 2 d). Even though 100% response was obtained when the concentration was increased, the number of shoots reduced and also shown stunted growth. It was seen that in nodal culture when the PGRs were tried separately maximum shoots were obtained in MS medium supplemented with 6.0 mg/l BAP (Table 2, Fig. 2 b). Maximum of 4 shoots were produced when 6.0 mg/l BAP was supplemented alone. When the hormone concentration was increased the multiple shoots were induced, but the shoot growth was stunted (Table 2, Fig. 2 c). The results obtained are in accordance with an earlier work reported in *C. jambhiri* where maximum response was obtained at 3mg/l BAP[24]. Also in *C. reticulata* multiple shoots were obtained when MS medium supplemented with BAP and KIN in combination[25]. When the lower concentration was used, the explants produced a single shoot with no indication of root formation. When Kinetin was used alone maximum of 4 shoots were obtained at 4 mg/l concentration.

Multiple shoot initiation from root explants

When the roots were used as the explants for multiple shoot formation, the best response was obtained when Whites root culture medium supplemented with 5mg/l BAP (5 shoots) (Table 2, Fig. 3b). When the hormone concentration was decreased the shoot length increased and maximum shoot length was obtained at 3.0 mg/l BAP (Table 2, Fig. 3a). Also when the hormone concentration was increased, number of multiple shoots formation also increased. Similar results for multiple shoot induction using BAP from root explants were obtained in *C. mitis*[26]. The histological observations indicated that the new shoots in root culture are regenerated from the cortical region of the roots. In root cultures of *Garcinia indica*, some of the cells of the cortical region became meristematic and produced shoot primordial and were pushed outside the epidermal layer[12].

Root initiation and hardening of regenerated shoots

The regenerated shoots from both nodal and root culture were further subcultured into MS medium supplemented with IAA and IBA to obtain individual plantlets. The maximum rooting was observed in MS medium supplemented with 3.0mg/l IAA (Fig 3 c,d, 4). In earlier works also it was found that MS medium supplemented with IAA was found to be the ideal for rooting[27]. Rooted plantlets were acclimatized and about 80% of the rooted plantlets survived in the greenhouse conditions (Fig. 3e). In summary, this work represents an ideal protocol for micropropagation of *A. monophylla*. The plant regeneration from meristems is one of the most promising ways for multiplying a species true to its type. Such plants are genetically similar showing the same agronomic characteristics. Further studies are needed to check the genetic uniformity and comparison between *in vitro* grown and field-grown plants in terms of their secondary metabolites and biological activities.

Figure 1
Habit of A. monophylla

Figure 2 (a)

Shoot induction from nodal explants when MS Media supplemented with 3mg/l BAP, (b) Multiple shoot induction from nodal explants when MS Media supplemented with 6mg/l BAP (c). Multiple shoot induction from nodal explants when MS Media supplemented with 7mg/l BAP, (d) Multiple shoot induction from nodal explants when MS Media supplemented with 3.5mg/l BAP +1mg/l KIN.

