

2.3 Genetic Improvement

2.3.1 Overview

Genetic improvement through traditional breeding methods is absolutely essential and important for improving productivity of plantations and quality of timber. However, because of long life cycles of many trees and low genetic gains per generation of breeding, it takes many years to achieve major genetic gains. Productivity of tree crops on agricultural lands in India is much lower than in many other countries (like Brazil and Indonesia). This seriously undermines the real potential of agro-forestry/tree farming in India. While productivity of a tree crop is determined by a variety of factors, the most basic determinant is quality of planting stock availability. Clonal plantations exploit existing natural variation for fast and immediate genetic gains, taking full advantage of superior genetic qualities of field tested clones. Field tested genetically superior clones of *Eucalyptus* and poplars have revolutionized the productivity and profitability of plantations in many countries and results from Indian endeavors are quite encouraging.

Indian Council of Forestry Research and Education is working on the genetic improvement of different forest tree species including economically important species required by the farmers, forest department and industries across the country. Basic and applied research in the field of genetics, clonal forestry and biotechnology is being carried out to as per the requirement of the stakeholders. Realizing the importance of strategic activities for Forest Genetic Resources (FGR) evaluation and improvement presently being undertaken by various Institutions, a long term research program and species specific tree improvement and tree breeding strategy to be adopted across the country has been taken up by ICFRE. Accordingly a National Coordinated Research Programme has been developed on 'Forest Genetic

Resource Management and Tree Improvement' aiming to improve the productivity of plantation forest through, use of quality seeds and seedlings obtained through tree improvement & breeding programme for meeting the demands of farmers, industries and forest department. This will also help in networking of activities by various forestry research institutes, state forest departments, universities, and putting their efforts in place for the benefit of stakeholders.

Eucalyptus

Artificial hybridization between *E. pellita* and *E. urophylla* was carried out and inter-specific F1 hybrids were also developed and planted in the field for their evaluation.

Dalbergia sissoo

Eleven populations of shisham were analysed for genetic variation and it was observed that the populations of Jammu & Kashmir (J&K) and Himachal Pradesh (HP) have more genetic diversity compared to the populations from Uttarakhand. A highly productive and resistant (against wilt disease) clone of *Dalbergia sissoo* (FRI-14) has been released for commercial cultivation by the Variety Releasing Committee of Ministry of Environment and Forest, Government of India.

Melia composita

Selection and characterization of 230 candidate plus trees was carried out using index method and plus trees were identified. Genetic evaluation of 21 families is being carried out in six geographical locations to analyze stability and adaptability in different eco-climatic conditions.

Bamboo

An efficient protocol for micro propagation was developed for *Thamnocalamus falconeri* (Dev Ringal).



In-vitro Rooting



Rooted Culm Cutting

The propagules for Dev Ringal developed through various path ways were planted in the field at Magra (1834 amsi) Mussoorie and evaluated for growth and adaptability for one year.

Tecomella undulata

The progeny trial of *Tecomella undulata* established in 2008 with 40 Candidate Plus Trees (CPTs) of Bikaner and Jodhpur, revealed better growth performance of progenies in Jodhpur.

Prosopis cineraria

The genetic improvement programme of *Prosopis cineraria* was executed and 21 new Candidate Plus Trees were selected from different locations in Rajasthan. The variation in pod parameters from various plus trees was also studied.

Tectona grandis

- Two orchards, one each in Karnataka (Janganamatti, Dharwad) and Andhra Pradesh (Achuthapuram, Rajamundry) were selected to standardize flower induction schedule in clonal seed orchards of *Tectona grandis*.
- 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

- 15 phenotypically superior trees of Teak selected and genetic variation among half sib families was studied, genetic gains were computed and inheritance of growth traits investigated.

Pterocarpus santalinus

Progeny trials of Red sanders have been established at Hyderabad and Bangalore. Early growth performance evaluation of the progenies shows the superiority of CPT's collected from Karnataka esp. Kushalnagar, Kodagu.

Azadirachta indica

- Evaluation of 8 years old Neem progeny trial established targeting high azadirachtin at Govindpura, Jaipur revealed superior growth performance of Progenies of CPT 7, CPT 4, CPT 11 & CPT12. Progenies of CPT 3, 4, 5, 6 and 7 were frost tolerant. Whereas, progenies of CPT 4 and 7 were superior in growth parameters as well as frost tolerant.
- *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 μ M BA and 125 mg l⁻¹ casein hydrolysate. Shoot regeneration with leaf pieces resulted in maximum number of shoots (3.56) on 0.44 μ M BA and 162.86 μ M Ads. HPLC method was standardized for quantification of azadirachtin from seeds.

Pinus gerardiana

Survey of Chilgoza pine populations in Kinnaur and Bharmour areas of Himachal Pradesh revealed that due to the biotic pressure, rights of the local people for seed collection, the regeneration has been negligible. The isoenzyme protocols were developed for three populations for genetic variation studies in *P. gerardiana*.



Forest Genetic Resource Evaluation & Conservation

Calophyllum inophyllum is an important tree borne oilseed used for biofuels and medicinal purpose. Selection of high fruit yielding trees of *Calophyllum* carried out. Germplasm bank has been established at Panampally research station.

- *Sapindus emarginatus* is another potential NTFP species of Tamil Nadu which is in high demand. Established germplasm bank with high fruit yielding trees and saponin content.
- Field germplasm banks of the important indigenous fodder tree species viz. *Grewia optiva* (2.0 ha at Dudhli Lachiwala Range, Dehradun Forest Division) and *Quercus leucotrichophora* (1.50 ha at Magra, Jhonpur Range, Mussoorie Forest Division) were established.
- In order to develop germplasm bank of a fodder tree species *Bauhinia variegata*, selection of the promising genotypes was made in the states of Uttarakhand & Himachal Pradesh.
- The germplasm of *Diospyros melanoxylon* was collected from four locations in three agroclimatic zones of Chhattisgarh.
- A germplasm bank consisting of 50 progenies of *Aquilaria malaccensis* was established in RFRI Jorhat campus.
- 29 districts were surveyed covering 10294 ha for the mapping distribution and density of *Commiphora wightii* (Guggal) and collected germplasm from 105 superior plants for *ex situ* conservation.
- For domestication, mass multiplication and popularization of *Moringa oleifera*, having higher leaf nutritive and cytokinin content', CPTs/ superior seed sources were identified in Jharkhand, West Bengal, Bihar and Orissa. Superior genotypes were clonally multiplied and maintained in germplasm bank.
- Laboratory protocols are being developed for extraction of dyes from *Mallotus philippensis* and *Wrightia tinctoria*.

- For conducting studies on pollarding and propagation in Kusum (*S. oleosa*) for lac cultivation., collection of stem cuttings and scion of plus trees of Kusum from Basia, Simdega, Bano etc was carried out and air layering procedure was successfully standardized

Molecular Characterization for Breeding Programme

- A programme initiated on timber forensics for applications of molecular markers in tracing origin of the stolen timber or log back succeeded in isolating DNA from the wood of *Cedrus deodara*.
- Germplasm of *Eucalyptus citriodora*, *E. pallida*, *E. hybrid* and three commercially used clones of eucalyptus was collected and analyzed for determination of marker constituents conferring CLSB resistance to the foliage.
- Molecular taxonomy facilities for the molecular characterization of selected bamboo species, established at FRI Dehradun. Initially the work has been started in five bamboo species.

