

Standard Operating Procedure for Micropropagation of *Meliadubia* Cav.: An Important Fastgrowing Tree

ABSTRACT

Due to increasing demand to meet out industrial requirement as a raw material, soft wood forest species are under tremendous pressure across the globe. The demand of fast growing *Meliadubia* is one of them. The usual approach of regeneration for this plant is through seed is unable to produce large scale plants. The present investigation aimed to develop the Standard Operating Procedure through Tissue culture method for mass multiplication of *M. dubia* using nodal segment. Results showed that the highest shoot initiation response (86.6%) was recorded in Murashige and Skoog medium supplemented with additives, NAA (0.1 mg l^{-1}) and Kinetin (0.5 mg l^{-1}). Maximum response of shoot multiplication with highest shoot length of 5.5 cm was obtained in MS medium supplemented with combinations of Ascorbic acid (50 mg l^{-1}) and Kinetin 1 mg l^{-1} . For rhizogenesis, MS + 3.0 mg l^{-1} IBA (93.3%) demonstrated superior in terms of the percentage of cultures with root induction, the average number of roots, and the average length of roots per explant. In conclusion present study ensures the successful mass multiplication of *M. dubia*, demonstrating the importance of tissue culture in the expansion of this economically significant multipurpose tree.

Key words: -*M. dubia*, Shoot initiation, Multiplication, Response, Explant

1. Introduction

“One of the most important species of industrial trees, *Meliadubia*, belongs to the family Meliaceae, has recently spread quickly across the Indian subcontinent. *M. dubia* is a native of tropical and subtropical regions. *M. dubia* occurs in the tropical moist deciduous forests of the Sikkim, Himalayas, North Bengal and upper Assam, the Khasi hills of North East India, Orissa, Deccan and the Western Ghats, at altitudes of 1,500-1,800 meters” (Mandang, 2003). “The species is cultivated largely for its usefulness in industry and medicine. The wood of *Meliadubia* is utilized as a primary resource for plywood, paper pulp, and secondary lumber.

Due to its multipurpose uses in bioenergy production, manufacture of pencils, furniture making (match boxes, packing cases, cigar boxes, ceiling boards), building materials and constructions, agricultural instruments making musical and splints, *Meliadubia* is gaining more popularity and is in high demand” (Bhimi Ram *et al.*, 2014; Mandang, 2003; Suprapti *et al.*, 2004; Parthiban *et al.*, 2009; Chinnaraj *et al.*, 2011).

The various extracts from different parts of the plant are known to have pharmacological importance which includes, antiviral, antibacterial, antifungal, antidiabetic, antineoplastic, antihelmintic and antileprosy properties (Pettit, 2002; Nagalakshmi *et al.*, 2003; Vijayan *et al.*, 2004; Gerige and Ramjaneyulu, 2007; Susheela *et al.*, 2008; Sukumaram and Raj, 2010; Sharma and Arya, 2011).

In medicine, the Nilgiri tribes use the trees to treat a variety of diseases. The research suggests that *Meliadubia* fruit is effective in treating colic, dermatitis, and anthelmintic diseases. The tetranotriterpenoids composition and compositolide are said to be present in this tree's leaves and seeds. New options for small and medium biomass, such as lop & top, for power generating projects have emerged as a result of the creation of a new cloned propagation of *Meliadubia* plantation (Malabar Neem tree- a very fast growing, with high calorific value of wood tree). This plant is widely grown by farmers in Tamil Nadu and other southern Indian states. It is a tree with quick growth and excellent yield that is replacing eucalyptus in local farmers' fields. Eucalyptus is known to have an allelopathic effect on soil, yet this species doesn't have any detrimental effects.

