Vegetative Propagation of Mature Teak Trees (*Tectona grandis* L.)

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Summary

Clonal propagation technology for mature teak trees (63 year-old) has been achieved for the first time. Indole butyric acid (IBA) was found to be the most effective auxin tested. Cuttings from coppice shoots of mature trees rooted between 74% to 91% with a 2000 ppm IBA treatment, while the cuttings from 1 to 2 year-old root-stocks (stumps) rooted between 79% to 100% with 1000ppm IBA applied at different times of the year. Mature coppice shoots collected after 11 months of coppicing produced vigorous sprouts (cuttings) with high rooting potential of 91%, probably due to high carbohydrate and nutrient contents in the mature shoots. Adventitious root formation and root system of cuttings collected from planted coppice shoots of 63 year-old mature trees were similar to those of one year-old root-stocks, suggesting the juvenility of coppice shoots regarding rooting. In teak, rooting depends upon the physiological status of the cutting, and season is no longer a major barrier in rhizogenesis.

Key words: Adventitious rooting, clonal propagation, coppice shoots, mature trees, *Tectona grandis,* vegetative propagation.

Introduction

Vegetative propagation can play a key role in tree improvement programmes as a means of large-scale multiplication of superior clones or tested plus trees. Teak (*Tectona grandis* L.) is one of the important high quality timber yielding species in the world, however, most of the planting stock of teak is still produced from seeds of unselected sources. Low quality seed and poor germination rates affect the availability of planting stock. It is well established that clonal plantations of genetically improved *Eucalyptus* raised through cuttings enhanced the productivity 4 to 7 fold compared to plantations of unimproved seed origins (Zobel and Ikemori, 1983; Lal *et al.,* 1993). However, a similar technique is not available for mass propagation of superior mature trees of teak and therefore clonal plantations of teak are not possible. Branch cuttings of mature trees of teak resulted in moderate (10% to 60%) rooting in cuttings collected in a particular season (Bhatnagar and Joshi, 1978; Nautiyal *et al.*, 1991, 1992; Palanisamy *et al.*, 1995) and this technique is not suitable for mass multiplication. Monteuuis *et al.* (1995) reported good rooting in cuttings from young teak trees (5 to 15 year-old). However, the rotation period of teak tree is about 50 years and superior trees can be identified only at the age of more than 30 years (Emmanuel and Bagchi, 1988). Therefore the aim of the present study is to develop a technology for large-scale multiplication of selected superior mature trees.

Materials and Methods

Rooting of cuttings from coppice shoots of mature trees

The superior trees of teak were selected from a single plantation of 63 year-old trees at Nilambur, southern India. The trees were felled at 20 cm to 30 cm (approximately) above the ground level in February 1999. The coppice shoots emerged 2 to 3 weeks after felling. There are two steps involved in the propagation of cuttings from mature trees : (i) collection and planting of coppice shoots for cuttings production ; and (ii) the rooting of cuttings produced on the planted coppice shoots. Coppice shoots (1.0 cm to 1.5 cm diameter and 50 cm to 100 cm length) were collected from selected trees during different seasons. The leaves were excised and from each coppice shoot consisting of either single or double nodal cuttings (15 cm to 20 cm length) were prepared. They were dipped in a fungicide (0.1% aqueous Bavistin [2-(Methoxycarbamoyl)-benzimidazole]) for 3 minutes followed by rinsing with distilled water. The top end of the cuttings was sealed with paraffin wax to reduce the water loss and then the cuttings were planted in polythene bags (20 cm x 10 cm) containing coarse sand and kept in polytunnel under high humidity (80% to 90% relative humidity [RH]) conditions (polytunnels were placed on moist sand in the shade house of 29% natural radiation). Cuttings were manually sprayed with water 4 times daily. New sprouts emerged from the planted coppice shoots within a week and cuttings (4 cm to 6 cm length and 0.2 cm to 0.35 cm diameter) with 2 or 3 pairs of leaves were collected after 15 to 20 days of planting. Larger leaves were reduced to half of their size to avoid excessive transpiration. Cuttings were sterilized with 0.1% Bavistin for 3 minutes, subsequently rinsed with distilled water and treated with 2000 ppm IBA (in talcum powder) and then planted in root trainers (150 cc) filled with composted coir fibre as rooting media and kept in polytunnels at 80% to 90% RH under shade house conditions for rooting. The cuttings were misted with distilled water 4 times daily.

