

## 2.3 Genetic Improvement

### Overview

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. Genetic improvement process increases the value of a tree species by (1) selecting the most desirable trees from natural stands or plantations, (2) breeding or mating these select trees and (3) testing the resulting progeny. ICFRE institutes have been adopting this process for the selected tree species in different eco-climatic zones across the country. The entire program of selection, breeding and testing at different generations has shown improvement in various tree species. In this process, the production populations in the form of seed orchards and vegetative multiplication gardens have been established to meet commercial planting demands for seed and vegetative material. The quality of seed planted in the nursery is of crucial importance, since seeds are the most basic input into any planting programme. Improved seeds from Clonal Seed Orchards and Seedling Seed Orchards of Casuarina, Dalbergia, Eucalyptus and Neem are already made available by the ICFRE Institutes for planting to user agencies.

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, improve productivity. Therefore, selection of superior trees, clonal multiplication and establishment of clonal plantation has been taken up in ICFRE institutes. Reproductive biology, fruit setting and qualitative characteristics of seed and seedlings are important for production of improved seeds in tree improvement programme. DNA based tools and

techniques are being used for population and conservation genetics of natural forests, identifying trait specific markers, timber forensics and molecular based taxonomy.

### Summary of the Achievements Under the Theme

#### Eucalyptus

Through systematic selection and multilocation testing, one clone of *Eucalyptus* hybrid (*Eucalyptus camaldulensis* Dehn. X *E. tereticornis* Sm.) developed by FRI, Dehradun has been recommended by the Regional Variety Testing Committee for release. Through controlled crossing work at FRI, F1 hybrids of *E. pellita* x *E. urophylla* were produced and are being evaluated in the field. IFGTB, Coimbatore has also made a comparative study and released four clones of *Eucalyptus camaldulensis* for cultivation in farmlands. Demo clonal plantations of the released four clones have been established. Efforts are also made to improve the quality of the seeds through progeny testing and establishment of Clonal and Seedling Seed Orchards. To facilitate registration of these new clones, guidelines for Distinctness, Uniformity and Stability (DUS) testing in Eucalyptus have been developed.

#### Casuarina

In Casuarinas, the major thrust is to move the ongoing breeding programme at IFGTB to the second generation and disseminating the output of one generation of breeding to all stakeholders especially to the farming community. Genetic gain tests conducted with seeds of first generation orchards revealed that they are capable of yielding 13 to 28% 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. To facilitate



registration of these new clones, guidelines for DUS testing in Casuarina have been developed and the guidelines are being validated with all available clones. Intra and inter specific hybrids of Casuarina (*C. equisetifolia* and *C. junghuhniana*) have been successfully generated and are being field-tested. A new concept of developing Community Seed Orchards has been developed to meet the enormous seed demand by involving farmers, traditional nursery workers and local forest department officials. Three such Casuarinas orchards have already been established in Tamil Nadu and Puducherry.

### ***Dalbergia sissoo***

Genetic improvement programme of *Dalbergia sissoo* is being executed in ICFRE institutes to cater the problem of poor stem form (crooked stem), forking, ramicorn branching and susceptibility to the dieback. The clonal trials established in northern India in previous years at Bithmeda (Haryana), Pantnagar (Uttarakhand), Hoshiarpur (Punjab) Ludhiana (Punjab) and Patiala (Punjab) were evaluated for various morphometric and wood traits. One productive and resistant (against wilt disease) clone of *Dalbergia sissoo* developed by FRI Dehradun has been recommended by the Regional Variety Testing Committee for release.

### ***Melia composita***

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 field trials of different progenies were established at various locations to evaluate their performance of different economic traits. Six such family evaluation trial were established at different locations of Punjab, Haryana, U.P. and Uttarakhand.

### ***Pongamia pinnata***

Promising genotypes of *Pongamia pinnata* were identified 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. Amenability of vegetative propagation and effect of maturation in Candidate Plus Tree of *P. pinnata* through rooting of stem cuttings/grafting treated with varying concentrations and combinations of auxins or other additives has been studied. DNA extraction protocol in *Pongamia pinnata* standardized for molecular based characterization of the germplasm.

### **Teak**

Investigations on improvement of *Tectona grandis* on the aspect of genetic evaluation and reproductive biology have been carried out. Variation and inheritance of fruit and seed traits of teak from Dang region of Gujarat was studied for the first time. Genetic variation amongst half sib-families was studied. Estimates of heritability and genetic gains were computed and inheritance of growth traits was investigated. Selected 10 phenotypically superior trees of teak and established progeny trial of 28 families at Sajjangarh, Rajasthan.

### **Ailanthus**

Tree improvement programme has been initiated in the indigenous species for boosting the productivity, yield and development of clones/genotypes. A Vegetative Multiplication Garden (VMG) of *Ailanthus excelsa* was established and research infrastructure created.

### **Red Sander**

Survey taken up in natural Red Sanders bearing areas. Leaf samples were collected for population genetic analysis. Core samples have been collected from different aged populations in Southern part of Karnataka. Studies revealed that heartwood formation occurred in this highly endemic tree species in regions away from its natural habitat.



## Bamboo

For establishments of Demonstration Plantations, multi-locational trials of *Arundinaria falcata* and *Thamnochlamus spathiflorus*, commonly known as Hill Bamboos having three replications each of the 22 identified clones were established in two sites at Naggar Range of Kullu Forest Division and in Kumarsain Range of Kotgarh Forest Division (H.P.). The clones were selected based on various morpho-metric traits.

A demonstration plot (50ha) of *Dendrocalamus hamiltonii* raised using both Tissue Cultured (TC) and cutting raised plants was evaluated and revealed that the survival percentage of TC raised plants was 88 % whereas that of the plants raised through cuttings was up to 95%. Optimised techniques for macro- and micro-propagation of bamboos of local importance with respect to nursery environment, propagation media, pot size and irrigation schedule etc. at Chotanagpur (Jharkhand).

## *Aquilaria malaccensis*

Identified plus trees of *Aquilaria malaccensis* for production of quality agar-wood. The seeds from the plus trees were collected for the establishment of progeny trials.

## *Azadirachta indica*

Progeny trials of neem were evaluated at AFRI Jodhpur. Progeny No. 327 was found to be tolerant against frost, whereas progenies of CPT No. 12 were severely (41.2%) affected during winter. Among the progenies of the 17 CPTs, progeny of CPT 7 has shown best performance based on the survival and growth parameters.

## *Hardwickia binata*

Survey carried out in different parts of Karnataka, Andhra Pradesh and Tamil Nadu to identify the populations of *Hardwickia binata*. Preliminary morphological observations have been recorded and core samples have been collected from different aged plantations/natural populations to document the variability for tree traits. Variability in seed traits has also been recorded.

## *Prosopis cineraria*

Surveyed different area of Khejari and selected 20 candidate plus trees.

## Molecular Characterization for Breeding Programmes

- Molecular characterization of Himalayan pines, *Cedrus deodara*, Eucalyptus and shisham germplasm has been completed.
- Three ramets each of 97 plus trees of *Tectona grandis*, maintained at National Teak Germplasm Bank, Chandrapur representing 12 teak growing states and 15-31 progenies (half sib families) of nine plus trees were validated using AFLP and STMS markers.
- Experiments were conducted (DNA extraction) for molecular characterization of 80 accessions of three important bamboo species viz. *Bambusa balcoa*, *B. tulda* and *B. nutans*.
- Application of regression equations to ISSR analysis of genomic DNA for total/polymorphic amplified bands of selected sandal plants of Seoni plantations predicted, 2.56% oil content by TFRI reference trees and 1.4% oil content by IWST reference trees, at the plantation age of 25-30 years.
- DNA extraction protocol standardized for *Pongamia pinnata* (L.) Microsatellite primers were designed for the analysis of genetic variability in selected germplasm of this species.