The primary issue with this species is the very low rate of seed germination and viability, with reports of only 20–30% seed germination from various researchers. In nurseries, this species cannot grow great numbers of seedlings using typical regeneration techniques. The demand for this species as high-quality planting material can, however, be met through *in vitro* methods of propagation. The primary benefit of tissue culture is the year-round production of a large number of plants that are true to type in a small amount of time and space. With this technique, we can get disease-free plantlets in a short amount of time. Tissue culture plants (TCPs) could flower sooner, have better branching, be more vigorous, and produce more. In this context, the aim of this work is to develop the standardized protocol for micro propagation of *M. dubia* .

2. Materials and Methods

2.1 Explants source & sterilization

The current study was carried out in the tissue culture lab RRL at the Department of Plant Molecular Biology and Biotechnology in the College of Agriculture, IGKV, Raipur (CG) during 2021 to 2023. Explants were taken from an *M. dubia* tree that had been grown for four years and had been planted in the Research Farm, Department of Forestry, College of Agriculture, IGKV, Raipur, Chhattisgarh. In order to surface sterilize the explants, 2.5 to 3.0 cm long nodal segments were excised after the leaves were removed. After being placed in a bottle with a cap, the explants (nodal segments) were thoroughly cleaned under running water. Explants were treated with 0.1% (v/v) Polyoxyethylenesorbitanmonooleate (Tween - 80; Himedia, India) liquid solution for 15 minutes followed by 3 to 4 times thorough washes in double-distilled water and then 0.1% (w/v) solution of Bavistin (Carbendazim 50% WP- a systemic fungicide) for 30 minutes, followed by 5 to 6 thorough washes in double-distilled water, after that explants were treated with 0.1% (w/v) HgCl₂ solution for 5 min. New options for small and medium biomass, such as lop & top, for power generating projects have emerged as a result of the creation of a new cloned propagation of *Meliadubia* plantation (Malabar Neem tree- a very fast growing, with high calorific value of wood tree). This plant is widely grown by farmers in Tamil Nadu and other southern Indian states. It is a tree with quick growth and excellent yield that is replacing eucalyptus in local farmers' fields.

2.2 Culture initiation

Prior to use, the laminar air flowchamber was sterilized by UV light for roughly 20 to 30 minutes. In the laminar air flowchamber, the floor was cleaned with 70% alcohol, and sterilized forceps and scalpels were employed. Then the sterilized *Meliadubia* nodal explants were placed in culture bottles containing 40 ml of MS basal medium that had been supplemented with additives and various growth hormones. For shoot initiation various treatments comprised of Murashige and Skoog basal media, Kinetin (0.5 mg l⁻¹), NAA (0.1 mg l⁻¹), IAA (0.1 mg l⁻¹) along with additives Ascorbic acid (50 mg l⁻¹), Casein hydrolysate (500 mg l⁻¹) and Adenine sulphate (25 mg l⁻¹) were used in various combination. After that, cultures were incubated in a

culture room, at $26\pm 2^{\circ}\text{C}$, humidity at 70-80% and under a photoperiod of 16 hours light and 8 hours dark for a period of 15–21 days while incorporating fresh media in the same cultural contexts. Each treatment had three replicates, each of which contained ten explants. The data was recorded at the end of 2nd week. To study the effect of PGRs on shoot initiation various concentration and combinations of cytokinins and additives viz. Ascorbic acid, Adenine sulphate, Casein hydrolysate were used.

2.3 Shoot multiplication

After 21 days, explants with started shoots were moved to glass bottles containing a new multiplication media. Plant growth regulators with varying doses and additives were evaluated in MS basal medium for multiplication. MS media, Kinetin (0.5 mg l^{-1} and 1 mg l^{-1}) along with additives Ascorbic acid (50 mg l^{-1}), Casein hydrolysate (500 mg l^{-1}) and Adenine sulphate (25 mg l^{-1}) were used in various combination. When the shoots had fully utilized the explants' stored nutrients able to independently absorb nutrients from the nutrient medium, the shoots were separated from the explant. Every 15 to 21 days, sub-culturing with basal media was done. The dried leaves, damaged cells/material, shoots, and sheath were removed during each subculture to provide room for the micro shoots to expand. Each treatment had three replicates, each of which contained ten explants.