Micro and Macropropagation

- Success has been achieved in the development of clonal propagation technique for *Saraca asoca*, a commercially important medicinal plant species using different rooting media, growth hormones and season of rooting.
- Shoot multiplication medium for *Dalbergia latifolia* and *Pterocarpus santalinus* has been standardized.
- Endogenous levels of auxin on Indole Acetic Acid (IAA) in selected trees of *Dalbergia latifolia* from Jabalpur, Chandrapur and Jagdalpur showed significant seasonal and genotypic variation. Juvenile and mature shoot cuttings of *Dalbergia latifolia* had 36% and 11% rooting response, respectively.
- Vegetative propagation protocol of selected bamboo species was developed. Methods have been developed for shoot multiplication and root induction in *Dendrocalamus hamiltonii*.



- Under *in vitro* conditions, the best shoot multiplication in *Salvadora persica* was 2.5 fold on MS medium supplemented with BAP (5.0 mg/l) and IAA (0.1 mg/l).
- In *Capparis decidua*, the best shoot multiplication was on MS media supplemented with BAP (2.0 mg/l) and IAA (0.5 mg/l).
- Somatic embryo derived plants were produced from 3 years old (long term maintained) cultures of *Commiphora wightii* and were found genetically uniform based on six RAPD markers found genetic stability.
- For the standardization of propagation method and germplasm conservation of *Machilus villosa* (Roxb.) and *Quercus lineata*, 10 plus trees of *M. villosa* and 9 of *Q. lineata* has been selected and marked.
- For conducting studies on variability in rooting proficiency in selected genotypes of *Pongamia pinnata*, scions were collected from 23 trees and variability in grafting success among plus trees was observed.
- For optimization of protocol for *in vitro* propagation and conservation of *Embelia ribes*, germplasm was collected from Nagpur, Maharashtra, Lower Subansiri, Arunachal Pradesh. Explants were collected from this source and cultures were established at tissue culture laboratory.

Distinct Uniform and Stability (DUS) Testing

Five populations of *Pinus roxburghii* viz. Chabbal forest, Banethi, Nurpur forest, Nerva and Dibkan forests and five populations of *Cedrus deodara* viz. Chopal, Cheog, Chail and Kinnaur were surveyed for distinct traits. The observations with regard to needle length and colour were found to vary considerably. The distinctness in traits for cone size and crown shape in both the species are also being looked into to identify distinct genotypes. The genotype of deodar in Chopal Forest division has been identified with distinct drooping branches.

Seed Science and Technology

Studies on seed viability in three recalcitrant species *Dipterocarpus retusus* Bl. (Hollong), *Shorea assamica* Dyer (Makai) and *Aquilaria malaccensis* Lamk. (Agar) has been carried out. Seed biology of *Abroma augusta* L.f. has also been undertaken.

Project under the Theme			
Projects	Completed Projects	Ongoing Projects	New Projects Initiated During the Year
Plan	21	62	19
Externally Aided	06	16	04
Total	27	78	23

2.3.2 Conservation of Forest Genetic Resources

NTFP species

At FRIs field research station, Khirsu (Pauri Garhwal) some medicinal plants namely Atees (*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



Methodology and parameters for selection of superior genotypes of five NTFP species viz. *Pongamia pinnata*, *Aegle marmelos*, *Pterocarpus marsupium*, *Strychnos Nux-vomica* and *Sloanea suaveolens* have been developed.



TFRI Staff along with Maharashtra Forest Official During Field Visit



Superior Plant of *S. Suaveolens* (Padal) in Chandrapur

Quercus leucotrichophora

Banj oak (*Quercus leucotrichophora*) is the most common broadleaf tree in the mid-elevational central Himalaya in India. It is the most preferred tree species in the temperate region, mainly used for fodder, fuel, and small timber. *Q. leucotrichophora* forms an extensive belt along the middle elevation (1200–2200 m) facing excessive pressure for existence. In order to study the genetic diversity and population structure of Himalyan Ban Oak forests, ten populations each with 30 individual trees covering Himachal Pradesh and Uttarakhand have been sampled for DNA marker based study. DNA extraction techniques from oak leaves have been standardized. Polymorphic ISSR/RAPD markers screened for molecular characterization of populations.



Trees Selected for Sample Collection in Ban Oak Forests of Gadach (Jhungi)



Dalbergia sissoo

Genetic diversity analysis of eighty six clones of *Dalbergia sissoo* was carried out using randomly amplified polymorphic DNA (RAPD) markers by selecting 10 decamer primers. The maximum similarity was found between clone 218, 1003, 10 and 12 and the most dissimilar clones were 7006, 59, 9032 and 5030 with minimum similarity coefficient. The average genetic diversity was recorded 0.21. Clone 5022 was found to be the most divergent clones which could be used for number of combinations to be established in the seed orchards and as a parent in hybridization programme.

Mangroves

Mangroves are fast disappearing assets of our country. The species *Bruguiera sexangula* grows landward and is very rare in India. It occurs in only three restricted patches of India namely South Andamans, Ernakulam region of West Coast and in Bhitrakanika, Odisha. In the Ernakulam region of West Coast, it was presumed to be extinct and was rediscovered only during 2004. The species is obligatorily ornithophilous and becoming globally rare. Considering the urgent need for conservation of this species the project is undertaken. Current achievement of the project is



Bruguiera sexangula Fruits



Raising of Seedlings for Re-introduction



Planting of *Bruguiera sexangula* in Degraded Areas



Established *Bruguiera sexangula*

reconnaissance survey of *Bruguiera sexangula* Populations, molecular characterization and control pollination. Reconnaissance survey was carried out in Panangad and Kumbalangi at Ernakulam region. 30 individuals were GP documented. Control pollination was attempted in Panangad and Kumbalangi. About 400 individuals were transplanted in the month of June, 2011. Two populations are being quantified for diversity estimation using DNA markers. Reproductive biology of the species is studied.

The CPT selections of *Calophyllum* have been conserved as field germplasm bank at Panampally Research Station as clone bank and half-sib progeny trial. Seedlings raised from *Sapindus* CPTs are maintained in the nursery for assembling in germplasm bank.

Pterocarpus santalinus

Population genetics and phylogeography of *Pterocarpus santalinus* and its *ex situ* conservation is being investigated through biotechnological interventions. DNA was extracted and purified from the collected population samples for the population genetic analysis work. For the development of the germplasm bank, land preparation has been taken up.

Tectona grandis

Molecular assessment of breeding patterns in clonal seed orchards of Teak in Andhra Pradesh is being investigated. Seeds were collected from the short listed clones and were put for germination and nursery being raised.

