



Research Journal of **Forestry**

ISSN 1819-3439



Academic
Journals Inc.

www.academicjournals.com

Mass Multiplication of Mature Trees of *Gmelina arborea* Roxb. Through *ex vitro* Rooting of Rejuvenated Bud Sprouts

¹Sanjay Singh and ²S.A. Ansari

¹Molecular Biology and Tree Physiology Laboratory, Institute of Forest Productivity, Aranyodaya, NH-23, Lalgutwa, Ranchi, 835303, India

²Genetics and Plant Propagation Division, Tropical Forest Research Institute, P.O. RFRC, Mandla Road, 482021, Jabalpur, India

Corresponding Author: Sanjay Singh, Molecular Biology and Tree Physiology, Laboratory Institute of forest Productivity, Aranyodaya, NH-23, Lalgutwa, Ranchi, 835303, India Tel: 91-651-2484057 Fax: 91-651-2546044

ABSTRACT

Clonal multiplication of selected superior genotypes of *Gmelina arborea* is difficult owing to maturity related loss in adventitious root potential which constitutes a serious constraints in their *ex situ* conservation and mass multiplication for sustainable use. The present study reports a new procedure for optimizing adventitious rhizogenesis through rejuvenation in the parent material taken from mature trees. Miniature bud sprouts (1-1.5 cm long with 1-3 unexpanded leaves) produced on semi-hardwood shoot cuttings taken from 15 year old trees were evaluated in comparison with juvenile seedling cuttings, coppice shoot cuttings and semi hardwood cuttings. The cuttings were administered with a quick dip treatment for 30 sec of water (T₁) or 5 mM (T₂) and 10 mM IBA (T₃) before planting and observations were recorded on various rooting parameters after 3 weeks. The mini-cuttings exhibited significantly superior adventitious rhizogenesis (94%) with about 2 and 4.7 times more rooting than seedling cuttings and semi-hardwood cuttings, respectively. The treatment of 5 mM IBA resulted in significantly superior adventitious rhizogenesis. The findings indicate rejuvenation of parent material in the procedure which may be employed for large-scale clonal multiplication and *ex situ* conservation of mature ortets of *Gmelina arborea* having desirable traits.

Key words: Auxin, mini-cuttings, rooting, rejuvenation, sprouts

INTRODUCTION

Gmelina arborea Roxb. (Family: Verbenaceae) is a moderate sized, deciduous, fast growing tree species of multiple utilities which occurs world-wide in different geographical and ecological conditions. It extends to Pakistan in the west, throughout India, Nepal, Bangladesh, Thailand, Laos, Cambodia, Vietnam and southern provinces of China (Yunan and Kwangsi Chuang) in the east spanning approximately a range of latitude of 50-30° North and range of longitudes of 70-110° East, i.e., nearly 3000-4000 km (Lauridsen *et al.*, 1995). *G. arborea* reaches its largest dimensions in mixed forests of moist region as in Assam and Myanmar; but extends to comparatively dry regions as in Central India (Troup, 1992). *G. arborea* has been introduced extensively to tropical countries, with West Africa and Central America housing the largest plantations.

G. arborea is a valuable tree and considered one of the best and most reliable timbers in India used variously for furniture, planking, paneling, carriage, carving, tea chest plywood, papermaking and match making. All parts of the tree possess medicinal properties (Kirtikar and Basu, 1980; Jain and de Fillipes, 1991). Due to ease of establishment, rapid early growth, early returns and excellent wood characteristics, *G. arborea* is a very promising species for afforestation programmes. However, the species bears the constraint of limited seed availability, poor germinability (Surendran, 1990) coupled with viability of only about 12 months. In its propagation through clonal means, ontogeny plays a significant role as mature ortets of over 15 years age do not exhibit marked root induction even after treatment with root inducing growth regulators (Singh, 2005). Hence the present study was attempted to evolve method for mass multiplication of mature ortets of *G. arborea* involving rooting of miniature bud sprouts.

MATERIALS AND METHODS

Plant material: Three types of shoot cuttings were employed—seedling tip cuttings (C_1), seedling cuttings (C_2) prepared from one-year-old seedlings and miniature bud sprouts or mini-cuttings (1-1.5 cm long with 1-3 unexpanded leaves) produced on semi-hardwood shoot cuttings (C_3). For production of bud sprouts semi-hardwood shoots were collected from 15 year old trees and planted in polythene bags containing soil, sand and farm yard manure in 1:1:1 ratio a month prior to start of experiment. These shoots were provided regular irrigation twice a day till collection of bud sprouts.

