



Shoots rooting of different genotypes of *Dipterocarpus retusus* bl. *Syn macrocarpus*: An endemic timber species of north eastern region

Papori Phukan Borpuzari^{1*}, Jonaki Kachari²

^{1,2} Rain Forest Research Institute, Biotechnology and Genetics Division Jorhat, PB -136, Assam, India

Abstract

Dipterocarpus retusus Bl. syn *D. macrocarpus* is an economically important tree species of tropical wet evergreen forest of Assam. The tree is locally known as 'hollong' belongs to the family Dipterocarpaceae. It is available in a very few forest areas of Arunachal Pradesh, Assam, Manipur, Meghalaya, Nagaland, Tripura and West Bengal. The species has a tremendous genetic diversity in the state of Assam and Arunachal Pradesh. Taken into account of genetic diversity of the region and for genetic potential of future plantation programme primary work on survey, selection (more than 100 plus trees) and establishment of the trees has been completed as 'planting stock improvement programme' under World Bank project. Among them, 23 half-sib progenies has been raised as hedge garden at RFRI, campus and present investigation was conducted with these planting materials. Due to very low coppicing ability of the species sprouting of the genotypes in the hedge garden were recorded at 15 days interval up to 135 days i.e. to the level of end days of four months age of the shoot cuttings as per the experiment laid out. Statistically analyzed data by measuring trend lines shows different coppicing ability. Among the genotypes coppicing value is highest in JRP-4 (0.98) and lowest in JKG-3 (0.71). Shoot cutting trials were tested for two seasons, January to April and May to August with four different hormonal concentration viz., 200, 500, 1000 and 2000 ppm of IBA. Experiment carried out to study the effect genotypes, type of cuttings, hormonal concentration and best seasons for optimum rooting. Observation recorded from May to August as the best collection period for rooting of propagated shoot cuttings. Both apical and non apical shoots responded in rooting within 6-8 weeks and highest percentage of cuttings rooted after 4 weeks. Among the genotypes mean percentage of rooting performed best in apical cuttings, highest percentage is recorded in DMP-9 (59.2±7.45) treated in 1000 ppm and lowest is (25.1±14.38) in control. Mean survival percentage is recorded (63.09 ± 4.6). Recorded data of rooting of shoot cuttings were analyses for two-way ANOVA and the results were significant at 0.01% level of significance within the genotypes and concentration. Hormonal treatment did not varied significantly for rooting. As the findings out of twenty three genotypes fourteen (14) genotypes with one check has been established at RFRI, campus.

Keywords: *Hollong*, shoots rooting, selected genotypes, apical, non apical cuttings

Introduction

Dipterocarpus retusus commonly known as 'hollong' is the state tree of Assam lies under the family Dipterocarpaceae. Owing to its qualitative characters it is the most wanted commercial 'A' quality timber for plywood timber, straight round cylindrical bole with more than 50 meter height and more than 3 meter dbh (Anon, 1997)^[4], (Rajput, 1996)^[29]. The tree is endemic to north east India and economically important of Assam tropical wet evergreen forest by (Champion and Seth, 1968)^[6]. Family Dipterocarpaceae constitutes most of the hard wood tree species of Southeast Asian rain forest (Aston, 1982)^[5]. The plant is a native of Southeast Asia to China, Indonesia, Malaysia, Myanmar, Thailand and Viet Nam. In India, it is available a very few of certain forest areas in Arunachal Pradesh, Assam, Manipur, Meghalaya, Nagaland, Tripura and West Bengal between 92-96° E longitudes, 26° - 28°N latitude. Propagation of the species is difficult due to non-availability of genetically superior seeds and when they do fruit they produce low quality seeds because of frequent insect and fungal attacks. It is therefore difficult to predict the yield and quality of seeds for an afforestation

programme. Seed collection and storage of *D. retusus* possesses only 15 days viability and studies on seed maturity, germination and storage were conducted at RFRI and findings recorded the possibility of storing of seed for a longer period of time in future and the chilling sensitivity of the seeds which supports recalcitrant nature (Kundu and Chanda, 2001)^[16]. Vegetative propagation of tropical species by stem cuttings is an important alternative for production of high quality and uniform planting stock for large-scale afforestation. They save time and labour in seed collection and storage and produce uniform planting material and the genetic purity of superior parent stock. The family lies in difficult to root category (Adjers and Otsamo, 1996)^[1]. Propagation through rooting of shoot cuttings is also restricted to juvenile material from seedlings or coppice shoots of younger trees (Kantarli, 1995)^[14]. The need of vegetative propagation of the family has been emphasized by (Hall & Kamil 1981)^[9], (Srivastava and Penguang 1981)^[35]. A very little success has been reported in dipterocarp genera. Hence, the initial project works was completed for species improvement under World Bank Free project with survey, selection and collection of genotypes

from different areas of Assam and Arunachal Pradesh. Where, plants were selected from natural forest with definite selection criteria and were considered for improvement of planting stock as plus trees. In order to perpetuate a broad genetic base, with selected 23 genotypes a hedge garden was established at RFRI, campus. Therefore, in continuation, the present research work was conducted as follow up for clonal multiplication of the above mentioned selected genotypes to maintain the heritability of the species and will focus on ex-situ conservation and propagation for sustainable development of the species.