Boswellia serrata

In order to study the genetic diversity and population structure of *Boswellia serrata* through RAPD and ISSR markers, genomic DNA extraction procedure from the leaf samples has been standardized. Polymorphic ISSR primers were screened for genetic analysis. Phenological data revealed 100% leaf fall in this species. Similarly immature fruit fall is 11.3% and fruit fall is 23.8%.



Rauvolfia serpentina

Variation in reserpine content in some high yielding genotypes of *in vitro* and seedling raised *Rauvolfia serpentina* was studied for the effect of genotypes on culture establishment, effect of basal media and growth hormones on shoot multiplication. The results revealed the genotypes sensitivity to hormones. A significant effect of different levels of BA and NAA on the rate of shoot multiplication was recorded. Best and rapid multiple shoot potentiality was observed on MS medium, in which highest number of shoot (6.10) was observed within six weeks.

Azadirachta indica

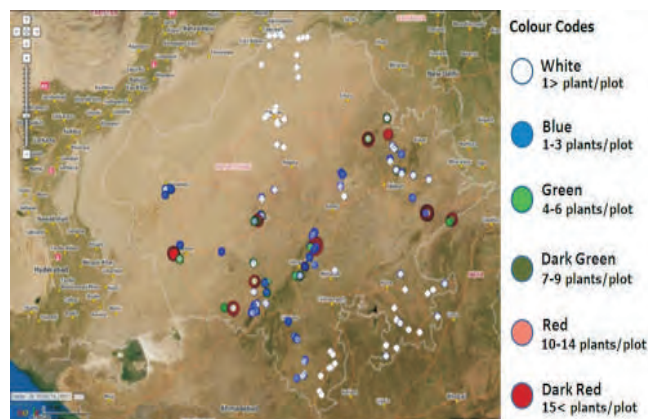
Variation with respect to *in vitro* azadirachtin production in selected high yielding populations of *Azadirachta indica* was studied. Isolation of azadirachtin from seeds and shoot cultures of different populations was carried out and quantified. Azadirachtin content was found to vary within and between populations of the species.

Bamboos

For inventorization and *ex-situ* conservation of Bamboo and Rattan resources of Mizoram, Tripura and Barak Valley of Assam, germplasm of *Calamus flagellum*, *Dendrocalamus hamiltonii*, *Dendrocalamus asper*, *Phyllostachys aurea* was raised. Besides, the germplasm of *Dendrocalamus hamiltonii*, *Phyllostachys manii*, *Dendrocalamus asper*, *Teinostachyum dullooa*, *Bambusa balcooa*, *Bambusa nutans* and *Bambusa wamin* was maintained in the nursery. Fifteen species of different bamboo species were collected from RFRI and multiplied through macro-proliferation technique in the Advanced Research Centre for Bamboo and Rattan (ARCBR) nursery for further plantation and establishment of Germplasm bank. Survey carried out and recorded the distribution of bamboo and rattan species in Lakhimpur.

Commiphora wightii (Guggul)

For investigating the population density and diversity for *in situ* and *ex situ* conservation of *Commiphora wightii* (Guggul) in Rajasthan, surveys were conducted in 29 districts of Rajasthan namely; Ajmer, Banswara, Baran, Barmer, Bharatpur, Bhilwara, Bikaner, Bundi, Chittorgarh, Churu, Dungarpur, Hanumangarh, Jaipur, Jaisalmer, Jalor, Jhalawar, Jhunjhunu, Jodhpur, Karauli, Kota, Nagaur, Pali, Pratapgarh, Rajsamand, Sawai Madhopur, Sikar, Sirohi, Sri Ganganagar and Udaipur. A total of 2660 sample plots in 141 sites in 29 districts were surveyed covering 10294 hectare where Guggul occurrence has been recorded. GPS data were loaded on Rajasthan map using software with colour codes for population densities.



Population Density of Guggul Plants Shown by Colour Code on Rajasthan map

Madhuca longifolia

For quantitative and qualitative improvement in flower seed and oil yield of *Madhuca longifolia*, field surveys were conducted for selection of CPTs at Allahabad, Pratapgarh, Jaunpur, Azamgarh, Mirzapur, Kaushambi, Kanpur, Unnao, Rai Bareilly, Varanasi, Sultanpur and Faizabad districts and 52 CPTs selected for screening. Collected corolla from selected CPTs, for qualitative analysis. Chemical screening of flower for total soluble sugars and protein has been analyzed and oil content from seed estimated.



Seed Storage

Seed water relation was studied and optimum storage conditions were standardized for seeds of *Bambusa bambos* and *Jatropha curcas*. Sorption isotherms were analysed by applying Brunauer Emmett and Teller (BET) theory, and status of water in the form of monolayer, primary and secondary water molecule was determined. The monolayer moisture content decreased with increase in temperature indicating that hygroscopic characteristics fall at high temperatures. In *J. curcas* seeds, the monolayer value was less than *B. bambos* at all the temperature. A strong agreement between experimentally obtained EMC and that obtained using BET theory validated the use of BET theory in elucidating the adsorption mechanism in seeds. The ideal RH for storing seeds of *J. curcas* is between 43 to 33.5% and for *B. bambos* it is between 33.5 to 23.5%. Best storage condition for *J. curcas* is 4.6% moisture content at 15°C temperature and for *B. bambos* is 6.5 % at 5°C temperature. The loss in viability was correlated with biochemical changes by using Fourier Transform Infrared (FTIR) Spectroscopy.

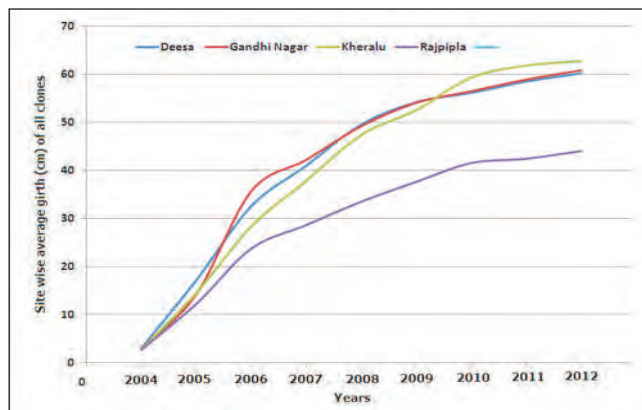
Tree Improvement

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 Uttar Pradesh, 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). The evaluation of earlier trials

consisting of 36 clones planted at Hoshiarpur, Ludhiana and Bithmeda were evaluated on various morphological and wood traits. A highly productive and resistant (against wilt disease) clone of *Dalbergia sissoo* (FRI-14) developed by FRI Dehradun was released for commercial cultivation by the Variety Releasing Committee of Ministry of Environment and Forests, Government of India.

