

NEW PROJECTS INITIATED DURING THE YEAR 2008-2009

PLAN PROJECTS

Project 1: Progeny testing of selected clones for establishment of clonal and seedling seed orchards in Eucalyptus (IFGTB/RP 55/ 2008-2014)

Status: The Institute has identified about 110 clones from the first generation seed orchards of Eucalyptus. These clones have been tested for their growth superiority in three different locations established during 2000. In this project, the genetic worthiness of their progenies need be tested and Clonal Seed Orchards (CSO) be established with tested clones for production of genetically superior seeds. During this year, the clonal trials of Karunya Nagar, Sathyavedu and Kulathupuzha were assessed and identified about 50 best performing clones. Seeds of these clones were collected and their progenies were raised in the nursery. About 25,000 seedlings belonging to 50 families were raised for establishment of progeny test in two different locations. A Vegetative Multiplication Garden (VMG) was established for mass multiplication of these best performing clones. Shoots were collected from the VMG and multiplied clonally for establishment of CSOs. About 10,000 clonally multiplied plants belonging to 50 clones were raised for establishment of CSOs in 10 ha area in Tamil Nadu and Andhra Pradesh.

Project 2: Developing Genetic Improvement of *Casuarina* Species through Second Generation Orchards [IFGTB/RP 56/2008-2014].

Status: Selected 250 outstanding trees in terms of growth, form and wood traits from 8 first generation orchards located in Andhra Pradesh, Puducherry and Tamil Nadu. Collected open-pollinated seeds from the selected trees and raised seedlings with family identity. Established two progeny trials-cum-second generation seedling seed orchards with 150 families each at Pugalur (Tamil Nadu) and Tirupati (Andhra Pradesh). These trials were planted in incomplete block design with 6 replications and each family was represented by a 4-tree plot in each replication. Assessed all the plants in both the trials for survival and growth at 6 months' age. The survival has been above 90% in both the trials with a mean height of 2.5 m in Pugalur and 2 m in Tirupati.

Project 3: Developing Cloning Techniques for Raising High Yielding Clonal Plantations of *Casuarina equisetifolia* L. [IFGTB/RP57/2008-2011]

Status: Identified the experimental sites at Karunya Nagar and Panampally and initiated the studies. Selected trees were coppiced at various heights (15, 30, 45, 60, 100 and 150 cm from the ground level) and applied 3 treatments (fertilizer, mulching and growth regulator application). Coppice shoot initiation was observed in 89 per cent of the trees felled. Coppicing at 45 and 60 cm was found to be better for shoot induction. Cladodes and needles collected from these coppice shoots were kept for rooting studies in the model nursery. Rooting could be observed in both cladode and needle explants. Attempts are being made to standardize the technique to develop plantlets from individual needles.

Project 4: Allelic diversity of CCR gene in *Casuarina equisetifolia* [IFGTB-60/RP-2008-2011]

Status: 50 clones of *Casuarina equisetifolia* wood samples were collected using increment borer from IFGTB Model nursery. Cellulose content was estimated using UV/VIS spectroscopy method. Based on the absorption mean, twenty five clones were selected for the proximate analysis. An estimation of hollo cellulose and lignin content was evaluated for 25 selected clones. The range between the hollo cellulose was 74% to 80%, whereas in lignin, content was 24% to 53% among the twenty five clones. Nucleotides of CCR gene in Eucalyptus, Leuceana, Pines and populus species were downloaded from NCBI data Library. 22 CCR gene primers were designed and synthesized.

Project 5: Identification of biochemical markers linked to sex determination in *Casuarina equisetifolia* [IFGTB-61/RP-2008-2011]

Status: 12 Isoenzymes (AAT,ADH, EST, PPO,POD,GDH, IDH,SOD, MDH, ME,LDH,G6PDH) were optimized in *Casuarina equisetifolia* clones. Casuarina young needles were collected from IFGTB model nursery and leaf tissues were extracted using 0.1M Tris Hcl extraction buffer with additives. Isoenzymes were profiled in 10% polyacrylamide gel electrophoresis system, stained with specific substrates for 12 different isoenzymes and the images were documented. Observations were made on the banding patterns of male female individuals, five enzymes (AAT, POD, IDH, LDH, GDH) were shown distinct banding profiles between male and female clones.

Project 6: Quantitative trait locii (QTL) mapping in eucalypts for salinity tolerance and adventitious rooting [IFGTB/RP 62/ 2008-2011]

Status: Pollen and seed parents of *E.camaldulensis*, *E.tereticornis* and *E.grandis* were selected for the development of inter-specific hybrids. Controlled pollination work on *E.camaldulensis* X *E.grandis* was completed to generate F1 hybrids for the establishment of mapping population. Twenty microsatellite primer sets were synthesized and their transferability to *E.camaldulensis* was assessed.

Project 7: Identification of secondary xylem specific cellulose synthase genes from *Eucalyptus tereticornis* [IFGTB/RP 63/ 2008-2011]

Status: Nucleotide and protein sequences of Cellulose synthase were assembled from public domain database, aligned and primer pairs were generated. The primer pairs targeting different groups of cellulose synthase were amplified in the wood tissues of *E. tereticornis* with complementary DNA as the template. Amplicons generated were sequenced and their similarity with the existing genes was determined.