2.4 Root induction

Long shoots originating from the node were transferred to $\frac{1}{2}$ MS medium supplemented with different concentrations of IBA and IAA for rooting. The rooting medium consisted of $\frac{1}{2}$ MS with 3% sucrose and solidified with 0.7% of agar. IAA (1 mg l^{-1} , 2 mg l^{-1} and 3 mg l^{-1}) and IBA (1 mg l^{-1} , 2 mg l^{-1} and 3 mg l^{-1}) were used individually or in combination. The percentage of rooted shoots, the total number of roots and the root length were evaluated after 6 weeks of growth on the rooting medium.

2.5 Statistical analysis

All the studies were done in laboratory under well-defined aseptic conditions of the medium, growth, temperature, and light, therefore for experiment the Completely Randomized Design (CRD) as described by Panse and

Sukhatme(1995)wasemployedandthedatawasanalyzedusingSAS9.3(StatisticalAnalysisSystemV9.3).

3.Results and Discussion

3.1 Shoot induction

Explants showed the presence of high levels of phenolic compounds after inoculation. Exfoliation of phenolic compounds from the shoot tip has resulted in the medium being completely brown and shoot tip explants have not been able to survive in most cultures. Cultures inoculated with nodal explants were observed after two weeks, in which highest shoot initiation percent (86.66%) was obtained on MS media supplemented with Ascorbic acid (50 mg l⁻¹), NAA (0.1 mg l⁻¹) and Kinetin (0.5mg l⁻¹) highest shoot initiation percent (86.66%) was obtained. MS media devoid of PGRs and additives showed very less shoot initiation percent (26.66%)(Table-1). The rate of in vitro shoot induction was accelerated by the addition of cytokinins (Kn) and modest concentrations of auxins (NAA & IAA). When NAA (0.1 mg l⁻¹) and Kinetin (0.5mg l⁻¹) were added to the MS medium, the in vitro shoot initiation increased significantly (from 5.3 to 8.6)(Table 1). However, addition of IAA was less effective than NAA for initiating shoot induction. According to Mascarenhas *et al.*(1982), kinetin is a typical natural cytokinin which is frequently utilized in plant tissue culture. The beneficial effect of cytokinin was noticeable in a variety of tree species, including *Buteamonosperma* (Kavitha *et al.*, 1996), *Azadirachta indica* (Su *et al.*, 1997, Mallika Vanangamudi *et al.*, 1997), *Albizia procera* (Majumdar *et al.*, 1998), *Eucalyptus tereticornis* (Parthiban *et al.*, 1999).

Table 1: Effect of growth hormone on Shoot initiation in *M. dubia*.

Treatments	Mean±SE	Initiation %
MS+ Ascorbic acid(50 mg l ⁻¹)+NAA(0.1 mg l ⁻¹) + Kinetin (0.5mg l ⁻¹)	8.6±0.33	86.66%

MS+ Ascorbic acid(50 mg l ⁻¹)+IAA(0.1 mg l ⁻¹)+ Kinetin (0.5mg l ⁻¹)	7.6±0.33	76.66%
MS+ Casein hydrolysate(500 mg l ⁻¹)+NAA(0.1 mg l ⁻¹)+ Kinetin (0.5mg l ⁻¹)	5.3±0.33	53.33%
MS+ Casein hydrolysate (500 mg l ⁻¹)+IAA(0.1 mg l ⁻¹)+ Kinetin (0.5mg l ⁻¹)	4.6±0.33	46.66%
MS+ Adenine sulphate (25mg l ⁻¹)+NAA(0.1 mg l ⁻¹)+ Kinetin (0.5mg l ⁻¹)	7.3±0.33	73.33%
MS+ Adenine sulphate (25 mg l ⁻¹)+IAA(0.1 mg l ⁻¹)+ Kinetin (0.5mg l ⁻¹)	6.6±0.33	66.66%
MS (control)	2.6±0.33	26.66%
SE(m)=0.33		
CD (0.01)=1.021		