## Gene Isolation and Functional Analysis

- Identified salt tolerant plant *Lepidium sativum* was hydroponically grown. Four genes (NHX1, SOS1, HKT1 and ClC-c) were shortlisted and gene sequence alignment work was completed.
- An *in-vitro* process for development of transgenic composite plants in Eucalyptus for rapid functional analysis of genes and promoters was developed.
- Two pathogen defense-related genes were isolated, cloned and characterized from *Casuarina equisetifolia*.



- Study on salt tolerance through gene expression pattern analysis has also been initiated.

### Micro and Macro propagation

- *In-vitro* aseptic cultures of *Azadirachta indica* were established from five populations of Madhya Pradesh. Significant increase in shoot length (2.28 cm) was obtained on 0.5  $\mu$ M BA and 125 mg l<sup>-1</sup> casein hydrolysate. Shoot regeneration with leaf pieces resulted in maximum number of shoots (3.56) on 0.44  $\mu$ M BA and 162.86  $\mu$ M AdS.
- Aseptic *in-vitro* cultures of five genotypes of *Rauvolfia serpentina* viz., GO-MN, OR-AG, KL-PB, GO-SG, KL-AJ, were established on MS medium supplemented with 1.5 mg l<sup>-1</sup> BA.
- Maximum rooting of 44% was obtained on WP medium supplemented with 1.5 mg/l IBA for GBW 4 clone of *Dalbergia sissoo*. One field trial was established comprising 5 clones following RBD design and 81% survival in FZB 1 clone was recorded.
- Endogenous auxin (IAA) in selected trees of *Dalbergia latifolia* from Jabalpur, Chandrapur and Jagdalpur showed significant seasonal/genotypic variation. Juvenile and mature shoot cuttings of *Dalbergia latifolia* had 36% and 11% rooting response, respectively.
- 0.3% HgCl<sub>2</sub> and 3% Bavistin® significantly produced highest aseptic cultures in *Albizia procera*. BA and kinetin were the best source of cytokinin for shoot multiplication and kinetin was better than BA for preventing callus formation at the base of the *in-vitro* shoots.
- Shoot multiplication medium for *Dalbergia latifolia* and *Pterocarpus santalinus* has been standardized.
- In *Dipterocarpus retusus*, vegetative propagation protocol has been standardized and a micro-propagation protocol for this species is being developed.
- On-farm innovation in macro-proliferation technique and promotion for commercial plantation of an edible bamboo shoot species (*B. balcooa*).
- Micro-propagation technique was standardized for clonal multiplication of *Aquilaria malaccensis* Roxb.
- Micro propagation of *Capparis decidua* have been initiated and axillary bud break was achieved and *in-vitro* shoots were multiplied.
- For micro-propagation of *Salvadora persica*, auxiliary bud break was achieved on MS medium supplemented with BAP and IAA. Two and half fold shoot multiplication was obtained on MS medium supplemented with 5.0mg/l BAP.
- *Jatropha curcus*, plants were produced from somatic embryogenesis and hardened. The problem of bacterial infection of cultures was also remedied.
- Somatic embryo based plant production protocol was scaled up for *Commiphora wightii* and tissue culture raised hardened plants were planted in field where survival rate was 100%.
- In bamboo, efficient micro-propagation protocol in economically important species viz. *Bambusa tulda* was standardized.
- Inventorization for *ex situ* conservation of Bamboo and Rattan resources from different regions of Northeast India was carried out.
- In order to study the effect of humic acid on rooting behaviour in selected tree species, organic humic acid production method and purification method was standardized in the laboratory.
- Leaves and flowers of genuine *Embelia ribes* were collected from Bangalore and culturing on different media in the TC Lab is going on for conservation and priming.





### Identification of DUS Traits

Three populations of *Pinus roxburghii* and *Cedrus deodara* were surveyed for distinct traits. The observations with regard to needle length and colour have been found to vary considerably in Lahhul forest of deodar and Plateau forest for Chir pine.

### Seed Technology

- Seed germination studies for both *Canarium strictum* and *Hydnocarpus pentendra* revealed that both the species were dormant in nature. Natural regeneration was very poor in *Hydnocarpus pentendra*.
- Viability of seeds of *Jatropha curcus* and *Bambusa bamboos* could be prolonged for 24 months if seeds stored at 15<sup>0</sup> C.
- Based on the 2009-2010 year's results on flower induction, chemicals viz. paclobutrazol (3g/tree), ALAR-85 (250 ppm), salicylic acid (200 ppm), potassium nitrate (2%) and PEG (10%) were found to be best performing when tested in non flowering trees in test orchards.
- Studies were carried out on seed viability in three recalcitrant species viz. *Dipterocarpus retusus* Bl. (Hollong), *Shorea assamica* Dyer (Makai) and *Aquilaria malaccensis* Lamk for moisture content and storage.

### Non Wood Forest Product (NWFP) Species

- Calophyllum inophyllum* is an important Tree Borne Oilseed used for biofuels and medicinal purpose. Tree improvement of *Calophyllum* through selection of high fruit yielder has been initiated.
- The germplasm of *Diospyros melanoxylon* was collected from four locations in three agroclimatic zones of Chhattisgarh. Morphometric field data on number of leaves per shrub, leaf area (cm<sup>2</sup>) and leaf dry weight were recorded.
- Under the project entitled “Domestication, mass multiplication and popularization of *Moringa oleifera* genotypes with superior leaf

nutritive and cytokinin content”, cuttings were collected from identified CPTs/ superior seed sources from different locations of Jharkhand, West Bengal, Bihar and Orissa and clonally multiplied and maintained as germplasm garden.

- For selection and improvement of Natural Dye Yielding Plants, laboratory protocols are being developed for extraction of dyes from *Mallotus philippensis* and *Wrightia tinctoria*.

### Projects under the Theme

Projects	Completed Projects	Ongoing Projects	New Projects Initiated During the Year
Plan	18	40	25
Externally Aided	9	9	9
<b>Total</b>	<b>27</b>	<b>49</b>	<b>34</b>

### 2.3.2 Conservation of Forest Genetic Resources

#### In-situ conservation of Medicinal Plants

At Field Research Station, Khirsu (Pauri Garhwal) some medicinal plants namely ateas (*Aconitum heterophyllum* wall ex Royle), brahmi (*Bacopa monnieri* L. Pennell), chirata (*Swertia chirata* Buch – Ham), dalchini (*Cinnamomum verum* Presl.) and daruhaldi (*Berberis asiatica* Dc.) are being conserved.



Conservation of Medicinal Plants at Khirsu



### *Cedrus deodara*

The genetic diversity and population structure of Himalyan deodar forests was investigated using micro-satellite markers. A total of twenty one large contiguous deodar populations/forests were selected for the investigation that includes forests from Uttarakhand, Himachal Pradesh and Jammu and Kashmir. Using 10 chloroplast simple sequence repeats (cpSSRs) markers, a maximum of 5 cpSSR alleles and 46 cpSSR size variants were found among 21 populations. The total gene diversity ranged from 0.60 to 0.71 with a mean of 0.67 and the within population gene diversity ranged from 0.43 to 0.57 with a mean of 0.53. The highest genetic diversity of 0.67 was recorded in population from Batote (Jammu & Kashmir). Most of the variation in *Cedrus deodara* occurs within populations and small (16.28%) portions of the variation existed between populations. Estimated gene flow between the populations was moderate ( $N_m=1.89$ ). The high differentiation among natural populations and moderate gene flow indicates inefficient long-distance gene flow or hindrance in the gene flow among populations resulting in heterogeneous genetic structures.