Materials and Methods

Source of cutting materials and preparation

Present study of shoot cutting trials from hedge garden was tested for two seasons, one in January to April and another is May to August. Within this period continuous collection were made depending upon the availability of coppice as apical and non apical shoots to study the effects for the selection of optimum root initiating hormones individually with different hormonal concentration of indole - butyric acid (IBA). Cuttings were collected from juvenile plants sprouts on stumps as coppice or juvenile shoots of 4 and half months old material (Plate-A, Fig.1-2). Collection was made during cool hours in the morning before the sun light intensity is high. Two node cuttings and apical with single node were prepared, leaves were trimmed and reducing the leaf area approximately 1/3 of the original area (Plate-A, Fig.3) based on the methods of Leakey *et al.* (1982) [18] and Smits (1983) [34]. Firm shoots with slight lignifications with auxiliary buds were selected and shoots were put in a bucket of water immediately after collection to avoid water loss and also kept in shade till collection work is not completed and sprayed with water immediately after collection. Transportation time was maintained as minimum as possible. The apical cuttings were about 8-10 cm long with few leaves retained. Non apical cutting varies in length from 12 cm - 14cm, consists at least two nodes and basal cut usually just below the node and the top cut is 2 - 3 cm above a node (Hartmann *et al.* 1993) [11]. Terminal buds were clipped off and the lateral buds active or dormant were retained along the entire length of the cuttings. Using a very sharp pruning shear cut at the basal end was made oblique angle to ensure a greater surface for absorption of hormone and more area for callus formation. Cuttings are immediately put in a tub containing fungicides like bavestin solution for 5 to 8 minutes (0.1%) in which cuttings to be immersed. After fungicides treatment basal part of the cuttings treated with auxin solution to a length of 1/2 inch and upper part were treated with hot waxed to avoid aeration and inserted in the potting media i.e. in coarse sand and pressed firmly around the cuttings. In order to keep the cuttings turgid water sprayed repeatedly given to the cuttings while preparing it. They were grouped together of each genotype and randomly distributed on the polythene bags. Experiments was opted for IBA with four different hormonal concentration viz., 200 ppm, 500 ppm, 1000 ppm and 2000 ppm and treated for liquid pulse treatment.

Preparations of polythene bags and watering schedule

Polythene bags of 7x9" size were used in order to avoid root

coiling and supply greater amount moisture to the seedlings. Bags were properly pierced for proper drainage. The growing medium contained only 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. Rooted cuttings were transplanted in polythene bags filled with 1:1:1 soil: sand: FYM and were placed under shade inside the greenhouse, watered regularly and gradually prepared for field planting after proper growth. 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 by means of three manually controlled misting heads placed in the propagation unit. During the first seven days after planting, misting was done for five minutes in every half an hour between 8:00 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 about 80 -85% and the minimum and maximum temperatures were 30-35°C in the mist chamber.