Rooting of Cuttings from Juvenile Plants

Teak seedlings were raised in a nursery bed in a local nursery. The 1 to 2 year-old seedlings (30 cm to 60 cm shoot and 20 cm to 30 cm root length approximately) were uprooted, and the leaves, lateral roots, a portion of shoot and tap root were removed and the root-stocks (stumps) with 6 cm shoot and 16 cm tap root length were prepared. The root-stocks were planted in polythene bags containing sand, soil, farmyard manure and coir fibre in the ratio of 1:1:1:1 and kept in a mist chamber. Intermittent mist was supplied to the root-stocks for 30 seconds at 30 minutes intervals with an automatic control device. The RH was between 60% and 70% and natural diffused radiation was 7% inside the mist chamber. New sprouts (young cuttings) emerged from these root-stocks within a week. In another experiment, the root-stocks were planted in nursery beds in open conditions and watered daily where they also produced new sprouts (cuttings). The young cuttings (7 cm to 9 cm in length and 0.2 cm to 0.3 cm diameter) with 2 or 3 pairs of leaves (larger leaves reduced to half) were collected from root-stocks both from mist chamber and nursery bed 15 days after planting, sterilized with 0.1% Bavistin for 3 minutes followed by distilled water rinses, treated with 1000 ppm IBA and planted in root trainers filled with coir fibre and kept in polytunnels for rooting. The juvenile cuttings were maintained in the same environmental condition (80% to 90% RH) as the coppice cuttings of mature trees. The root-stocks were used as stock plant and cuttings were collected in most months from April 1999 to March 2000. The rooted cuttings were transplanted to polythene bags containing sand, soil, farmyard manure and coir fibre (1:1:1:1) and again kept in the polytunnel with regular watering. After 20 days they were transferred to shade house condition (29% natural radiation) for hardening off.

Sampling and Statistical analysis

Three replicates each of thirty cuttings (bulked collection of different genotypes) were used in rooting treatments for coppice shoot or juvenile root-stock cuttings. The rooting characteristics such as percentage of rooting, root length and number of roots were observed 50 days after planting. Data were subjected to analysis of variance (ANOVA) and F-tests for significance. Critical difference (CD) values were calculated for comparing the treatment means at $p = 0.05$.

Results

Adventitious root formation occurred in the cuttings collected from planted coppice shoots within 20 to 25 days after planting (*Fig. 1*). Between 74% to 91% rooting was observed at different times using 2000 ppm IBA (*Table 1*). The number of cuttings produced by mature coppice shoots (after 11 months) was greater and more vigorous when compared to those of juvenile coppice shoots (after 2 and 4 months). There was a gradual increase in the rooting percentage of the cuttings in relation to age of the planted coppice shoots in which the cuttings were collected. The cuttings collected from 2 month-old coppice shoots gave 74% rooting and those from 11 month-old showed a 91% rooting (*Table 1*). The root length was maximum in January and June, but there was no significant difference in rooting percentage and number of roots in different months (*Table 1*). The survival rate of rooted plant after hardening off was >90%. Hardened off plants were planted in the nursery bed as stock plant for further multiplication. The growth characteristics of the cuttings were similar to those of seedlings.

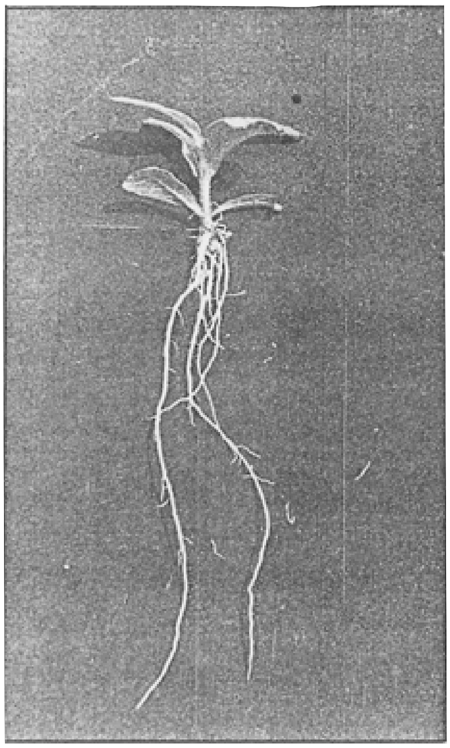

Fig. 1. – Rooted cuttings from coppice shoots of 63 year-old teak trees.

