

## 2.3 Genetic Improvement

### Overview

Realizing the importance of production forestry, strategic activities for tree improvement are in progress in ICFRE. The primary consideration to bring about genetic improvement in a particular species is the development of a sound scientific programme based upon the available genetic variability and application of appropriate breeding methods to utilize the variability. Applied and basic research in the field of genetics, clonal forestry and molecular biology is being carried out to meet the requirement of the stakeholders particularly state forest departments, industries and farmers. Developing appropriate strategies for tree improvement and integrating them with the tree genetics, activities of the state forest departments are crucial. In this approach, the emphasis is on species oriented tree improvement programme in collaboration with state forest departments. ICFRE institutes during the last several years have been working on comprehensive strategies for tree improvement programme for species like *Eucalyptus*, *Casuarina*, *Acacia*, Teak, *Ailanthus*, Tamarind, Poplars, *Dalbergia*, *Melia*, *Buchnanian lanzan*, *Sclleichera oleosa*, *Jatropha curcas*, Bamboos and medicinal plants like *Asparagus racemosus*, *Rauwolfia serpentin*, *Saraca indica*, *Tinospora cordifolia* and *Acorus calamus* to establish Seed Production Areas (SPAs), Clonal Seed Orchards (CSOs), Seedling Seed Orchards (SSOs) and also selection of productive accessions.

Clonal propagation is one of the indispensable components in tree improvement programme by means of mass multiplication of superior genotypes for clonal forestry programme and thereby improvement of productivity. Therefore selection of superior trees, clonal multiplication and establishment of clonal plantation has been taken up in the different tree species.

ICFRE institutes also impart training to officials of the state forest departments of various levels on field application of various techniques of planting stock improvement, forest genetics and clonal propagation. The short term trainings are provided to the students of different universities and educational institutions on tree improvement and biotechnology.

#### ***Eucalyptus* spp.**

Through systematic selection and multilocation testing of more than hundred clones, four high yielding clones of *Eucalyptus* spp. have been released for commercial cultivation. The seed orchards and vegetative multiplication gardens for production of improved seeds and vegetative material have also been established.

#### ***Casuarina* spp.**

Genetic gain tests with seeds of first generation orchards revealed an improvement of 13 to 28 % in yield with respect to additional wood in plantations depending upon the site conditions. Through systematic selection and multilocation testing, four high yielding clones of *Casuarina* have been released for commercial cultivation. Produced intra and interspecific hybrids of *Casuarina* (*C. equisetifolia* X *C. junghuhniiana*).



A 2 years old Hybrid Progeny Trial of *Casuarina* at Panampalli, Kerala



The interspecific hybrids between *C. equisetifolia* and *C. junghuhniana* (right) in a 2 years old hybrid progeny trial of *Casuarina* at Panampalli, Kerala have shown substantial superiority in growth and tree form and disease resistance over either of parent species (left).

#### ***Dalbergia sissoo***

New sets of clones have been developed to cater the problem of poor stem form (crooked stem), forking, ramicorn branching and susceptibility to the dieback. Five clones of *Dalbergia sissoo* viz. GBW, JB, FXB, FZK and RB were multiplied to study the genetic variation for *in-vitro* morphogenetic potential and evaluation of their field performance. Investigations were also carried out to find out endogenous auxin level and its relationship with adventitious rooting potential in *D. latifolia*.

#### ***Melia* spp.**

A total of 230 Candidate Plus Trees (CPTs) of *Melia composita* were selected from different geographical regions and analyzed for index value based on height, diameter at breast height, straightness, clear bole height, crown diameter and knots. Further, evaluation trials of 21 most suitable families was established in six geographical locations of Haryana, Punjab, Uttar Pradesh and Uttarakhand. A progeny trial of (10 plus trees each) *Melia azedarach* and *Melia dubia* has been established at Forest Research Centre Campus, Hyderabad.

#### ***Pongamia pinnata***

The plantations of *Pongamia pinnata* were surveyed in the states of Punjab, Uttarakhand, Uttar Pradesh and Haryana and identified promising genotypes for higher seed productivity and oil content. Field trials have been raised with 49 selected families at Jhumpa (Haryana) and Pantnagar (Uttarakhand) for testing stability, adaptability and growth performance.

#### ***Tectona grandis***

Understanding the bottlenecks related to reproductive success in teak seed orchards limiting their usefulness was developed. Variation and inheritance of fruit and seed trait of Gujarat teak was studied for the first time and identified five good general combiners.

#### **Phyllodinous Acacias**

Comprehensive genetic improvement programme of *Acacia auriculiformis* and *Acacia mangium* was initiated. Breeding population having wide genetic base has been established and considerable improvement in tree form has been observed through progeny trials. Work has been initiated for development of advanced generation seed orchard using the selections from the existing breeding populations.

#### ***Ailanthus excelsa***

Tree improvement programme has been initiated in the indigenous species for boosting the productivity, yield and development of clones/genotypes. *Ailanthus excelsa* seeds were collected from Tamil Nadu, Rajasthan, Gujarat, Uttarakhand, Madhya Pradesh and Andhra Pradesh and germplasm assemblage was done in three different locations in Andhra Pradesh and Tamil Nadu, as base population for the future breeding programme.

#### **Tamarind**

Tree improvement programme has been initiated to select and conserve the rare phenotypic variants by conducting survey in different parts of southern India and identified 47 red and 30 sweet tamarinds in various parts. The conservation of tamarind genetic resources was carried out by establishing germplasm banks of red and sweet tamarinds.

#### ***Buchnanania lanzan***

Seeds of *Buchnanania lanzan* collected from phenotypically superior candidate plus trees and progeny trial has been established.



### Bamboo

- Thirty five clones of *Bambusa tulda*, 18 of *Bambusa balcooa*, 23 of *Bambusa nutans*, 14 of *Bambusa pallida* and 23 of *Dendrocalamus hamiltonii* have been collected from different places of northeast India and established in bamboo germplasm bank of RFRI. In all 115 clones and 188 rhizomes collected from selected superior mother clumps of target bamboo species are conserved in the gene bank.
- Field trials of tissue culture plants of *Bambusa bambos* and *Dendrocalamus strictus* were established at three places, two in Rajasthan and one in Gujarat. *D. strictus* produced 16% more new culms and *B. bambos* produced 74% more new culms/clump at Dahod almost after three years with fertilizer treatments.
- Keeping in view the ecological significance and socio-economic relevance of *Arundinaria falcata* and *Thamanocalamus spathiflorus* (hill bamboos), conducted survey in Kullu, Shimla and Sirmaur districts of Himachal Pradesh for the identification of their populations. Accordingly, 22 sites in these districts for further collection of the germplasm and establishments of the seed orchards have been identified.

### *Gmelina arborea*

Reproductive biology of *Gmelina arborea* has been studied for generating base line information for improvement programmes. Rain Forest Research Institute has assembled 119 elite clones of *Gmelina arborea* selected from various provenances of North-Eastern states and West Bengal.

### *Tecomella undulata*

A progeny trial with 40 candidate plus trees was established at Bikaner and Jodhpur during September 2008. The progeny from

Chohtan (Barmer) demonstrated good growth at Jodhpur and progeny from Mohangarh (Jaisalmer) at Bikaner.

### *Azadirachta indica*

Evaluation of 7 years old Neem progeny trial established targeting high azadirachtin at Govindpura, Jaipur revealed that very low rate of seed setting took place in a few progenies mainly due to frost during the past two years.

