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Standardization of Sterilization Protocol for Explants of *Melia dubia* Cav.–An Important Short Rotation Tree

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ABSTRACT: Soft wood forest species are under extreme pressure all over the world as a result of growing demand to meet out industrial requirements as a raw resource. One of these is the rapidly expanding demand of *Melia dubia.* Large-scale plants cannot be produced through means of seed for this species. Therefore, the aim of the current research work was to create a protocol for *M. dubia* multiplication using the tissue culture approach. The key to establishing an aseptic culture is to standardize sterilization procedure, in view of this present investigation was conducted. Nodal explants, taken from mature *M. dubiatrees*, were subjected to experiments to see how well they could be disinfected. Maximum explants survival percentage 86.66% was obtained with 0.01% $HgCl_2$ for 5 min followed by 73.3% percent with 1% NaOCl for 3 min. Contamination per cent was also least with these two treatments 13.33 % contamination was recorded with 0.01 % $HgCl_2$ for 5 min and 20 % contamination was recorded with 1% NaOCl for 3 min. Overall 0.01% $HgCl_2$ for 5 min produced the best results for sterilization of nodal explants of *Melia dubia*. The developed sterilization protocol has potential to be successfully employed for micropropagation of *M. dubia.*

Keywords: *M. dubia*, Sterilization, Response, Explant, Fedility, survival.

INTRODUCTION

The *Melia dubia*, often known as Malabar neem, is a member of the Meliaceae family, which has the fastest-growing tree species. The plantation can be harvested in 6-8 years. The plywood industries have a healthy demand for the wood. It has a quick growth rate and is employed in industrial plantations. The cultivation of trees outside of forest areas has been encouraged using a variety of tree species, including *Melia dubia.* It is a tree species with multiple uses, including as a primary component of plywood, livestock feed, and secondary lumber. The wood is utilised for making splints, cigar boxes, ceiling boards, building materials, agricultural implements and pencils. This species is widely grown by farmers in Tamil Nadu and other southern Indian states. It is a very productive and quick-growing tree, quickly displacing eucalyptus from farmers' fields and taking its place as a substitute in the region.

M. dubia is found in tropical moist deciduous forests at elevations of 1,500–1,800 metres in Sikkim, the Himalayas, North Bengal, higher Assam, the Khasi highlands of North East India, Orissa, the Deccan, and the Western Ghats. A minimum profit of Rs. 40,000 per year from an acre may be obtained by the farmers due to the plywood industry's high demand for *Melia dubia* wood.

According to the literature, *Melia dubia* fruit is useful for treating anthelmintic, dermatitis, and colic conditions. According to reports, this tree's leaves and seeds contain the tetranotriterpenoids composition and compositolide. However, new opportunities for small and medium biomass, such as lop & top, for power generation projects have emerged with the development of new cloned propagation of *Melia dubia* plantation (Malabar Neem tree- a very fast growing, with high calorific value of wood) which can be used as a fire wood for power generation. The fact that eucalyptus has an allelopathic effect on soil is widely known, however *Melia dubia* species doesn't seem to be exerting any harmful effects. The main issue with this species is the relatively low seed viability and germination rate, which has been found by numerous researchers to be only 20-30%. Due to this problem, this species cannot generate significant numbers of plants in nurseries using standard methods of regeneration. However, in vitro methods of propagation hold great promise for supplying the high demand for this species as planting material. The primary benefit of tissue culture is the year-round, mass production of exact replicas of plants in a limited amount of time and space. In this method we may obtain disease-free plantlets within short time and space. Tissue culture

plants (TCPs) may have improved branching, early flowering, greater vigour and higher yield. Micropropagation always has been linked with microbial contamination, to avoid this contamination, the explants must be gently surface sterilized before inoculation on to culture medium, in view of this the present investigation was conducted.

MATERIALS AND METHODS

A. Explants source

The present investigation was conducted during 2021-23 in the tissue culture laboratory RRL, Department of Plant Molecular Biology and Biotechnology in the College of Agriculture, IGKV, Raipur (CG). Explants were collected from four year old healthy and disease free tree of *M. dubia,* planted at Research farm, Department of Forestry, College of Agriculture, IGKV, Raipur, Chhattisgarh. After the leaves were removed, 2.5 to 3.0 cm long nodal segments of the explants were taken for surface sterilization.