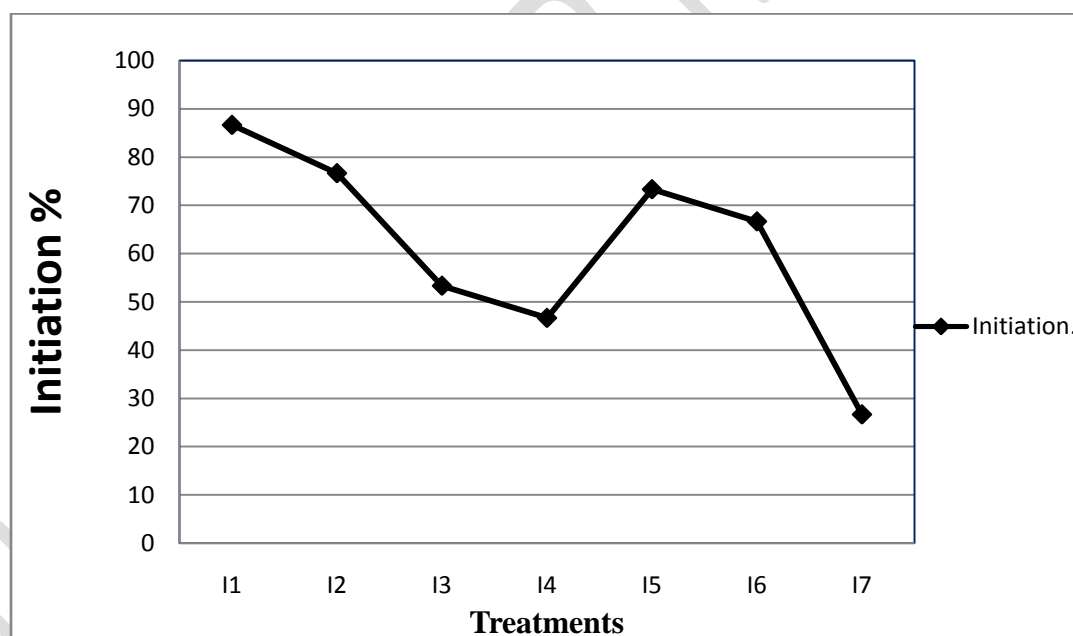


Fig 1:- Effect of growth hormone on shoot initiation in *Meliadubia*

3.2 Shoot multiplication

Sub culturing was found essential within 2-3weeks period on fresh shoot multiplication medium to regenerate more number of shoots and for shoot elongation. Shoot clump of 2-3 shoots proved better than single shoot or shoot segment for further shoot multiplication. MS supplemented with Ascorbic acid (50 mg l⁻¹), Kinetin (1mg l⁻¹) recorded (13.33) higher number of shoots per explants with highest shoot length 5.5 cm whereas, lowest number of shoots per explants (6.33) and lowest shoot length 2.4 cm was obtained in MS basal media devoid of PGRs (Table-2). The analysis of variance for shoot multiplication of *M. dubia* showed significant results because F-calculated value is greater than F-tabulated value at 1% level of significance. Due to their anti-oxidative qualities, Ascorbic acid, Adenine sulphate, and Casein hydrolysate in the medium had an effect on the frequency of shoot multiplication rate and length of shoot, which indirectly had an axillary effect on shoot multiplication and shoot growth. Ascorbic acid serves as a best precursor to cytokinins or derivatives as compared to other additives. The strategy of using Ascorbic acid as an adjuvant has been adopted effectively for many other plant species including *Tectonagrandis* (Devi *et al.*, 1994), *Acacia catechu* (Kauret *et al.*, 1998), *Pterocarpus marsupium* (Husain *et al.*, 2008), *Melia azedarach* (Husain and Anis 2009; Senet *et al.* 2010).