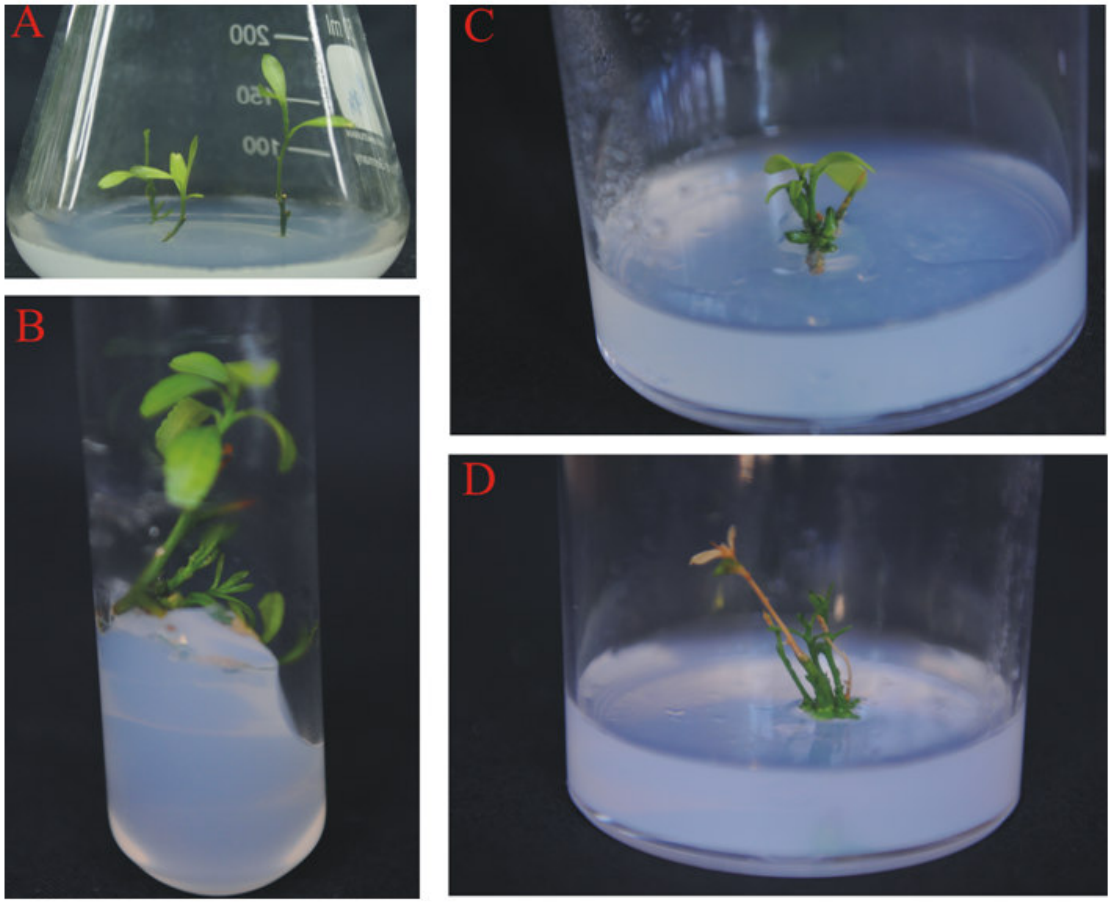

Figure 3 (a)

Shoot induction from root explants when Whites root culture media supplemented with 3.0mg/l BAP, (b) Multiple Shoot induction from root explants when Whites root culture media supplemented with 5.0mg/l BAP, (c) Root initiation in regenerated shoots when MS media supplemented with 3.mg/l IBA, (d) Root initiation in regenerated shoots when MS media supplemented with 3.mg/l IAA, (e) Two weeks old acclimatized plant.

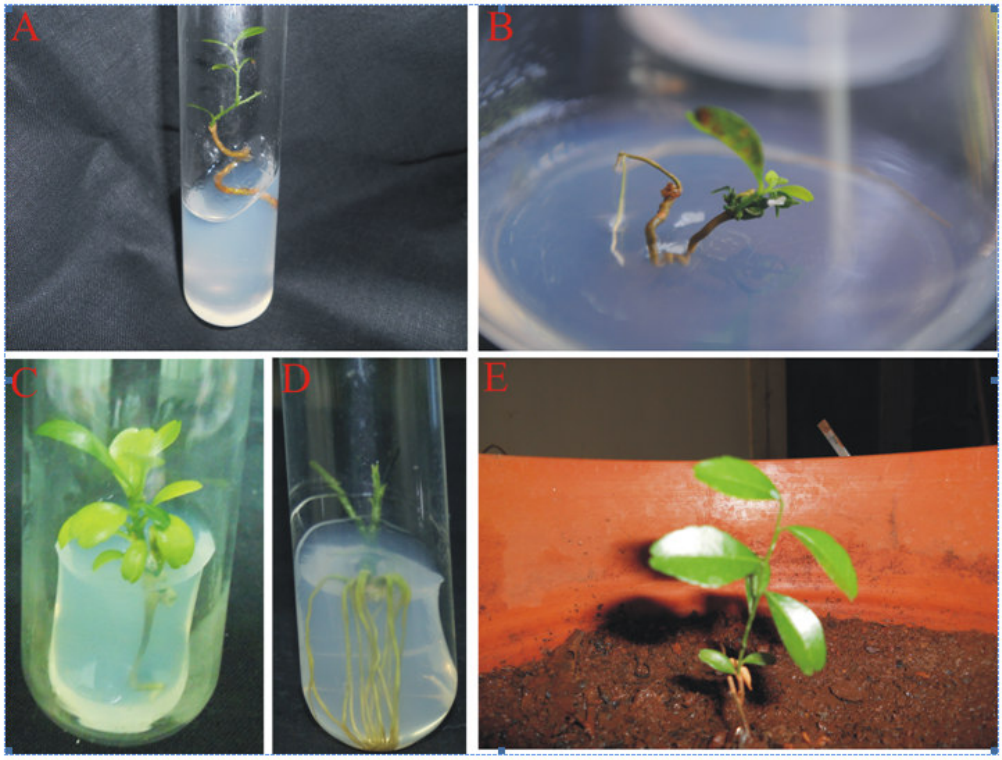

Figure 4

Effect of MS medium supplemented with IAA and IBA on root formation (values are mean ± SD of 6 replicates)

