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Clonal propagation of *Gmelina arborea* Roxb. An important multipurpose tree species of north eastern region

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Abstract
The present investigation was targeted to conduct the study of rooting behaviour of different genotypes of *Gmelina arborea.* Rooting of different genotypes shows the effect of collection time of planting material, type of cuttings and also the effect of growth regulatory substance. Nodal sprouting in different genotypes was very good and healthy in all most all type of cuttings. In branch cuttings rooting observed in IBA200ppm and followed by NAA200ppm. In green cuttings NAA100 and 200ppm shows rooting.

Keywords: *Gmelina arborea*, clone, propagation, branch cuttings, green cuttings

1. Introduction
Gmelina arborea (Roxb.) is a tropical medium sized deciduous forest tree species reaches up to 30-35 m in height and 3-4 m diameter under the family Verbenaceae. The species is commonly known as white teak grwon extensively in India and other Southeast Asian countries for timber, fodder and industrial purpose. The species is fast growing and distributed in South-East Asia and occurring naturally throughout India, Nepal, Sikkim, Bangladesh, Sri Lanka, Myanmar, Thailand, Laos, Cambodia, Vietnam and the southern provinces of China. In India the tree is distributed naturally Assam and west Bengal to Orissa. Poor germination of seeds in *G.arborea* is reported by many authors due to unknown region (Omoyiola 1974, Okoro 1983, Hartman and Koster, 1975) [34, 33, 11]. and also reported for this inability of seeds germination may be due to over ripeness or over fermentation of the pulpy part of the fruit which causing death of the embryo. Besides this, *Crespedonta leayana* (leaf defoliator) the mostly attacked insect pest of the species have constantly been a hurdle for a successful plantation. The insect has been responsible for complete abondoning of plantation in very large areas. This is considered a useful multi - purpose species and wood is used as raw material for cellulose (Foelkel *et al*., 1978) [6], firewood (Lugo *et al*., 1988) [22], pole wood (Moya, 2004a) [25], particle board (Chew and Ong, 1989)[2], veneer (Sicad, 1987)[45] and structural uses (González *et al*., 2004)[8].The species has been cultivated commercially for timber as furniture, pellets boxes, veneers, industrial wood etc. Timber wood is excellent for its durability, lack of shrinkage and distortion, smooth finish and for general construction, in making furniture and for making agriculture implements, paneling, carriages, carpentry, boxes, pulp and plywood industries. Leaves are used as a green fodder due to the presence of high protein content. Its multipurpose utility has gained the attention of farmers, industries and forest departments for commercial cultivation in farmlands. Besides this, it has also gained widespread acceptance as a plantation species for source of pulp and fiber production and for various farm and agro forestry schemes (Doat 1976) [5]. The species comprises high medicinal value of leaf, flower, root bark and fruit are used in many Ayurvedic preparations. It is also grows rapidly and can be harvested under short rotation, hence progressively becoming an alternative to the species harvested from natural forests (Alfaro and De-camino, 2002) [1].
Work on the genetic improvement of *G. arborea* has been initiated by the Indian Council of Forestry Research & Education (ICFRE) institutes through assembling divergent populations in the gene banks from different parts of the country and the work was started by Rain Forest Research Institute, Assam during 2003 and assembled different divergent Populations by using various conventional methods in gene bank of the species and clonal seed orchard were established at Naharoni, Assam. These orchards age is more than 12-13 years. Vegetative propagation by rooting stem cuttings is a simple and comparatively less expensive method for clonal multiplication of genetically superior trees. Further, propagules produced by vegetative means retain the genetic constitution of parent plants without segregation. Hence, it has been

practiced for a long time in forestry and horticulture to produce planting stock of desired genetic constitution in tree improvement programmes.

Besides this, vegetative propagation can be used for regeneration of tree species having problems of irregular seeding habits, long flowering and fruiting intervals, poor seed setting, low percentage of germination and undesirable short or long period of seed dormancy (Surendran and Seethalakshmi, 1987) [53]. Though the species has number of promising attributes, it also exhibit major drawback of poor stem form, low productivity and susceptibility to the insect pests etc. An understanding of the natural variation of the species it is important to conserve traits is essential to protect the species for improvement programs.

Hence, the present research work has been carried out to study for the propagation of improved planning stocks for future research programme.

2. Materials and Methods

Following the methodology among the various methods of vegetative propagation, rooting of cuttings was adopted, and the most convenient, less expensive and already been proved in several species for successful for multiplication of selected trees (Romero, 2004) [48]. The present experiments were conducted at Rain Forest Research Institute, Jorhat, campus (elevation 116 m; 94.2026° E longitude and 26.7465° N latitude) in the month of January to September. To study the effect of season on rooting of branch cuttings, green and coppice planting material was collected in the three different seasons and conducted under nursery condition.