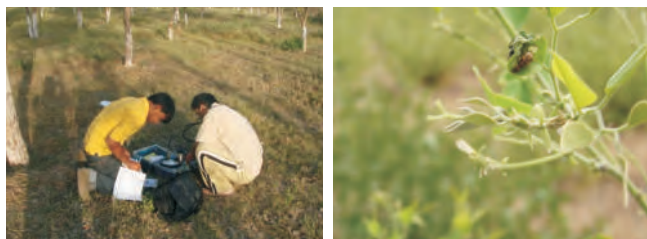
Multilocal trials of *D. sissoo* clones established in 2003 at four locations in Gujarat namely; Deesa, Gandhinagar, Kheralu and Rajpipala revealed a stable performance of the clones G2, 15 and 35 across the sites as demonstrated by their better growth (height and girth parameters).



Effect of Site on Girth Parameter of *D. sissoo* Clones

Natural populations and old plantations of the species have been surveyed and screened in Jharkhand and Odisha for selection of candidate plus trees on the basis of morphological characteristics, physiology and health.

HFRI Shimla studied the selected clones of shisham for genetic variation through isozymes analysis and also for stress resistance and insect-pest tolerance. Isozyme analysis revealed considerable genetic variation in clones which was also confirmed by their growth data at two sites. The tolerance of clones against stress and insect-pests showed difference in the resistance pattern. The advance generation orchards of shisham are maintained and growth data being recorded periodically.



Studies on Stress and Insect Resistance in the Field

Melia composita

The natural forests and the plantations of *Melia* were surveyed in different states. A total of 230 candidate plus trees (CPTs) were selected by FRI from different geographical regions and analyzed for index value based on height, diameter at breast height, straightness, clear bole height, crown diameter and knots. Evaluation trials of 21 most suitable families were established applying lattice design in six geographical locations of Haryana, Punjab, Uttar Pradesh and Uttarakhand. Genetic evaluation trials have also been established in different locations with 42 families to analyze stability and adaptability and screening of suitability of genotypes for arid and semi-arid regions. Early results have demonstrated absolutely remarkable progress for straightness, growth and diameter as well as disease and insect incidences. In fact, few of the progenies have touched a height of 9 m and circumference of 0.60 m in less than two years of age. Some of the progenies show high degree of tolerance to high temperature of around 49°C and have survived for three years under drought conditions.

Thirty plus trees, each of *Melia dubia* and *Melia azedarach* respectively were selected by IFGTB in the southern part of the country based on the morphometric traits to identify suitable seed sources with high oil content.

- The seed of *Melia azedarach* collected from plus tree number PAK-9 of Dharwad sources was estimated to have the higher oil content as compared to other sources.
- The seed of *Melia dubia* collected from plus tree number PDT-12 of Thalamalai sources was

estimated to have the higher oil content as compared to other sources.

- Seed germination study also revealed that de-pulping the fruits and soaked in normal water for 168 hr significantly enhanced seed germination percentage.
- Seed germination study also revealed that the seeds collected in the month of January to February gave the best results in *Melia dubia*.
- The progeny traits were established using seeds of plus trees from Karnataka, Tamil Nadu and Andhra Pradesh at Forest Research Centre, Hyderabad and Nallal Field Research Station, Bangalore. The survival percentage was not found to be 100% in both the species.
- Under field evaluation studies, growth parameters were recorded and many analyses of variance indicated significant difference among the seed sources for all the characters studied.
- RAPD studies for both the species provided the basic information that there is variation among the plus trees population. This will be used as a base to initiate the advanced studies in tree improvement programme.

Evaluation of growth performance of progeny trial has been carried out at FRC, Hyderabad and Nallal, Bangalore. Coppicing was done to carry out vegetative propagation in *Melia dubia* and it was found that it is a good coppicer and further experiment are being carried out for rooting in stem cuttings. Among various auxins tested for rooting in *M. dubia*, IBA at 3000ppm found best to induce rooting.

Pongamia pinnata

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



Candidate plus trees of *P. pinnata* screened for phenotypic characters in different states, and progeny trials conducted. Oil content ranged from 27.89 to 41.43 %, and 33 progenies profiled for fatty acids.



Selected Superior Tree of *Pongamia pinnata* at Bhavanisagar, Tamil Nadu



Rooted Cuttings of Selected Tree



Hardened Clones of *Pongamia*

A total of 74 candidate plus trees of *Pongamia pinnata* were selected from 23 districts in different agro-climatic zones of Tamil Nadu and being multiplied clonally. Flowering and fruit production in selected trees were recorded. Seeds were collected from 10 selected trees for analysis of oil content.

Eucalyptus

Controlled crossing was done in *E. pellita* and *E. urophylla* for production of F1 hybrids. Hybrid seeds were collected, raised and established in the field for their evaluation. *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. Few ramets of F1 hybrids of *E. pellita* x *E. urophylla* have been produced through rooting of branch cuttings. The ramets have been planted in vegetative multiplication garden for production of coppice shoots for mass propagation.

- The Potted Seed Orchards (PSO) were established using top 5 clones of *E. camaldulensis*. The early flowering in PSOs was induced through the treatment of Paclobutrazol. This will help in inter clonal hybridization and will save resources in seed production.



Mapping Population Trial at Sathyavedu

- IFGTB Coimbatore identified best performing 50 clones based on three clonal trials in Karunya Nagar, Coimbatore and Sathyavedu. The poor 25 clones present in the trials were culled and seeds were collected from the best performing 50 clones and progeny trials were established in Puthukottai (2 Ha) and ANGRAU, Hyderabad (2 Ha). At the same time, the same clones were mass multiplied



Progeny Trail of Eucalyptus



Application of Paclobutrazol to Enhance Flowering

and were established in CSOs at Salem (2 Ha) and Nellore (9 Ha). Seedling Seed Orchards at Coimbatore (2 Ha) and Chennai (3 Ha) are also established.

- A VMG of a hybrid of *E. tereticornis* x *E. grandis* has been established at Pugalur TNPL campus premises.

- For characterization of eucalyptus clones for physiological and nutritional parameters, field trials have been established in four locations viz., Pudukottai, Tirunelveli, Sivagangai and Coimbatore. For the short-listed 30 Eucalyptus clones, parameters like chlorophyll a, chlorophyll b, total chlorophyll and total leaf area were worked out. Observations on physiological parameters and growth parameters are being made at regular intervals in four locations.
- For assessing the suitability of *Eucalyptus tereticornis* and *E. camaldulensis* clones for various agroclimatic zones of Southern India, twelve clonal trials have been established at Karaikkal (Puducherry), four locations in Andhra Pradesh viz., Warangal (Jakaram), Rajmundhry, Hyderabad (Mulug) and Tirupathi (Sreevarimetta), three in Karnataka viz., Badami, Gangargatti (Dharwad), Halbhavi (Belgaum), four in Tamil Nadu viz., Nachiarpettai (Ariyalur), Amaravatipudur (Karaikudi), Thyagadurgam (Kallakurichi), Marakkanam. From the data 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. Maximum height is 4.61m and maximum girth is 11.72 cm.
- Multilocal trials of *E. camaldulensis* clones established in 2003 at four locations in Gujarat namely; Deesa, Gandhinagar, Kheralu and Rajpipala revealed a stable performance of the clones A3, 10 and 105 across the sites as demonstrated by their better growth (height and girth parameters).

