vation of the genetic resource will be important to ensure ongoing access to a broad base for the breeding program.

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# Clonal Propagation of Teak (*Tectona grandis* Linn. f.): Effect of IBA Application and Adventitious Root Regeneration on Vertically Split Cuttings

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## Summary

The experiment was conducted on leafy mono-nodal cuttings of 1-year-old seedlings. Each cutting was split vertically into two equal halves. The auxin treatments included 1000 and 2000 ppm IBA. The cuttings were planted under mist for rooting. Even untreated vertically split cuttings rooted profusely

and 62.67% rooting was recorded in the controls. However, maximum per cent rooting (88.00%) was observed in 2000 ppm IBA followed by 1000 ppm IBA (80.00%). Furthermore, IBA treatment increased per cent sprouting, mean number of leaves, shoots and their length, mean number of roots and their length; the effectiveness of IBA increased with its increas-

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ing concentration. It is concluded that vertically split mononodal leafy, softwood cuttings of teak can be successfully used to mass multiply the clonal planting stock more rapidly.

Key words: Vertically split cuttings, adventitious root, IBA, Tectona grandis.

#### Introduction

Teak (*Tectona grandis* Linn. f.) can be considered to be the noblest of all woods not simply because of its golden hue and wonderful texture, but even more because of its durability. It occurs naturally only in India, Myanmar, Laos and Thailand, and it is naturalized in Java, Indonesia, where it was probably introduced some 400 to 600 years ago. In addition, it has been established throughout tropical Asia, as well as in tropical Africa (including Côte d'Ivoire, Nigeria, Sierra Leone, the United Republic of Tanzania and Togo), Latin America and the Caribbean (Costa Rica, Colombia, Ecuador, El Salvador, Panama, Trinidad and Tobago and Venezuela). Teak has also been introduced in some islands in the Pacific region (Papua New Guinea, Fiji and the Solomon Islands) and in northern Australia at trial levels.

Though relatively unimportant in terms of the volume of world timber production, yet because of its strength and aesthetic qualities teak is the tropical hardwood most in demand for a specific market of "luxury" applications including furniture, shipbuilding and decorative building components. It is thus of major importance in the forestry economies of its main producing countries. In the recent past, rapid destruction in natural teak forest had taken place owing to over-exploitation to meet timber requirement. There is an urgent need to raise teak plantation in order to reduce pressure on natural forest. This can be achieved with the improvement of quality of planting stock, which give more quality timber in shorter rotations. Teak forests are generally regenerated artificially by seed. Its seeds are abundant but natural regeneration is variable and is often affected by a number of eco-climatic factors (TEWARI, 1992). Furthermore, the number of seeds per fruit and germinability of teak seed are variable for different seed sources and low per cent germination caused by several factors (Gupta and Kumar, 1976; Dadwal and Jamaluddin 1988; Kaosaard, 1996; Indira et al., 2000). It can also be vegetatively propagated by traditional methods such as grafting, cutting etc. as well as by the in vitro techniques (HUSEN and PAL, 2001 and 2003; MASCARENHAS et al., 1993). Further, there are reports on successful propagation of teak by using mono-nodal softwood cuttings (Husen, 2002). Juvenile teak shoots show opposite and decussate phyllotaxy, that makes two oppositely placed buds are available at each node. Splitting the normal cutting vertically can make available two vertically split cuttings, each having a node and a leaf. If high success rate can be obtained in rooting such split cuttings, then theoretically twice as many clonal propagules can be obtained as are possible if normal (unsplit) cuttings are used. But information on rooting response of vertically split mono-nodal cuttings is lacking. Therefore in this study our objective was to find out rooting behaviour of vertically split mono-nodal softwood cuttings of teak treated with indole-3-butyric acid (IBA).

## **Materials and Methods**

Plant material

The donor plants (seedlings) were raised in nursery beds using seed of FG11 clones, collected from teak seed orchard at New Forest campus, Forest Research Institute, Dehra Dun, India. After germination these were transplanted in polybags (9" x 6") filled with 1.50 kg of rooting medium containing soil,

sand and farmyard manure in 2:1:1 ratios. These were maintained by regular watering and weeding under normal environment prevailing at New Forest campus. Complete protection was provided against diseases and insects by foliar spray with insecticides and fungicides, as and when required. After one year, mono-nodal leafy softwood cuttings were prepared from 200 seedlings. The terminal portion comprising about 3 nodes was excised and used for making cuttings. Each cutting retained a pair of opposite leaves and each leaf was cut so as it retained only 25 cm² leaf area. Total length of cutting was about 4.0 cm which comprised of 1.0 cm internodal portion above the node and 3.0 cm below it. The shoot was green with little lignification. Each mono-nodal cutting was split vertically into two equal halves. The cuttings thus obtained were used for experimentation.