2.1 Source of cutting materials and preparation

Branch cutting trials were conducted with matured branches of 12 years old clonal trial established at Naharoni, Golaghat, Assam (Fig.1). Cuttings were prepared from brown wood (Fig.2) with dormant bud in about 1.5 to 2 cm diameter 16-18 cm long. Green wood coppice cutting materials were collected from established VMG of RFRI (Fig.3 a-b).Coppice materials were collected at the age of 4 and half months (Fig.4 a-b). In case of green wood cuttings leaves are trimmed down to 1/3 of original size, and small new leaves below the terminal buds are kept as usual. Coppice and green cuttings were tested in the mist chamber and matured branch cutting were under nursery condition. Experiment was conducted to study the effect of different seasons, growth regulators concentration, the best season for collection of planting materials for rooting etc. Trials were carried out during January to September.

Collection of planting materials was made during morning before the sun light intensity is high and was brought to working field as soon as possible. Shoots were put in a bucket of water immediately after collection to avoid water loss and kept in shade till collection work is not completed. Prepared branch cuttings were consists of at least two nodes with 17-18 cm long and leaves retained where it is possible but half of the leaf blades were trimmed and reducing the leaf area approximately 1/3 of the original area (Leakey *et al.* 1982 and Smits, 1983) [21, 55]. Terminal buds were clipped off, but the lateral active or dormant buds were retained where along the entire length of the cuttings. The basal cut usually just below the node and the top cut is 1.5cm - 2.5 cm above a node (Hartmann *et al.* 1993) [15]. Using a very sharp pruning shear cut at the basal end and made oblique to ensure a greater surface for absorption of hormone and more area for callus formation and subsequent rooting. Coppice shoot tips were taken with single node 5-6 cm in length.

All cuttings were put into tub containing fungicides solution like bavestin for 10 to 15 minutes. 20 gm of bavestin was dissolved in 10 liters of water (0.1%) in which cuttings to be immersed. The basal end of the cutting was dipped in water immediately to prevent air bubbles entering the vascular system which may later interfere with the absorption of growth regulating substances. After fungicides treatment basal part of the cuttings treated with auxin solution to a length of 1 inch and upper part of the shoot cuttings were treated with hot waxed to avoid aeration and were inserted in the potting media coarse sand for green wood cuttings and coppice shoots, sand and soil: sand: FYM 1:1:1 for branch cuttings.

Fig 1: Selected clones of *G. arborea* at Naharoni, Golaghat, Assam

Fig 2: Branch cuttings

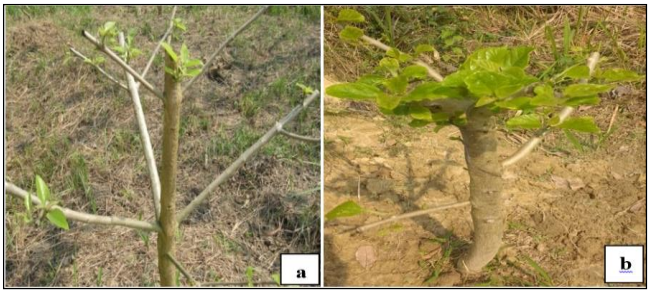

Fig 3: a-b Coppice and green wood materials of vegetative multiplication garden (VMG)