Gmelina arborea

Candidate plus trees of *Gmelina arborea* identified from North East India, West Bengal and other areas of Southern India with less knots and straight boles. Evaluation of the CPTs will reveal their worth in further breeding programmes and productivity enhancement.



Selected CPT of *Gmelina arborea*

A progeny trial was established at Gudalur Research Station, Kancheepuram District, Tamilnadu.

Also intensive surveys were conducted in the natural forests of Siruvani, Anaikatti, Anthiyur, Sathiyamangalam, Dindugal, Kodaikanal, Sirumalai, Theni and farmers plantation in Pudhukottai. Selected 50 CPTs based on growth superiority, clear bole and freedom from pest and diseases. The reproductive traits like flowering phenology, pollen fertility, pollen germination on stigma and pollinator interaction of *Gmelina arborea* have been studied on the selected CPTs. Wood samples (core) were collected and analyzed for wood parameters

Calophyllum inophyllum

Identified populations of *Calophyllum inophyllum* in Tamil Nadu, Kerala, Puducherry and Andamans.

Trees with high fruit count per foot length branch was taken as selection criteria. Stem cuttings and raised clones of CPTs for establishing germplasm bank collected. Tree-wise oil content quantified to rank the clones.

Sapindus emarginatus

Populations of soapnut in Tamil Nadu were identified in Mettupalayam, Pillur, Palani, Dhimbham, Thengumarada, Thalavadi, Sarkarpathy, Hogenakkal, Aliyar, Srivilliputtur and Rajapalayam. Collected seeds from Pillur and Thengumarada, processed and conducted germination test. The number of fruits per meter length of branch was taken as selection criteria. Measured seedling parameters in the germinated seedlings. Raised stock in nursery and maintenance done for field establishment. Quantified tree-wise saponin content, to rank the CPTs.

Casuarina sp.

The major work done in *Casuarina* improvement is establishment of second generation seed orchards of *Casuarina equisetifolia* and *C. junghuhniana* to increase seed production and genetic gain. So far 16 ha of progeny tests have been established in various locations including 2 ha established near Chennai during the current year.

All the test plots are intensively managed and data on growth, tree form and flowering have been recorded



A Wind-hardy and Fast-growing *Casuarina* hybrid Clone under On-farm Testing in Cuddalore Dt, Tamil Nadu



periodically. Ranking of all trees through index selection method has been carried out for two test plots which have attained the age of 3 years (mid-point of 6-years' breeding cycle). Inferior families and individuals have been marked for culling for conversion into second generation seed orchards.

For evaluation and characterization of clones of *Casuarina* with reference to yield, tree form, biomass, pulping characteristics and key nursery pests; 95 clones were shortlisted for field testing and prepared their planting stock. Established field trials in Tamil Nadu at Mayiladumparai, Karur district, in a sodic site at Pugalur and in a casuarina belt at Sirugramam, Cuddalore district. Biometric / qualitative observations are being recorded from these trials at regular intervals. The top 10 ranking genotypes are Clone 01, Clone 12, Clone 11, Clone 31, Seed lot 01, Seed lot 02, Clone 83, Clone 21, Clone 49 and Clone 29. At Sirugramam, the better performers 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.

A total of 220 clones of *Casuarina* were maintained in a nursery trial. The trial was screened for natural incidence of the targeted pests, *Icerya purchasi* and *Eumeta crameri*. Observations on the incidence and intensity of attack of these pests on the clones, recorded at 15 days intervals revealed variations among the clones for tolerance. Clones free from attack were shortlisted. 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*.

Study was undertaken to select phenotypes of *Casuarina* which are suitable for developing a windbreak agroforestry system in western zone of Tamil Nadu. Twenty five superior trees have been selected by adopting 'Point Grading Method' of selection by giving greater scores for more branchiness. All the selected phenotypes have been assembled in germplasm bank at IFGTB. These selected phenotypes

were further multiplied and 4 replicated field trials have been established in farmers' field for evaluation of selected phenotypes for their efficacy in minimizing wind speed. Superior clones will be popularized for establishing windbreak agroforestry systems.

Acacia mangium

The advanced generation seed orchards of *Acacia mangium* were created based on biomass and wood density at Mundur (Palakkad). Maintenance works were carried out in the planted trials.



Six Month Old Progeny Trial of *A. mangium* at Palode

Acacia auriculiformis

In order to select promising clones of *Acacia auriculiformis* with desirable stem form and wood properties for short rotation timber production, superior trees of *A. auriculiformis* based on stem form and growth have been selected in Wadkkancherry (15), Panampally (30) and Nilambur (17) Palode (25). Trees have been initially coppiced in Panampally for young shoot production for further rooting. Superior trees (61) selected in Wadkkancherry, Panampally and Nilambur Palode, based on stem form and growth have been multiplied vegetatively and 7000 rooted cuttings made ready for planting in clonal trial and VMG.

Pterocarpus santalinus

A project was undertaken to study variation in *Pterocarpus santalinus* for growth and heartwood content according to edaphic and climatic factors in



A Selected CPT of *Pterocarpus santalinus* (Red Sanders)
in Vellore Division, Tamil Nadu

Tamil Nadu. Soil samples were collected and analyzed from five plantations in Ammoor in Vellore Forest Division. Growth data were also recorded from these plantations and core wood samples were collected. Heartwood presence was observed in all the trees sampled. Thirty two CPTs were selected based on heartwood presence and other growth characters.

Study was undertaken for collection of quantitative field data through rapid assessment of population, growing stock and natural regeneration status. Around 400 stratified random sample plots of 0.1 ha were laid out in natural red sander forest areas and data on population structure of red sanders, regeneration were collected, compiled and analyzed. Data on Removals and level of regeneration were also recorded. Threat factors to natural populations have been identified. An



Visit to Red Sanders Depot in Rajampet

NDF report based on CITES guidelines was prepared and submitted to MoEF during February 2012.

Ailanthus excelsa and *Ailanthus triphysa*

In order to study the reproductive biology and breeding systems in *Ailanthus excelsa* and *Ailanthus triphysa*, germplasm bank of *A. triphysa* has been established at Panampalli Field Research Station. Karyotyping work was initiated with root tips. Pollen viability and male and female structural variation in *Ailanthus triphysa* has been studied. Key pollinator have been identified as Indian Honey bee (*Apis cerena indica*) and Dammar bee (*Trigona iridipennis*). Long term pollen storage is being standardized.

For developing clonal technology for raising clonal plantation of indigenous species viz. *Ailanthus excelsa* and *Ailanthus triphysa* in Tamilnadu and Kerala, 170



A Plus Tree of *Ailanthus excelsa*



CPT's of *Ailanthus excelsa* and 120 CPT's of *Ailanthus triphyssa* have been identified. Standardization of the vegetative propagation of *Ailanthus excelsa* is in progress.