Plate- A

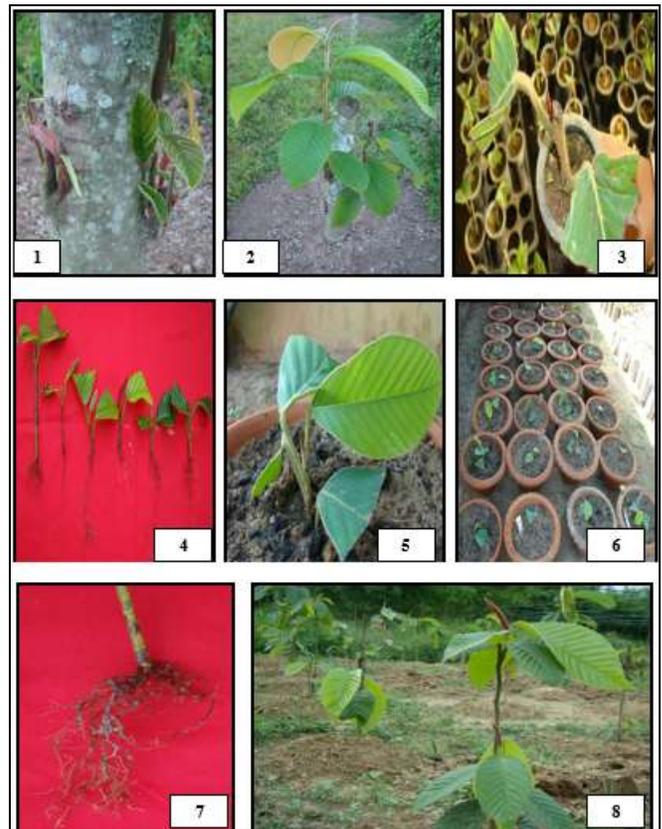


Plate A: Vegetative propagation through rooting of shoot cuttings of *Dipterocarpus retusus*: 1-2 Sprouting of shoots in the hedge garden, 3 Budding of shoot cuttings during trials under mist chamber, 4 Rooting of shoot cuttings, 5 Single rooted plants transplanted into earthen pot, 6 Several rooted plants transplanted into earthen pot, 7 Rooted plants before transplanting into the field, 8 Established clonal hedge garden of *D. retusus*

Statistical analysis

In case of sprouting of buds observations were recorded on 15

days interval of different genotypes in the hedge garden. The data were analyzed statistically by measuring trend lines of coppicing ability up to 135 days i.e. to the level of end days of four (04) months age of the shoot cuttings as per the experiment laid out. In case of rooting, data recorded for effect of hormonal concentration of different genotypes of apical and non apical cuttings. Number of shoot cuttings used for each experiment was designed for 25 numbers. Statistical analysis represents two way ANOVA for 1.0 and 5.0% level of significance (Table 2). Hormonal treatment did not varied significantly for rooting.

Results & Discussion

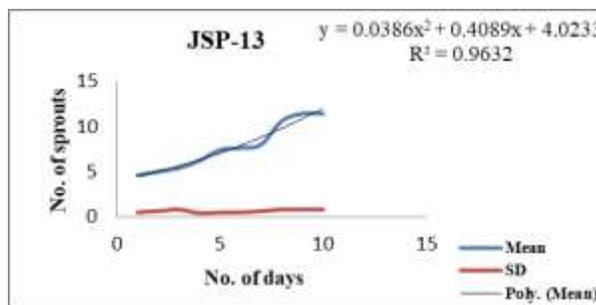
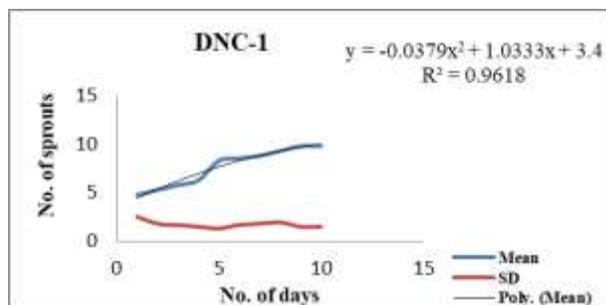
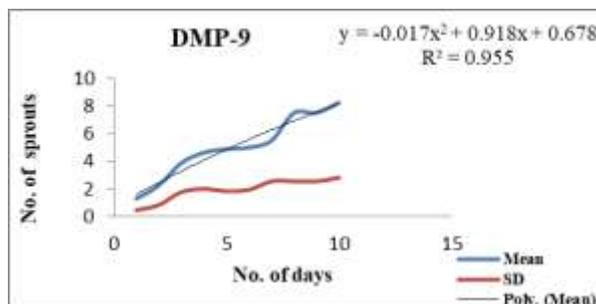
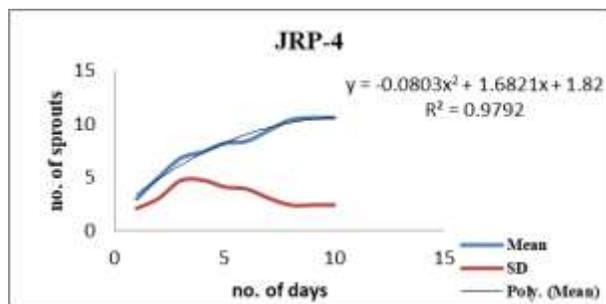
Best suitable season of collection of shoot cuttings