Fig 4: a-b Bulk of coppice shoots and after preparation

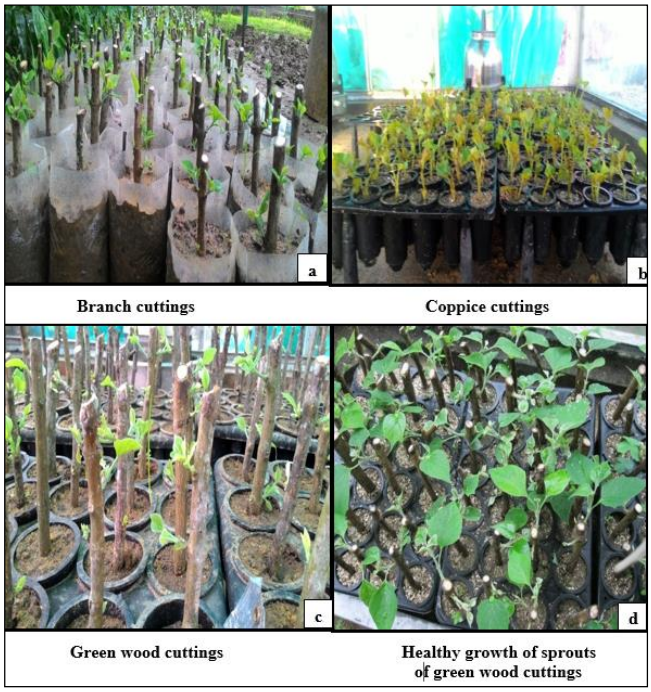

Fig 5: Sprouted cuttings of *G. arborea* under nursery and mist condition

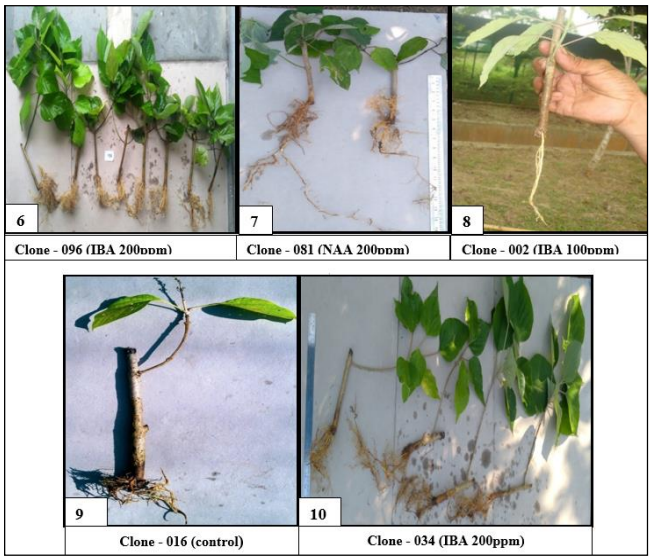

Fig (6, 7, 8, 9, 10): Rooting of branch cuttings of *G. arborea*

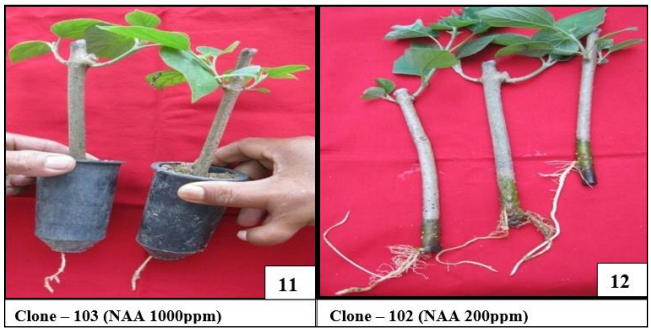

Fig (11, 12): Rooting of green cuttings of *G. arborea*

2.2 Preparations of polythene bags and watering schedule
Polythene bags of 9x7 inch size were used to supply greater amount of moisture to the cuttings. Bags were properly pierced for proper drainage. Growing medium contained coarse river sand and was sterilized by making heap on a polythene sheet, moistens and covered with black polythene sheet for 8-12 days in direct sun turning the heap after every 2 days. After planting, watering was carried out immediately to create high humidity around the cuttings and ensure an adequate amount of moisture in the rooting zone. Mist was created manually controlled misting heads placed in the propagation unit. During the first seven days after planting misting was done 9:00 a.m. and 5:00 p.m. In the next 15 days, misting was done at hourly intervals for five minutes. The mean relative humidity was around 80% with 30°- 35° temperatures. In the nursery watering is also carried out regularly in the winter morning and evening and in the rainy season, from mid of April to July was done as per their requirement.

2.3 Preparation of Stock solutions
To prepare 1000 ppm stock solution of IBA, IAA and NAA accurately weigh out 1.0 gm of rooting hormone and dissolve in few drops of full strength ethyl alcohol and make this solution up to 1000 ml using distilled water. The stock solution can be diluted to provide lower concentrations e.g., if the stock solution needed the following concentrations can be made by diluting as indicated,
1000 -ppm - 1 pt stock solution
500 -ppm – 1/2 pt stock solution + 1/2 pts distilled H_20
200 -ppm - 2 pt stock solution + 8 pts distilled H_20
100 -ppm - 1 pt stock solution + 9 pts distilled H_20
50 -ppm – 1/2 pt stock solution + 9 and 1/2 pts distilled H_20
The chemical is diluted in water to targeted concentrations and basal parts of the cuttings were soaked in the solution for overnight and pulse treatment.