The investigation revealed that majority of the deodar forests of Himachal Pradesh are very rich in genetic diversity, particularly, the forests viz. Rampur (Bhurja, Punan), Karsog (Seri range), Wildlife Chamba forest division (Kalatop), Chopal (Sarain range), Anni (Luhri and chowai range) and Theog forest divisions (Cheog range). On the other hand, some forests particularly Chopal (Chopal range), Binno, Kullu (Naggarjhir and Phetavan) and Churah (Khani reserve forest) have considerably low level genetic diversity. These forests need special attention for their scientific management for increasing diversity level. Also these forests will be more susceptible to environmental variations. The forests of deodar of Uttarakhand possess considerably low level of genetic diversity in comparison to there of

Himachal Pradesh and Jammu & Kashmir. Deodar forests of Gangotri and Jageshwar depicted lowest level of genetic diversity in the Uttarakhand. The deodar forest of Batote (J&K) revealed maximum level of genetic diversity in comparison to all the studied forests and hence was identified a main reservoir of genetic variation and can be used for genetic conservation programme.

### *Acorus calamus*

*Acorus calamus* L. (family Acoraceae), commonly known as sweet flag, is an important medicinal and aromatic plant used in several drugs of the Unani and Ayurvedic health care systems. The essential oil obtained from the rhizome called calamus oil contains ' -Asarone', which is found carcinogenic in nature. The genetic diversity and population structure of fifty populations of *A. calamus* from different geographical regions of its range of distribution in India was studied through morphometric traits and DNA markers (RAPD and chloroplast microsatellite). Genetic diversity was found high among populations in comparison to among individuals within populations. The collected sources were also evaluated for -Asarone content. From the investigation, six sources have been identified bearing low -Asarone content (16–25 %) in their rhizome. Out of the identified low -asarone containing populations, one was identified as diploid while other five were identified as hexaploids. The frequency of diploid and hexaploid populations from all the collected sixty-five populations was found less i.e. only 1:65 and 5:65 respectively. Utilization of the identified high valued low -asarone containing populations in medicines as well as in the food-stuffs will enhance the economical and medicinal value of Indian *A. calamus*.

### *Pterocarpus santalinus*

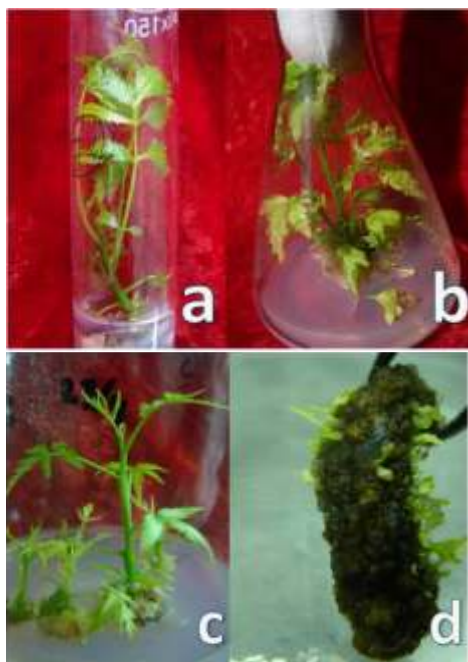
Field surveys were taken up in the natural Red Sanders bearing areas falling under eight forest ranges of Chittoor and Kadapa districts of Andhra Pradesh. Leaf samples were collected



from 8 populations and preserved for genetic analysis. Sixteen hundred Red Sanders seedlings were raised for establishment of germplasm bank. Core samples have been collected from different aged populations in Southern part of Karnataka. Studies revealed that heartwood formation occurred in this highly endemic tree species, in regions away from its natural habitat. Considerable variability for heartwood content was recorded in 20 years old plantation suggesting that while selecting superior genotypes, taking into account heartwood content is also essential. Few wavy grained genotypes were also identified.

#### ***Azadirachta indica***

Studies on variations with respect to *in-vitro* azadirachtin production in selected high yielding populations of *Azadirachta indica* were carried out in the plant material collected from five locations of M.P., viz., Chhatarpur, Katni, Sehore, Khandwa and Bargi. Aseptic conditions were standardized for the micro-propagation. Method for isolation and quantification of azadirachtin was also standardized. Azadirachtin content of samples collected from different places of Madhya Pradesh was quantified.



Micro-propagation of *Azadirachta indica*

#### **Medicinal plants**

Seed germination studies for both *Canarium strictum* and *Hydnocarpus pentendra* revealed that both the species are dormant in nature. Natural regeneration was very poor in *Hydnocarpus pentendra*. Seed storage studies revealed that *Canarium strictum* seeds can be stored up to one year and *Hydnocarpus pentendra* seeds have low viability.

In order to study the variation in reserpine content in some high yielding genotypes of *Rauvolfia serpentina* produced through *in-vitro* and seeds, the shoot cultures of five genotypes namely, GO-MN, OR-AG, KL-PB, GO-SG, KL-AJ were established for *in vitro* multiplication.

#### **Bamboos and Rattans**

- Viability of seeds of *Bambusa bamboos* lost very rapidly (by 1 to 4 months) for the seed stored at high RH of 95%, 88% and 75% 35°C. Seeds stored at 15°C remained viable for 24 months.
- For the inventorization and *ex situ* conservation of Bamboo and Rattan resources of Mizoram, Tripura and Barak Valley of Assam, some of the potential Rattan growing areas were surveyed. Inventorized rattan species viz. *Calamus acanthospathus*, *C. khasianus*, *C. flagellum*, *C. gracilis*, *C. tenuis*, *C. erectus*, *C. guruba*, *C. inermis*, *C. latifolius*, *Daemonorops jenkinsianus* and *Salaaca secunda* and collected for introduction in the Canetum at the ARCBR campus. The locations and geo-coordinates were recorded for future reference. Established bamboo nursery for mass propagation through culm cutting and branch cutting from the selected superior clones. Germ plasm of 20 different bamboo species collected and conserved at ARCBR Campus. Some of the species conserved are: *Melocana baccifera*, *Dendrocalamus hamiltonii*, *Dendrocalmus longispathus*, *Bambusa bamboos*, *Bambusa balcooa*, *Bambusa tulda* and *Bambusa nutans* etc.





Demonstration Plantation of Hill Bamboos (*Arundinaria* spp.) in Chhichhar Forest of Kumarsain Range, Kotgarh



Establishment of Clonal Banks of Hill Bamboos (*Arundinaria* spp.)

- For the ecological assessment, collection, multiplication and establishment of clonal banks of superior genotypes of economically important Hill Bamboos (*Arundinaria* spp.) in Himachal Pradesh, three districts namely; Kullu, Shimla and Sirmour were selected for further detailed investigations and exact occurrence and extent of these species. It was observed that the *A. falcata* form moderately dense to dense under growth in the ban oak and deodar forests especially where the tree canopy has a natural break. *T. spathiflorus* forms big patches in the fir, spruce and moru oak forests. In general, both these species have been found to occupy moist, cool niches along streams or on steep slopes forming origin of many hill streams. A total of

22 locations in three districts viz. Kullu, Shimla and Sirmour were identified for collection of superior genotypes for establishment of Clonal Banks. Two sites measuring 2 ha each, have been established as Clonal Banks with selected 22 genotypes of hill bamboos in the forest areas adjoining Bruhandhar Research Station falling in the Naggar Range of Kullu Forest Division and in Kumarsain Range of Kotgarh Forest Division.

### 2.3.3 Tree Improvement

#### *Jatropha curcas*

Performance of different accessions of *Jatropha curcas* in un-reclaimed sodic soils was found to be unsatisfactory. At four and half years of age, the plants exhibited poor survival and growth. None of the accessions could record growth comparable to plantation of this species on a good site. Seed yield has been negligible. In Dehradun valley, at three years of age and 2m x 2m spacing, higher seed yield was recorded.

#### *Populus deltoids*

A nursery of the selected clones was raised for the establishment of multilocation clonal trials and study wood anatomical properties and cellulose content clones of *Populus deltoids*. Germplasm bank was re-established and maintained. Wood anatomical properties of 10 clones have been studied.

#### Bamboo

Enriched field germplasm bank of *Dendrocalamus strictus* (Lathi Bans) and Hill Bambusetum (Field Germplasm Bank of Temperate and high altitude Bamboo species). The clonally multiplied ramets of *Dendrocalamus strictus* have been prepared at Shatabdi Van Vigyan Kendra, Forest Research Institute, City Centre, Dehradun for field planting.