B. Sterilization of explants

The explants (nodal segments) were transferred to a bottle with a cap and then thoroughly rinsed under running water. Explants were treated with 0.1% (v/v) Polyoxyethylene sorbit an monooleate (Tween - 20; 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 different concentration of $HgCl_2$ and NaOCl solution for different exposure time. Explants were washed five to six times with autoclaved double distilled water following each treatment to get rid of any remaining residues of sterilizers.

In the present investigation, to reduce leaching, the cut ends of the surface sterilized explants were trimmed aseptically before being vertically inoculated on culture medium. The explants were incubated in a culture room, where the temperature was maintained at 26±2ºC, humidity at 70-80% and under a photo period of 16 hours light and 8 hours dark. Data on contamination and survival percentage were recorded. The following formula has been applied to calculation of contamination, survival and drying percentage.

$$\text{Contamination \%} = \frac{\text{Number of explants contaminated}}{\text{Total number of explants inoculated}} \times 100$$

$$\text{Survivability \%} = \frac{\text{Number of explants survived}}{\text{Total number of explants inoculated}} \times 100$$

$$\text{Drying \%} = \frac{\text{Number of explants dried}}{\text{Total number of explants inoculated}} \times 100$$

C. Data analysis

A completely randomized design was used for present experiment. Sterilization results carefully calculated based on the number of explants used. The data of all experiments were statistically analyzed and expressed as Mean ± Standard Deviation.

RESULT AND DISCUSSION

Micropropagation always has been linked with microbial contamination. Multiplication of microbes, their competition with explants growth for nutrients and altering the culture environment e.g. pH by releasing chemicals inhibits explants growth or cause mortality. To avoid this contamination ,the explants must be gently surface sterilized before inoculation on to culture medium. Two common sterilants *viz.*, Sodium hypochlorite (NaOCl) and Mercuric chloride ($HgCl_2$) at various concentrations and durations were used in standardizing sterilization procedure for obtaining contamination free cultures. The sterilants at various concentration and durations used were presented in Table 1.

Maximum survival of explants 86.66% percent was recorded with treatment 0.01% $HgCl_2$ for 5 min followed by 73.3% in treatment with 1% NaOCl for 3 min. Per cent contamination was also least with these two treatments 13.33 % contamination was recorded with 0.01 % $HgCl_2$ for 5 min and 20 % contamination was recorded with 1% NaOCl for 3 min. In all other treatments the contamination observed was ranged between 23.3 to 40.00 percent.

Similar findings reported by Kumar (2011) in *M. dubia* that the optimal method for sterilizing explants was 0.01% $HgCl_2$ for 5 minutes, which led to a maximum survival rate. Similar reports were observed in *Pterocarpus marsupium* (Rajkumar, 1998), *Eucalyptus globules* (Pattanaik, 1995), *Bambusa bamboos* (Arya and Sharma 1998), *Azadirachta indica* (Arya and Arya 1998), *Simarouba glauca* (Sekar, 2003; Geethanjali, 2004), *Acacia mangium* (Sumana *et al.*, 1998) and *Robinia pseudoacacia* (Kanwar *et al.*, 1995).

Surface sterilization of explants with mercuric chloride and sodium hypochloride may be relatively easy, but the systemic infection/endophytic contamination may be relatively difficult to eliminate (Thorpe *et al.*, 1991). This could be the probable reason for 13.33 percent contamination recorded in the best treatment i.e. 0.1 % $HgCl_2$ for 5 min.

With regard to drying of explants, the treatment 0.01 % $HgCl_2$ for 5 min expressed least value (0%) . Whereas highest drying % was recorded in treatment with 0.5% $HgCl_2$(5 min) 13.33%, this may be due to higher conc. of mercuric chloride. In all other treatments, the drying was ranged between 6.66 percent and 16.66 percent (Table 1).