### *Cedrus deodara*

The Seed Production Areas (SPAs) of Deodar, by enumerating 50 ha seed stands and marking lists of finally selected stands were prepared and submitted to the State forest department to obtain culling permission.

### Medicinal Plants

- On the basis of index value of the four most important traits for the twenty seed sources of *Asparagus racemosus*, six sources viz. Panthnagar (Uttarakhand), Jodhpur (Rajasthan), Dehradun (Uttarakhand), Chandigarh (Punjab), Jammu (Jammu and Kashmir) and Solan (Himachal Pradesh), were found promising for root production at Dehradun, Uttarakhand.
- Out of the screening of fifty populations of *Acorus calamus* representing Uttarakhand, Himachal Pradesh & Jammu Kashmir, five sources have been identified bearing low -Asarone content.
- A vegetative propagation centre has been established in College of Forestry, Ponnampet for propagation of *Dendrocalamus brandisii* and *Dendrocalamus asper* and to promote cultivation of these two species in Coorg District, Karnataka.
- *In-vitro* regeneration of plantlets and their genetic fidelity in a vulnerable medicinal plant species, *Saraca indica*, showed a





maximum of 35.2% sprouting of auxiliary buds in summer season in 2-3 years old plants.

### Molecular Characterization for Breeding Programmes

- Simple Sequence Repeat (SSR) markers were developed for *Eucalyptus tereticornis* to assess the genetic structure in association mapping population targeting adventitious rooting traits.
- The genetic diversity estimates and relationship between the eight populations of *Cedrus deodara* with 10 chloroplast micro-satellite (cpSSR) markers were worked out.
- To identify genetic determinants of salt stress tolerance in *Casuarina*, 82 *Casuarina equisetifolia* clones were screened for highly salt tolerant and susceptible conditions.
- One hundred and fifty clones were profiled using RAPD markers. Four SSR target primers were developed from ISSR PCR product of the *Casuarina equisetifolia*.
- The clones of *Casuarina equisetifolia* were evaluated for their holocellulose and lignin content, which ranged from 75 to 80% and 24 to 54% respectively among the clones. Wood proximate chemical analysis (% ash, AB extractives, water solubility, NaOH solubility) and CCR enzyme activity was assessed. The results indicated consistent variation among the clones.
- Twelve isoenzymes (AAT, ADH, EST, PPO, POD, GDH, IDH, SOD, MDH, ME, LDH, G6PDH) were optimized in *Casuarina equisetifolia* clones. *Casuarina* young needles from 24 male and 23 female clones were screened for five distinct gender specific enzymes (AAT, IDH, ADH, LDH, POD). Peroxidase profiles were found to be very distinct in gender discrimination.

- Molecular characterization of 125 accessions of three important bamboo species viz. *Bambusa balcooa*, *Bambusa tulda* and *Bambusa nutans* was carried out.
- Molecular characterization of 28 clones of *Jatropha curcas* was completed using ten RAPD primers.

### Gene Isolation and Functional Analysis

- Class I chitinase gene with approximate size of 1.4 kb was identified with ORF size of 867 bp, 3'UTR of 240 bp and 5'UTR of 293 bp encoding a protein of 289 amino acid with Glycoside hydrolase family 19 chitinase domain was characterized.
- Six transcript representing six classes of cellulose synthase were characterized from *Eucalyptus tereticornis*.
- For rapid functional analysis of genes involved in salt tolerance, parameters critical for development of *E. tereticornis* composite plants with transgenic hairy roots were identified.

### Micro and Macro propagation

- To reduce the *Aegle marmelos*, developed cell culture protocol for the species and tested the efficacy of the metabolites produced in culture against pathogens.
- Enhancement of root-ability and planting stock production of selected high yielding *Eucalyptus* clones through micro and mini cutting technique was achieved. Rooting percentage of 13 clones was enhanced using mini cutting technique.
- In *Aquilaria malaccensis*, vegetative propagation protocol through *in-vitro* and *ex-vitro* techniques has been developed.
- In *Dipterocarpus retusus*, vegetative propagation protocol has been standardized.



- Developed basic protocols for *in-vitro* propagation of *Jatropha curcas* and *Commiphora wightii*.
- A demonstration plot (50ha) of *Dendrocalamus hamiltonii* was established using both Tissue Cultured (TC) and cutting raised plants.
- Extensive exploration was carried out to select 30 superior trees of Shorea from the whole lac producing region in eastern part of the country, represented by three states, West Bengal, Jharkhand and Orissa. Besides stem cutting, other macro-propagation methods like air-layering and grafting experiments were carried out. More than 50% rooting was observed through these methods. DNA extraction method from juvenile leaves of clones has been standardized with the aim to test the clonal fidelity.

**Projects under the theme**

Project	Projects Completed During the Year	Ongoing Projects	New Projects Initiated During the Year
Plan	17	39	19
Externally Aided	14	10	09
<b>Total</b>	<b>31</b>	<b>49</b>	<b>28</b>

**2.3.2 Conservation of Forest Genetics Resources**

***Pinus roxburghii***

*Pinus roxburghii* Sarg. an evergreen conifer species belonging to the family Pinaceae is known for its valuable timber and oleoresin. The oleoresin obtained from pine is an important source of revenue for the Northern Himalayan region of India. Considerable genetic variation exists within the *P. roxburghii* species for resin

production across the growing region. It is difficult to identify high resin yielder at early stages of growth and development. In order to characterize and establish DNA marker association with high resin production, a total of 73 genotypes (high and low resin yielder) of *P. roxburghii* from (Uttarakhand, Himachal Pradesh and Jammu) were characterized using RAPD and ISSR markers. The number of polymorphic loci was 120 (82.76%). The total gene diversity was 0.289 and Shannon's information index was 0.431 for the analyzed genotypes. Within population gene diversity and proportion of total genetic diversity between populations was 0.273 and 0.053 respectively. Three RAPD loci (M-186-48, M- 186-49 and OPA-6-76) and one ISSR locus (ISSR – 7-108) showed significant association with resin yield. Of which, OPA-6-76 was in high occurrence (80.64 %) for high resin yielder (resin yield higher than 2.5kg/ annum using bore hole tapping method) and lower occurrence (38.09 %) in low resin yielders (less than 1 kg/ annum). The results indicated possibility of using these loci for development of molecular markers for high resin production.

***Cedrus deodara***

*Cedrus deodara* (deodar), also known as Indian cedar is the most important among the Indian conifers though the species is in great demand for its timber, has been subject to over exploitation for centuries. Natural deodar forests are under great pressure not only due to human impact but also due to the changing weather conditions. In order to study the genetic diversity and population structure of Himalyan deodar forests, twenty populations each with 50 individual trees covering Jammu & Kashmir, Himachal Pradesh and Uttarakhand were sampled for DNA marker based study. DNA extraction and



Trees Selected for Sample Collection in Deodar Forests of Manali



Seed Production Area (SPA) of Deodar

SSR fingerprinting techniques have been standardized. Polymorphic SSR markers screened and a total of 8 populations with each of 10 cpSSR markers were fingerprinted and genetic diversity estimates worked out. The genetic relationship between the eight populations was also established. The fingerprinting of other populations is under progress.

With the objective of establishing Seed Production Area and raising progeny trials of *Cedrus deodara*, marking lists of finally selected and enumerated 50 ha seed stands were submitted to SFD to obtain culling permission from the competent authority. The progeny trial was initiated in the nursery during January 2007 and, thereafter, these progenies were maintained in the nursery with one pricking done during August 2007. Progenies, thus, raised and maintained were planted out in the field using Randomised Block Design during August 2009 and growth data recorded periodically.