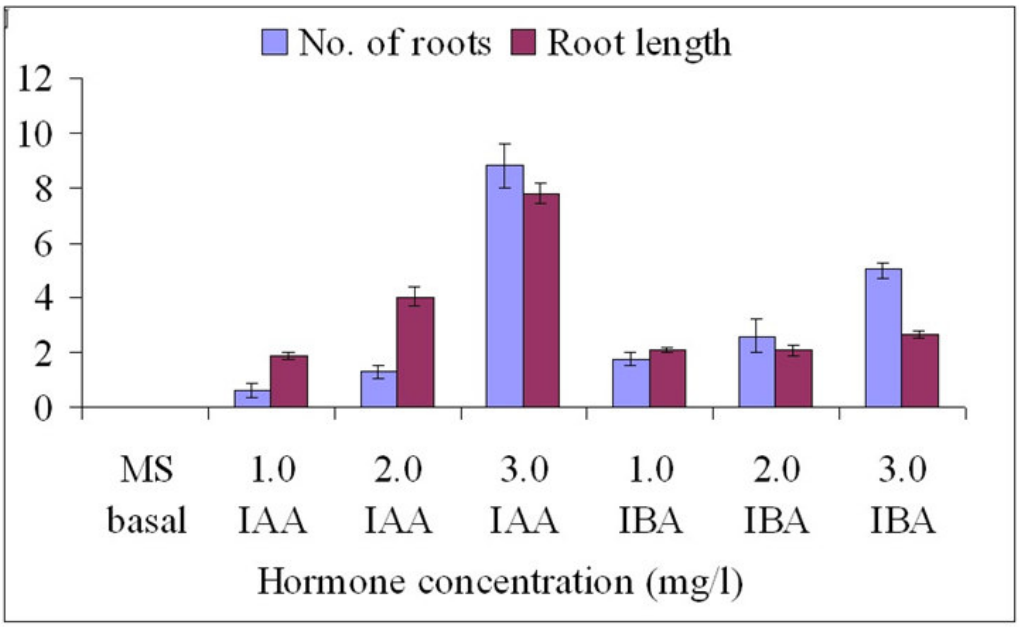

Table 1

Multiple shoot induction using MS media supplemented with different concentrations of BAP and KIN (values are mean ± SE of 6 replicates). Mean values with different alphabets are statistically significant at P<0.05.

BAP (mg/l)	KIN (mg/l)	Percentage of Response	Number of shoots	Shoot length (Cm)
1.0	-	0.0	-	-
2.0	-	33	1.0±0.00^{j}	2.25±0.15^{d}
3.0	-	50	1.0±0.00^{j}	3.63±0.15^{c}
4.0	-	67	3.33±0.33^{g}	3.20±0.41^{d}
5.0	-	67	3.4±0.25^{f}	2.49±0.31e
6.0	-	83	4.2±0.2^{d}	6.80±0.1^{a}
7.0	-	83	5.4±0.24^{c}	0.91±0.02^{i}
-	1.0	0.0	-	-
-	2.0	67	1.0±0.0^{j}	0.45±0.05^{j}
-	3.0	67	1.0±0.0^{j}	0.52±0.08^{j}
	4.0	83	3.6±0.25^{e}	3.19±0.14^{d}
	5.0	100	2.25±0.25^{i}	1.38±0.07^{g}
1.0	1.0	67	1.0±0.0^{j}	0.45±0.05^{j}
1.5	1.0	100	1.0±0.0^{j}	0.95±0.05^{h}
2.0	1.0	83	3.0±0.0^{e}	1.43±0.14^{g}
2.5	1.0	83	3.25±0.25^{h}	2.45±0.42^{f}
3.0	1.0	100	4.33±0.33^{d}	5.23±0.05^{b}
3.5	1.0	83	7.4±0.4^{a}	6.63±0.07^{a}
4.0	1.0	100	6.67±0.33^{b}	5.22±0.06^{b}

Table 2

Response in White's root culture medium supplemented with different concentrations of BAP (values are mean ± SE of 6 replicates). Mean values with different alphabets are statistically significant at P<0.05.

Hormone concentration (mg/l)	Percentage response	No. of shoots	Shoot length (cm)
1.0 BAP	0.0	-	-
2.0 BAP	0.0	-	-
3.0 BAP	27.7	1.0±0.00^{c}	5.23±0.58^{a}
4.0 BAP	47.2	2.33±0.21^{b}	2.75±0.1^{b}
5.0 BAP	69.4	4.17±0.31^{a}	2.36±0.24^{b}
6.0 BAP	19.4	2.33±0.33^{b}	1.01±0.06^{c}

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