2.4 Hormonal treatment
The experiments were laid down with different hormonal concentrations of IAA, IBA and NAA. For branch cuttings trial IAA, IBA and NAA were treated with 200ppm overnight dipping, green cuttings were treated with 500ppm, 1000ppm IAA, IBA and NAA for 25 min. Coppice cuttings were treated for pulse treatment in 50ppm, 100ppm for 1min.
Green and coppice cuttings were kept under the mist condition. Branch cuttings were kept under nursery condition and top area was covered by agro shed net to reduce the direct sunlight. Cuttings were grouped together of each genotype and randomly distributed in the rooting trays. Basal 0.5cm portion was dipped in the aqueous solution for all the cuttings and were inserted into the rooting medium and pressing firmly around them.

3. Results
Rooting of the species shows effect of season and type of cuttings that is branch and green cuttings. Results also indicate the factors of growth regulating substance (GRS) and their concentration in induction of rooting. In case of sprouting, branch cuttings possess similar type of results in all the concentrations i.e. IAA, IBA and NAA (200ppm) (Fig.5a). In green cuttings, bud sprouting was also observed very well (Fig.-5c) and nice healthy growth was continued up to 3-4 months (Fig.-5d). Coppice material, possess no rooting and leaves were green and healthy under the mist chamber (Fig. 5b) and gradually fall off within 1-2 weeks.
Branch cutting collected during the period of January to March was most effective for rooting of the species. The hormonal treatment of overnight dipping in NAA 200ppm (Fig.6 & 10) shows better rooting and followed by IBA 100 and 200ppm (Fig.7&8). In green cuttings, planting material collected in the month of February to March shows rooting

NAA 1000ppm (Fig.11) and NAA 200ppm (Fig.12). Rooting also observed in control GA016 (Fig.-9). Though, the age of the collection source of planting material is a most important factor the results indicate that optimum conditions for rooting with species which is depend on the age and nature of cuttings used for rooting experiments. In the present investigation with the treatment of various GRS, rooting was observed during seven months. Maximum number of roots 07 developed with a treatment of NAA 200 ppm followed by 200ppm IBA. More number of roots was also observed in cuttings treated with auxin 200ppm IBA as compared to the control. Uprooting was done during July. In control, rooting was observed only in cuttings collected during January and March with a maximum response of 16%.

Present study reveals that spring season was most effective for sprouting and rooting of the species. This season was followed by rainy season. In the branch cuttings within the season IBA was most effective growth hormone in comparison to NAA. In spring season IBA resulted highest rooting percentage with 34.66% in GA096 and in rainy season it was maximum with 20% in GA034. IBA 100 ppm resulted 18% of rooting in the rainy season in GA002. Comparing the different concentrations of the growth regulators, 200 ppm IBA was the best considering the all season. In the green cuttings, minimum percentage of rooting also observed in NAA 1000 ppm of GA103 and in NAA 200ppm for GA102. Rooted cuttings were transplanted in polythene bags filled with 1:1:1 soil: sand: FYM and were placed under shade inside the greenhouse and watered regularly.

4. Discussion

Rooting of cuttings and root formation is generally depends on the nutritional status of cuttings. It is generally observed that cuttings with high carbohydrate content shows better rooting than those with low carbohydrate content. The difference in rooting ability of adult and juvenile cuttings, have also been attributed to the difference in their nutritional status. The seasonal variation was observed in rooting potential of cuttings from the species may be either due to physiological nature of the cuttings or variation in external factors or interaction of both. These factors have a profound effect on the success of rooting to stem cuttings (Pal 1990, Joshi, *et al.* 2002, Nautiyal *et al.* 1991, 1992) [27, 11, 23, 24]. Several workers have also reported the effect of season on vegetative propagation of different tree species. Rooting depends on season of collection of cuttings, treatment of the cuttings and environmental conditions. In our experiment, response of rooting spring season was observed as best This was also reported by Palanisamy and Kumar (1997) [28] in *Pongamia pinnata*, Verma and Puri (1996) [31], Palanisamy *et al* (1998) [29] in *Azadirachta indica*, Shamet and Dhiman (1991) [37] in *Grewia optiva*, Uniyal *et al.* (1995) [44] in *Dalbergia sericea* and observed that cuttings rooted well in spring season. Similarly, in case of green wood cuttings Modgil and Nayithal 1998 [18] was observed sprouting during spring season in *Anogeissus latifolia* while, cutting failed completely to root. In *Dalbergia sissoo* Gupta *et al.* (1993) [6] found that the cuttings of rooted well in rainy season. Gurumurthi *et al.* (1994) [7] also reported for rooting in *Acacia nilotica* during in the same season and cuttings failed to root during winter season.