#### *Dalbergia sissoo*

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, 36 and 24 clones respectively have been established at different locations following proper statistical design in the states of Punjab, Haryana, Uttarakhand and Uttar Pradesh. The earlier trials consisting of 36 clones planted at Hoshiarpur, Ludhiana and Bithmeda were evaluated as per schedule on various morphometric and wood traits.

One productive and resistant (against wilt disease) clones of *Dalbergia sissoo* developed by FRI Dehradun have been recommended by the Regional Variety Testing Committee for release.

Eleven populations from the states of Himachal Pradesh (HP) and Jammu & Kashmir (J&K) were analysed for genetic variation and it was observed that the populations of J&K and HP have more genetic diversity compared to the populations from Uttarakhand. The selected clones of Shisham in CSO were analysed for genetic variation and the stress resistance. The selected clones planted at Jawalajee (HP) and Basanterbela (J&K) were evaluated for growth and adaptability.

### ***Melia composita***

The natural forests and the plantations of the species were surveyed in different states to select plus trees. A total of 230 Candidate Plus Trees (CPTs) were selected from different geographical regions covering the northeast 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. Further, the field trials of different progenies were established at various locations to evaluate the performance of different economic traits over the locations.

Ten plus trees, each of *Melia composita* and *Melia azedarach*, were selected from Karnataka, Tamil Nadu and Andhra Pradesh. Progeny trials of both species were established at Hyderabad and Bangalore. Morphological attributes and oil content of seeds of *M. azedarach* and *M. composita* were studied and recorded. Growth performance evaluation of the progenies showed the superiority of CPT's collected from Karnataka (Kushalnagar, Kodagu).

### ***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.

### ***Eucalyptus***

Through systematic selection and multilocation testing, one clone of *Eucalyptus* hybrid (*Eucalyptus camaldulensis* Dehn. X *E. tereticornis* Sm.) developed by FRI, Dehradun has been recommended by the Regional Variety Testing Committee for release. Through controlled crossing work at FRI, F1 hybrids of *E. pellita* x *E. urophylla* were produced and are being evaluated in the field. A few promising F1 hybrids have been clonally multiplied and established in vegetative multiplication garden. IFGTB has also made a comparative study and released four clones of *Eucalyptus camaldulensis* for cultivation in farm lands. Demo clonal plantations of the released four clones have been



established in three different locations. About 30 clones are being planted and tested in different regions. Efforts are also being made to improve the quality of the seeds through progeny testing and establishment of Clonal Seed Orchards (CSO) and Seedling Seed Orchards (SSO).

Best performing 50 clones were identified based on three clonal trials in Karunya Nagar, Coimbatore and Sathyavedu and the poor clones present in the trials were culled. Seeds were collected from the best performing 50 clones and progeny trials were established in Puthukottai and ANGRAU, Hyderabad. At the same time, the same clones were mass multiplied and CSO using these clones were established in Salem and Nellore. SSOs at Coimbatore and Chennai were also established.

To facilitate registration of these new clones, guidelines for DUS testing in Eucalyptus have been developed under a project funded by the Protection of Plant Varieties and Farmers Rights Authority (PV & FRA), Government of India and the guidelines are being validated with all available clones.

In order to evolve methods for enhancing seed production in seed orchards, experimental results showed that three cycles of watering accompanied by soil working enhanced the seed production in CSO. Culling operation in SPA was also found to improve seed production in Eucalyptus.

For characterization of Eucalyptus clones for physiological and nutritional parameters, field trials were established at four locations viz., Pudukottai, Tirunelveli, Sivagangai and Coimbatore. The short-listed 30 Eucalyptus clones are being evaluated for physiological and nutritional parameters.

Suitability evaluation of various clones of *Eucalyptus tereticornis* and *E. camaldulensis* under multilocation tests for various agro-climatic zones of Southern India revealed that based on height and girth from the trials raised in

Andhra Pradesh at Hyderabad, Tirupathi, Warangal, Rajamundhry and (PAJANCOA) Karaikal, six clones viz., 9, 10, 66, 123, 124, 196, are maintaining superiority over other clones.

Development of micropropagation protocol for promising mature superior recombinants of F2 generations of *Eucalyptus* hybrid (*E. citrodora* Hook. x *E. torelliana* F.v. Muell.) is under the process of development for the multiplication of the genotype and its deployment in the field.

### Casuarinas

In Casuarinas, the major thrust is to move the ongoing breeding programme at IFGTB to the second generation and disseminating the output of one generation of breeding to all stakeholders especially to the farming community. The goal is being achieved through a NFRP project under which many second-generation breeding populations have been established with selections from the first generation orchards and infusions from unrelated populations. Genetic gain tests conducted with seeds of first generation orchards revealed that they are capable of yielding 13 to 28 % additional wood in plantations, depending upon the site conditions. The task of converting this genetic gain into economic gain for farmers was achieved through supply of seeds collected from orchards. A new concept of developing Community Seed Orchards has been developed to meet the enormous seed demand by involving farmers, traditional nursery workers and local forest department officials. Three such Casuarinas orchards have already been established in Tamil Nadu and Puducherry.

Through systematic selection and multilocation testing, four high yielding clones of Casuarina have been released for commercial cultivation during February 2010. Mass multiplication of these clones is being done for making them available to farmers with a buy back guarantee from the paper industries. To facilitate registration of these new clones, guidelines for



A Community Seed Orchard and Nursery of *Casuarina Equisetifolia* at Valluvamedu Village, U.T. of Puducherry  
Managed by Traditional Nursery Operators under  
AusAID-Funded Project

DUS testing in *Casuarina* have been developed and the guidelines are being validated with all available clones. Following validation, the DUS guidelines will be notified in the Gazette of India.

Intra and interspecific hybrids of *Casuarina* (*C. equisetifolia* and *C. junghuhniana*) have been successfully generated. The hybrid progeny have been subjected to field-testing in contrasting environments and outstanding individuals have been selected and vegetatively propagated. At present, the hybrid clones are under multilocation testing and the most outstanding of them will be released for commercial cultivation in the next few years.

The second-generation progeny tests established during the previous years at Nellore, Puducherry, Salem and TNPL were assessed for growth and stem straightness. Significant differences were observed among progeny of different first generation orchards for these traits. In general, progeny from Karunya orchard performed better than entries from other orchards. Data recording will continue in the third year based on which ranking and thinning of the progeny tests will be undertaken.

Short-listed 95 clones of *C. equisetifolia* for field testing and prepared the planting stock. Considerable variation was observed with reference to the rooting percentage. Assessed the survival in all the field trials established in the previous years. So far, the top 10 ranking

genotypes are Clones 01, 12, 11, 31, 83, 21, 49, 29 Seed lot 01 and Seed lot 02. At Sirugramam, the better performing clones were CE 2003/5, CE 9, S 88, CE 268, S 90, CE 224, TN 111, CE 219, S89, CH 3001, CE 332, CE 73, CE 2002/1, TNCS 1 and TCR 060101.

For developing cloning techniques for raising high yielding clonal plantations, nine years old trees of *C. equisetifolia* were coppiced at various heights (15, 30, 45 and 60 cm). Pollarding at 1 and 1.5 m height from the ground level was also carried out. Coppice shoot initiation was observed in 89% of the cases within 21 days. Stumps cut at 45 and 60 cm from the ground level were found to yield significantly higher number of coppice shoots when compared to those coppiced at 15 and 30 cm. The results could be successfully replicated in selected clones maintained by the Tamil Nadu Newsprint and Papers Limited at Kagithapuram, near Pugalur, Tamil Nadu. Experiments with pollarding did not yield promising results. Cladodes and needles collected from the coppice shoots were treated with IBA 2000 mg/l and placed in moistened vermiculite grade IV. Needles recorded a maximum rooting percentage of 65, whereas, for sprigs, it was above 90. Attempts were made to develop plantlets from individual needles for mass multiplication of limited rejuvenated plant materials and succeeded in developing plantlets from individual needles rooted through the cost effective hydroponic technique.

