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STANDARDIZATION OF MACROPROPAGATION TECHNIQUE FOR *DALBERGIA LATIFOLIA* ROXB. THROUGH ROOT CUTTINGS, ITS APPLICATION IN PRODUCTION OF QUALITY PLANTING STOCK AND CONSERVATION OF GENETIC RESOURCES

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ABSTRACT

The Indian Rosewood (*Dalbergia latifolia* Roxb.), is one of the precious timber tree species categorized as "Vulnerable" in the Red Data Book of IUCN. The fast depletion of its valuable genetic resources due to over-exploitation and low regeneration has been a matter of great concern. In this context, a study on the macropropagation of this species through root cuttings was undertaken. The root cuttings of *D. latifolia* were maintained in polytunnel condition to produce the sprouts which were used for rooting. The effect of sprout lengths (5 – 10 cm, 11 – 15 cm and 16 – 20 cm), IBA forms (powder and liquid) and IBA concentrations (2000 ppm and 4000 ppm) on the rooting of sprouts as well as suitability of different rooting media were investigated. The results showed that IBA form and its concentration have significant impact on rooting. But, sprout lengths did not significantly influence the rooting. The liquid form of IBA at 2000 ppm concentration resulted in highest rooting (65-70%) compared to powder form and higher IBA concentration. The water and vermiculite were found to be the best rooting media compared to sand, red soil, coir pith and vermicompost. Initial rooting using water medium and further transplanting to vermiculite (water + vermiculite medium method) resulted in better rooting of sprouts. The study revealed that the right IBA form, its optimum concentration and appropriate rooting media play a crucial role in successful macropropagation, which could be useful for the production of quality planting stock and conservation of genetic resources of this threatened species.

Key words: Indian Rosewood, macropropagation, quality planting stock, genetic resource conservation

INTRODUCTION

Dalbergia latifolia Roxb. (Indian Rosewood) is a medium sized to large deciduous leguminous tree which is naturally distributed in the Indo-Malaysian region. It has high wood quality and hence considered as one of the economically important timber yielding trees (Boga *et al.* 2012; Fatima *et al.* 2018; Sasidharan *et al.* 2020). The timber is used to make furniture, carvings, decorative plywood and veneers. Because of the high wood demand, the trees growing in natural forests have been logged indiscriminately. The natural regeneration of the tree is also very low due to the poor seed viability, competition from weeds, forest fire and other environmental factors (Pradhan *et al.* 1998; Fatima *et al.* 2018; Sasidharan *et al.* 2020). Therefore, the natural populations of the tree in the forest areas have declined significantly (Boga *et al.* 2012; Sasidharan *et al.* 2020). Hence the species has been categorized as "Vulnerable" in IUCN Red list (Asian Regional Workshop, 1998). Vegetative propagation techniques are increasingly applied for domestication of tropical tree species as a means of producing planting stock and capturing genetic variation (Leakey *et al.* 1990; Ezekiel, 2010) and therefore, this technique could be effectively used to multiply and conserve the genetic resources of *D.latifolia*.

The influence of hormones on rooting behaviour of *Dalbergia sericea* stem cuttings and the seasonal variations in rooting response were elucidated by Uniyal *et al*. (1993 & 1995). Amri *et al.* (2010) conducted vegetative propagation of another valuable timber species, *Dalbergia melanoxylon* (African Blackwood) and standardized the protocol. Singh *et al.* (2011) studied the effect of mother tree age, different rooting media, light conditions and auxin treatments on rooting behavior in branch cuttings of *Dalbergia sissoo* and standardized the protocol. They reported vegetative propagation as an alternate strategy, which could avoid the serious die back disease encountered by *D.sissoo* when the plants are raised from seeds. Washa *et al.* (2012) carried out rooting experiments with softwood, semi-hardwood, hardwood and root cuttings of *D.melanoxylon* and reported that the cutting type and soil type used had significant impact on rooting. Puri and Verma (1996) attempted rooting of mature hardwood and softwood cuttings of *D.sissoo* and reported that auxins at lower concentrations triggered / enhanced rooting, while at higher concentrations, they inhibited or slowed down the rooting ability of cuttings. Rasheed *et al.* (2017) undertook vegetative propagation studies with different sizes of *D.sissoo* cuttings and different auxins / concentrations and noticed significant difference in sprouting percentage with reference to size of shoot cuttings, type of auxins well as well as their concentrations.