### *Asparagus racemosus*

A field trial of 20 seed sources of *Asparagus racemosus* was established at the Forest Research Institute, Dehradun (Uttarakhand) to evaluate their performance based on different economic traits. Maximum genotypic and phenotypic variance was observed in shoot height among the shoot-related traits and root length among the root-related traits. The maximum genetic advance and genetic gain were obtained for shoot height among the shoot-related traits and root length among the root-related traits. Based on index values, six sources viz. Panthnagar (Uttarakhand), Jodhpur (Rajasthan), Dehradun (Uttarakhand), Chandigarh (Punjab), Jammu (Jammu and Kashmir) and Solan (Himachal Pradesh), were found promising seed sources for root production.

### *Acorus calamus*

*Acorus calamus* is an important medicinal and aromatic plant used in several drugs of the Unani and Ayurvedic health care systems. The genetic diversity and population structure of fifty populations of *Acorus calamus* from different geographical regions of its range of distribution in India was studied through morphometric traits and DNA markers (RAPD and chloroplast micro-satellite). The collected sources were also evaluated for -Asarone content. Five sources





have been identified bearing low -Asarone content in their rhizome.

#### ***Rauvolfia serpentina* and *Tinospora cordifolia***

Germplasm of *Rauvolfia serpentina* and *Tinospora cordifolia* were gathered from 39 locations across the country to provide a broad base of the gene pool. The germplasm is being maintained and multiplied by developing various propagation techniques. The multilocation trials were established at Raigarh (Chhattisgarh), Chandrapur (Maharashtra) and Jabalpur (M.P.), and revealed considerable variation. The growth performance was recorded and root samples collected and tested for active principal contents and, based on DUS characters, these have been marked to arrive at the most promising genotypes.

#### ***Pterocarpus marsupium***

The morphological variations were categorized within and between the populations of *Pterocarpus marsupium* in Tamil Nadu and Kerala, 17 and 14 populations were short listed on Eastern and Western aspects of Western Ghats, respectively. Field surveys were carried out in the Eastern aspect (Tamil Nadu) of Western Ghats and 579 representative trees were tagged and their morphological and phenological parameters documented. Also 214 herbarium specimens were collected from the identified trees. From the short-listed sites in the Western aspect of Western Ghats (Kerala), 219 trees were tagged and their morphological and phenological parameters documented and also 115 herbarium specimens collected.

#### ***Bruguiera* spp.**

Study on reproduction of mangroves constituting phenology, floral biology, pollination, and reproductive success were made in the RET species *Bruguiera cylindrica*,

*B. gymnorrhiza* and *B. sexangula*. Detailed studies were conducted in 6 locations across the East and West Coasts. In the West Coast, Kannur and Ernakulam districts in Kerala were surveyed and found that *B. cylindrical* in East Coast flower during April–May, whereas, in West Coast, it flower during October–November. Both sunbirds and insects pollinate *B. gymnorrhiza*. *B. cylindrica* pollinated by thrips shows the highest reproductive success. *B. sexangula* is exclusively pollinated by sunbirds and exhibits the lowest reproductive success.



Bird Pollination in *Bruguiera gymnorrhiza*

#### **Sandal (*Santalum album* L.)**

Produced 1.3 lakh seedlings of sandalwood during 2006-09 and provided to the SFDs, farmers, sandal based industries and NGOs from all over India. Four sandal stakeholders meeting programmes conducted in Murdeswar, Kolar and in Shimoga districts in Karnataka. Two on- farm demonstration trials of sandal-based agroforestry have been established in Mantralaya (A.P), Bevanahalli, Mudelahalli and in Chikmagalur (Karnataka).

#### ***Chloroxylon swietenia***

*Chloroxylon swietenia* is an important species, which falls in vulnerable category as reported by IUCN. Regeneration status of this species was documented. Three seed stands and three candidate plus trees have been identified.



### ***Canarium strictum* Roxb. and *Hydnocarpus pentendra***

Trees of *Canarium strictum* and *Hydnocarpus pentendra* were identified and marked for periodic phonological observations. Both the species were sparsely distributed and very few trees were fruiting. Maturation of fruit is staggered. Natural regeneration was reported to be very poor in both the species of *Hydnocarpus pentendra* and *Canarium strictum*. Though germination was found to be good, field survival of seedlings at four months was less than 5%.

### **Bamboos**

- Species-cum-clonal evaluation trials of *Bambusa tulda*, *Bambusa balcooa*, *Bambusa nutans*, *Bambusa pallida* and *Dendrocalamus hamiltonii* were established at 6 selected sites in Assam (2 sites), Mizoram (2 sites), Nagaland (1 site) and Tripura (1 site). Monitoring of survival, growth and performance of species-cum-clonal trial plantations established at all the 6 sites in different northeast states have been carried out. As per observation and data recorded till May 2009, *Bambusa balcooa* and *B. nutans* showed highest survival followed by *D. hamiltonii* and *B. tulda*. Height of *B. nutans* (166.5 cm) is recorded higher than others followed by *B. balcooa* (147.5cm), *B. tulda* (145.7cm) and *D. hamiltonii* (126.9 cm). Overall growth of new shoot recorded higher in *D. hamiltonii* followed by *B. nutans*, *B. tulda* and *B. balcooa*. In Kamrup, *B. balcooa* performs satisfactory in all growth parameters and clone No.11 (C-11) showed better result. In Jalukie, *B. nutans* performs well and Clone No. 9 (C-9) is found to be better. In Teliamura, *B. nutans* performs well and Clone No. 11 (C-11) is found to be well followed by Clone 10 (C-10). In Aizawl, Kolasib and Hailakandi, *B. nutans* was found to be a better performing species. In general performance of trial plantation is best in Tripura (Teliamura) and West Kamrup (Assam), moderate in Aizawl and Vairengte. The growth parameters showed very poor performance in Nagaland (Jalukie) and Hailakandi. *Bambusa nutans* at Teliamura attained most vigorous growth. Among all species, survival of *Bambusa nutans* was found to be the highest.
- A germination trial on seeds of *Melocanna baccifera* collected from different provenances was conducted, which ranged from 42 to 95%. Seed from Agartala and Dharmanagar (Tripura) showed the highest germination, whereas, lowest germination was recorded in seeds collected from Kohima (Nagaland).
- Trial plantations of *M. baccifera* for staggered flowering were established on permanent plots in RFRI campus. These plantations comprised seedlings raised from the seeds of four flowering years i.e. 2001, 2003, 2007 and 2008. Hundred percent survival in all blocks of plantation raised from seeds collected in four different years was recorded. Among the plantations, saplings raised of the seed collected from flowering years 2001 and 2003 showed more vigorous growth.
- Study was undertaken to evaluate the accessions assembled in the bamboo (*Bambusa balcooa*, *Bambusa tulda* and *Bambusa nutans*) germplasm bank by marker technology to generate information related to management strategies like selection of a genetically distant accessions for mass multiplication, addition of new genotypes to increase genetic diversity, identification of duplicate accessions for culling etc, and also to generate accessions specific RAPD





fingerprints that can be used for planting stock certification purpose. Though the study is continuing, genomic DNA of 70 bamboo accessions has been extracted for reproducibility test and primer screening work.