Table 1 : Effect of different sterilants and exposure time on response of explants in *M. dubia*

Sterilization treatments of explants	Survivability%	Contamination%	Drying%
0.1% $HgCl_2$(3 min)	66.6%	23.3%	10%
0.1% $HgCl_2$ (5 min)	86.6%	13.33%	0%
0.5% $HgCl_2$ (3 min)	50%	40%	10%
0.5% $HgCl_2$ (5 min)	56.6%	30%	13.33%
1%NaOCl(3 min)	73.3%	20%	6.66%
1% NaOCl (5 min)	60%	33.3%	6.66%
3% NaOCl (3 min)	56.6%	26.6%	16.66%
3% NaOCl (5min)	53.3%	40%	6.66%

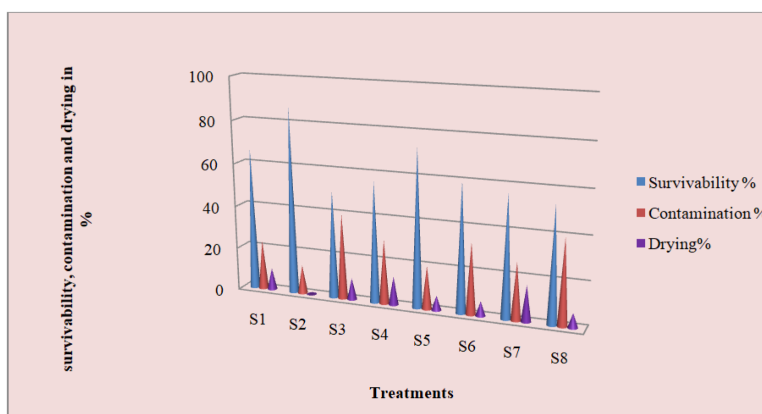

Fig. 1. Graphical representation of survivability, contamination and drying % for sterilization treatment in *M. dubia*.

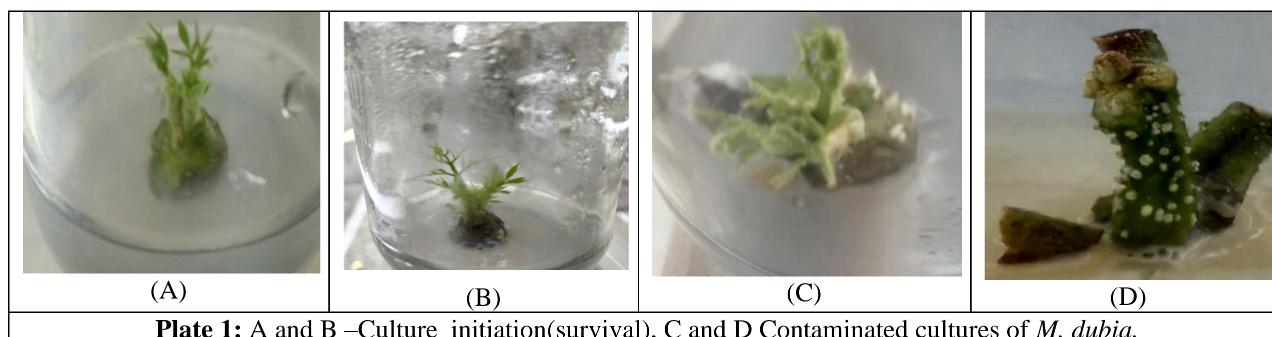

Plate 1: A and B –Culture initiation(survival), C and D Contaminated cultures of *M. dubia*.

CONCLUSIONS

There were differences between the explant responses to various type & concentrations of sterilization agents. For nodal explants sterilization in *M. dubia* 0.01%$HgCl_2$ was found best & effective sterilizing agent. Over sterilization increased tissue mortality of explants and hence to overcome to this problem, optimized concentration of sterilizing agents & duration is required.

In case of *M. dubia* nodal explants surface sterilization with 0.1% (v/v) Polyoxyethylene sorbitan monooleate (Tween - 20; Himedia, India) for 15 min., then 0.1% (w/v) solution of Bavistin (Carbendazim 50% WP- a systemic fungicide) for 30 minutes followed by treatment with 0.01% $HgCl_2$ for 5 min. proved most effective for maximum survival percentage, minimum contamination and no drying of explants, followed by treatment with 1% NaOCl for 3min (Table 1 and Fig. 1).

FUTURE SCOPE

This developed sterilization protocol has potential to be successfully employed for in vitro propagation of *M. dubia* by nodal explants for mass multiplication of this species to meet out requirement for industrial & agroforestry programmes.

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