Even though there is lot of information available on the silvicultural aspects of *D.latifolia*, the macropropagation method through root cuttings has not been attempted and hence the present study was carried out to standardize the technique.

MATERIALS AND METHODS

i) Collection of root cuttings

Five phenotypically superior trees of *D. latifolia* were selected from the forest area of Sadivayal, Coimbatore Forest Division and the root cuttings of about 30 cm to 40cm length and 15cm to 20cm girth were collected by digging the soil around the trees. The collected root cuttings were labeled properly and covered with wet gunny bags in the field to avoid desiccation. The root cuttings were brought to shade house facility of the institute at Coimbatore.

ii) Production of sprouts from root cuttings

The root cuttings were buried horizontally in standard nursery beds filled with sand and covered with polytunnels, under shade house with 50 percent shade. Regular watering of the root cuttings was done to maintain a relative humidity of 80 to 90 percent. Chlorpyriphos (3 ml/L) treatment was given fortnightly as soil drench to prevent termite attack. Bavistin (2g/L) was sprayed regularly to prevent fungal infections. The root cuttings started sprouting after 46 ± 20 days and these sprouts were used for the rooting experiments.

iii) Collection of sprouts and auxin treatment

The different lengths of sprouts (5 – 10 cm, 11 – 15 cm and 16 – 20 cm) were collected, washed thoroughly in clean water, followed by dipping the cut end in 0.1 percent solution of Bavistin to protect it from pathogens. Subsequently, the sprouts were treated with IBA at concentrations of 2000 ppm and 4000 ppm, both in powder form (IBA + talcum powder) and liquid form (water + sodium hydroxide pellet + IBA). The above methods are known as basal dry-dip method and basal quick-dip method of rooting hormone treatments respectively. Twenty sprouts of a particular length were used for each treatment and altogether 360 sprouts were used for the whole experiment. The treated sprouts were placed in vermiculite medium and maintained in polytunnels, under shade house. The talcum powder and water served as control for IBA powder form and IBA liquid form respectively.

iv) Testing of different rooting media for root development and plant survival

Vermiculite, water, water + vermiculite (i.e. initially sprouts were placed in water for callus formation and root development and subsequently transferred to vermiculite for further root development), sand, coir pith, vermicompost and red soil were tested as rooting media for the production of adventitious roots from sprouts under polytunnel condition. The root trainers or hycopots were used for the vermiculite and coir pith rooting media. The plastic water cups were used for testing water, sand, vermicompost and red soil rooting media. Twenty sprouts were treated with 2000 ppm IBA in liquid form alone and placed in each rooting media. A total of 140 sprouts were used for the whole experiment. In the case of sprouts kept in hycopots, the root development was observed at the bottom of hycopots. Observations on the root developments in sprouts placed in plastic water cups were made after washing the sprouts to remove the medium, whereas in sprouts placed in plastic cups with water medium, the root development was observed directly.

v) Data collection and analysis

The sprouts were observed periodically for the presence of adventitious roots up to 120 days. The sprouts producing at least one primary root (> 1 mm length) were considered as rooted. The data on rooting was analyzed using Z-test and significance found out.

RESULTS

a) Effect of sprout length, IBA concentration and IBA form

All sprouts, irrespective of length, treated with different forms and concentrations of IBA showed callusing at the basal portion. Hence, the callus formation did not show any difference based on sprout length, IBA concentration and IBA form. But significant differences were observed in rooting. The liquid form of IBA resulted in significantly high percentage of rooting (40 – 70 %) compared to powder form of IBA (15 – 50 %). The sprouts treated with powder form of IBA and liquid form of IBA showed marked difference in position of root initiation. In the former, the root initiation was noticed at the treated cut end of the sprouts, while in the latter, root initiation took place slightly above the cut end. The IBA 2000 ppm concentration in liquid form produced more number of rooted sprouts (65 – 70 %) compared to 4000 ppm IBA in liquid form (40 – 45 %). Even though the sprouts treated with IBA 4000 ppm produced the larger sized callus, root differentiation did not take place. The sprouts of different lengths did not show any significant difference in rooting. No rooting was noticed in sprouts maintained as control (Table-1).