The 1 to 2 year-old juvenile root-stocks planted in a mist chamber or nursery bed produced, on average, 6 cuttings every month. The juvenile cuttings were collected periodically and rooted between 79% to 100% in 1000 ppm IBA treatment and 30 to 97 % rooting in auxin-free control during most of the months (*Table 2*). In the IBA treatment, rooting was between 95% to 100%, and more roots were produced in March, April, July and August. Root length was maximum in February, March, July and September (*Table 2*). In juvenile root-stock cuttings rooting occurs within 20 to 25 days after planting (*Fig. 2*) similar to cuttings of coppice shoots.

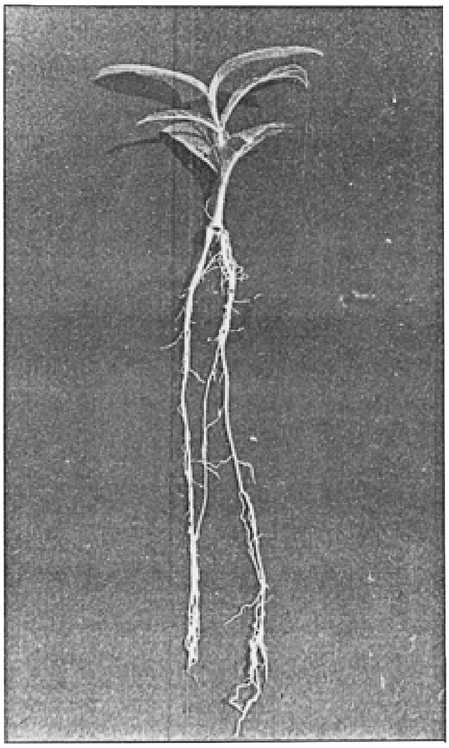

Fig. 2. – Rooted cuttings of 1 to 2 year-old teak root-stocks (stumps).

Table 1. – Rooting response and root characteristics of cuttings collected from planted coppice shoots of mature trees (coppice shoots were collected from 63 year-old trees in different seasons and planted. The young sprouts produced in the planted coppice shoots were used for rooting) with 2000 ppm IBA treatment at different months. Results are the mean values of three replicates.

Month of Coppice Shoot Collection	Age of the coppice shoots used for cutting collection (months)	Percent of rooting	Total number of main roots	Maximum root length (cm)
April '99	2	74	4.13	9.26
June '99	4	75	4.11	15.33
July '99	5	81	2.73	12.91
November '99	9	83	2.06	11.07
January 2000	11	91	2.53	15.77
CD at $p = 0.05$		NS	NS	2.5

Table 2. – Effect of 1000 ppm IBA and auxin-free control on rooting response and root characteristics of cuttings collected from 1 to 2 year-old teak root stocks (stumps). Results are the mean values of three replicates.

Month	Treatment	Percent of rooting	Total number of main roots	Maximum root length (cm)
April '99	IBA	97	4.13	8.37
	Control	62	2.93	6.70
July '99	IBA	95	4.93	15.83
	Control	NC*	NC	NC
August'99	IBA	98	3.33	14.90
	Control	89	2.07	13.73
September '99	IBA	86	2.60	15.37
	Control	60	3.00	14.33
October '99	IBA	89	2.80	13.53
	Control	61	3.07	10.17
November '99	IBA	79	2.60	11.03
	Control	30	2.20	7.47
December '99	IBA	86	2.47	14.83
	Control	78	2.13	14.60
January 2000	IBA	87	2.87	9.40
	Control	83	1.80	10.80
February 2000	IBA	85	2.33	15.89
	Control	89	2.33	13.63
March 2000	IBA	100	4.93	17.63
	Control	97	3.07	14.37
CD at $p = 0.05$		13.8	0.6	1.6