A total of 220 clones of *Casuarina* were screened for natural incidence of the targeted pests, *Icerya purchasi* and *Eumeta crameri*. Biology of the pests of *Casuarina* was studied and completed on live host. Clones free from attack of these pests short listed and controlled condition studies, in respect of determination of true and pseudo resistance, with *E. crameri* was completed for 70 of the 88 short listed clones. Analysis of biochemical parameters such as Phenol and Tannin was completed for 10 short listed clones,





showing different levels of tolerance for *Eumeta crameri* and for 3 clones showing high susceptibility for the scale insect *Icerya purchasi*.

### ***Madhuca longifolia***

For quantitative and qualitative improvement in flower seed and oil yield, field surveys were conducted in various parts of Uttar Pradesh viz. Allahabad, Pratapgarh, Jaunpur, Azamgarh, Mirzapur, Kaushambi, Kanpur, Unnao, Rai Bareilly, Varanasi, Sultanpur and Faizabad districts for selection of superior trees. A total of 52 candidate plus trees selected for screening. Corolla of flowers from the selected CPTs were collected for qualitative analysis. After drying and processing and samples have been prepared for chemical screening. Samples are being analysed at Chemistry of Forest products Division, FRI. Seeds from selected CPTs have also been collected for qualitative analysis.

### ***Acacia mangium***

For the development of advanced generation, seed orchard of *A. mangium*, based on biomass and wood density, seeds were collected from 125 selected superior trees in Nilambur, Karunya, Panampally and Palode. The half-sib progenies were evaluated in nursery for variation in growth and establishment of seed orchard.

### ***Acacia auriculiformis***

For the selection of clones of *Acacia auriculiformis* with desirable stem form and wood properties, wood samples of various families from a progeny trial were extracted and sent to IWS, Bangalore for evaluation. Superior trees based on stem form and growth selected in Wadkkancherry (15), Panampally (30) and Nilambur (17) Palode (25). Trees were initially coppiced in Panampally for young shoot production for further rooting. Initial rooting experiments have shown that good percentage of rooting can be obtained with 2-3 months old terminal cuttings obtained by coppicing.

### ***Pterocarpus santalinus***

To study the variation in *Pterocarpus santalinus* for growth and heartwood content according to edaphic and climatic factors in Tamil Nadu, a mixed plantation of *Albizia* and *Pterocarpus santalinus* near Mettupallyam was visited and growth data recorded. Soil samples were collected in two plantations in Amrethi and three plantations in Banavaram in Vellore Forest Division for analysis.

### **Teak**

Teak improvement programme through selection, quality seed production, hybridization and clonal evaluation is being executed in ICFRE Institutes. Teak fruits were collected from 230 selected trees in different SPAs at Parmbikulam, Topslip, Konni, Tholpatty and Nilambur. The morphological character of fruits and seed filling per fruit analysed. Germination studies were carried out for 82 trees. Flowering and fruiting was assessed in 475 trees in Walayar CSO and 170 trees in Panampalli CSO. Assessed all clones for flowering and fruiting behavior in three CSOs and one SSO (1500 trees). Pollinator visitation per unit time per tree was assessed in both the CSOs. The progeny trial established in Nilambur was evaluated. Trees were selected at Walayar for carrying out the control crosses. Following partial diallele crossing, crosses were made in teak at Panampally field trial (TNT19 x TNT10, TNT20 x TNT10 TNT19 x TNT20). Control Pollinated (CP) and Open Pollinated (OP) fruits were collected and morphological differences between CP and OP fruits analyzed. Seed setting of CP and OP were assessed using X-ray radiography technique. Significant differences were noted between CP and OP fruits in both morphological traits and seed setting.

Plus trees were selected and their coppices were collected. The selected clones were planted in the vegetative multiplication garden and being multiplied for establishing clonal trials. The rooting performance of different clones was



studied. A clonal trial of teak has been established at Salem (TN), which showed outstanding growth performance.

For realizing the genetic gain from teak seed orchards with regard to enhanced seed production and out crossing through cultural and ecological interventions, three teak orchards were visited and flower and fruit production assessed in 900 trees and seeds collected from 60 selected trees. Seeds were then subjected to X-radiography to determine seed filling. Based on flowering and seed filling status, 50 trees in each orchard were identified for imposing flower induction treatments. Flower-inducing plant growth regulator paclobutrazol was applied to non-flowering and low-flowering clones. Apiaries have been introduced in the orchard to increase the frequency of pollinator visits to teak flowers to promote cross-pollination and fruit and seed setting.

For standardization of flower induction schedule in *Tectona grandis* CSO and its impact on fruit set, out of 9 chemicals used in the previous year the best performing chemicals like, Paclobutrazol (3g/tree), ALAR-85 (250 ppm), Salicylic acid (200 ppm), Potassium nitrate (2%) and PEG (10%) were applied in different combinations to the low and non flowering trees in test orchards. The observations on flower induction and fruit set have to be taken at both the test orchards.

Genetic analysis of fruit and seed parameters in teak was investigated at AFRI Jodhpur. Open pollinated seeds from, 80 phenotypically superior trees from different locations were collected. Fruits from 41 trees of Dang region of Gujarat from this collection were used to carryout studies on seed morphological parameters. Investigation revealed highly significant variation for all the fruit and seed parameters in Gujarat teak except number of locules. Fruit and seed characters were found to be

least affected by environmental variations. The investigation also revealed that, treated stone length, stone weight and treated stone weight were found to be the most heritable, whereas, stone length, stone width, and number of unfilled chambers were moderately inherited.

Genetic analysis of progeny trial and investigation on inheritance of growth traits in Gujarat Teak was carried out on a progeny trial established in 2008 at Shivrajpur (Gujarat) with 16 half-sib families of Teak. Height and girth exhibited very high estimates of narrow sense heritability at individual as well as family level. Family heritability values were considerably higher for all the traits suggesting effectiveness for family selection. Genetic advance estimates for these traits also followed similar trend. Study indicated the presence of both additive and non additive gene action in Gujarat teak.

A progeny trial comprising of 28 families in RBD with four replications was established in 2010 at sujjangarh, Udaipur, Rajasthan. Data on survival and growth were collected. In addition to this, ten new CPTs of teak were selected in different location of Gujarat



Candidate Plus Trees of Teak

### ***Ailanthus excelsa* and *Ailanthus triphysa***

In order to study the reproductive biology and breeding systems in *Ailanthus excelsa* and *Ailanthus triphysa*, seeds were collected from Kerala Forest Research Institute and Palakkad



Division, KFD. *Ailanthus triphysa* germplasm bank has been established at Panampalli field research station. Karyotyping work of *Ailanthus triphysa* has been initiated. Pollen viability and male & female structural variation in *Ailanthus triphysa* have been studied. Key pollinator (*Apis cerana indic*) Indian Honey bee) and Dammar bee (*Trigona iridipennis*) have been identified. Long term Pollen storage is being standardized.

For developing clonal technology for raising clonal Plantation of *Ailanthus excelsa* and *Ailanthus triphysa* in Tamil Nadu and Kerala, survey and selection of trees (37 male and 20 female trees of *Ailanthus excelsa*) have been made. A total of 120 CPT's of *Ailanthus triphysa* have been identified in Kerala and Tamil Nadu. Standardization of the vegetative propagation of *Ailanthus excelsa* is in progress.

### Tamarind

Evaluated Tamarind orchards located at Neyveli, Thoppur, Theni & Mullangaddu for flowering and fruiting. Recorded very low percentage of fruit setting. Soil samples were collected from four different tamarind orchards and analyzed for micro and macro nutrients. Estimation of phenol, carbohydrates, protein and CN ratio is under progress. The data on flowering and fruiting behaviour have been recorded. The tamarind orchards were imposed to ploughing, mulching, light shoot pruning, heavy shoot pruning, root pruning, girdling and notching. The orchards are also subjected to organic, inorganic, micro nutrient and spraying of  $KNO_3$ ,  $K_2HPO_4$ , SADH, Thiourea and Cultar in different concentration for improving flowering and fruiting.