- Germplasm collection of *Dendrocalamus strictus* was carried out from seventeen states. Various technologies on Bamboo species were developed for demonstration to the farmers and forest officers. Clonal material was developed in Clonal Nursery City Centre, FRI Dehradun and distributed to the six states i.e. Haryana, U.P., Punjab, Uttarakhand, Chandigarh and Delhi. Germplasm collection for hill bamboos was also carried out from all the parts of India.
- A brief account of two important species of Hill Bamboo viz. *A. falcata* and *T. spathiflorus* growing in the state of Himachal Pradesh was prepared/compiled. Germplasm from 22 locations was collected and established at Research Stations, HFRI at Baragaon, Shimla Hills and at Bruhandhar, Near Manali, Kullu valley, Himachal Pradesh.
- Survey, identification, collection and preservation of germplasm was carried out in *Dendrocalamus strictus*, *Bambusa bambos* and *B. tulda* in East and West Midnapore districts of West Bengal.

#### ***Moringa oleifera***

Superior seed sources were identified in Jharkhand, West Bengal, Bihar and Orissa and 15 candidate plus trees were marked. Cuttings collected from identified CPTs/seed sources and clonally multiplied. Shoot cuttings planted employing auxin and non-auxin growth regulators in summer season.

#### **Conservation of Medicinal Plants**

Surveyed Darjeeling Hills and foot hills and collected germ plasm of 6 species of medicinal plants viz. *Rauvolfia serpentina*, *Withania somnifera*, *Stevia rebaudiana*, *Asparagus racemosus*, *Gymnema sylvestre* and *Abolmoschau moschatus*.

#### **2.3.3 Tree Improvement**

##### ***Dalbergia sissoo***

The Forest Research Institute, Dehradun has been working on the genetic improvement programme of *Dalbergia sissoo* since 1990. Though this species has a number of promising attributes, it exhibits poor stem form (crooked stem), forking, ramicorn branching and susceptibility to the dieback. In genetic improvement programme of the species, a number of plus trees from various locations have been selected and assembled in the gene/clone bank. Initially the selection of promising trees was carried out in the states of undivided UP, Rajasthan, Bihar, Nepal and other Shisham growing regions, the genetic worth of these genotypes is being tested in the field. The field trial consisting of 49 clones has been established at three locations each at Bithmeda (Haryana), Pantnagar (Uttarakhand) and Hoshiarpur (Punjab) following lattice design. The evaluation of earlier trials consisting of 36 clones planted at Hoshiarpur, Ludhiana and Bithmeda were evaluated as per schedule on various morphometric and wood traits. The wood samples have been collected and are being tested for anatomical and wood properties.

Multilocational (4 locations) clonal trials of *D. sissoo* clones were also established in Gujarat. Significant variation has been observed between the clones for most of the growth traits across the locations.



Seven Years Old Clonal Trials of *D. sissoo* in Gujarat

The clones selected on growth performance were subjected to stress resistance and insect pest resistance studies. The genetic variation using isozyme techniques with five stable enzyme systems namely MDH, 6PGDH, IDH, SKDH and MNR was studied. The stress resistance of these 16 selected clones was studied by for water potential. Experiments were also laid out to test the insect pest resistance in the field and identified defoliators were studied in the lab. These selected clones were field planted at Jawalajee in the state of HP and at Basanterbela in the state of J&K during August 2009.

### **Melia**

The natural forests and the plantations of *Melia composita* were surveyed. A total of 230 Candidate Plus Trees (CPTs) were selected from different geographical regions and analyzed for index value based on height, diameter at breast height, straightness, clear bole height, crown diameter and knots. The mean index value for CPTs was calculated to 44.39, however, the trees with index value of more than 75 (58 trees) were considered as plus trees with average index value of 81.81, an improvement by 82 % over the CPTs. Further, evaluation trials of 21 most suitable families was established applying lattice design with seven replications in six geographical locations of Indian states of Haryana, Punjab, Uttar Pradesh and Uttarakhand.

Ten plus trees, each of *Melia dubia* and *Melia azedarach* have been selected from Karnataka, Tamil Nadu and Andhra Pradesh. Seeds were collected and nursery rose. A progeny trial has been established for *M. azedarach* and *M. dubia* at FRC, Hyderabad. Morphological attributes and oil content of seeds of *M. azedarach* and *M. dubia* were studied and recorded. DNA extraction from leaf samples is in progress to study genetic variation among progenies of the selected plus trees.

### **Pongamia pinnata**

The plantations of *Pongamia pinnata* were surveyed in the states of Punjab, Uttarakhand, Uttar Pradesh and Haryana and identified promising genotypes for higher seed productivity and oil content. Field trials have been raised with 49 selected families at Jhumpa (Haryana) and Pantnagar (Uttarakhand) for testing stability, adaptability and growth performance.

### **Maduca longifolia**

Selected 40 CPTs of mahua from Uttar Pradesh, for qualitative analysis flowers and seed were collected for chemical analysis.

### **Casuarina**

Second generation seed orchards were established with progeny raised from the promising trees of first generation orchards of *Casuarina equisetifolia* and *C. junghuhniana*. A 15 months old second generation progeny trial of *Casuarina equisetifolia* was established with progeny of the best 5% of the first generation breeding populations. Early growth data of



A 15 months Old Second Generation Progeny Trial of *Casuarina equisetifolia* at Karur, Tamil Nadu



second generation seedlets showed significant improvement over that of the first generation (Location: TNPL Campus, Karur, Tamil Nadu).

Short listed 10 best performing clones of *Casuarina equisetifolia* based on initial observations of multilocation field testing of 87 clones at (1) Mailladumparai, Karur district, Tamil Nadu, (2) a sodic site at Pugalur, Tamil Nadu and (3) at Sirugramam, Cuddalore, Tamil Nadu was established during 2008. Considerable variations were observed for growth and stem form.



Clonal Trial at Mailladumparai

In order to developing cloning techniques for raising high yielding clonal plantations of *Casuarina equisetifolia* L., coppicing experiments were conducted 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). Stumps cut at 45 and 60 cm. from the ground level were found to yield more coppice shoots. Cladodes and needles collected from these coppice shoots were kept for rooting studies. Significant variation was observed between rooting in cladode and needle explants. Needles recorded a maximum rooting percentage of 65 where for cladodes was above 90.



Coppicing Ability



Rooting Ability

### Eucalyptus

A breeding arboretum of eucalypts was established in open pollinated mating design for production of inter and intra-specific hybrids. Two species viz. *E. pellita* and *E. urophylla* were taken up initially for the study of their breeding behavior and cross compatibility. *E. pellita* is a frost resistant and moderately resistance to stem cankers due to *Cryphonectria cubensis* and has fast growth. *E. urophylla* has higher productivity and adaptability but is susceptible to *Cryphonectria cubensis*. Both the species are used for pulp and paper. The reproductive biology of these two species was studied and controlled crossing between them was attempted. The F<sub>1</sub> hybrid of *E. pellita* x *E. urophylla* was produced and planted in the field, which show high degree of heterosis at the initial stage.

The clonal trials were evaluated and poor clones were culled to improve the quality of the seed orchards. After culling, seeds were collected from 60 selected clones and about 25000 seedlings were raised to establish progeny trials at Hyderabad and Puthukottai. The selected clones were also multiplied clonally and about 2 seed orchards were established at Salem and Nellore.