Treatments and propagation environment: Quick dip treatment for 30 sec of water (T_1), 5 mM Indole-3-butyric acid-IBA (T_2) or 10 mM IBA (T_3) were given to all types of cuttings before planting in inert rooting medium, Soilrite-TC (Keltech Entergics Ltd., Bangalore) in root trainer. The root trainers were maintained in aluminium frame polyhuts (4×2.5×2.5) mounted on sunken beds of soil and sand saturated with water by flooding and provided with 75% shade with agro-shade net. Conditions of temperature and humidity were recorded manually that ranged 30-35°C and 60-80%, respectively.

Experimental design, data recording and analyses: The experiment was laid out according to randomized block design with three replicate per treatment each of twenty cuttings. Observations on adventitious rooting parameters viz., rooting (%), root number per cutting, length of longest root (cm), root fresh and dry weight (g) were recorded after 3 weeks. The data were statistically analyzed employing analysis of variance (ANOVA), Fⁿ-test to ascertain significance at $p \leq 0.05$ and least significant difference (LSD_{0.05}) values to separate means in different statistical groups (Gomez and Gomez, 1984). The percentage data were transformed employing arc sine square root transformation prior to statistical analysis.

RESULTS

The types of shoot cuttings, IBA treatments and their interactions revealed significant effect of these factors on induction and growth of adventitious roots. Significantly superior adventitious rhizogenesis was exhibited by sprout cuttings (C_3) which had 2 times more rooting than seedling tip cuttings (C_1) and seedling cuttings (C_2) (Fig. 1). Also, sprout cuttings proved significantly optimum for all the growth characteristics of adventitious roots (Table 1).

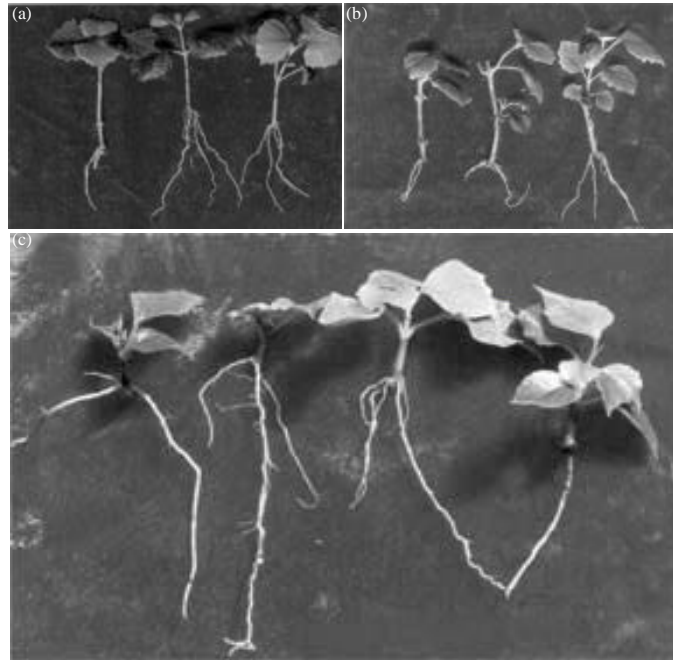


Fig. 1(a-c): Adventitious rooting in different types of *G. arborea* cuttings, (a) Seedling tip cuttings; (b) Seedling shoot cuttings and (c) Sprout cuttings

Table 1: Influence of cutting types and IBA treatments on induction and growth of adventitious roots in *G. arborea*

Variables	Characteristics				
	Rooting (%)	Root No.	Root length (cm)	Fresh weight (g)	Dry weight (g)
Cutting type (C)					
Seedling tips (C ₁)	33.10	3.46	3.65	0.449	0.045
Seedling cuttings (C ₂)	33.50	5.18	9.01	0.776	0.080
Sprout cuttings (C ₃)	63.00	10.40	10.89	1.322	0.227
LSD _{0.05}	9.07	2.73	1.31	0.340	0.028
IBA Treatments (T)					
Control (T ₁)	34.00	5.17	7.79	0.634	0.069
5 mM IBA (T ₂)	61.50	7.43	8.54	0.871	0.129
10 mM IBA (T ₃)	35.10	6.43	7.32	1.041	0.153
LSD _{0.05}	7.03	0.72	0.57	0.230	0.047

The treatment of shoot cuttings with 5 mM IBA resulted significantly superior for rooting, number of roots and root length and those with 10 mM IBA for root fresh and dry weight. The 5 mM IBA recorded about 2 times enhanced rooting over the control (T₁) and 10 mM IBA (T₃). The Treatment also enhanced root number by 43.7 and 15.5% and root length by 9.6 and 16.7% over T₁ and T₃, respectively (Table 1).

