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Micropropagation of selected teak clones for improvement of planting stock

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Abstract of Project Proposal

1. Title of the Project : **Micropropagation of selected teak clones for Improvement of planting stock**

2. Principal Investigators & Co-Investigators

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3. Date of commencement : April 2003
4. Date of completion : March 2006 (Discontinued in March 2004)
5. Funding agency : KFRI Plan Grants

6. Objectives:

i. To clonally propagate selected plus trees through micropropagation
ii. To evaluate the vigor and health of plantlets produced through the above method.
iii. To establish a suitable field trial to compare the performance of the propagules Produced by tissue culture with those produced by rooted cuttings.

ABSTRACT

This study envisaged the rapid micropropagation of selected clones of teak derived from plus trees of the plantations of the Kerala Forest Department. The project was discontinued when an externally funded research project was approved with the same objectives. The results of the studies carried out for a year on micropropagation of teak clones is summarised here. A basic protocol for micropropagation of teak from buds collected from adult trees of teak was standardised during the project period.

Shoot cultures were established by using shoot tips of epicormic shoots that sprouted from branch cuttings of the selected trees maintained in the mist chamber in polybags. Excised shoot tips surface sterilized with $HgCl_2$ solution were inoculated on a modified MS medium with various levels of Benzyl aminopurine (BA) and Kinetin (Kn) in test tubes. Multiple shoot formation was obtained in third subculture onwards on the same medium. Thereafter subcultures were carried out at 4-6 week intervals by separating clusters of 2-5 shoots and transfer to fresh media in bottles or plastic containers. Rooting was achieved *ex vitro* by treatment of microshoots of at least 3 cm length with IBA and transfer to vermiculite and maintenance of high humidity either in individual plastic cups or in tray with a plastic cover. Hardening was obtained in two weeks and transfer of plantlets to soil was done after 4 weeks. High levels (> 95 %) of survival were obtained in most batches using this method. Further studies based on the basic protocol were continued in another study.

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INTRODUCTION

Teak is the tree species of choice for forestry plantations in Kerala and many of the states in India. It is gaining popularity in other regions of the world where it has been introduced in recent decades. As with other important tree crops of the world, genetic improvement programmes are in progress around the world as well as in the country.

Plus tree selection has been done and clonal seed orchards established in locations such as Chandrapur (Maharashtra), Walayar (Kerala), and Top Slip (Tamil Nadu). In Kerala the Kerala Forest Research Institute (KFRI) has established CSO's at Nilambur, Palapilly and Arippa. The general observation has been that the CSO do not perform to expectations, poor flowering and fruit formation being common across all clones. This aspect is being studied in great detail at KFRI through a multidisciplinary approach. One of the reasons for the poor performance could be the result of the grafting process itself. Even when graft incompatibility has not been a problem and trees show normal growth, any influence on the physiology cannot be ruled out. With the advent of tissue culture technology and improved vegetative propagation methods, grafts are no longer the only means of establishing a CSO. A comparison between different propagules for suitability as clones in CSOs has however not been carried out.

Several laboratories have carried out tissue culture of teak successfully and the published protocols are available (Mascarenhas *et al*., 1987; Monteuuis, 1994). It is however well known that genotypic variation in the in vitro response exists in tree species and therefore the protocol needs to be standardized for individual clones of teak. Cloning through rooting of stem cuttings although simple and cheap has the disadvantage of low multiplication rates and a serious limitation is in the continuous availability of cuttings. Collection of cuttings directly from the tree is difficult and damaging to the tree. Establishment of hedge gardens as a source of the cuttings is therefore being envisaged. However in situations where a very large number of plants are required, the method has limitations to meet the demand. Micropropagation of the clones is clearly the only option.

The use of tissue culture for propagation has the advantages of very high multiplication rates and other advantages like potential for production of uniform, disease and pest free plants throughout the year. The technology for micropropagation has been standardized in several labs in India and elsewhere (Gupta *et al*., 1980; Devi, 1994; Mascarenhas *et al*., 1987; Monteuuis, 1994; Tiwari *et al*., 1995, 2002; Bonal and Monteuuis, 1997). Superior clones of teak can now be produced commercially through enhanced axillary bud proliferation and rooting.

The application of tissue culture and propagation through rooted stem cuttings for production of propagules for raising plantations is another area of great potential. Here also the question of relative performance of the different propagules remains unresolved.