Progeny Testing of Eucalyptus Clones at Pudukkottai



Clonal Seed Orchard at Salem





The suitability of *Eucalyptus tereticornis* and *E. camaldulensis* clones was studied at four locations in Andhra Pradesh viz., Warangal, Rajahmundry, Hyderabad and Tirupati and one location in Karaikal. Multiplication of the clonal material is still under progress for further trials in Tami Nadu and Karnataka. Clonal multilocal trials of *E. camaldulensis* were also established in Gujarat, which revealed significant variation between the clones of both the species for the growth traits across the locations.



*Eucalyptus* Clonal Trial at Warangal raised by IFGTB



Vegetative Multiplication Garden of Short listed *Eucalyptus* Clones

### *Acacia mangium*

The advanced generation seed orchard of *Acacia mangium* were created based on biomass and wood density and evaluation of families in Seedling Seed Orchards (SSOs) in Nilambur and Karunya. Outstanding families have been delineated for seed collection.

Keeping in view the potential of the species to grow successfully in North-Eastern region of the country, 16 plus trees were selected following point grading selection method. Seeds

of plus trees were collected and their half-sib progenies were raised in the nursery. After evaluation, two progeny trials were established at Satra and Melang Grant, Assam.

### *Tectona grandis*

Twenty clones were studied for reproductive biology and tagged for hybridization. All the clones were assessed for flowering and fruiting behavior in three CSOs and one SSO (1500 trees). A clonal trial was established at Salem (TN). Clones KLN2 KLN 4 & TNT 20 were found to be superior performers in Nilambur progeny trial. A total of 56 superior teak trees were selected in different parts of Kerala, multiplied through coppice shoots and established in the Vegetative Multiplication Garden (VMG). The rooting performance of different clones was also studied.

In order to assess realized genetic gain from teak seed orchards, flower production and reproductive phenology of different clone and families in two clonal seed orchards and one seedling seed orchard were assessed periodically. Nursery experiments are underway to estimate germination and seedling vigour as an indicator of the extent of out-crossing in seed orchards.

Impact of continuous moisture on growth, flowering, seed production and wood characteristics of canal teak plantations in Tamil Nadu was studied. The canal teak showed outstanding growth and attained the harvestable size within 20 years. The percentage of fruit setting was 5% in canal areas, and generally, fruit setting in canal teak is late as compared to other natural teak growing areas. Thirty five superior teak trees were selected in Tiruvarur and Thanjavur areas for clonal multiplication. The damages and natural decay symptoms such as hollowness in different parts on the tree, top dying, early forking, illicit pruning, bulging,



rotten branches and presence of any fungal structures were also studied.

The flower induction was studied in two clonal seed orchards of *Tectona grandis*, two one each in Karnataka (Janganamatti, Dharwad) and Andhra Pradesh (Achuthapuram, Rajamundry). The effect of chemicals viz. Paclobutrazol, Chloroethyl-trimethyl-ammonium chloride, 2,3,5-triiodo benzoic acid (TIBA), Salicylic Acid, Succinic acid 2, 2-Dimethyl Hydrazide (ALAR-85), Naphthylene Acetic Acid (NAA), Potassium Nitrate, Poly Ethylene Glycol 6000 (PEG) and 2, Chloroethyl posphonic acid (ETHERAL) was studied in 36 different treatments on flower induction. Apart from cultural treatments like girdling, shoot training, shoot pruning and root pruning were also employed. A new method for stem injection of chemicals was devised during the experiment.

The magnitude, type of genetic variation and direction of association among different seed and fruit parameters was studied in dry teak (5A/C 1b & 5A/C 1a). The investigation revealed highly significant variation for all fruit and seed parameters in Gujrat teak. The stone length, treated stone length (mm), stone weight (gm), and treated stone weight were found to be highly heritable, whereas, stone width, treated stone width and number of unfilled chambers were moderately inherited.

Genetic analysis of eleven years old progeny trial (18 half-sib families) on inheritance of growth traits in Gujarat Teak was carried out. Individual and family heritability values ranged 4 to 26 and 12 to 43 per cent for apical dominance and Clear Bole Length (CBL), respectively. Height and CBL exhibited moderately high, Girth at Breast Height (GBH) and volume exhibited moderate and basal area and apical dominance showed low estimates of narrow sense heritabilities. Values of narrow sense heritability

coupled with moderate to low estimates of genetic advance indicated presence of both additive and non-additive gene action.

Amongst the tested parents, seven parents showed positive General Combining Ability (GCA) for height and girth. Five parents exhibited positive GCA for all the traits. These parents with positive GCA values are expected to harbor constellation of desirable alleles, and expected to produce good progenies through recombination. It was recommended to use these parents for establishing advanced generation seed orchards.

#### *Acacia auriculiformis*

Genetic improvement program of *Acacia auriculiformis* through half-sib progeny selection is being executed at IFGTB, Coimbatore. Tree selection was carried out in two seedling seed orchards established by IFGTB, at Karunya and Panampally, one Seed production area established by Kerala Forest Department at Chettikulam in Kerala and two seed orchards established by Mysore Paper Mills Bhadravathi at Hosanagara in Karnataka. Altogether 132 trees were selected based on stem form, branching habit and growth in these orchards. Single tree seed collections from selected trees were made for raising half-sib progenies. Two progeny trials were raised one at Panampally and other at Pondicherry. Two more progeny trials of 1 ha each consisting 132 half-sib families of selected superior trees were established at Palode (Trivandrum division) and Vadakkancherry (Thrissur Division)



Thinning of Two and Half Years Old Progeny Trial at Palode









**Hardwickia binata**

Survey was carried out in different parts of Karnataka, Andhra Pradesh and Tamil Nadu to identify the populations of *Hardwickia binata*. Preliminary morphological observations were recorded and core samples were collected from different aged plantations in these states to document the variability for tree traits. Studies on these core samples are being carried out. Variability in seed traits is also being recorded.

**Tecomella undulata**

Forty Candidate Plus Trees (CPTs) of *T. undulata* were selected after a vast survey in Jaisalmer, Barmer and Jodhpur districts of Rajasthan. Seeds were collected from 40 CPT's and seedlings were raised in AFRI nursery, Jodhpur. Progeny trials were established at Bikaner and Jodhpur during August 2008. The survival percentage was 91% at Jodhpur as compared to only 66% at Bikaner. The progenies from Chohtan (Barmer) and Mohangarh (Jaisalmer) gave best growth at Jodhpur (45 cm) and whereas at Bikaner, the progeny from Mohangarh (Jaisalmer) showed best growth.

**Buchnanian lanzan**

A total of 33 phenotypically superior candidate plus trees were selected in Chhindwara, Gondia, Shahada and Raigarh Forest Divisions of Madhya Pradesh. Seeds were collected from *Buchnanian lanzan* Seedlings 25 candidate plus trees and progeny trials were established at the Centre for Forestry Research and Human Development campus, Chhindwara.



**Gmelina arborea**

Trials were conducted to know the reproductive biology in clonal seed orchards of *Gmelina arborea*. Periodical observations were made on vegetative and reproductive events. Data of flower and inflorescence recorded from

randomly selected ramets and assessed. Out of 70 clones, flowering was recorded in 57 clones. Bud initiation started in last week of March and reached to peak in mid of April. Thereafter formation of buds decreased and ended by April and fruit set occur in 22 flowers per branch.

**Aquilaria malaccensis**

Studies on selection of desirable genotypes has been undertaken to establish field gene bank of *Aquilaria malaccensis*. Germplasm collected from various places of Assam and SSO was established. Clonal multiplication protocol through rooting of shoot cutting was standardized.