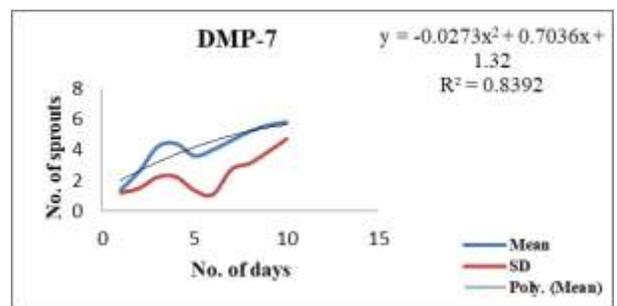
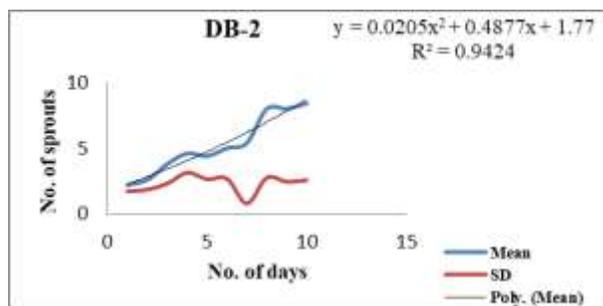
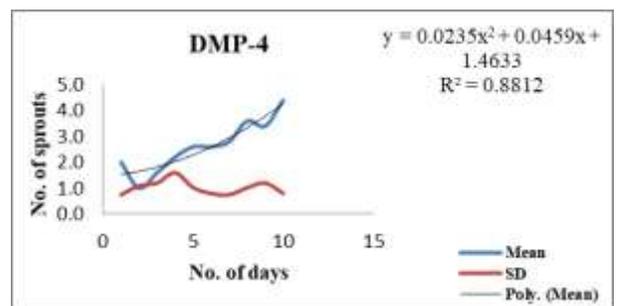
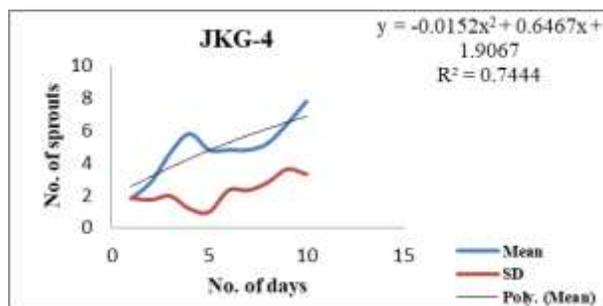
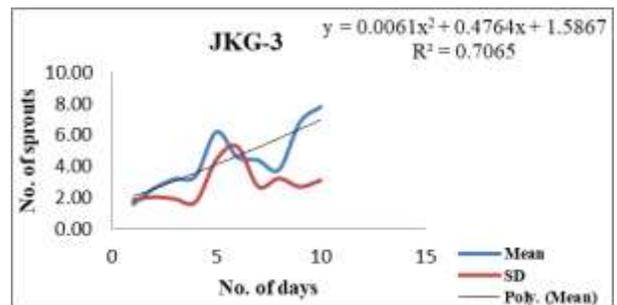
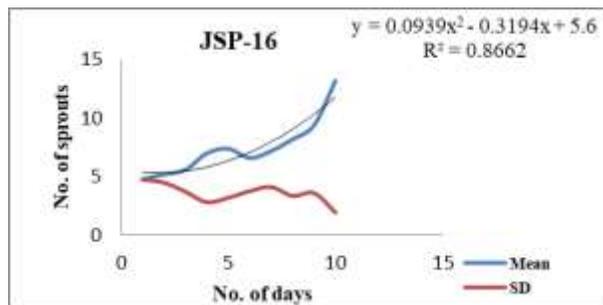
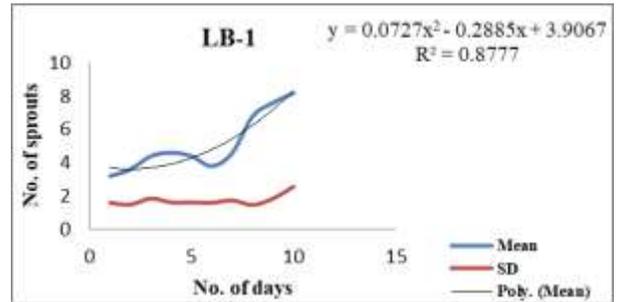
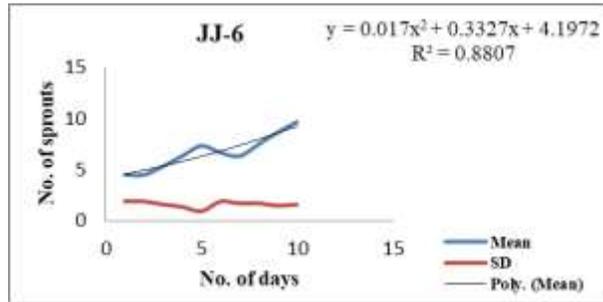
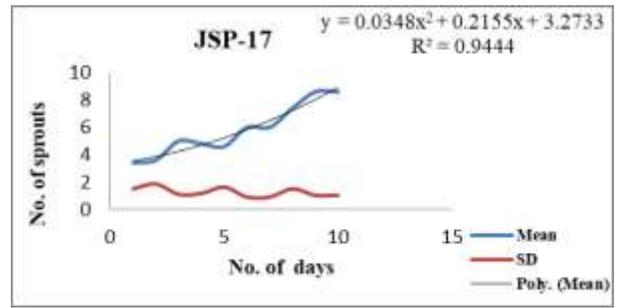
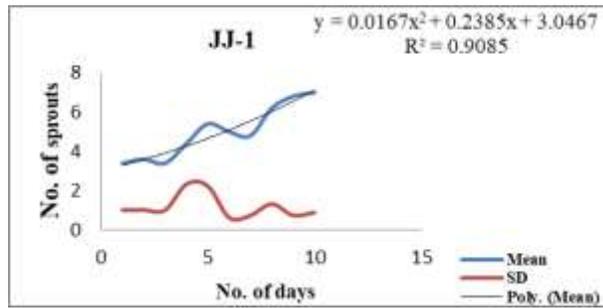
The species was observed very low coppicing ability (Fig.1-2, Plate-A) Hence, the collected shoot material is also very less. An average of 20-30 numbers of shoots has been available for