An earlier study conducted in KFRI assessed the field performance of teak micropropagated plantlets in comparison with stumps but the plants used were obtained from commercial sources and controls were or different genetic origin (Muralidharan and Pandalai, 2000). Only aspects like plant health and early growth were studied here but the observation pointed to the need for

proper nursery management to ensure quality of the plantlets before planting in the field is ensured.

This study envisages the micropropagation of the teak clones that have shown good performance in field trials carried out with rooted stem cuttings.

MATERIALS AND METHODS

A. Initiation of in vitro cultures:

Shoot cultures were established by using shoot tips of epicormic shoots that sprouted from branch cuttings of the selected trees maintained in the mist chamber in polybags. Young shoots between 2-6 cm in length were used as source of shoot tip explants. Excised shoot tips were washed in distilled water and the expanded leaves removed and the tip of about 2 cm were used as explants. Surface sterilization was done with a treatment under the laminar flow bench with 0.1% (w/v) $HgCl_2$ solution for 6-10 minutes followed by three rinses with sterile distilled water.

Explants were inoculated on a solid shoot initiation media consisting of the mineral salts and vitamins of the Murashige and Skoog (1962) media supplemented with 2 % sucrose and the plant growth regulators Benzyl aminopurine (BA) and Kinetin (Kn) at 0.15 mg/l each. Agar was added at 0.6 % to solidify the media after adjusting the pH to 5.6. Initiations of shoots were done in test tubes containing 15 ml of media. Cultures were initiated from buds of plus tree T1, T9, T10, T36 and T49.

B. Multiplication

Shoot cultures induced by the above method resulted in multiple shoot formation after 2-3 passages on the media of the same composition. Each explants thereafter consisted of a cluster of 2-4 multiple shoots. The subsequent cultures were carried out in glass bottles with polypropylene closures consisting of a double layer of clear PP sheets held in place with a rubber band or in larger PP containers. Subcultures were carried out every 4-6 weeks depending on the multiplication rates obtained in each clone. Dead and senescent leaves and tissues were removed at each subculture. One clump of shoots was used as explants per bottle and upto four such explants were placed in the larger vessels. Fluorescent lighting was provided with a 12 hr light and 8 hr dark photoperiod and temperatures maintained at 25 °C$\pm$ 2.

C. Rooting and plantlet regeneration

Microshoots of 2-4 cm were excised from clusters of multiple shoots of different clones growing on the multiplication medium for rooting and plantlet regeneration. The rooting treatment consisted of dipping the lower part of the stem in 4000 mg/l IBA solution (w/v, prepare in ethyl alcohol) for 2-3 seconds and drying in air, followed by planting the shoot in vermiculite in trays. Trays were placed under intermittent mist in a mist chamber for two weeks by which time root development had taken place. After four weeks the plantlets were transferred to soil and removed to the nursery.

RESULTS AND DISCUSSION

A. Initiation of in vitro cultures:

Buds on shoot tip explants inoculated on media supplemented with 2 % sucrose and the plant growth regulators Benzyl aminopurine (BA) and Kinetin (Kn) at 0.15 mg/l each sprouted and formed shoots in about three weeks. Browning of explants and the media was overcome by shifting the position of the explants within the tube or by transfer to fresh media in the first few days of culture.

B. Shoot Multiplication

Shoot cultures induced by the above method resulted in multiple shoot formation after 2-3 passages on the media of the same composition. Each explants thereafter consisted of a cluster of 2-4 multiple shoots. The subsequent cultures were carried out in glass bottles with polypropylene closures consisting of a double layer of clear PP sheets held in place with a rubber band or in larger PP containers. Subcultures were carried out every 4-6 weeks depending on the multiplication rates obtained in each clone. Dead and senescent leaves and tissues were removed at each subculture. One clump of shoots was used as explants per bottle and upto four such explants were placed in the larger vessels. Fluorescent lighting was provided with a 12 hr light and 8 hr dark photoperiod and temperatures maintained at 25 °C$\pm$ 2.

C. Rooting of microshoots

Microshoots of 2-4 cm were excised from shoot clusters in from 5th passages onwards to test for their rootability. High rates (95%) of rooting and plantlet formation was obtained through the ex vitro rooting treatment and hardening in mist chamber. Plantlets after 4 weeks of hardening could be successfully transferred to soil in plastic bags and shifted to the nursery for maintenance.

Conclusion

A successful protocol for rapid multiplication of teak clones was demonstrated during the period of study. High rates of rooting and plantlet survival were obtained however the scope for high shoot multiplication rates and reduction in costs exists in teak. Further experimentation with optimizing the procedures was continued in a different project that followed.

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