Among the interactions, 5 mM IBA (T₂)×sprout cuttings (C₃) exhibited significantly higher rooting which was 76 and 184% more than that recorded in the interaction of the same cutting type

Table 2: Interaction of cutting types and IBA on adventitious root induction and growth in *G. arborea*

Cutting type (C)	IBA treatments	Characteristics				
		Rooting (%)	Root No.	Root length (cm)	Fresh weight (g)	Dry weight (g)
C ₁	T ₁	43.3	3.73	4.77	0.470	0.045
	T ₂	38.8	4.10	4.23	0.487	0.050
	T ₃	21.7	2.53	1.97	0.390	0.041
C ₂	T ₁	26.2	4.00	9.40	0.777	0.079
	T ₂	43.2	5.53	8.73	0.776	0.080
	T ₃	31.7	6.00	8.90	0.773	0.081
C ₃	T ₁	33.0	7.77	9.23	0.655	0.085
	T ₂	93.9	12.67	13.63	1.349	0.258
	T ₃	53.4	10.77	10.11	1.961	0.337
LSD _{0.05}		11.71	1.26	ns	0.250	0.130

with 10 mM IBA and control (Table 2). Overall this treatment resulted in significantly superior increase in all parameters except root length in sprout cuttings. It optimized rooting (94%), root No. (12.67) and root length (13.6 cm). The higher IBA dose i.e., 10 mM IBA exhibited the best root fresh weight and dry weight even though the root induction was modest. However, shoot tip cuttings treated with both IBA treatments proved supra-optimal to inhibitory for root induction. For root growth characteristics, interaction C₃T₂ proved significantly optimum for root number. In contrast, C₃T₃ accumulated significantly better root biomass. Seedling shoot tip cuttings administered with 10 mM IBA (C₁T₃) significantly inhibited root induction and growth (number and biomass). Further, seedling shoot cuttings treated with both IBA doses (C₂T₂ and C₂T₁) had no influence on root induction and growth (Table 2).

DISCUSSION

The nature and type of cutting has a vital impact on adventitious rhizogenesis in tropical trees (Leakey, 1983; Dick and Aminah, 1994). Both types of seedling cuttings recorded almost equal amount of rooting but seedling shoot cuttings influenced significantly better root number, length root weights compared to seedling tip cuttings. However, in control condition the seedling tip cuttings provided the best root induction among all three types of cuttings indicating synthesis of root promoting substances; auxin/rhizocaline in shoot tips. Enhanced endogenous concentrations of these substances due to basipetal movement may have resulted in better rooting without exogenous auxin but became supra-optimal (more than required) in 10 mM IBA treatment. In contrast, exogenous Auxin (IBA) treatment affected enhanced rooting in seedling shoot cuttings as well as sprout cuttings. The effectiveness of IBA for adventitious rhizogenesis in shoot cuttings of tropical woody perennials has been well documented (Leakey, 1992; Mundt, 1997; Tchoundjeu and Leakey, 2000; Singh *et al.*, 2006). IBA provides more rooting than IAA due to relative stability and insensitivity to the auxin degrading enzyme systems and retention near the site of application (Mullins, 1972; Nickell, 1982).

A reason for low rooting success in seedling cuttings could be insufficient lignifications of these tender cuttings adversely affecting adventitious rhizogenesis and *ex vitro* acclimatization. In *Prosopis julifera* cuttings, Dick *et al.* (1994) have reported that an increase in lignified tissues decreases respiration rates, saving energy and assimilates for rooting process. Lignified cuttings from 2 year old seedlings of *G. arborea* have been recorded to show good rooting response (Zakaria and Ong, 1982; Sandum *et al.*, 1986).