**Developing Strategies for Describing, Testing and Registering Varieties of Forest Tree Species**

Studies were conducted in the base populations of different provenances for developing DUS descriptors and DUS test guidelines for *Eucalyptus* and *Casuarina*. Variation in morphological characters in leaf, stem, bark and reproductive structures was recorded. Studies were also conducted in replicated clonal trials for quantifying the



	Clome no. 14	Clome no. 19	Clome no. 94
Live bark	Light green	Yellow	Yellow
Dead bark	Gray	Brown	Green
Peeled bark	Brown	Dark brown	Brown

Variation in Bark Characters of Different Clones of *Eucalyptus*



uniformity and stability of the selected morphological traits. In *Casuarina*, the cladode, leaf, bark, branch and reproductive structures were taken as DUS characters based on the study conducted in 300 clones. About 49 different descriptors were given for discrimination of clones. Basic discriminating characters for *C. equisetifolia* and *Casuarina junghuhniana* are also given. In *Eucalyptus*, clones placed in two locations, namely Coimbatore and Sathyavedu were studied. The leaf, bark, branch and reproductive characters were taken as DUS characters. About 31 different descriptors were given for discrimination of different *Eucalyptus* clones. Detailed DUS descriptors and DUS test guidelines have been developed for these two species and submitted separately.

Plan of action for validation of the DUS characters have been developed and possible DUS characters of *Eucalyptus* and *Casuarina* have been finalized. Clonal plantation with known identity is selected in different locations for validation of the DUS characters. Scoring sheets are formed for recording the DUS characters in the field.

To improve the accessibility and affordability of improved seeds from breeding programs to benefit large numbers of small holder tree farms and rural communities, three community seed orchards were established involving farmers, traditional nursery operators and field staff of forest department. These orchards will be maintained as model orchards to spread awareness on the need for genetically improved planting stocks. Capacity building programmes are underway to improve the skills of farmers, nursery operators and forest department staff in developing and managing seed/seedling production system. Quality seeds to the growers are being provided through seed bank at FRI Dehradun where seed of good quality with proven identity is collected and maintained.

### 2.3.4 Vegetative Propagation

- Vegetative propagation of *Dalbergia sissoo* and *Eucalyptus* clones was carried out. In *Dalbergia sissoo*, 75 clones were multiplied and about 15000 plantlets were produced. The propagated plants were established in clonal trials at different locations of Haryana, Punjab, U.P and Uttarakhand. Similarly in *Eucalyptus*, about 5000 plantlets were produced for experimental purpose.
- Rooting trials were conducted to study the response of productive clones of *Eucalyptus* using conventional two noded cutting methods. On the basis of rooting response, clones were categorized as good, moderate and poor rooters. Attempts were made for enhancing rooting and planting stock availability through mini cutting technique and micro cutting technique. Hedge garden with 22 clones was established and maintained using vegetatively propagated ramets. Propagules of eight clones produced by the tissue culture route were hardened and planted in beds to establish a hedge garden for the micro cutting technique route.
- As regards fast growing species, twenty different combinations of growth hormones of *Melia dubia*, individually and in combination were tested for induction of shoots under *in-vitro* conditions. Benzyl Amino Purine singly or with low concentrations of Kinetin has been identified as the most suited growth regulators for bud initiation.



Multiple Shoot Induction in *Melia dubia*



- A Vegetative Propagation Centre (VPC) at CoF Ponnampet was established with capacity for raising 50,000 rooted cuttings of bamboo for commercial cultivation of bamboos.
- Genetic variation for *in-vitro* morphogenetic potential of *Dalbergia sissoo* clones studied.
- Significant seasonal/genotypic variation was recorded in endogenous auxin (IAA) level in



Adventitious Rooting in Shoot Cuttings of *Dalbergia latifolia*

*Dalbergia latifolia* trees from Jabalpur. IAA was minimum ( $1.742 \mu\text{g g}^{-1}$  fresh weight) in March and maximum ( $3.640 \mu\text{g g}^{-1}$  fresh weight) in July. Significant genotypic variation in endogenous auxin was also recorded among selected trees from Chandrapur (Maharashtra) and Jagdalpur (Chhattisgarh). Experiments conducted for testing rooting potential in selected trees of Jabalpur, Chandrapur and Jagdalpur. A basal dip treatment of 5mM IAA for 4 hours promoted adventitious rooting up to 11.33% compared to 1.33% in control in cuttings of selected trees of Jabalpur. Literature reveals only up to 5% rooting and categorized the species as “very difficult to root”.

- *In-vitro* regeneration of plantlets in *Saraca indica* Linn. a vulnerable medicinal tree was carried out. Highly significant effect of seasons was observed on sprouting of axillary buds with maximum sprouting (35.2%) obtained in summer season in 2-3 years old plants. Sterilizing treatments also had significant effect on sprouting of buds with 0.2 %  $\text{HgCl}_2$  treatment resulting in maximum sprouting (38.9%), which was at par with sprouting (27.8%) obtained with 0.1 %  $\text{HgCl}_2$  treatment. Season and sterilizing treatments did not have significant effect on sprouting of terminal buds from 20 years old mature tree.  $\text{B}_5$  medium supplemented with  $2.2 \mu\text{M}$  BA was screened out as the most suitable medium for bud sprouting and elongation of regenerated shoots from nodal segments. On modification of  $\text{KNO}_3$  in  $\text{B}_5$  medium, the modified strengths of  $\text{KNO}_3$  had statistically significant effect on sprouting of buds and number of shoots after four weeks of culture.
- Macro propagation studies were carried out for 23 selected genotypes of *Dipterocarpus retusus*. Rooting of coppice shoot cuttings has been found suitable method for macro-propagation of the species.
- Standardization of nursery techniques of *Bambusa pallida* through a media containing equal proportion of soil, sand and FYM was found to be suitable for propagation through culm cuttings. 20-25% survival was observed in case of culm cutting in the treatment 300 ppm of IBA solution. In the case of macro-proliferation technique, 75%-85% survival was recorded.





- Demonstration plots of three bamboo species viz. *B. balcooa*, *B. nutans* and *D. hamiltonii* at eight sites (20 ha X 8 ha) in North-East were established to demonstrate the field performance of tissue cultured plants.
- Grafting techniques was standardized for propagation of selected adult male and female plants of *Ailanthus excels.* Success rate of grafting increased up to 50% over the earlier method (where success rate was 10% only) developed by AFRI. The plants produced were also used for field evaluation.
- *In Scleichera oleosa*, besides stem cutting, other macro propagation methods like air-layering and grafting were carried out. On an average more than 50% rooting was observed through these methods.
- Extensive trials were conducted on refinement of macro propagation of common bamboos of eastern India. The role of culm segments, rooting hormones, diverse propagating medium, shading etc. and diversity of rooting and rhizome genesis of different bamboo species was evaluated.
- Propagation of hill bamboos (ringal) was undertaken for mass multiplication through *in-vivo* and *ex-vitro* techniques. Offset planting of *A. falcata*, *T. falconeri* and *S. jaunsauensis* revealed 90, 85 and 70% success. Macro-proliferation of Dev and Gol ringal were successfully performed. The species could be multiplied 8-10 times by these techniques in six months period.
- Tissue culture technology for *Swertia chirata* through two regeneration pathways namely, axillary bud proliferation and adventitious bud differentiation was developed.
- Inter and intra-clonal variations were studied with respect to age of the VMGs. Wide inter and intraclonal variations were observed in first coppice shoot production, number and length of coppice shoots. Clone C9 produced maximum shoots. The effect of age on shoot initiation, production, rooting, sprouting, root initiation, time of harvestable shoots was prominent. The best response was in 10 years old hedges. Subsequent growth of propagules was also affected by age of hedges. Older hedges depicted plagiotropy.