Table-1. Effect of IBA form, IBA concentration and sprout length on rooting of *D. latifolia* sprouts in vermiculite medium

IBA form	IBA Concentration (ppm)	Sprout length (cm)	No. of sprouts treated with IBA	No. of sprouts rooted	Rooting percentage*
Powder	Control (Talcum powder)	5 to 10	20	0	0[d]
		11 to 15	20	0	0[d]
		16 to 20	20	0	0[d]
	2000	5 to 10	20	10	50[a]
		11 to 15	20	9	45[a]
		16 to 20	20	8	40[a]
	4000	5 to 10	20	4	20[b]
		11 to 15	20	5	25[b]
		16 to 20	20	3	15[b]
Liquid	Control (Water)	5 to 10	20	0	0[d]
		11 to 15	20	0	0[d]
		16 to 20	20	0	0[d]
	2000	5 to 10	20	13	65[c]
		11 to 15	20	14	70[c]
		16 to 20	20	13	65[c]
	4000	5 to 10	20	8	40[a]
		11 to 15	20	8	40[a]
		16 to 20	20	9	45[a]

*Rooting percentage followed by same alphabet is not significantly different ($P>0.05$) from each other as per Z-test.

b) Effect of rooting media on root development and plant survival

D. latifolia sprouts treated with IBA 2000 ppm concentration in liquid form and placed in vermiculite, water, water + vermiculite and sand showed root formation. The highest rooting of 85 percent was obtained with water + vermiculite medium, which gave the inference that this media combination is very effective. The rooting of sprouts was very poor in sand medium, compared to vermiculite and water medium. The IBA treated sprouts placed in coir pith, vermicompost and red soil exhibited heavy casualty with no root formation and hence these media were not considered to be suitable for rooting (Table-2).

Table 2. Performance of *D.latifolia* sprouts treated with IBA 2000 ppm in liquid form in different rooting media

Rooting media	No. of sprouts	No. of sprouts rooted	Rooting percentage	No of days required for rooting Mean ±SD)	No. of rooted sprouts survived after transplantation to nursery bags	Survival percentage
Vermiculite	20	13	65[a]	80 ± 40	12	92[c]
Water	20	16	80[a]	20 ± 10	9	56[d]
Water + Vermiculite	20	17	85[a]	25 ± 5	16	94[c]
Sand	20	4	20[b]	35 ± 5	0	0[e]
Coir pith	20	0	0[b]	-	0	0[e]
Vermicompost	20	0	0[b]	-	0	0[e]
Red soil	20	0	0[b]	-	0	0[e]

* Rooting percentage and survival percentage followed by same alphabet is not significantly different (P>0.05) from each other as per Z-test.

D. latifolia sprouts in water medium produced the roots quickly, compared to vermiculite medium. But, the roots produced in water medium were highly fragile, compared to those developed in vermiculite medium. Because of this nature, the rooted sprouts in water medium were prone for root damage during transplanting to nursery bags. So, both the media have their own advantages and disadvantages. In order to overcome this issue, the IBA treated sprouts were initially placed in water until roots developed ($\leq$ 3 cm) and subsequently they were transferred to vermiculite medium in hycopots. In hycopots, the sprouts were allowed for further root development before transplanting to nursery bags. The water + vermiculite medium method significantly reduced the duration for rooting, increased the rooting percentage and survival of rooted sprouts after transplantation to nursery bags (Fig-1).