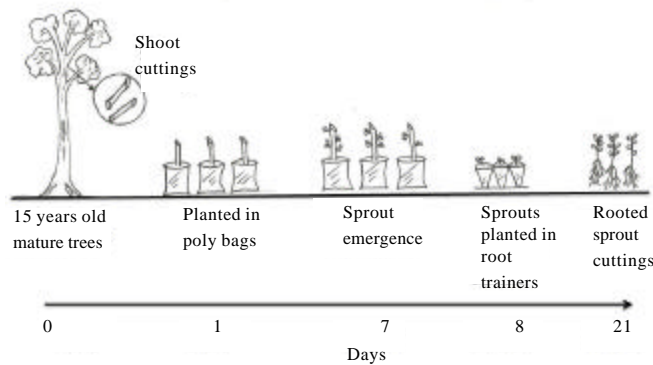


Fig. 2: Process of mass multiplication of mature trees of *G. arborea*

Decline in adventitious rhizogenesis with maturity in trees is the main hindrance in development of clonal forestry in many species. In *Eucalyptus* the development of the microcutting technique i.e., use of *in vitro* rejuvenated plants as sources of progagules (Assis and Rosa, 1992; Xavier and Comerio, 1996) and the minicutting technique, similar to earlier but devoid of *in vitro* stage (Xavier and Wendling, 1998; Wendling *et al.*, 2000) led to considerable gains, mainly derived from increasing the proportion of plants that rooted and shortening the time required to produce. Minicuttings have also been used in propagation of some other species e.g., pines (Andrejow and Higa, 2009; Majada *et al.*, 2012), guava (Marinho *et al.*, 2009), *Liquidambar styraciflua* (Wendling *et al.*, 2009) and Malaysian landscape trees including *Dillenia philippinensis*, *Lopanthera lactescens* and *Xanthestemon chryanthus* (Awang *et al.*, 2009).

In present investigation a different procedure was employed by collecting sprout cuttings produced on semi-hardwood shoot cuttings derived from mature ortets of 15 year age which provided significantly superior adventitious rooting in terms of rooting percentage, number, length and biomass compared to seedling tips and seedling shoot cuttings of 1 year old seedlings of *G. arborea* indicating towards rejuvenation in the cloning process. The procedure is rapid and efficient for mass multiplication of mature ortets with time span of 3 weeks (Fig. 2). Similar effect of “rejuvenation” of the clones with low rooting ability with the use of micro-cuttings has been reported in *Eucalyptus grandis* (Titon *et al.*, 2006). The sprouts emerging from the planted coppice shoots of mature trees (63 year-old) of *Tectona grandis* provided rooting response, speed of rhizogenesis and the root system morphology (the number of roots and root length) similar to those of cuttings from 1 to 2 year-old juvenile rootstocks (Palanisamy and Subramanian, 2001). Thus, rejuvenation of mature parent material is possible in sprout cuttings. Overall, the procedure of rooting of sprout cuttings appeared as a simple, inexpensive and efficient for mass clonal multiplication of mature superior/elite genotypes of *Gmelina arborea*.

In conclusion, the new procedure involving optimal rooting of rejuvenated sprout cuttings produced on semi-hardwood shoot cuttings derived from mature trees provides a means for mass clonal multiplication of mature ortets of *Gmelina arborea* having desirable traits for large-scale afforestation/plantation and *ex situ* conservation of the species.

REFERENCES

- Andrejow, G. M.P. and A.R. Higa, 2009. Rooting potentiality of *Pinus taeda* L. mini-cuttings collected from apical coppicing of young seedlings. *Floresta*, 39: 897-903.