one time collection from 3-4 plants. In case of sprouting of buds recorded data of different genotypes possesses different coppicing ability. But, the similarity among all of the genotypes was recorded as increasing trends in number of coppice regeneration. Number of coppicing ability of each genotype recorded up to 135 days and results illustrated in the (Table 1). Statistically, trend line estimated coppicing value of adjusted R² is highest in JRP-4 (0.98) and lowest in JKG-3 0.71 (Fig. A 1-17). Analysis of recoded sprouting data, the value of adjusted R² is polynomial equation which is found to be highest than other equations. Hence, this equation has been selected as best equation for representing the data for sprouting of buds. Comparing between two different seasons observation recorded May to August was best suitable collection period as compared to January to April. The period between September to December is treated as bud dormant period.

Table 1: Response of sprouting of different genotype of *Dipterocarpus retusus* of hedge garden at 15 days of interval

Clone No.	1D	15D	30D	45D	60D	75D	90D	105D	120D	135D
DMN-9	1.3±0.46	2.3±0.83	3.8±1.76	4.6±2.00	4.8±1.83	5.0±1.94	5.5±2.55	7.5±2.55	7.5±2.55	8.25±2.82
JRP-4	3.0±2.10	5.0±3.03	6.8±4.71	7.4±4.76	8.2±4.12	8.4±3.93	9.4±3.07	10.4± 2.42	10.6± 2.42	10.6±2.42
DNC-1	4.7±2.49	5.2±1.79	5.7±1.64	6.3±1.48	8.2±1.30	8.5± 1.66	8.7± 1.79	9.3± 1.92	9.7± 1.48	9.7± 1.48
JSP-13	4.6±0.49	5.0±0.63	5.4±0.80	6.2±0.40	7.4±0.49	7.6± 0.49	8.0± 0.63	10.6± 0.80	11.4± 0.80	11.4± 0.80
JSP-17	3.4±1.50	3.6±1.85	5.0±1.10	4.8±1.17	4.6±1.62	6.0± 0.89	6.0± 0.89	7.4± 1.50	8.6± 1.02	8.6± 1.02
JJ-1	3.4±1.02	3.6±1.02	3.4±1.02	4.4±2.33	5.4±2.24	5.0± 0.63	4.8± 0.75	6.2± 1.33	6.8± 0.75	7.0± 0.89
JJ-6	4.5±1.89	4.5±1.89	5.3±1.60	6.3±1.37	7.3±0.94	6.6± 1.80	6.3 ±1.70	7.5± 1.71	8.67± 1.49	9.6± 1.60
LB-1	3.2±1.60	3.67±1.5	4.4±1.85	4.6±1.62	4.4±1.62	3.8± 1.60	4.6± 1.74	6.8± 1.47	7.6±1.85	8.2± 2.56
JSP-16	4.8±4.75	5.2±4.49	5.6±3.72	7.0±2.83	7.4±3.20	6.6± 3.77	7.2± 4.12	8.2± 3.37	9.4± 3.61	13.2± 1.94
JKG-3	1.6±1.85	2.6±2.06	3.2±1.94	3.4±1.74	6.2±4.45	4.6± 5.28	4.4± 2.73	3.8± 3.25	6.8± 2.71	7.8± 3.12
JKG-4	1.8±1.83	2.8±1.72	4.6±1.96	5.8±1.17	4.8±0.98	4.8± 2.32	4.8± 2.32	5.2± 2.79	6.4± 3.61	7.8± 3.31
DB-2	2.2±1.72	2.6±1.85	3.8±2.32	4.6±3.14	4.4±2.65	5.0± 2.68	5.4± 0.80	8.0± 2.76	8.0± 2.45	8.4± 2.58
DMP-4	2.0±0.75	1.0±1.10	1.6±1.20	2.2±1.60	2.6±1.02	2.6± 0.80	2.8± 0.75	3.6± 1.02	3.4± 1.20	4.4± 0.80
DMP-7	1.4±1.20	2.6±1.50	4.2±2.23	4.4±2.24	3.6±1.36	4.0± 1.10	4.6± 2.73	5.2± 3.12	5.6± 3.88	5.8± 4.71
LK-3	4.2±4.07	3.8±3.87	5.7±3.49	4.4±3.50	3.5±3.11	5.6± 3.26	5.0± 2.83	6.4± 3.50	6.75±2.68	9.6± 3.50
JKG-2	1.6±1.02	1.6±1.02	1.2±0.75	0.8±0.75	0.6±0.80	1.4± 1.36	1.8± 1.60	1.4± 1.36	2.4± 1.36	2.8±1.33
LB-2	1.4±1.50	2.2±1.60	3.4±3.20	2.2±2.04	2.6±1.62	5.0± 1.26	7.8± 1.72	10± 2.10	7.0± 3.58	10± 5.40