Tamarindus indica

For evaluation and identification of optimal parameters for flowering and fruit set in Tamarind (*Tamarindus indica* L.), orchards located at Neyveli, Thoppur, Theni and Mullangaddu were evaluated for flowering and fruiting. Soil samples were analyzed for micro and macro nutrients. Analyzed phenol, carbohydrates, protein and CN ratio from non-flowering and flowering trees. The tamarind orchards were imposed with 30 different treatments for improving flowering and fruiting. Among different treatment soil drenching of Cultar @3000 ppm and spraying of 2% KNO₃ found positive implication on enhancing fruit productivity.



Application of Flower Inducing Plant Growth Regulator in an Unproductive Tamarind Orchards

Thespesia populnea

A study was undertaken to select and screen germplasm of *Thespesia populnea* for improving productivity. Extensive field surveys were undertaken in the Western, North Western, Cauvery Delta and Southern Zones of Tamil Nadu and selected 80 CPTs of *Thespesia populnea* for further improvement. Clonal accessions of 80 CPTs selected mainly based on growth and tree form and assembled at IFGTB.

Community Seed Orchards

A new concept of establishing "Community Seed Orchards" was developed through which farmers and self-help group members were encouraged to establish their own seed orchard in government / village land with inputs from IFGTB in the form of planting material and technical support. The cost of maintaining the orchards for two years is taken care of by the Institute in addition to capacity building of farmers in orchard management. The seeds produced is used by the farmers themselves and any surplus will be sold to others like wood-based industries with quality assurance from IFGTB. Three model orchards have been established in Tamil Nadu and Puducherry which are at present producing seeds.

Tectona grandis

Study was undertaken for realizing genetic gain from teak seed orchards and enhancing seed production through cultural and ecological interventions. Two teak seed orchards (one SSO and a CSO) were evaluated for flowering, fruit and seed production. Organized seed collection with individual tree identity and studied for morphological, germination and seedling characteristics. Studied the impact of flower-inducing and seed-set promoting treatments like paclobutrazol application and introduction of apiaries on the orchard output. Studies initiated for estimation crossing rates in seed orchard in comparison to seed production area. These investigations are expected to yield precise information on the current status of teak seed orchards in terms of seed production and the extent of genetic gain possible through use of orchard seeds.

Identified more than 200 outstanding trees in different teak-growing areas of Kerala and Tamil Nadu and undertook open-pollinated seed collection with individual tree identity. These seedlots will be utilized to raise broad-based breeding populations. The Walayar Teak CSO has been systematically studied for annual flowering and seed production dynamics. Successfully produced fullsibs through control



pollination, involving clones with high general combining ability observed in a 20-year old progeny test. Plus trees were selected and 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.

Two orchards one each in Karnataka (Janganamatti, Dharwad) and Andhra Pradesh (Achuthapuram, Rajamundry) were selected to standardize flower induction schedule in clonal seed orchards. Six chemicals like Paclobutrazol, Salicylic Acid, Succinic acid 2, 2-Dimethyl Hydrazide (ALAR-85), Potassium Nitrate, Poly Ethylene Glycol 6000 (PEG) were used in 12 different treatments to study their effect on flower induction. New method for stem injection of chemicals was devised during the experiment wherein ALAR, stem injection along with combination of paclobutrazol and salicyclic acid showed encouraging results in both the CSOs.

Genetic variation and inheritance pattern of Western Indian Teak was investigated. The materials for this investigation came from three progeny trials established with 16, 28 and nine half-sib families of teak at Shivrajpur, Sajjangarh and Jodhpur, respectively. The Shivrajpur trial was established in 2008, under the jurisdiction of Silviculture Division, Rajpipla by the SFD, Gujrat, while the other two trials were established in 2010. Analysis of variance of these trials revealed that variation due to families was highly significant for height and collar girth indicating scope for family selection, except in case of Jodhpur trial, where these differences were non significant at one year, but was significant at nine month growth. Height and collar girth exhibited high to moderate estimates of narrow sense heritability, respectively at individual as well as family level.

Family heritability values were considerably higher for both the traits suggesting effectiveness for

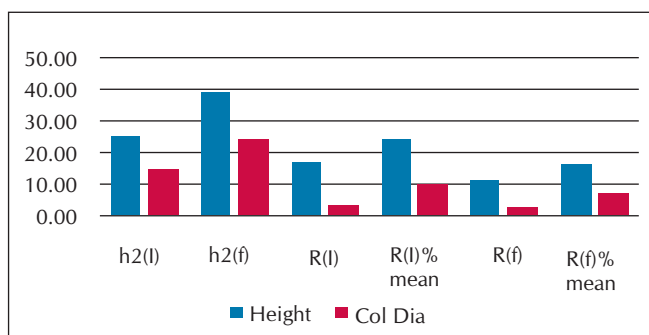
family selection. Genetic advance estimates for these traits also followed similar trend and ranged from 6.41 to 24.32 percent. In addition to this, 15 new phenotypically superior trees were selected from different locations of Rajasthan and Gujarat. Open pollinated seeds from 70 trees from Gujarat and other areas of the country were collected, weathered and seedlings were raised in the nursery.



General View and Close Up view of Sajjangarh Progeny Trial of Teak



CPT of Teak Selected in Gujarat

Heritability and Genetic gain estimate Sajjanganrh Trail (1st year)

Validation of DUS Testing Guidelines for Casuarinas and Eucalyptus

The Protection of Plant Varieties and Farmers' Rights Authority entrusted the Institute of Forest Genetics and Tree Breeding (IFGTB), Coimbatore with the responsibility of developing DUS test guidelines for two widely cultivated forestry crops viz., Casuarina and Eucalyptus. IFGTB has brought out a draft set of guidelines in 2009 followed by its validation with varieties of these two species available with different stakeholders like industries, forest departments, Universities and Research Institutions. A stakeholders meeting on Validation of Descriptors of Casuarinas and Eucalyptus was organized on 15.6.2012 with the funding support of the PPVFR Authority to complete the validation process of the draft DUS guidelines. Stake holders representing paper mills, forest departments, research organizations and universities attended the meeting. After the completion of the validation process, the draft guidelines will be submitted to PPVFR Authority for examination by a Task Force followed by notification in the Plant Variety Journal of India.

Neolamarkia cadamba

Neolamarkia cadamba is being viewed as an alternative species for Pencil, Match Wood and ply wood industries. A project was undertaken to improve *Neolamarkia cadamba* through selection and mass multiplication of superior genotypes. Selection of candidate plus trees has been done in two plantations of

Coppice Shoots of *Neolamarkia cadamba*
Get Rooted through IBA Treatment

Tamil Nadu (Narasipuram and Devarayapuram) and in natural forest at Konni, Parambikulam, and Nilambur forest divisions of Kerala and in South and Middle Andaman. Till date, 72 Candidate Plus Trees have been identified. Standardization of Seed handling technique is in progress.

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 have been collected from different aged plantations. Considerable variations have been recorded with reference to girth, height, bark, sapwood, heartwood and specific gravity.