## Treatments and planting

The split cuttings were treated with 1000 and 2000 ppm concentrations of IBA in talcum powder which also contained 0.05% Bavistin (BASF India Ltd. Mumbai). Preparation of IBA mixture and treatment with IBA performed following the method described HUSEN (2002). The control cuttings were treated with talcum powder (this powder was used for 1000 and 2000 ppm IBA preparation) containing Bavistin only. After treatment the cuttings were planted into plastic trays, which were filled with sterilized vermiculite (pH 7.0). The vermiculite was presoaked in tap water for 24 hrs. before filling it up in the trays. The cuttings were planted immediately after the treatment with auxins and these were kept inside a mist chamber where the relative humidity was maintained at  $85 \pm 2\%$  and maximum and minimum day/night temperature at  $32 \pm 10$  °C to  $26 \pm 10$  °C respectively.

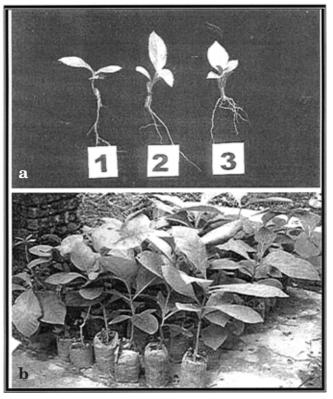
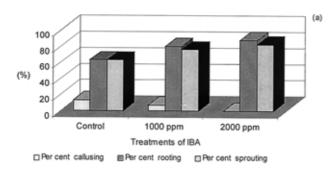
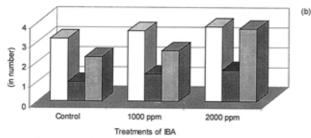


Figure 1 (a). – Rooting response of vertically split mono-nodal softwood cuttings

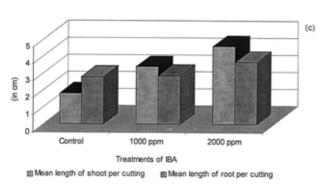
1. Control, 2. 1000 ppm IBA and 3. 2000 ppm IBA

(b). – Teak propagules raised using vertically split mono-nodal softwood cuttings





☐ Mean number of leaves per cutting 
☐ Mean number of shoots per cutting
☐ Mean number of roots per cutting
☐ Mean number of roots per cutting
☐ Mean number of shoots per cutting
☐ Mean number of leaves per cutting
☐ Mean number of shoots pe



 $\it Figure~2.-$  Effect of IBA application on rooting response of vertically split cuttings of teak.

## Observation on rooting response

The cuttings were carefully removed from the rooting medium 45 days after planting. Wound in the bark regions had healed in almost all cuttings by that time. Observations were recorded on callus formation, sprouting, rooting, number of shoots per cutting and their length in cm, number of leaves per cutting, number of roots per cutting and their length in cm (Figure 1).

#### Statistical analyses

The experiment was laid as per completely randomized design. Each treatment was replicated 5 times with 50 cuttings per replicate. The data recorded in percentage were transformed to arcsine √p value (ANDERSON and McLean, 1974). However, other analyses were performed on untransformed data. All analyses were conducted using SPSS (Statistical Package for Social Sciences). The analysis of variance (ANOVA) procedures were used to test for significant effects of the treatments for the response variables measured. However, for the comparison of different means of different treatments, the critical difference (CD) was calculated based on the student t-test at 5 per cent probability.

### **Results and Discussion**

Data on rooting response of vertically split cutting of teak are presented in Figure 2a, b and c. Application of IBA exhibited significant effect on per cent callusing. Thus, control cuttings showed maximum (13.33%) per cent callusing followed by 1000 ppm IBA (6.67%) and 2000 ppm IBA (0.00%) application (Figure 2a). Per cent rooting was significantly affected by IBA application. Increasing the concentration of IBA increased per cent rooting also (Figure 2a). Hence, maximum per cent rooting (88.00%) was observed in 2000 ppm IBA followed by 1000 ppm IBA (80.00%) and minimum in untreated control (62.67%). IBA application had a significant effect at P<0.05 level on per cent sprouting. Increase in per cent sprouting was obtained with the increasing concentration of IBA. Hence, maximum per cent sprouting was observed by the application of 2000 ppm IBA (82.67%) followed by 1000 ppm IBA (76.00%) and minimum in untreated control (62.67%) cuttings (Figure 2a).