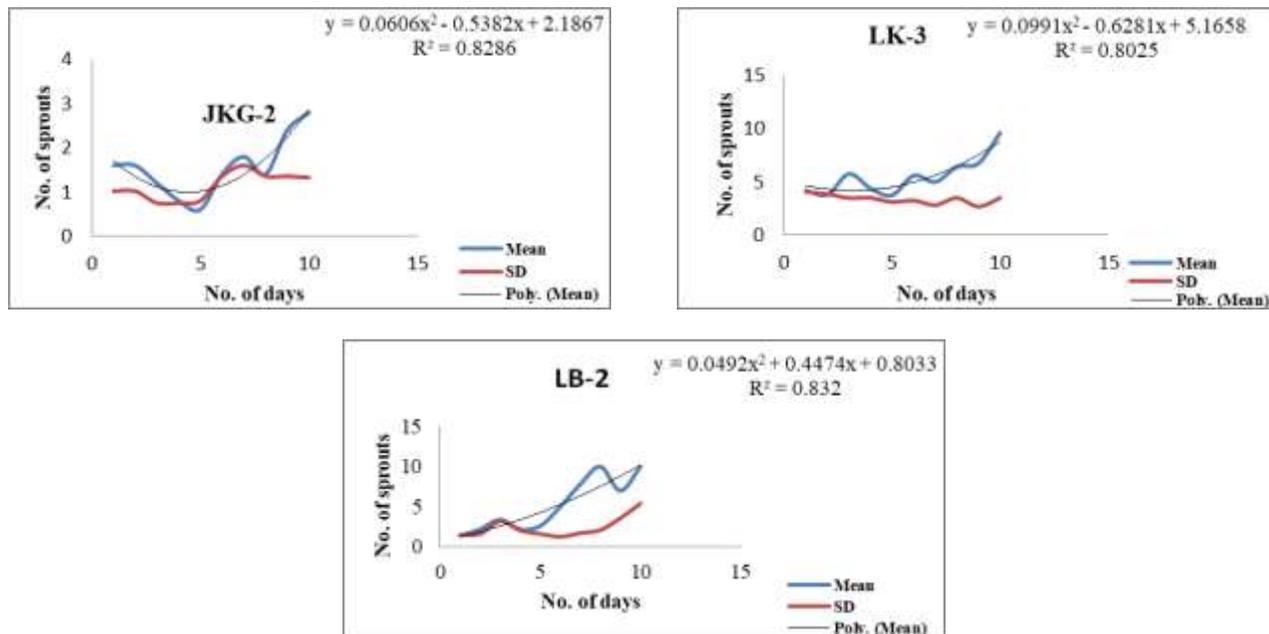


Fig A: Response of sprouting in different genotypes of *D. retusus* hedge garden

Selection of optimum root initiating hormones

To induce faster rooting among the different hormonal treatments such as NAA, IBA and IAA were used for initial trials were studied. In case of IBA treatment cuttings possesses bud within 3 weeks of incubation in the mist chamber (Fig. 3, Plate-A) and both types of tender shoot cuttings responded in rooting within 6-8 weeks. Considering the results recorded among the hormonal treatments of IBA concentration, best response was recorded at 1000ppm in tender apical shoot than non apical cuttings IBA (Fig.1and 2) and followed by 500, 200 and 2000 ppm concentration. Cuttings possesses bud within 3 weeks of incubation in the mist chamber (Fig. 3, Plate-A). Among the different genotypes DMP-9 (59.2±7.45), DMP-7 (55.2 ± 17.2), JKG-3 (51.2 ± 14.6) performed best rooted genotypes of apical cuttings. An overall survivability of the rooted cuttings of the genotypes were recorded as 63.09 ± 4.6. In case of percentage of rooting, high percentage of cuttings rooted after 12 weeks in the misting unit. It appeared that 12 weeks was the minimum time necessary for reaching a high percentage of rooting, since a much lower percentage was observed after 8 weeks and months during May to August were found to be best for rooting. Prominent callus tissue was developed and observed at the basal end of non apical cuttings, and new shoots appeared much earlier than roots. An average of 91% cuttings produced green buds between the sixth and seventh weeks. In case of comparison of percentage of rooting apical cuttings shows better than the non apical cuttings. The treatment with 500 ppm IBA also produced most abundant and uniform root system and followed by the treatments with 200 and 2000ppm. After rooting, cuttings were transferred and showed very good health under greenhouse condition as possesses well developed roots (Fig. 7, Plate-A) and in the field condition shows nice growth (Fig. 8, Plate-A). The