Orthotropic Shoot Production

### 2.3.5 Biotechnology

#### DNA Profiling

- DNA fingerprinting using SSR markers was carried out to understand the complex genetic structure of trees with particular reference to delineation of provenances and study of genetic diversity, molecular characterization of germplasm, inheritance pattern and establishment of genotype and species specific markers. Presently molecular characterization of Himalayan pines, *Cedrus deodara*, *Tectona grandis*, *Eucalyptus* and shisham germplasm is in progress.
- The genetic and molecular evaluation of 28 clones have been done in sandal. Oil content, carbohydrate, and protein estimation of seeds were completed of all the selected clones.



Some clones with as high as 42% content were recorded. Molecular characterization of all clones using ten RAPD primers (Bangalore Genie Ltd.) has been completed with high numbers of polymorphic bands.

- Development of SSR markers and DNA profiling through Randomly Amplified Polymorphic DNA assay were carried out in *Casuarina equisetifolia*. RAPD assay was optimized and six polymorphic primers used for profiling one hundred and fifty clones of *Casuarina equisetifolia*.
- The short term trainings were provided to approx.100 M.Sc. (Biotechnology) students of different universities and educational institutions on genetics and biotechnology during the year.

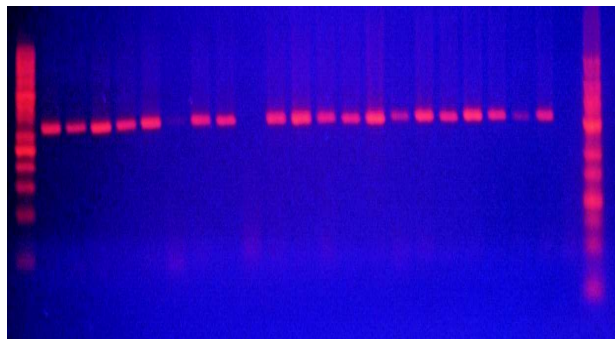
### Use of Molecular Markers in Breeding Programmes

#### Association Analysis of Traits Using STS Markers

Attempts were made to identify potential STS markers for rooting in *Eucalyptus tereticornis* using association analysis approach. *E. tereticornis* exhibits rooting variability from 10- 90 %. Thirty three STS markers linked with rooting, 10 mapped Simple Sequence Repeat (SSR) markers and 10 Expressed Sequence Tag-Simple Sequence Repeats (EST-SSRs) were cross amplified in *E. tereticornis* 30 phenotypes with contrasting rooting potential. The linkage disequilibrium (LD) value ( $D'$ ) ranged from 0.49 to 0.80 and LD decay ( $r^2$ ) varied from 0.02 to 0.13, which has provided the basic information to select candidate STS markers for identifying marker-trait associations in *E. tereticornis*.

Attempts are also in the process for the identification of molecular marker associated with

wood quality traits in *Tectona grandis* L. for which PCR conditions have been standardized for STMS analysis.



STMS Amplification of Genomic DNA of Teak plus Trees with Tg19 SSR (L-R): 50bp DNA ladder, APKEA-23, APNPL-9, AC-II, G+10, ST-17, ST-, KLK-1, PT-41, PT-3, MHSC-A3, MHSC-A1, ORBUB-11, ORPBU-6, TNT-8, TNT-10, UP-M, UP-A, WB, WB+4, AI-N, AI-1 and 25bp DNA ladder

### Evaluation and Prediction of Oil-bearing Capacity of Sandal (*Santalum album* L.)

Nitrate reductase activity was determined in leaves of 27 selected trees from TP and 3 trees from TO plantation area. TP-1 from TP selection and TO-1 from TO selection had maximum enzyme activity. On the other hand, the lowest enzymatic activity was recorded in TP-6, TP-7 and TO-17 trees among their selections. Peroxidase activity was determined in leaves of selected 30 trees each from TP and TO plantation area. Trees from TP selection exhibited more enzyme activity than those from TO selection.

### Quantitative Trait Loci (QTL) Mapping in Eucalypts

Controlled pollination leading to combinations of *E.camaldulensis* x *E. grandis* and *E. tereticornis* x *E. grandis* was completed to generate  $F_1$  hybrids for the establishment of mapping population. Traits such as salt tolerance, adventitious rooting and wood property were chosen in this hybridization work to generate genetic linkage maps. Salt tolerance levels in *E. camaldulensis* clones were assessed at



different stages of sodium chloride treatment for various enzyme systems like super oxide dismutase, catalase, glutathione peroxidase and ascorbate peroxidase to ascertain tolerance/susceptible levels. Ninety five microsatellite primer sets were tested for their transferability to *E. camaldulensis*, *E. tereticornis* and *E. grandis*, which on successful amplification in parents and F1 hybrids will be used for linkage map construction.

#### **Allelic Diversity of Cinnamoyl CoA Reductase Enzyme Gene in *Casuarina equisetifolia***

Lignin and holocellulose content in wood samples was evaluated in twenty five clonal accessions aged twelve years. The range of holocellulose was 75-80% and lignin was 24-54%. Wood proximate chemical analysis (% ash, Alcohol benzene extractives, water solubility, NaOH solubility) was also done. In twenty five clonal samples Cinnamoyl CoA Reductase (CCR) enzyme activity was assessed using Phenyl Ammonia Lyase assay where inconsistent variation was noticeable. Four out of the twenty two CCR (forward & reverse) primers yielded amplified products.

#### **Sex Determination in *Casuarina equisetifolia***

Twelve Isoenzymes Aspartate Amino Transferase (AAT), Alcohol Dehydrogenase (ADH), Esterase, (EST), Polyphenol Oxidase (PPO), Peroxidase (POD), Glutamate Dehydrogenase (GDH), Isocitrate Dehydrogenase (IDH), Superoxide Dismutase (SOD), Malate Dehydrogenase (MDH), Malic Enzyme (ME), Lactate Dehydrogenase (LDH) Glucose 6-Phosphate Dehydrogenase (G-6-PDH) were optimized in *Casuarina equisetifolia*. Twenty four males and twenty three female casuarina clones were screened for five distinct gender specific enzymes (AAT, IDH, ADH, LDH and POD). Peroxidase profiles were found to be very distinct in gender discrimination.

#### **Salinity Tolerance in *Casuarina equisetifolia***

Efforts are on to identify suitable biochemical markers to enable screening of clones of *C. equisetifolia* developed and maintained by IFGTB. Based on the tests, clones suitable for saline and non-saline areas can be categorized. Experiment was conducted to identify the salinity range for exposing the *Casuarina equisetifolia* clones.

#### **Gene Isolation and Functional Analysis**

##### **Identification of Secondary Xylem Specific Cellulose Synthase Genes from *Eucalyptus tereticornis***

In silico research was conducted to catalogue and assemble nucleotide and protein sequences of cellulose synthase (CesA) genes and primer pairs were designed and synthesized targeting the CSR II domain of the CesA. The primer pairs were screened and six transcripts representing the six families of CesA were identified in the developing secondary xylem tissues of *E. tereticornis*. Studies are in progress to identify the 3' downstream sequences of the CesA genes.