Project 8: Studies on response of important tree species to elevated CO₂ levels under simulated temperature and moisture regimes at seedling stage [IFGTB/RP 69/ 2008-2009]

Status: Project was initiated in July 2008. Fabrication of Open Top Chambers (OTC) with CO₂ analyzer and the SCADA platform-the controlling device is in progress. After the OTC becomes operational, the experiments will be initiated in June 2009.

Project 9: Studies on the suitability of *Eucalyptus tereticornis* and *E. camaldulensis* clones for various agroclimatic zones of Southern India [IFGTB/ RP-58/2008-2013]

Status: Shoots of thirty clones of *Eucalyptus tereticornis* and *E. camaldulensis* were collected from Sathyavedu and Karunyanagar clonal trials and raised in the root trainer. With these ramets Vegetative Multiplication Garden (VMG) was established in Bharathiar University during September, 2008. Forest departments of Puducherry and Andhra Pradesh and the Agricultural College at Karaikal have allotted land for the trials. Land has been inspected in six locations, viz., Puducherry, Karaikkal, Warangal, Rajmundhry, Hyderabad and Tirupathi. The multiplication of the clonal material is in progress.

Project 10: Status and influence of microbial inoculants associated with *Eucalyptus* clones in established breeding populations. [IFGTB/RP 65/ 2008-2011]

Status: Rhizosphere soil samples collected from 31 clones of eucalyptus at three different clonal trials of eucalyptus located at Sathyavedu (Andhra Pradesh), Karunya (Tamil Nadu) and Kulathupuzha (Kerala) were analysed for presence of AM fungi and PGPRs . AM fungi, *Glomus viscosa* and *G. geosporum* and the PGPRs, *Bacillus megaterium*, *Pseudomonas striata*, *Azotobacter insignis* and *Azospirillum brasilense* isolated from the soil samples of certain clones were multiplied in sterilized soil and artificial media respectively. Interestingly, specific association of certain PGPRs with certain clones was observed. These PGPRs were multiplied in nutrient broth as well as carrier material (Charcoal) as shelf life forms.

The PGPR, *Bacillus megaterium* was tested on *Eucalyptus* stem cuttings and found that it induces rooting in stem cuttings. Stem cuttings of 17 selective clones collected from Sathyavedu and Karunya clonal trials and inoculated with PGPRs. Stem cuttings treated with IBA were kept as control. The results obtained showed significant variations on rooting % age and time taken for rooting among the clones tested. The rooting percentage was observed to vary from 45-81 while the time taken for rooting varied from 14 – 20 days..

Project 11: Characterisation and evaluation of allelochemical profiles of some economically important, insect pest resistant tree species. [IFGTB/RP 68/ 2008-2010]

Status: Regular field screenings of *Acacia mangium*, *A. auriculiformis* and *Ailanthus excelsa* populations for selection of insect pest resistant candidates were carried out and level of incidence and extent of damage caused by the pests, *Mylocerus* sp. on *Acacia* and *Eligma narcissus* and *Atteva fabricella* on *Ailanthus* seed sources/ provenances were assessed. In order to understand the biochemical basis of resistance in Casuarina, teak and eucalypts against the targeted key pests estimation of chemical constituents like Tannins, phenols lipids in the leaves of teak and eucalypts and bark and wood of casuarinas carried out.

Project 12: Characterization of Eucalyptus clones for Physiological and Nutritional parameters [IFGTB/RP-59/2008-2011]

Status: For establishment of field trials to evaluate the Eucalyptus clones for water and nutrient use efficiency, vegetative multiplication garden was established and the production of ramets is under progress. Fields were identified in Tirunelveli, Sivagangai and Pudukottai for establishment of field trials. For the short-listed 30 Eucalyptus clones, parameters like chlorophyll A, chlorophyll B, total chlorophyll and total leaf area were worked out. For carrying out the carbon isotop discrimination studies for estimation of water use efficiency of 30 clones, wood samples were collected and powdered for analysis at University of Agriculture Sciences, Bangalore.

Project 13: Some phytochemical, toxicological, pharmacological investigations of *Aegle marmelos* for a new product (IFGTB/RP-66/2008-2011)

Status: The different tissues, leaf, ripened & unripened fruits of *Aegle marmelos* were sequentially extracted with aqueous and other organic solvents. Extracts fractionated through XAD- 16 resin packed column were analysed for chemical analysis such as primary nutrients, phenols, alkaloids, flavonoids, tannins, saponins, enzymes like PO, PPO, PAL, TAL, individual fatty acids etc. in order to evaluate the tissue specific pharmacological screening of the extracts. And, assessed the efficacy on CUMS (Chronic unpredictable Mild stress) especially on stress, antioxidant, behaviour etc. The dried micro-sieved crude powder of those tissues tested for preliminary pharmacological screening on animal showed, that leaves shown less antioxidant activity in super oxide and nitric oxide scavenging activity, ripen and unripen fruits exhibited similar antioxidant activity in super oxide and nitric oxide scavenging activity and all the three crude extracts shown similar reducing power activity. Among the three antioxidant activity studied super oxide scavenging activity found to be better. The observation has to be further confirmed and compared with the extracts. The work is in progress.

Project 14: Bioinformatic approach to data mine wood forming genes of *Eucalyptus* (IFGTB/RP 64/ 2008-2011)

Status: Sequences have been downloaded for the following wood formation genes PAL (Phenylalanine ammonia-lyase), 4CL (4-Hydroxycinnamoyl CoA ligase), C3H (p-Coumarate 3-hydroxylase): Identification of conserved regions and primer designing carried out for the above genes