### Seed Improvement

For prolonging the seed viability of three recalcitrant species viz. *Dipterocarpus retusus* Bl. (Hollong), *Shorea assamica* Dyer (Makai) and *Aquilaria malaccensis* Lamk. (Agar), fruits were collected from 6 different localities in Assam. Evaluation was done on the basis of moisture

content on seed longevity and consequences of storage condition on it. The effect of grading *Dipterocarpus retusus* (Hollong) fruits into big, medium and small on germination, indicated that smaller fruits should be discarded for nursery practices, as germination was poor in smaller seeds as compared to that of medium and big fruits. The viability of *Aquilaria malaccensis* (Agar) seeds fall with the reduction in moisture content. The seeds with 25% moisture content can be stored for a longer period when stored in polythene bags at 7°C. Storage of *Dipterocarpus retusus* (Hollong) fruits in mud pots embedded in moist sand bed was found to be better in retaining the viability for a longer period as compared to in paper or cloth bags at room temperature, in polythene bags at 10°C and by treating with liquid paraffin wax.

### Validation of DUS Testing Guidelines for Casuarinas and Eucalyptus

DUS characters and guidelines developed for Casuarinas and Eucalyptus are being validated with other Paper industries, Universities, Forest Department and Research institutions. In *Casuarina*, methods were developed for recording the colour of the needles. Most of the clones are discriminated in two colours viz. dark green and yellowish green. Vegetative characters like deciduous branchlet, bark and lenticel characters are able to discriminate. In reproductive character, the anther of the male inflorescence discriminates three different colours viz Pink (CJ22, CJ30, CJ64, CJ41, CJ54CJ 56), reddish pink (CJ8, CJ10, CJ25, CJ68) and yellow (CJ23, CJ69). Similarly, the position of male inflorescence and cone and seeds characters were categorized for all IFGTB clone. In case of Eucalyptus, as per the recommendations of the technical workshop, the juvenile leaf characters were studied in 45 clones. All the clones available with IFGTB (75 clones) were characterized for the draft DUS characters. The commonly planted five ITC clones in Tamil Nadu were assessed for their uniformity with respect to all the DUS





characters. Studies in two regions of the Tamil Nadu Forest Plantation Corporation have completed.



Field Visit to Red Sanders Forest Areas in Andhra Pradesh for Making CITES Non Determent Study



Clonal Trial Raised at Nachiarpettai(Ariyalur) TN

Clonal Trial Raised at Kallakurichi

### *Neolamarkia cadamba*

In order to improve *Neolamarkia cadamba* through selection in the natural population and existing plantation in different parts of Tamil Nadu, Kerala, A & N Island and North-Eastern states (Assam), candidate plus trees were selected in two plantation of Tamil Nadu



Candidate Plus Tree Selection in the Plantation

(Narasipuram and Devarayapuram) and in natural forest at Konni forest division of Kerala. Till date, about 50 Candidate Plus Trees have been identified. The coppice shoots were collected and tried for rooting and it was found that treatment of cutting with 2000 ppm of IBA gave better results.

### *Hardwickia binata*

Survey has been carried out in different parts of Karnataka, Andhra Pradesh and Tamil Nadu to identify the populations of *Hardwickia binata*. Preliminary morphological observations have been recorded and core samples collected from different aged plantations/ natural populations. Using the cores collected, variability has been observed for girth, sapwood, heartwood content and specific gravity. Calorific value is being estimated. Variation for seed and seedling traits are being recorded.

### *Diospyros melanoxylon*

The germplasm of Tendu in the form of root suckers was collected from three agro-climatic zones of Chhattisgarh that produce good quality Tendu leaves. The root suckers were planted in field conditions for further growth.

### *Buchnanian lanzan*

Through extensive surveys, 33 phenotypically superior candidate plus trees were selected from Chhindwara, Gondia and Raigarh. Seeds were collected from 25 Candidate Plus Trees and progeny trial established at the Centre for Forestry Research and Human Development.

### *Aquilaria malaccensis*

The range of distribution of *Aquilaria malaccensis* (plantation/natural region) was surveyed in North-East and phenotypically superior trees (CPT) were selected from different provenances on the basis of height, DBH, crown area, wood density and degree of infection. Methodology for extraction of DNA from the leaves was also developed.



## Bamboo

Genomic DNA of 80 Bamboo accession has been extracted and checked by running a 1% agarose gel. PCR protocol was optimized for *B. balcooa* for fingerprinting of its accessions.

## *Azadirachta indica*

Progeny trial of Neem, established in the year 2002 at Govindpura, Jaipur with 17 CPTs of high Aza content revealed significant variation in flowering and fruiting at the age of 8 years. Overall fruiting and flowering was very poor. Moreover conversion rate of flowers into fruit was also very poor. Data analysis revealed that overall 16.5% trees of total progeny trial were affected by frost and progenies of CPT No. 3, 4, 5, 6 and 7 were tolerant to frost.



Neem Progeny Trial Established at Govindpura, Jaipur  
Exhibiting Poor Leaf Biomass.

## *Tecomella undulata*

Progeny trials of *Tecomella undulata* with 40 CPTs were established at Bikaner and Jodhpur during August 2008. The survival percentage was high at Jodhpur (90%) as compared to that at Bikaner (60 %) at the age of 30 months. The progeny of CPT-19 from Chohtan (Barmer) gave best growth at Jodhpur with the height of 102.5 cm and minimum is CPT-2 (Mohangarh) of 73.06 cm in height at Jodhpur. At family level, highest survival (97.2%) was found in CPT-15 (Daichu) and minimum (75%) in progeny of CPT-23 (Chohtan). In Bikaner, CPT-3 (Mohangarh) exhibited highest survival (75%) and minimum

(36%) in progenies of CPT-4 (Mohangarh). In general, growth performance of progeny trial was poor at Bikaner as compared to Jodhpur.



Progeny Trial of *T. undulata* at Bikaner after 30 Months



Progeny Trial of *T. undulata* at AFRI after 30 Months

## *Moringa oleifera*

Superior seed sources were identified at 25 different locations (8 in West Bengal, 6 in Orissa, 7 in Jharkhand & 4 in Bihar). Accessions from the identified sources were clonally multiplied and maintained in Lagutwa germplasm garden.

## *Gmelina arborea*

Potential seed source of *Gmelina arborea* were identified in Jharkhand and Orissa. Seeds have been collected from selected CPTs for progeny trial. Clonal trial of stem cuttings of 25 CPTs has been initiated at FRC, Mandar.





### Identification of Distinct Traits for DUS for Conifers

Three populations of *Pinus roxburghii* viz. Platu forest, Nihari and Malan forests and *Cedrus deodara* viz. Rohanda forest, Cheog and Madgraon forest in Lahul valley were surveyed for distinct traits. The observations with regard to needle length and colour have been found to vary considerably in Lahul forest of Deodar and Platu forest for Chir pine. More populations in Chopal, Karsog Chail and Kinnaur are also being surveyed for identification of individuals with distinct traits.



Bark Pattern of *Pinus roxburghii*

### Selection and Improvement of Natural Dye Yielding Plants

A total of 17 superior dye yielding trees of *Mallotus philippensis* and 6 of *Wrightia tinctoria* were selected. Fruits were collected and dyes were extracted from *Mallotus*. Laboratory protocols are being developed for extraction of dyes from *Mallotus philippensis* and *Wrightia tinctoria*.

#### 2.3.4 Vegetative Propagation

1. Vegetative propagation of *Dalbergia sissoo* and *Eucalyptus* clones carried out. In *D. 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 500 plantlets were produced for experimental purpose. Disease tolerant clones were assessed for morphological characters, biomass production, and nodulation behaviour, nitrogen fixation and nitrogen assimilation activity. In all the clones, profuse nodulation was observed.