The effect of IBA on the mean number of shoots per cutting was statistically insignificant while, application of IBA on mean length of shoot was significant (*Table 1*). Increase in mean length of shoot per cutting was obtained with increasing the concentration of IBA. Thus, maximum (3.70 cm) mean length of shoot per cutting was obtained in 2000 ppm IBA, followed by 1000 ppm IBA (2.58 cm) and minimum (2.24 cm) with untreated control cuttings (*Figure 2b*). Effect of IBA application had a statistically insignificant effect on mean number of leaves per cutting (*Table 1*).

Effect of IBA application on mean number of roots was significant. Maximum (4.60) value mean number of roots per cutting was observed with the application of 2000 ppm IBA, followed by 1000 ppm IBA (3.40) and minimum in untreated control (1.80) cuttings (Figure 1 and Figure 2c). The effect of IBA application was significant on mean length of root per cutting; the effect increased with increasing the concentration of IBA application. Hence, maximum (3.70 cm) mean length of root

Table 1. – Level of significance and critical difference (at P < 0.05) on studied rooting parameters.

	Per cent callusing	Per cent rooting	Per cent sprouting	Mean number of shoots per cutting	Mean length of shoot per cutting	Mean number of leaves per cutting	Mean number of roots per cutting	Mean length of root per cutting
Effect of IBA treatment (in ppm)	*	*	*	NS	*	NS	**	**
CD (0.05)	0.74	2.85	3.02	-	0.41	-	1.07	0.36

Where, \* and \*\* reflects significant at P < 0.05 and P < 0.01 level respectively and NS for insignificant.

per cutting was observed with the application of 2000 ppm IBA followed treatment with 1000 ppm IBA  $(2.87\ cm)$  and minimum in untreated control  $(2.80\ cm)$  cuttings (*Figure 2c*).

The results clearly show that vertically split cutting of juvenile 1-year-old seedlings of teak root easily. It is further evident that treatment with IBA, especially with 2000 ppm concentration, the percentage of rooting and sprouting, as well as growth of roots and shoots can be increased in teak cuttings. Although, promotion of rooting by IBA in teak and other species has been well known (Nanda, 1970; Husen and Pal, 2001; Husen and Mishra, 2001; Husen, 2002), no published information available on the rooting response of vertical split teak cuttings.

The bark portion of the wound healed up within 45 days and the entire wound zone of split cutting was overgrown by cambium and healed in about five months after the cuttings were out planted. Meanwhile, the shoot emerging from the axillary bud had already established itself on the main shoot and, further growth of the propagules was determined by this shoot only. Thus, the growth and wood quality of the trees propagated by split cutting method would be comparable to normal cuttings. Hence, such cuttings can be successfully used for rapid mass production of juvenile clonal planting material of teak.

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## Amplification of North American Red Oak Microsatellite Markers in European White Oaks and Chinese Chestnut

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## Summary

We examined the cross-species amplification success of thirty microsatellite markers developed from North American northern red oak  $(Quercus\ rubra)$  in other members of the family Fagaceae. Sixteen of these markers are newly developed and we report primer sequences and amplification conditions here. Twelve of the thirty (40.0%) red oak markers amplified and were polymorphic in the European white oaks  $Quercus\ petraea$  and  $Quercus\ robur$ . Five of the thirty loci (16.7%) also amplified and four were polymorphic in the phylogenetically distant Chinese chestnut  $(Castanea\ mollissima)$ . These markers should be

widely applicable to genetic studies of Quercus and other members of the Fagaceae.

Key words: Castanea mollissima, genetic diversity, marker, Quercus petraea, Q. rubra, Q. robur, SSR, transferability.

## Introduction

The family Fagaceae includes ecologically and economically important tree taxa such as oak (Quercus), chestnut (Castanea), and beech (Fagus). Until very recently, most microsatellite marker development in the Fagaceae has focused on two clades within the genus Quercus: the white oak group subgenus Quercus section Quercus ([Quercus petraea (Matt.) Liebl.; 17 microsatellite loci; Steinkellner et al., 1997a], [Quercus robur L.; 32 loci; KAMPFER et al., 1998], and [Quercus macrocarpa Michx.; 3 loci; Dow et al., 1995]), as well as the more basal cycle-cup oaks Quercus subgenus Cyclobalanopsis (Quercus myrsinifolia Blume; 9 loci; ISAGI and SUHANDONO, 1997). Several of these markers have been transferred successfully to other taxa including other white oaks (e.g., Quercus suber L., Gomez et al., 2001) and red oaks (Quercus subgenus Quercus section Lobatae) such as Quercus rubra L. and Quercus humboldtii Bonpl. (e.g., Fernandez et al., 2000). Some

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