- Assis, T. and O.P. Rosa, 1992. Goncalves SI: Propagacao clonal de *Eucalyptus* por microestaquia. Proceedings of the Congresso Florestal Estadual, Nova 7th, 1992, UFSM Anais Santa Maria, Prata, pp: 824-837.
- Awang, Y., N. Abdul Rahman and A.K. Sandrang, 2009. Adventitious rooting in microcuttings of selected indigenous landscape trees of Malaysia. Int. J. Agric. Biol., 11: 419-424.
- Dick, J.M. and H. Aminah, 1994. Vegetative propagation of tree species indigenous to Malaysia. Commonwealth For. Rev., 73: 164-171.
- Dick, J.M.P., D.G. Blackburn and C. McBeath, 1994. Stem respiration in leafy cuttings of *Prosopis juliflora* during the rooting process. New For., 8: 179-184.
- Gomez, K.A. and A.A. Gomez, 1984. Statistical Procedures for Agricultural Research. 2nd Edn., John Wiley and Sons Inc., Singapore, Pages: 704.
- Jain, S.K. and R.A. de Fillipes, 1991. Medicinal Plants of India. Vol. 2, Reference Publications Inc., Michigan.
- Kirtikar, K.K. and B.D. Basu, 1980. Indian Medicinal Plants. Vol. 3, Bishen Singh and Mahendra PalSingh, Dehradun, India.
- Lauridsen, E.B., E.D. Kjaer and M. Nissen, 1995. Second evaluation of an international series of *Gmelina provenance* trials/by Elmer B. Lauridsen, Erik D. Kjaer and Mads Nissen. Danida Forest Seed Centre, Humlebaek, Denmark.
- Leakey, R.R.B., 1983. Stock plant factors affecting root initiation in cuttings of *Triplochiton scleroxylon* K. Schum. an indigenous hardwood of West Africa. J. Horticult. Sci., 58: 277-290.
- Leakey, R.R.B., 1992. Enhancement of rooting ability in *Triplochiton scleroxylon* by injecting stockplants with auxins. For. Ecol. Manage., 54: 305-313.
- Majada, J., C. Martinez-Alonso, I. Feito, A. Kidelman, I. Aranda and R. Alia, 2012. Mini-cuttings: An effective technique for the propagation of *Pinus pinaster*. New Forestry, 41: 399-412.
- Marinho, C.S., L.M.A. Milhem, J.A. Altoe, D.G. Barroso and C.V. Pommer, 2009. Guava propagation through minicuttings. Rev. Bras. Frutic., 31: 607-611.
- Mullins, M.G., 1972. Auxin and Ethylene in Adventitious Root Formation in *Phaseolus aureus* Roxb. In: Plant Growth Substances, Carr, D.J. (Ed.). Springer Verlong, New York, pp: 526-533.
- Mundt, T., 1997. Vegetative propagation of teak (*Tectona grandis*) by cuttings. Teaknet Newslett., 7: 3-4.
- Nickell, L.G., 1982. Plant Growth Regulators-Agricultural uses. Springer-Verlag, New York.
- Palanisamy, K. and K. Subramanian, 2001. Vegetative propagation of mature teak trees (*Tectona grandis* L.). Silvae Genet., 50: 188-191.
- Sandum, A.K.I., P.B.L. Srivastava and M. Doraisingam, 1986. Trials of rooting of cuttings of *Gmelina arborea*. III. Effect of hormone treatment media and frequency of misting. Malaysian For., 49: 332-352.
- Singh, S., 2005. Studies on physiomorphological basis of adventitious rhizogenesis vis-a-vis clonal propagation of *Gmelina Arborea* Roxb. Ph.D. Thesis, FRI University, Dehradun.
- Singh, S., A.S. Bhandari and S.A. Ansari, 2006. Stockplant management for optimized rhizogenesis in *Tectona grandis* stem cuttings. New For., 31: 91-96.
- Surendran, C., 1990. Vegetative propagation in *Gmelina arborea* Roxb. through single noded cuttings. Indian J. For., 13: 162-164.
- Tchoundjeu, Z. and R.R.B. Leakey, 2000. Vegetative propagation of *Khaya ivorensis* (African mahogany): Effects of stockplant flushing cycle, auxin and leaf area on carbohydrate and nutrient dynamics of cuttings. J. Trop. For. Sci., 12: 77-91.

- Titon, M., A. Xavier and W.C. Oton, 2006. Clonal propagation of *Eucalyptus grandis* using the mini-cutting and micro-cutting techniques. *Scientia Forestalis*, 71: 109-117.
- Troup, R.S., 1992. *The Silviculture of Indian Trees*. ICFRE Publication, Dehradun.
- Wendling, I., A. Xavier, J.M. Gomes, I.E. Pires and H.B. Rade, 2000. Propagacao clonal de hibridos de *Eucalyptus* spp. por miniestaquia. *Revista Arvore*, 24: 181-186.
- Wendling, I., G.E. Brondani, L.F. Dutra and F.A. Hansel, 2009. Mini-cuttings technique: A new *ex vitro* method for clonal propagation of sweetgum. *New For.*, 39: 343-353.
- Xavier, A. and I. Wendling, 1998. Miniestaquia na clonagem de *Eucalyptus* (Minicutting cloning of *Eucalyptus*). Technical Information SIF, 11, Vicosa: SIF, pp: 10.
- Xavier, A. and J. Comerio, 1996. Microestaquia: Uma maximizacao da micropropagacao de *Eucalyptus* [Microcuttings: Maximization of *Eucalyptus* micropropagation]. *Revista Arvore*, 20: 9-16.
- Zakaria, B. and T.H. Ong, 1982. Vegetative propagation of yemane *Gmelina arborea* by stem cuttings. *Malaysian For.*, 45: 282-284.