2. Rooting has been achieved in some difficult to root commercially important tree species viz. *Diploknema butyracea* and *Adina cordifolia* through branch cuttings. However, *Lagerstroemia parviflora* and *Anogeissus latifolia* did not respond to air layering.
3. Vegetative propagation of *Arundinaria falcata*, *Thamnocalamus falconeri* and *Sinarundinaria jaunsarensis* revealed 65%, 65 % and 40% success respectively through culm cuttings with root promoting hormones.
4. Vegetative propagation of *Saraca asoca* performed just before monsoon through air layering/wiring, resulted in 70% success with IBA 500 ppm. The air layers/propagules obtained through wiring when transplanted in earthen pots revealed nearly 95% survivability.



FRI Wire Technique





Successful Air Layers

5. A total of 56 superior teak trees were selected in different parts of Kerala, multiplied through coppice shoots and established in a Vegetative Multiplication Garden (VMG). The clones were multiplied from VMG and a clonal trial has been established. The clones showed outstanding growth performance in the field.
6. Rooted cuttings from 25 clones of *Casuarina equisetifolia* produced to evaluate tolerance against salinity. The hardened cuttings were subjected to sodium chloride treatments of varying concentrations. Two experiments were conducted, one with fixed concentration of sodium chloride, and in the other, the concentration was gradually increased following which the plant samples were tested for morphological, physiological and biochemical responses. A demonstration trial to test the clonal performance on saline soil was also established.
7. A Vegetative Multiplication Garden (VMG) of *Ailanthus excelsa* was established in ½ ha area.
8. For the vegetative multiplication of *Calophyllum inophyllum* using stem cuttings, 1000 and 2000 ppm IBA have been found to be suitable for most of the populations.
9. Experiments conducted for testing rooting potential in selected trees of Jabalpur,

Chandrapur and Jagdalpur. Endogenous auxin (IAA) level was estimated. Significant seasonal/genotypic variation was recorded in endogenous auxin (IAA) level. Basal dip treatment of 5mM IAA for 4 hrs promoted adventitious rooting up to 11.33% compared to 1.33% in control. Study revealed a weak relationship between endogenous IAA and rooting potential of selected trees.

Experiment on Rooting Potential of Selected Trees of *Dalbergia latifolia*

Adventitious Rooting in Seedling Cuttings

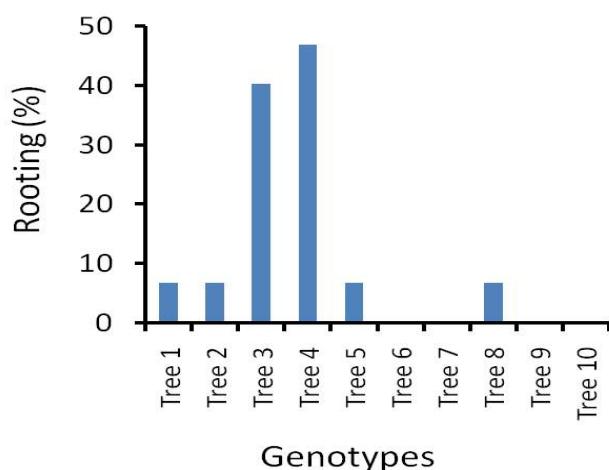
Hardening of Rooted Cuttings



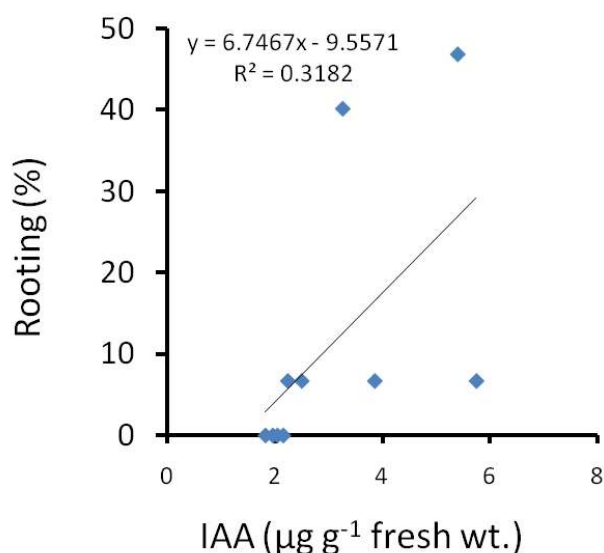
IAA: Variation in Progeny of Selected Trees



Experiment on Rooting of Seedling Cuttings



Genotypes



Rooting Response and Its Relationship with Endogenous IAA in Rosewood Genotypes

10. For development of vegetative propagation protocol of selected bamboo species viz. *Bambusa polymorpha*, *B. bamboos*, *Teinostachyum dullooa* and *Melocanna baccifera*, it has been observed that IBA at 200 and 300 ppm concentration was effective in root proliferation in cuttings.
11. Grafting technique for mature trees of *Ailanthus excelsa* developed (Grafting success 50%). At present, this grafting method is more efficient over any other clonal technique. Wedge grafting gives better success than patch grafting. This technique can easily be performed by farmers and field staff of SFDs.

Demonstration Trial of Male and Female *Ailanthus excelsa* (Grafted)

12. The micro-propagation method for *D. asper* has been refined and better rooting and shoot initiation has been achieved with Coumarin and Benzyl Amino Purine (BAP) respectively. Polypot size 16" x 20" favoured better propagation (culming intensity and culm growth) of out planted plants. For survival under agro-shade, weekly irrigation was essential for tissue culture raised *D. asper* and culm raised *B. nutans*. While *D. strictus* could withstand irrigation at 2-3 weeks interval.
13. Upto 30% success in rooting of mature stem cuttings of candidate plus tree of *P. pinnata* was observed in selected superior trees. Higher rooting was observed in cuttings taken





from seedlings. Variability in grafting response among plus trees was also observed.

14. Humic acid extraction method has been standardized using classic alkali/acid fractionation method. The preliminary results showed that humic acid had positive effects on rooting of cuttings collected from young trees/saplings of Shisham and Gamhar.
15. A hedge garden of selected clones of *Eucalyptus camaldulensis* was established. Successful studies on micro and mini cutting with a success of 80-100% rooting was recorded. Study on the rooting responses of 22 clones was carried out using mini cutting technique and resulted in significant improvement in rooting in 13 clones.



Juvenile Shoots of Eucalyptus



Rooted Cuttings of Eucalyptus

### 2.3.5 Biotechnology

#### DNA Profiling

- DNA fingerprinting using SSR markers is being 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. Molecular characterization of Himalayan pines, *Cedrus deodara*, Eucalyptus and Shisham germplasm has been completed.
- Sixty eight clones of *Casuarina equisetifolia* were profiled using ten RAPD primers. The Binary data was analysed using RAPD Distance and NTSYS softwares. Dendrogram was made for the profiled clones.
- Four SSR targeted primers were developed from the *Casuarina equisetifolia* through ISSR-PCR products. The new sequences were deposited in the NCBI library. Sixteen individuals in one population were screened with four SSR primers.
- For assessment of variability and genetic fingerprinting in *Pongamia pinnata* (L.), accessions of the species have been maintained in the Institute. DNA extraction protocol has been standardized using different plant material.

#### Use of Molecular Markers in Breeding Programmes

- Based on the association mapping, the SSR loci Embra40 was significantly associated ( $P=0.0012$ ) with low rooting percent in *E. tereticornis* accessions. Similarly, *Embra7* was correlated with shoot length. The loci *Embra67* and *Embra39* were correlated with root length and shoot length of the vegetative propagules in *E. camaldulensis* accessions.
- Efforts were made to develop Eucalyptus us hybrids between the species *E. camaldulensis* X *E. tereticornis*, particularly for salinity





tolerance traits. Considering the species differences and based on salinity stress studies, controlled hybridization was carried out and F1 hybrids were generated. For the development of genetic linkage map followed by localization of QTLs for the salinity tolerance traits, SSR markers were cross amplified and about 200 loci were tested for polymorphism between the parents. The mapping population, thus, generated will be used for phenotyping and genotyping for marker-trait associations.