optimum result was a clonal hedge garden of 14 genotypes with one (01) check has been established in RFRI Campus (Fig. 8, Plate-A).

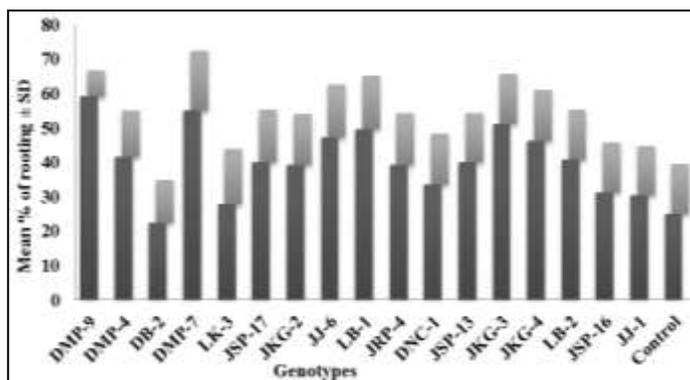


Fig 1: Rooting of apical cuttings in different genotypes of *D. retusus* in 1000ppm concentration of IBA

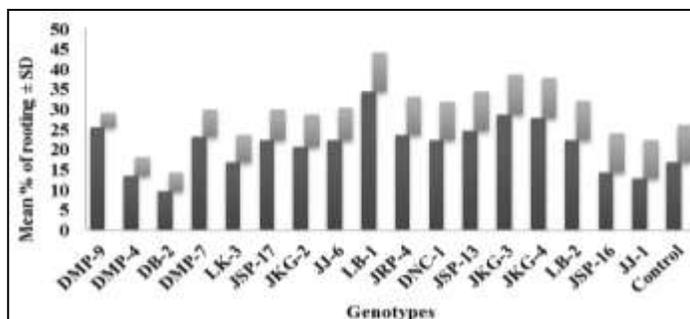


Fig 2: Rooting of non apical shoot cuttings in different genotypes of *D. retusus* in 1000ppm concentration of IBA

Table 2: ANOVA for apical and non apical cuttings of different genotypes of *D. retusus*

Parameters	Rooting range	Mean	S.D	F ratio	CD (0.05)
Apical cuttings (cm)	59.47 - 22.4	40.2	10.03	bet. conc. 92.62**	62.05
				bet. genotypes 2.79**	7.75
Parameters	Rooting range	Mean	S.D	F ratio	CD (0.05)
Non- apical cuttings (cm)	28.96 - 9.6	21.2	6.02	bet. conc. 55.76**	23
				bet. genotypes 3.61**	2.88

Age of the donor plant

Age of the stock plant or donor plant is a very important factor in rooting of stem cuttings. Steele *et al.* (1989) [36] in his experiment on effect of age of donor plant on rooting of cuttings of *sitka* spruce found that the rooting percentage decreased drastically with increase in age of donor. On the other hand, high percentages of rooting were also observed when juvenile material was used by (Aminah Hamzah, 1990a) [3]. In our rooting of shoot experiment it is adopted for juvenile coppice cuttings and results of rooting may surely may possesses good may be due to this juvenility. The source and kind of propagating material plays a vital role in the success of most of vegetative propagation of the species. Dick and Aminah, 1994 [7] and (Leakey *et al.*, 1994) [17] also found rooting in apical shoots of tropical hard wood species. Jacobs (1939) [13] observed that cuttings rooted more easily if the shoots from which they are taken have been partly broken and the treatment induces callus growth. In our experiment the cutting materials are taken from cut trees of the hedge garden, where new sprouts were collected for rooting trials may also induced roots of this species which belongs to very difficult to root category.