Diospyros melanoxylon

For selection and cloning of superior germplasm of *Diospyros melanoxylon*, sites were selected and marked for collection of plant materials. These sites were Lohattar (Bhanupratappur), Litipara (Gariaband), Kotadol (Korea) and Madanpur (Katghora) in Chhatisgarh. The germplasm collected from the above sites were maintained. The leaf area and total dry weight of the leaves collected from these localities were measured in order to screen superior germplasm. Significant differences were also observed between localities and sites for dry weight (mg) of leaves.



Collection of Germplasm of *Diospyros melanoxylon* (a) Demarcation of Area for Germplasm Collection (b) collection of Leaf Samples from the Field (c) Collection of Root Suckers from the Field (d) Pretreatment of Root Suckers in the Nursery of TFR I (e) Planted Root Suckers in the Polythene Bags (f) Sprouted Root Suckers.

Seed Testing

Studies were undertaken on seed viability in three recalcitrant species viz. *Dipterocarpus retusus*, *Shorea assamica* and *Aquilaria malaccensis*. Results revealed that the seed size effects seed germination and moisture content in Dipterocarps, and temperature affects the storage of seeds for longer period. In Agar, the storage period of seeds can be increased by storing the seeds at 7°C with 25% moisture content. Study was also undertaken on the Seed biology of *Abroma augusta*.

Aquilaria malaccensis

An Extensive survey was carried out in the North Eastern states to select Agar (*Aquilaria malaccensis*) trees from its natural habitats and also in home

gardens/plantations. 91 trees were selected from Assam, Tripura, Arunachal Pradesh, Nagaland, Manipur and Mizoram. Fruits were collected from the selected trees and progeny of 55 trees was raised. A germplasm bank consisting of 50 progenies of *Aquilaria malaccensis* was established in RFRI campus and preparations are underway to establish another germplasm bank in Arunachal Pradesh. The genetic relationship between the selected trees was analysed by using RAPD technology.

Bamboo

Studies were undertaken for the development of DUS descriptors on *Bambusa balcooa* and *Dendrocalamus hamiltonii*. Extensive field surveys were done to ascertain the availability of *B. balcooa* and *D. hamiltonii* in different parts of North East. Thirty two accessions were collected and multiplied in the nursery. Different morphological characters were studied, such as, Clump Characters, Culm Characters, Branching Characters, Leaf Characters and Culm Sheath.

Tecomella undulata

Candidate plus trees (47 nos.) were selected from various district of Rajasthan based on the quantitative traits (height, girth, clear bole and crown diameter) and qualitative traits (straightness, roundness and health). Progeny trials were established using seedling of 40 CPT's at Bikaner and Jodhpur in August, 2008. The progenies of CPT-23 from Chohtan (Barmer) exhibited best growth at Jodhpur, attaining height of 143 cm and minimum in progenies of CPT-4 (Mohangarh) of 87 cm (at Jodhpur). At family level, highest survival (97.2%) was found in progenies of the CPT-15 (Daichu) and minimum (75%) in progenies of CPT-23 (Chohtan) at Jodhpur, whereas, at Bikaner progenies of CPT-3 (Mohangarh) exhibited highest survival rate of 73% and minimum (36%) in progenies of CPT-4 (Mohangarh). At Bikaner, highest collar diameter was 1.09 cm in progenies of CPT-24 (Chohtan) and least collar diameter (0.70 cm) was of progenies of CPT-2



Overview of Progeny Trial of *Tecomella undulata* at the age of 3½ years at Jodhpur



Close up View of Progeny Trial of *Tecomella undulata* at the age of 3½ years at Jodhpur

(Mohangarh). In general, growth performance was poor at Bikaner as compared to that at Jodhpur.

Azadirachta indica

Studies were undertaken to screen oil and Azadirachtin content in progeny trial established in the year 2002 at Govindpura, Jaipur with seedlings of selected 17 CPTs. This trial is almost, now, 9 year old and significant variation in flowering and fruiting has been recorded. Seed collected from 33 trees belonging to 10 families showed high oil content (above 40%).

Prosopis cineraria

A coordinated project on integrated management of Khejri mortality for socio-economic upliftment in Rajasthan was undertaken by AFRI Jodhpur. Surveys

were conducted in Nagaur, Sikar, Churu and Jhunjhunu districts of Rajasthan and 21 candidate plus trees (CPTs) were selected. Pods collected from selected CPTs and parameters from individual pods of these CPTs like; length, width and weight have been recorded. Cutting and layering experiments were conducted on mature trees of *Prosopis cineraria* for their clonal propagation.



Selected CPTs of Khejri in farmer's field

Pinus gerardiana

A project was undertaken on isozyme variation in natural populations of *Pinus gerardiana*. Survey of chilgoza populations in Kinnaur and Bharmour areas of Himachal Pradesh was carried out for regeneration studies and selection of Plus Trees. It was observed that owing to biotic pressure and local people enjoying the rights of seed collection, the regeneration in these areas has been very negligible. There was very poor cones/seed production during the year 2010-2011. For



conducting genetic diversity studies in the dwindling populations of *P. gerardiana*, isoenzyme, protocols have been standardized.



Isozyme Variation Studies and Plus Tree of *Pinus gerardiana*



Regeneration Status in *Pinus gerardiana*

Identification of Distinct Traits for DUS for Conifers

Five populations of *Pinus roxburghii* viz. Chabbal forest, Banethi, Nurpur forest, Nerva, Dibkan and four populations of *Cedrus deodara* viz. Chopal, Cheog, Chail and Kinnaur were surveyed for distinct traits. The observations with regard to needle length and colour were found to vary considerably. The distinctness in traits for cone size and crown shape in both the species are also being looked into to identify distinct genotypes. The genotype of deodar in Chopat Forest division has been identified with distinct drooping branches. More populations of both the species are being surveyed for identification of individuals with distinct traits

Moringa oleifera

Information on natural populations/growing areas/ plantations of *Moringa* in Jharkhand, West Bengal, Bihar and Odisha collected. Superior seed sources have



Bark Pattern of *Pinus roxburghii* and *Cedrus deodara*

been identified at different locations and 25 potential areas (8 in West Bengal, 6 in Orissa, 7 in Jharkhand and 4 in Bihar) have been demarcated. Cuttings collected from identified sources and clonally multiplied and maintained in Lagutwa germplasm garden. The rooting season has more pronounced effect than growth regulators with planting of big size woody cuttings (30-50 cms) in open prior to monsoon and gives >90% success.

Kusum (*S. oleosa*) for Lac Cultivation

Studies were undertaken on pollarding and propagation in Kusum (*S. oleosa*) for lac cultivation. Stem cuttings and scion of plus trees of Kusum were collected from Basia, Simdega, Bano etc. and air layering on superior trees made. Ten years old Kusum trees were pollarded at height of 1m & 1.5m and observations on number of shoots / branches emerged are being recorded.

Natural Dye Yielding Plants

Study was undertaken on selection and improvement of natural dye yielding plants. Accordingly, 17 superior dye yielding trees of *Mallotus philippensis* and 6 of *Wrightia tinctoria* were selected. Laboratory protocols are being developed for extraction of dyes from *Mallotus philippensis* and *Wrightia tinctoria*.