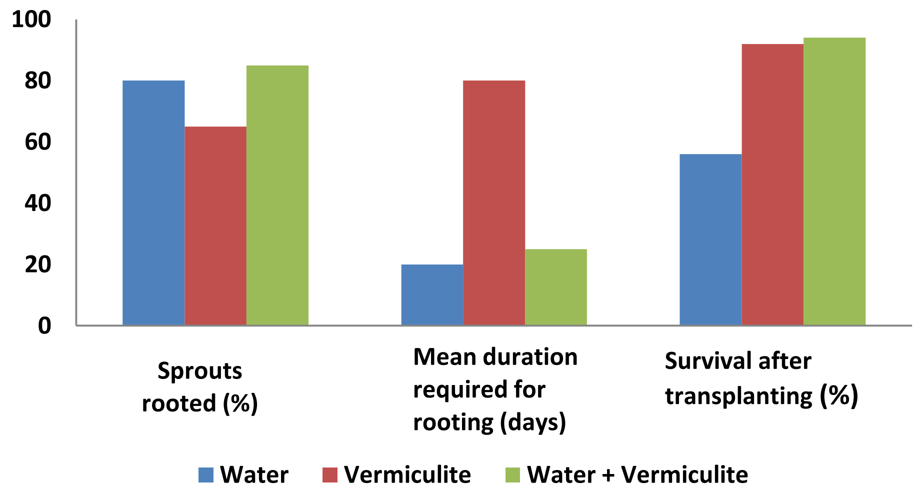

Fig 1.Performance of *D. latifolia* sprouts in different rooting media

DISCUSSION

Rooting of cuttings is a complex process involving ecophysiological, biochemical as well as anatomical factors controlling root initiation and development on stem cuttings (Uniyal *et al*. 1993). The cuttings treated with IBA, both in powder and liquid form produced callus and roots, compared to untreated control, during the present study. The stimulatory effects of IBA in rooting of stem cuttings of several woody plant species have been reported by many researchers and they found that IBA has an important role in the development of adventitious roots, increasing rooting percentage, improving quality of roots and uniformity in rooting of cuttings (Nagesh, 2002; Tchoundjeu *et al.*2002; Teklehaimanot *et al.*2004; Husen and Pal, 2007a; Opuni-Frimpong *et al.*2008). The process of root formation in root cuttings is intensified by IBA which influences polysaccharide hydrolysis, resulting in increased content of physiologically active sugar needed to provide energy for meristematic tissues and later for root primordia and root formation, as reported in Teak (*Tectona grandis*) (Husen and Pal, 2007b) and in *Dalbergia sissoo* (Husen, 2008).

The liquid form of IBA at 2000 ppm concentration was found to be the best for the development of adventitious roots in sprouts of *D.latifolia*, in the present experiment. According to Kroin (1992) IBA dissolved in water may be more effective than other solvents like alcohol. Husen (2004) reported maximum adventitious root formation in *D.sissoo* stem cuttings with application of 0.2 percent IBA, which is in agreement with the present study. But, interestingly, Singh *et al.* (2011) and Ullah Khan *et al.* (2012) reported higher rooting percentage in stem cuttings of *D.sissoo* with application of IBA at a much lower concentration of 200 mg L^{-1} which could be due to species specific variation in response to auxin concentration.

When the sprouts of *D.latifolia* were treated with IBA at 4000 ppm concentration, both in powder form as well as in liquid form, it resulted in lower rooting percentage, compared to that of 2000 ppm. Uniyal *et al.* (1993) reported lower rooting percentage with higher concentrations of auxins (IAA. IBA & GA) in *Dalbergia sericea*. An IBA concentration beyond the threshold of auxin tolerance will inhibit plant growth (Blazich, 1988). Rasheed *et al.* (2017) observed that hormones of concentration 400 mg L^{-1} have negative effect on sprouting percentage of *D.sissoo* cuttings with lower biomass. Though biomass of cuttings has not been a criterion in the present study, the lesser rooting percentage exhibited while treating with 4000 ppm IBA gives an indication that IBA at higher concentration is not suitable in the case of *D.latifolia*.

The present study revealed that water and vermiculite are the best suited media for rooting of *D.latifolia* sprouts. The rooting media can influence rooting percentage and type of root system developed (Long, 1932; Copes 1977; Wojtusik *et al.*1994). Apart from holding the cuttings in place, an ideal rooting medium has to perform certain essential functions like maintenance of high moisture content, good drainage, provide adequate air exchange at the base of the cuttings as well as it should be free from disease and insects (Hartmann and Kester, 1968). Perhaps, the other media used in the study like coir pith, vermicompost and red soil might not have been satisfying some these requirements and hence the cuttings did not produce roots. Venkataramanan *et al.* (1995) have shown that stem cuttings of *Eucalyptus* hybrid with low dose of IBA could result in 95 percent rooting in water medium, which corroborates the present findings.

CONCLUSION

The study has shown that treatment with 2000 ppm concentration of IBA in liquid form is the best method for rooting of *D.latifolia* sprouts. It also revealed that the water + vermiculite media combination is able to provide more rooted sprouts in a shorter period. The standardized macropropagation method by means of sprouts developed from root cuttings will go a long way in mass multiplication of superior trees, so as to produce quality planting stock for raising plantations and it could also help in conservation of valuable genetic resources of *D. latifolia*, which is considered as a "Vulnerable" species.