Treatments	Mean±SE	Shoot length(cm.)
MS+ Ascorbic acid(50 mg l ⁻¹) + Kinetin (0.5mg l ⁻¹)	11.33±0.66	5.0
MS+ Ascorbic acid(50 mg l ⁻¹) + Kinetin (1mg l ⁻¹)	13.33±0.66	5.5
MS+ Casein hydrolysate(500 mg l ⁻¹) + Kinetin (0.5mg l ⁻¹)	7.66±0.33	3.9
MS+ Casein hydrolysate(500 mg l ⁻¹) + Kinetin (1mg l ⁻¹)	8.00±0.57	4.0
MS+ Adenine sulphate (25mg l ⁻¹) + Kinetin (0.5mg l ⁻¹)	8.66±0.66	4.1
MS+ Adenine sulphate (25mg l ⁻¹) + Kinetin (1mg l ⁻¹)	9.33±0.33	4.4
MS (control)	6.33±0.33	2.4
SE(m)=0.509 CD (0.01)=1.586		

3.3 Root induction

In the present study, rhizogenesis was observed due to addition of auxins viz., IAA and IBA either singly or in conjoint addition between two auxins evaluated, all the treatments recorded significantly higher rooting per cent due to increasing concentrations of either IBA or IAA auxins. Highest root induction was obtained on ½ MS supplemented with 3.0 mg l⁻¹ IBA (93.3 %) with root length (5.46 cm) followed by ½ MS supplemented with 2 mg l⁻¹ IBA (90%) with root length (4.63 cm) and lowest rooting was observed in ½ MS (control), where no rooting response was obtained (Table 3). The positive effect of IBA on rooting of *in vitro* propagated plants had been established in many tree species, viz., *Simaroubaglauca* (Sekar, 2003), *Bambusa bamboos* (Kamala, 2009) and *Simaroubaglauca* (Nesamani, 2005) which are consistent with the result of present findings.

Table 3: Effect of different concentration of auxins on root induction in <i>M. dubia</i>			
MS media with different concentrations of IAA and IBA (mg l ⁻¹)	Mean±SE	Rooting (%)	Root length (in cm.)
½ MS +control	0.000±0.00	0%	0
½ MS +1 mg l ⁻¹ IAA	2.100±0.30	50%	1.46
½ MS +2 mg l ⁻¹ IAA	3.233±0.14	63.3%	2.76
½ MS +3 mg l ⁻¹ IAA	4.133±0.08	73.3%	3.46
½ MS +1 mg l ⁻¹ IBA	4.200±0.05	83.3%	4.03
½ MS +2 mg l ⁻¹ IBA	4.633±0.12	90%	4.30
½ MS +3 mg l ⁻¹ IBA	5.467±0.21	93.3%	4.60
½ MS +1 mg l ⁻¹ IAA+1 mg l ⁻¹ IBA	4.267±0.08	86.6%	4.10
½ MS +2 mg l ⁻¹ IAA +2 mg l ⁻¹ IBA	4.200±0.11	80%	3.90

½ MS +3mg l ⁻¹ IAA +3 mg l ⁻¹ IBA	4.000±0.05	76.6%	3.66
SE(m)=0.146			
CD(0.01)=0.433			

4. Conclusion

This study provides an efficient *in vitro* propagation method which could be commercially feasible for *M. dubia*. Present investigation showed that MS supplemented with Kinetin and low concentration of auxin i.e. NAA are essential to produce higher shoot initiation percent, and the highest number of micropropagated shoots obtained by the use of Ascorbic acid as additive, Kinetin (1mg l⁻¹) along with MS basal media for this fastgrowing tree species. Whereas, for high rooting percent, IBA was found most appropriate auxin. The standard acclimatization procedure was developed successfully to get high survival percent of micro-propagated plantlet of *M. dubia*. Thus it is concluded that the current investigation ensures the successful mass multiplication of *M. dubia*, demonstrating the importance of tissue culture in the expansion of this economically significant multipurpose tree.

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