Similar study was reported by Nautiyal *et al.* (1991) [23] they found maximum rooting response in *Tectona grandis* during rainy season while, no rooting was initiated during winter months. In our study we have used three types of cuttings that the nature which has a vital impact on adventitious rhizogenesis in tropical trees was reported Leakey 1983 [19], Dick and Aminah, 1994 [3]

4.1 Hormonal effect

Auxin is necessary for optimal production of roots, type of auxin and its concentration is also plays an important role in the success rooting. In general, rooting response was enhanced and the period for which rooting obtained was extended by treatment with GRS. Various external factors like humidity, light, temperature, rooting medium and internal such as factors endogenous growth regulating substances, level of nutrients, growth retardants and anti-metabolites are known to affect rooting in cuttings (Komissarov 1964, Nanda 1970, Hartman and Kestler 1976) [17, 29, 12]. Role of auxin to promote the rooting of shoot cuttings of many woody plants have been shown by Nanda1970, Hartmann and Kester 1983, Pal *et al.* 1994[29,13,36].

Depending on the endogenous level of GRS, application of exogenous GRS may be promotive, ineffective or even inhibitory for rooting of cuttings (Nanda, 1970) [29]. In our study superiority of hormone, IBA shows over NAA. Auxin like IBA and NAA were more effective is reported by (Nanda and Kochar, 1985, Ragnose *et al*., 1973, Verma and Puri, 1996 and Palanisamy *et al.,* 1998) [28, 41, 40, 38]. The effect of IBA was recorded as best auxin for inducing rooting by Palanisamy and Kumar 1997 [37] Palanisamy *et al.* 1998 [41]. Reddy *et al.* 1998 [42], studied that the IBA was the best rooting hormone as compare to lAA and NAA for the stem cuttings of *Acacia concina*. Where, Palanisamy *et al.* (1997 and 1998) [37, 41] recorded *Pongamia pinnata* with IBA 800 ppm and 1000 ppm, respectively. 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) [20, 27, 54, 50]. 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) [26, 32]. Mayavel *et al.* (2014) [23] reported a similar type of study on clonal propagation of *G. arborea* for multiplication of selected superior CPTs treated with 750 ppm Indole -3 Butyric Acid (IBA). In our study also 200ppm IBA shows best result over NAA. IAA produced no rooting in any season. Ujjwala *et al.* 2013 [55] reported on clonal propagation of *G. arborea* through the stem cuttings in 500-5000ppm NAA for 12 hours and showed highest percentage of response. In the present investigation 1000ppm NAA and 200ppm NAA treatment shows nice rooting in green cuttings.

4.2 Age of branch cuttings

In our study branch cutting materials were collected from matured clone. A similar type of study was reported by Surendran and Seethalakshmi, 1987 [53]. In his study he recorded the failure to induce rooting in cuttings as one of the reason for the tree species such as *H. cordifolia*, *H. parviflora*, *X. xylocarpa*, *M. dubia* and *S. macrophylla* and it may be possibly the effect of age branch cuttings were collected from mature trees more than 14 years old tree. Reduced rooting potential due to aging is attributed to the production of rooting inhibitors as in eucalypts (Paton *et al.,* 1970) [39] or reduction in rooting cofactors such as phenols in *Hedera helix* (Rigouard, 1969) [43]. Stem cuttings taken from young seedlings root much more easily than those taken from older plants (Gardner, 1929, Hitchcock and Zimmerman, 1932, Sax,

1962, Libby *et al.*, 1972) [7, 15, 47, 21]. Similarly, in *Prosopis julifera* cuttings, Dick *et al*. (1994) [4] have reported that an increase in lignified tissues decreases respiration rates, saving energy and assimilates for rooting process. Lignified cuttings of *G. arborea* have been recorded to show good rooting response (Zakaria and Ong, 1982; Sandum *et al*., 1986) [57, 46]. Surendran 1990) [52] reported on rooting of brown wood cuttings of *G. arborea* in 100 and 1000 ppm IBA. He also reported that the green wood cuttings did not root at all in *G. arborea* and this failure may be due to the immaturity of axillary buds. In case of our study also green wood cuttings possess very minimum rooting. Singh and Ansari, 2014[49] reported the reason for low rooting success in seedling cuttings, which could be insufficient lignifications of these tender cuttings adversely affecting adventitious rhizogenesis.

5. Conclusion

The species is suitable for rooting in IBA 200ppm and NAA 200ppm for branch cuttings. The different clone shows low rooting due to their maturity. Green cuttings show rooting under mist. Collection time and type of cuttings is also shows as important for rooting of the species. During spring season followed by rainy is best for rooting of branch cuttings under nursery. The result may possess genotypic difference of rooting, so further study is required for mass propagation.

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