- Efforts were also made for developing genetic linkage maps and identification of QTLs responsible for wood property and adventitious rooting, as they are the major industrial traits.

#### Gene Isolation and Functional Analysis

- The programme on gene discovery from woody perennials and medical plants is underway and isolation and characterization of genes, targeting the cellulose biosynthesis pathways and biotic defence-related pathways have been attempted.
- Two pathogen defence-related genes isolated, cloned and characterized from *Casuarina equisetifolia*, including the class I chitinase (CeChi1) and glucanase (Ceglu). They are the first report on full-length gene isolation from this species. Further, the expression pattern of several transcripts identified during the *Casuarina*–*Trichosporium* interaction was analyzed using qRT-PCR.
- A partial transcript of lectin was isolated and sequenced from the leaves of *Withania somnifera*. Research is in progress to isolate the full-length gene and characterize it for antifungal and antipest properties.
- Primer pairs for the gene responsible for vascular system development were synthesized and amplified in genomic DNA of *E. tereticornis*. The Amplicon size ranged from 1.5 kb-2.5 kb and the sequences showed

similarity to HD-Zip class III TF from *Malus domestica*, *Populus trichocarpa*, *Arabidopsis thaliana*, *Picea glauca* and *Medicago truncatula*. A putative conserved domain MEKHLA was identified in the sequence, which is characteristic Class III HD-ZIPTFs.

- In order to study the salt tolerance through gene expression pattern analysis, a list of genes based on clustal W2 multiple nucleic acid sequence alignment was prepared. These genes are; Salt Overly Sensitive (sos1), High Affinity Potassium Transporter (hkt1); Sodium Protein Exchanger (nhx1) and Chloride Channel –c (clc-c). Survey of Sambhar salt lake (saline wet land) region for selection of halophyte was conducted. *Lepidium sativum* has been finalized as the halophytic species for the studies in this project. An ultra low cost hydroponic system has been developed in-house and tested.
- An *in-vitro* process for development of transgenic composite plants in Eucalyptus for rapid functional analysis of genes and promoters was developed. Gene homologues for sodium transporters from salt tolerant tree species were also sequenced.
- Unravelling partial gene sequence of *LiCHS1* and *Liefl alpha* that represent the first genome sequence information for *L. invasa*.

#### Development of Micro-propagation Technique

- Rhizogenesis in five clones of *Dalbergia sissoo* viz. GBW 4, JB 1, FZB 1, FZK 1, RSK 1 was evaluated by conducting experiments. The explants inoculated on WP medium produced a maximum of 33% rooting followed by 22% rooting in MS medium. Similarly, 44% rooting was obtained on WP medium supplemented with 1.5 mg/l IBA. For studies on organogenesis from callus culture, 0.5 mM putrescine resulted with 58% organogenic callus formation. One field trial was established comprising 5 clones and 81% survival recorded with good growth of the plants.



- Endogenous hormones (IAA, GA3) were estimated for the samples of ten male and ten female *Casuarina* clones in flowering and non flowering stages through HPLC. Higher levels of IAA correlated with female sex expressions, whereas, a greater amount of, gibberellins favours the differentiation of male sex organs.
- Techniques for macro and micro-propagation were conducted in *Melia dubia*. Propagation ability of the material is found to vary with the girth size of coppice shoots. Best results, however, were obtained on MS + BAP (1 ppm) where 4-5 shoots regenerated from a single shoot apex.
- In Sandalwood, *in-vitro* rooting percentage and quality of plants improved by changing carbohydrate source from glucose to sucrose, by addition of polyamines and pulse treatment of shoots by IAA, IBA along with auxin conjugates. In *ex-vitro* rooting shoots pulse treated with 2500ppm IBA favoured 62% rooting in medium with 75% sand + 25% Soilrite. Use of mannitol (1%) and sucrose (1%) in liquid medium during maturation and mannitol 2% during germination, resulted in synchronized and higher conversion rate of embryos and better quality of plantlets.
- For development of micropropagation protocols for production of superior germplasm of *Dalbergia latifolia* Roxb. and *Pterocarpus santalinus*, performance of basal media like MS, B5 & WPM along with different plant growth regulators were compared. Two different explants types were compared for multiple shoot induction. 80% success in shoot initiation with 4.20 shoots per explant and maximum shoot length (1.50 cm) was obtained on MS medium supplemented with NAA (0.1 mg/l) + BAP (1.0 mg/l) within 4 weeks period.
- Three ramets each of 97 plus trees of *Tectona grandis* maintained at National Teak Germplasm Bank, Chandrapur representing 12 Teak growing states and 15-31 progenies (half sib families), were used for molecular characterization and identification of molecular marker associated with wood quality traits in *Tectona grandis*. Genomic DNA extracted from the selected genotypes and STMS analysis carried out in collaboration of TERI. STMS analysis validated ramets and progenies commensurate mostly with their plus trees.
- For *in-vitro* induction of essential oil components of Agar plant, a very efficient callus induction protocol along with cell suspension culture technique has been standardized. Cell suspensions and callus, thus produced, were treated with various elicitor molecules for induction of essential oil and/or oil components. Initial screening of the extracts from treated and untreated cell extracts with Thin Layer Chromatography (TLC) showed some difference which may be due to induction of essential oil components.
- For clonal multiplication of *Aquilaria malaccensis* Roxb. through *in-vitro* culture, methodology for production of aseptic culture from node, shoot tip and leaf from matured plant was standardized. Optimum shoot regenerating media with high frequency multiplication of healthy shoots were achieved from node explants. These multiplied shoots were rooted *in vitro* condition and also established outside the laboratory condition.
- Macro and micropropagation of selected clones of *Dipterocarpus retusus* Bl. Syn *D. macrocarpus* was carried out. The best suitable season for collection of shoot cuttings, optimum root initiating hormone, optimum root initiating media was standardized for 18 selected genotypes and propagated. Rooted shoot cuttings were established in field condition from mist



chamber. For micropropagation, establishment of aseptic culture has been standardized from leaf and petiole explants. Regeneration of somatic embryonic callus, distinct globular, torpedo and dicotyledonous embryos were observed from embryonic callus. Histology of callus showed the embryos.

- *In-vitro* shoot multiplication protocol has been standardized for *B. tulda* and *in-vitro* regenerated plantlets have been established in the green house.



*In-vitro* Grown *Bambusa tulda* Plantlet

- *In-vitro* shoot multiplication protocol has also been established for *Dendrocalamus hamiltonii*. Presently experiments to induce roots in the multiplied shoots are under progress.
- Studies on development of *in-vitro* regeneration system for *Albizia procera* revealed that MS medium supplemented with BA and Kinetin proved to be the best sources for shoot multiplication. However, the administration of kinetin prevented callus formation at the base of the *in vitro* shoots giving its preference over BA.
- Protocols of *in-vitro* plant propagation through somatic embryogenesis and axillary shoot proliferation were developed for *Jatropha curcas*. The protocols were based on somatic embryogenesis and using axillary bud proliferation pathway.

- In order to develop refined protocol for rapid and mass clonal production of plus trees/superior genotypes of *Salvadora persica*, experiments were conducted. MS medium supplemented with BAP (7.5 mg/l) proved the best and favoured multiple shoot induction (2-3 shoots/explants) in 4 weeks at 25<sup>o</sup> C temperature for 12 h photoperiod and 2500 lux intensity of light.



Shoot Initiation in *Salvadora persica*



Shoot Multiplication in *Salvadora persica*

### Bio-pulping of Bamboo Chips

On the basis of chemical analysis and SEM, pre-treatment of Bamboo chips and mechanically processed fibres with identified fungus *Trametes versicolor* kraft pulping was carried out. It was observed that there was 8-10 points gain in brightness over the control untreated chip's pulp, however, the pulp yield was slightly lower than the unbleached pulp. The physical strength properties were adequate in both the pulps.