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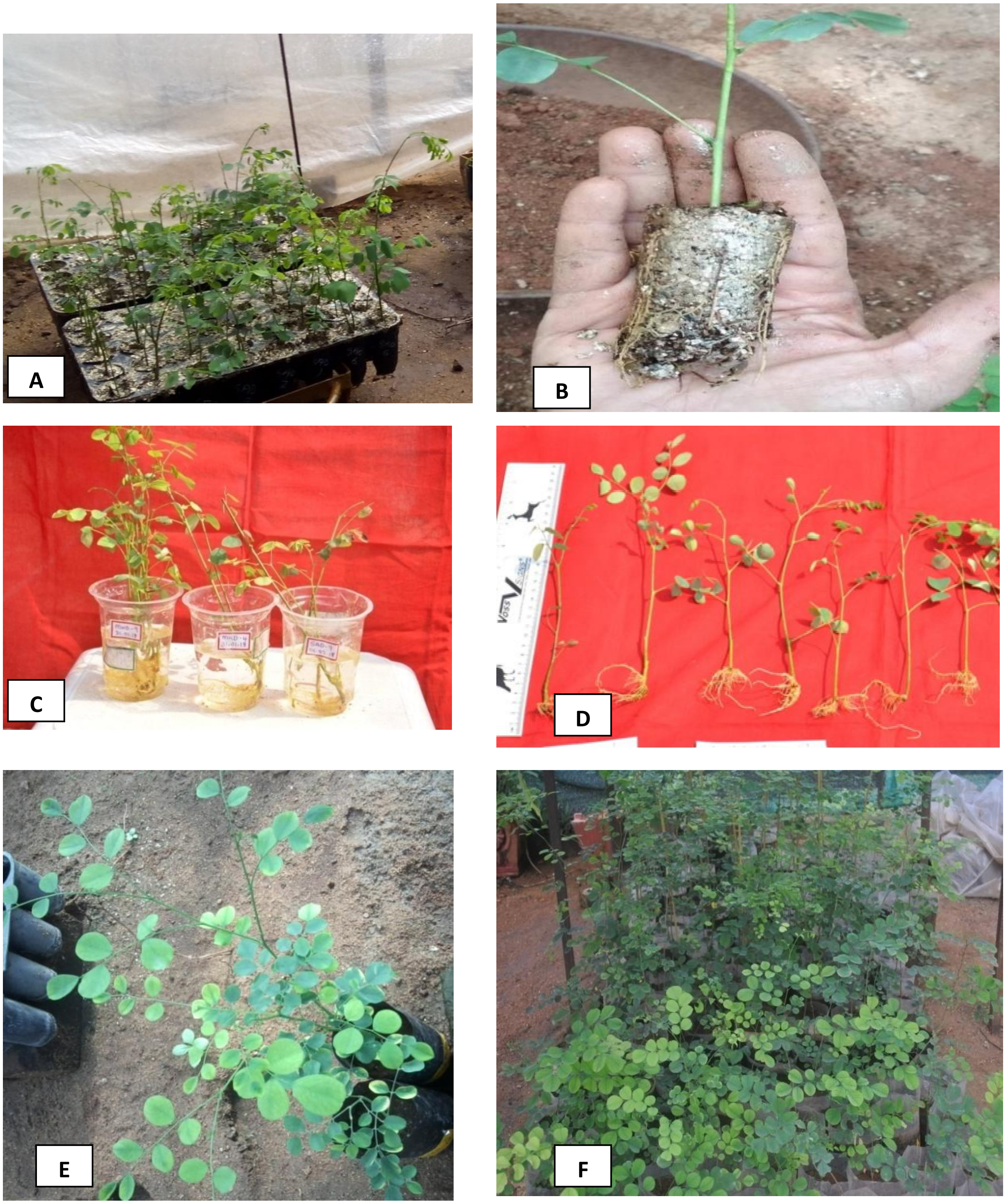
A-Superior tree of *Dalbergia latifolia* **B- Collection of root cuttings from tree**
C-Root cuttings placed in nursery bed **D- Sprouts developed from root cuttings**
E-Collection of sprouts from the nursery bed



A-Sprouts planted in hycopots with vermiculite medium **B- Rooted plant in vermiculite**
C-Sprouts kept in water medium **D-Root developed in water medium**
E- Plants shifted to nursery bags **F-Hardened plants in nursery**