Root promoting hormones

It has been confirmed that many times auxin is required for initiation of adventitious roots on stems, and indeed, it has been shown that divisions of the first root initial cells are dependent upon either applied or endogenous auxin Hartmann *et al.* (1993) [11]. In case of dipterocarp species the highest (80%) rooting percentage in *Ani soptera scaphula* was obtained in cuttings treated with 1000 ppm, IBA and best root systems were developed under 2000 ppm IBA. This is as similar to our study recorded highest response of rooting in 1000ppm IBA. Almost all cuttings developed prominent callus tissue at the basal end, and new shoots appeared much earlier than roots. Lo, 1985 [19] reported most successful rooting on apical stem cutting of *Shorea macrophylla*. In work with *Shorea talura*, *Vatica wallichii* and *Anisoptera scaphula*, (Momose, 1978) [23] obtained an overall rooting percentage below 50%. In indole-3-butyric acid (Halle and Kamil, 1981) [9] reported rooting of six dipterocarp species in (IBA) solutions. Srivastava and Penguang (1981) [35] reported rooting percentages between 8 and 56% for *Shorea leprosula* and *Dipterocarpus chartaceus* raised in different propagator beds and exposed to a range of concentrations of IBA (0 to 2000 ppm). Similar study also observed in the investigation where in case of hormonal treatment IBA shows best result. In case of other rooting hormones, it was reported by Moura- Costa & Lundoh, 1994 [24] that cuttings were treated with NAA showed that exogenous auxins did not improve rooting of juvenile cuttings of *D. lanceolata*.

Incubation condition

The rate at which cuttings form roots depends upon both the physiological condition of the cuttings and the environment in which they are placed. The closed chamber mist propagator used in this study and causes a reduction in leaf temperature, using this system relative humidity inside the mist chamber was maintained above may induce rooting of this difficult species. The low rooting percentages achieved by (Srivastava and Penguang 1981) [35] may have been due to the low relative humidity inside their propagation chambers. Cutting of the leaf size is also a good effort for the best rooting result because, percentage rooting of *D. lanceolata* cuttings was significantly higher when leaf area was reduced compared to cuttings with intact leaves Moura Costa & Lundoh, (1994) [24]. Genotypic differences in rooting have been documented in a number of woody species like *Acacia baileyana* Schwarz *et al.* (1999) [30] and *Eucalyptus resinifera* Mc Comb and Wroth (1986) [20]. The hardwood cutting was found to be the best type of cuttings when treated with IBA because it gave the best performance compared to semi-hardwood and soft-wood cuttings of *Jatropha curcas* Noor Camellia *et al.*, (2009) [26].

Influence of the season on root initiation

The selection of best rooting season in our experiment proves to several others report. As compared to the present experiments December or early January not responded and best result recorded in the period of May to August. Jacobs (1939) [13] and Fielding (1954) [8] found in *Pinus* cuttings taken in April, May or June rooting observed in late October to early November and dormant period recorded December to January. A number of investigators Melchior, 1963 [21], Ooyama and Toyoshima, 1965 [27] give evidence that hormone treatment particularly with IBA result in the production of more roots per cutting. Sherry (1942) [31] records cuttings taken and set in August begin to root in November. Pawsey (1950) [28] gives evidence that early rooting may be a clonal characteristic. Fielding (1954) [8] records wide differences in the type of root system that cuttings produce and associates variations in the parent tree rooting medium and climate. Jacobs (1939) [13], Sherry (1942) [31], Mirov (1944) [22], and Fielding (1954) [8] present evidence that cuttings can be rooted decreases as the parent trees age. Cuttings from old trees have been reported as being difficult to root because of insufficient quantities of stored carbohydrates, proteins, etc. to support metabolic processes during the period of root initiation hence, it is more directly under the control of nutritional, hormonal, and ontogenetic influences reported by Allen and McComb, 1955 [2], Nienstadt *et al.*, 1958 [25], Hartmann and Kester, 1959 [10], Sinnott, 1960) [33]. Reported by Melchior (1963) [21], Hoffman and Kummerow (1966) [12], Kummerow (1966) [15] and Sievwright (1967) [32] show that the use of IBA improves

both the rate at which roots form and the number that cuttings eventually produce. In case of misting condition, Jacob (1939)^[13] states that rooted cuttings appear to undergo a degree of rejuvenation and that sometimes primary leaves are produced as well as occasional juvenile buds. Hence, misting may enhance rooting results. (Fielding, 1954)^[8] finds that hedged trees remain physiologically juvenile in that cutting taken from these, roots more readily than cuttings from other trees of the same age. Ooyama and Toyoshima (1965)^[27] stated that cuttings from second generation trees root more readily than the original cuttings. Therefore age of the cuttings also plays a very vital role in case of rooting.

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