##### **Differential Analysis of Transcript Expression in *Casuarina-Trichosporium* Interaction to Isolate Defense-Related Genes**

Two pathogenesis related genes; chitinase and glucanase were cloned to *Casuarina equisetifolia*, and their expression during pathogen elicitation was studied. Further, major transcripts including cytochrome oxidase, proteasome, signal recognition particle and unknown transcripts with similarity to drought tolerant ESTs were demonstrated to be over expressed during pathogen elicitation.

##### **Web Enabled Database and Analysis of Gene Sequences Implicated in Abiotic Stress Tolerance for Screening Gene Homologues in Salt Tolerant Tree Species**

To identify genetic determinants of salt stress tolerance in *Casuarina*, 82

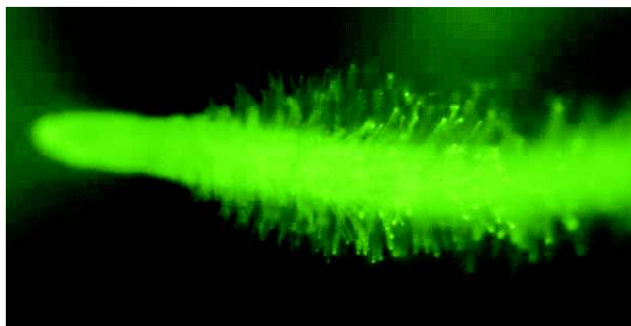




*Casuarina equisetifolia* clones were screened to identify highly salt tolerant and salt susceptible clones. The most susceptible clone could tolerate up to 400 mM, while the most tolerant clone survived for 45 days post 550 mM NaCl stress. While the root to shoot sodium content was close to one in resistant clones, in the case of susceptible clones, shoots accumulated 2 to 3.6 times more sodium than in the roots. The clones will be subjected to differential gene expression analysis.

### Development of Methods for Functional Analysis of Genes Involved in Salt Tolerance in *Eucalyptus*

For rapid functional analysis of genes involved in salt tolerance, parameters critical for development of *E. tereticornis* composite plants with transgenic hairy roots were analysed. Identified micropropagated plantlets as suitable explants for obtaining hairy roots and obtained GFP expression in hairy root cultures of *E. tereticornis* tissue cultured shoots. Suitable media for hardening of composite plants in lab environs was determined. Transformation experiments using *A. tumefaciens* harbouring the AtNHX constructs were continued.



Green Fluorescent Protein Expressing Transgenic Hairy Roots of *Eucalyptus tereticornis*

### Improvement of White Rot Fungus Strains

Pre-treatment of destructured bamboo chips and non-destructured bamboo chips with different strains of white rot fungi (*Schizophyllum commune*, *Coriolus versicolor*, *Ceriporiopsis subvermispora* and *Flavodon flavus*) was completed. Best fungus *Coriolus versicolor* (*Tremetes versicolor*) and *Flavodon flavus* after

ascertaining the optimum removal of lignin were selected for bulk inoculation and mass growth on chips and mechanical processed chips for further pulping experiments.

### Development of Micropropagation Technique

- Tissue culture technology was developed for *Dendrocalamus hamiltonii* and *Gigantochloa atter* through axillary bud culture. In *D. hamiltonii* optimal rooting, i.e. 66.13% was obtained on MS medium supplemented with 25 $\mu$ M IBA and in *G. atter* 47.67% rooting was obtained on MS + 35 $\mu$ M IBA, in four weeks. Rooted plantlets of *D. hamiltonii* were successfully hardened and later transferred for field plantation. Studies were also undertaken to develop an efficient micropropagation protocol for clonal multiplication of *Bambusa tulda*. In spite of the best efforts the frequency of *in-vitro* root induction is very less. Experiments are in progress to enhance the rooting percentage. Experiments are ongoing to study the genetic fidelity of the micropropagated plants of bamboo-*Bambusa bambos* and *Dendrocalamus stocksii*.
- In order to develop tissue culture technology for multiplication of economically important desert plant *Salvadora persica*, surveys of natural stands of *Salvadora persica* were done and CPTs were selected as a source of explants from Jodhpur (Bilara, Caparda and Badi Khurd), Pali (Rohat, Sanderav and Ranakpur) and Jalore (Pandagara, and Suryashwar Mahadev Temple). About 70 per cent bud break and multiple shoot induction (2-3 shoots/explant) were obtained on MS medium supplemented with 4.0mg/l BAP + 0.5mg/l NAA.
- Studies were conducted to scale up the protocols for *in-vitro* propagation,



hardening, production of cloned plants and establishment of field trials of Sandal wood (*Santalum album* L). Studies were carried out on the effect of auxins and their conjugates on *ex-vitro* rooting from the *in-vitro* shoots. IBA along with conjugate was found better for *in-vitro* as well as *ex-vitro* rooting.

- For development of micropropagation protocols for production of superior germplasm of *Dalbergia latifolia* and *Pterocarpus santalinus*, aseptic cultures of superior germplasm of *Dalbergia latifolia* and *Pterocarpus santalinus* have been established. Experiments for axillary bud proliferation are in progress.
- Attempts have been made to induce essential oil/oil components of Agar plant under *in-vitro* condition using tissue culture techniques. So far, an efficient callus induction medium has been standardized using leaf tissue. Cell suspension culture has also been established using the callus. At present, experiments are in progress to induce essential oil in this cell culture.
- Attempts were made to develop micro propagation technique for *Aquilaria malaccensis*. Auxiliary buds, as explants have been found best for culture initiation through direct regeneration. Through this technique up to eight (8)-fold shoot multiplication has been achieved. Efforts are being made to increase the rooting frequency in the multiplied shoots.
- *In-vitro* plantlet formation in *Jatropha curcas* has been achieved from 3 pathways viz, through axillary bud break, adventitious shoot induction through callus phase and direct somatic embryogenesis. The procedures are being optimized for low cost options for economizing the technology.



Different Methods of *in-vitro* Regeneration in *Jatropha curcas*: A. Progressive Stages of Somatic Embryogenesis, B. Axillary Bud Break from Mature Nodal Segments, C. Adventitious Shoot Regeneration from Leaf Derived Callus and D. Elongated Shoot

#### Field Trials of Tissue Culture Raised Plants

- Field trials of tissue culture plants of *Bambusa bambos* and *Dendrocalamus strictus* were established at three places, two in Rajasthan and one in Gujarat during July-August 2006 at 5m x 5m spacing. Average height of *Dendrocalamus strictus* was 10 % higher at Dahod (about 4 m) than Kushalgarh (about 4.3 m). In case of *Bambusa bambos*, average height was 50% higher at Dhaod. Similar trends were also observed for number of new culms per clump. Dahod site favoured better growth in terms of number of culms than Kushalgarh site. *D. strictus* produced 16% more new culms and *B. bambos* produced 74% more new culms/clump at Dahod almost after three years.
- Demonstration plot of 50 ha of tissue culture raised and stem cutting raised plants of *Dendrocalamus hamiltonii* was established during 2006 to 2008 by HFRI, Shimla. The tissue culture raised plants were provided to IHBT, Palampur. The growth data for culm length, culm diameter, number of nodes and inter-nodal length were recorded periodically. The survival percentage of TC raised plants was 88% whereas those of SC raised Plants was up to 95%.