*) NC – Experiment not conducted

Discussion

Auxins are effective in induction of adventitious rooting in most of the tree species (NANDA *et al.*, 1970; NANDA and KOCHHAR, 1984). The cuttings from coppice shoots of 63 year-old trees and 1 to 2 year-old root-stocks of teak rooted well (74% to 100%) using an auxin treatment (IBA) at different times of the year (*Table 1* and *2*). Adventitious rhizogenesis occurs within 20 to 25 days after planting. This is the first report of multiplication of mature teak trees through cuttings. KAOSA-ARD *et al.* (1998) reported 90% of rooting in stem cuttings of juvenile seedlings of teak. Earlier findings stated that rooting of branch cuttings of mature trees of teak is season specific, gave moderate rooting and also takes 2 to 3 months for adventitious root formation (BHATNAGAR and JOSHI, 1978; NAUTIYAL *et al.*, 1992; PALANISAMY *et al.*, 1995). MONTEUUIS *et al.* (1995) observed good (60%) and minimum rooting (40%) in 5 and 15 year-old teak trees respectively, while NAUTIYAL *et al.* (1991) reported 60% rooting in 16 year-old trees in a particular season and very poor rooting (10%) in 62 year-old trees. ISIKAWA (1968) and LIBBY and HOOD (1976) suggested that as the donor plant matures, the rooting rate decreases, the length of the time required for root formation increases and the quality of the root system decreases. The sprouts emerging from the planted coppice shoots of mature trees (63 year-old) appear juvenile because the rooting response, speed of rhizogenesis, and the root system morphology (the number of roots and root length) were similar to those of cuttings from 1 to 2 year-old juvenile root-stocks (*Table 1* and *2*). The results indicate that juvenility enhances the rooting potential, and adventitious root formation in teak is highly dependent upon the physiological maturity, the tissue characteristics and nutritional status of the cuttings. There was no significant variation in rooting percentage during different seasons in cuttings of mature trees or cuttings from 1 to 2 year-old juvenile root-stocks with the auxin treatment (*Table 1* and *2*) indicating that season does not have a major effect on rooting in teak, which is contradictory to the earlier findings (BHATNAGAR and JOSHI, 1978; NAUTIYAL *et al.*, 1992; PALANISAMY *et al.*, 1995). In mature trees the percentage of rooting in cuttings collected from planted coppice shoots gradually increased with the age of the coppice shoots (*Table 1*) possibly because the level of carbohydrate and nutrient content were higher in mature coppice shoots resulting in production of vigorous sprouts with maximum rooting potential. VEIERSKOV (1988) described a positive relationship between carbohydrate content and rooting of the cuttings. The auxin-free control treatment also induced good rooting in cuttings from 1 to 2 year-old root-stocks (*Table 2*), and 49% to 81% rooting in cuttings from coppice shoots of 63 year-old trees (PALANISAMY, unpublished), which is contrast to the findings of NAUTIYAL *et al.* (1991) that teak cuttings did not root without auxin treatment.

Conclusion

It is concluded that the cuttings of mature trees and seedlings of teak responded well to rooting. The rooting performance of cuttings from mature trees was similar to those of seedlings, and juvenility is the major factor on rhizogenesis in teak. Therefore, superior trees and quality seedlings of teak can now be mass propagated to establish clonal plantation.

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Multivariate Analysis of Allozyme and Morphometric Variability in *Racosperma auriculiforme* and *R. mangium*

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Summary

We investigated the levels and distribution of genetic variation of *Racosperma auriculiforme* (*Acacia auriculiformis*) and *R. mangium* (*A. mangium*), using multivariate analysis of allozymes and phenotypic attributes. The patterns of genetic variation based on allozymes were similar to those based on phenotypic attributes for *R. auriculiforme*. In *R. mangium*, there was, however, a lack of correspondence between phenotypic attributes and allozymes. For *R. auriculiforme*, these results suggest that initial isozyme surveys of a limited number of populations covering the species'geographic range could help define more efficient sampling strategies for intense seed collections and large scale provenance-progeny tests. For *R. mangium*, the results, however, suggest that we should rely mainly on genecological studies to establish guidelines for seed transfer in applied tree improvement programs. The allozyme diversity revealed that *R. mangium* was genetically depauperate compared to *R. auriculiforme*. The genecological diversity in quantitative traits over four sites indicated that *R. auriculiforme* is more plastic than *R. mangium*, both showing a geographical pattern of population differentiation. Genetic diversity parameters were negatively correlated with the latitude for *R. auriculiforme*, suggesting Papua New Guinea as a centre of diversity. On the other hand, genetic diversity parameters were negatively correlated with the elevation for *R. mangium*. Canonical correlation analysis revealed two and one significant canonical variates for *R. auriculiforme* and *R. mangium*, respectively. It also revealed significant association between geographic origins and some allozymes and adaptive quantitative traits. Both principal components and discriminant analyses revealed a clear pattern of population grouping related to taxon delineation and could be used to detect possible introgression between the two species. For both species, factor and discriminant variable scores, derived from principal components and discriminant analyses, exhibited strong relation with location variables: latitude, longitude and elevation.

Key words: Acacia, genetic variation, plantation forestry in the tropics, multivariate analysis, *Racosperma*, social forestry.

Introduction

In most tropical countries, migratory slash-and-burn agriculture, along with modern agriculture, fuelwood gathering, selective logging, mining, and bush fires, all intimately linked to a rapid expansion in human population, are reported to be the main causes of loss of forest biodiversity and environment degradation (KHASA et al., 1995a; KHASA and DANCIK, 1997). In

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