Vegetative Propagation

- Studies are underway on development of micro propagation protocol of *Rhododendron arboreum*. Both micro and macro methods of propagation is being applied. FRI wire technique applied on 15 *Rhododendron* trees near Surkanda. Experiment is still going on.
- Vegetative propagation of *Dalbergia sissoo* and Eucalyptus clones carried out. In *D. sissoo* 75 clones were multiplied and about 10000 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.
- Developed new technique on rooting of 3 node thin branch cuttings (less than pencil thickness) of *B. bambos* and achieved 46% of rooting with the treatment of IBA and Boric acid.
- The farmers were trained on clonal propagation of farm forestry species like *Eucalyptus*, *Casuarina equisetifolia* and *Bamboos* to make them self sufficient to satisfy their own requirements of superior planting stock.
- In *Calophyllum inophyllum*, standardized vegetative multiplication, using different concentrations of IBA on stem cuttings.
- In *Sapindus emarginatus*, various concentrations of IBA tried for root induction. Apical shoot cuttings using IAA and IBA combination gave rooting success of 25%.
- Seventy four superior *Pongamia* trees are being multiplied through branch cuttings and are being maintained for establishing clonal trial and clone bank.
- Experiments on *in vitro* rhizogenesis in five clones (GBW 4, JB 1, FZB 1, FZK 1, RSK 1) of *Dalbergia sissoo* were conducted. One field trial has been established with tissue culture raised plants of the 5 clones. A good growth of plants with 81% survival has been recorded depicting a maximum height of 79.57 cm in FZB 1 clone.



Developing the Process of Hardening is under Progress



Establishment of Field Trial of Five Clones of *D.sissoo*

- Studied endogenous auxin levels and its relationship with adventitious rooting potential in

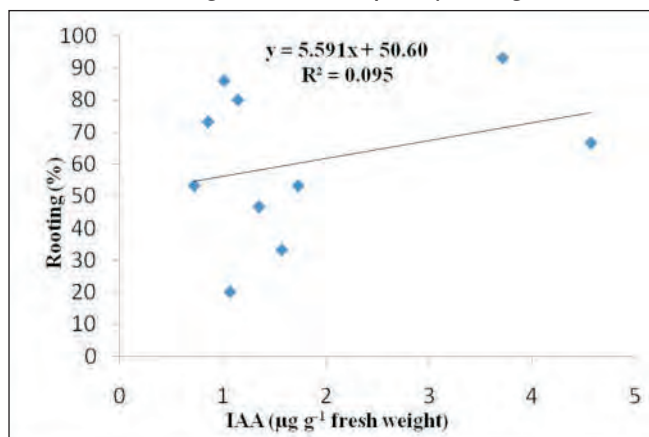
Dalbergia latifolia. Significant seasonal/genotypic variation was recorded in endogenous auxin (IAA) and in rooting potential of selected trees. Selected trees exhibited maximum rooting potential just after rainy season. Rooting potential of selected



Adventitious Rooting in *Dalbergia sissoo*



trees do not correspond with their endogenous IAA level. Exogenous IAA treatment helped in expression of the inherent rooting potential by way of local redistribution and lateral supply of endogenous IAA to the target cells in the pericycle region.



Relationship Between Endogenous IAA and Adventitious Rooting

- Studies were undertaken on development of vegetative propagation protocol of selected bamboo species viz., *Bambusa polymorpha*, *B. bambos*, *Teinostachyum dullooa* and *Melocanna baccifera* using different growth hormones. It was observed that 200 ppm IBA for *Melocanna baccifera*; 300 ppm IBA for *Bambusa bambos*; 100 ppm IBA + 50 ppm NAA for *B. polymorpha* and 50 ppm IBA + 100 ppm NAA for *Teinostachyum dullooa* gave best results for maximum shoot production. Observations also revealed that the standard basal media was Soil: Sand: FYM (1:1:1) in case of *Bambusa bambos* and *B. polymorpha*.
- Grafting technique for mature trees of *Ailanthus excelsa* developed (grafting success \approx 50%), which is presently more efficient over any other clonal technique. Wedge grafting gave better success than patch grafting. This technique can be handled easily by farmers and field staff of SFDs.
- Variability in rooting proficiency in selected genotypes of *Pongamia pinnata* was investigated. Scions were collected from 23 trees. Up to 30% success in rooting of mature stem cuttings was observed. Whereas, >70% rooting was observed

in cuttings taken from seedling. Variability in grafting success among PTs was also observed.

- For clonal propagation of *Salvadora persica* from Rohat, Sanderav, and Balotra, different concentrations of auxins (500-4000 ppm) were tested. IBA 2000 ppm was found to be the best for rooting from stem cuttings. Rooted cuttings were transferred to polybags consisted of mixture of FYM: Sand: Soil in the ratio 1:1:1 (V/V), and kept in agroshade net for one month. Among the rooting media studied (viz; Sand, Soil, Vermiculite) sand proved the best rooting medium.
- For development of technique for multiplication of economically important desert plant *Capparis deciduas*, three types of cutting of *Capparis deciduas* were used for rooting viz, softwood, semi hard wood and hard wood. Semi hard wood cutting gave better response. 1000 ppm IBA was found to be the best for rooting from stem cuttings. Experiments for rooting of stem cutting were conducted in April to March. Cutting harvested during the month of March-April and August-September-October were found to be best for rooting. Sand:Soil:FYM (2:1:1) was found to be the best for rooting of stem cutting.

2.3.3 Biotechnology

Use of Molecular Markers in Breeding Programmes

- Eucalyptus camaldulensis* and *E. tereticornis* are the most commonly cultivated for industrial plantations in India. Both the species are closely related and hybridized naturally hence discrimination of these species based on morphological features is challenging. Authentication of species identity is highly essential in genetic improvement program, particularly during the establishment of seed orchards and progeny trials. Efforts were made to use microsatellite markers and discriminate the species and their putative hybrids. Fifty five microsatellite loci belonging to 11 linkage groups



and 7 unmapped loci were amplified in 93 individuals, belonging to two species and landraces. Fifty nine microsatellite markers (95%) were polymorphic and 3 loci were monomorphic, across all the three groups. Analysis of 38 microsatellite loci (61%) for the presence of most common alleles with GDA software showed that 36, 27 and 39 SSR alleles were specific for *E. tereticornis*, *E. camaldulensis* and landrace respectively.

- In an attempt to estimate the outcrossing rate in *Acacia* seed orchards, fifty three families of *Acacia auriculiformis* in the orchard (Panampalli) were studied for the diversity analysis using ten ISSR primers.

Quantitative Trait Loci (QTL) Mapping in *Eucalyptus*

Efforts are being made to develop linkage and QTL maps in *eucalypts*, wherein SSR genotyping of parents such as *E. camaldulensis*, *E. tereticornis* and *E. grandis* was carried out to select polymorphic loci. In the present study, a cost effective M13- tailed three primer strategy was optimized for SSR polymorphism studies in *Eucalyptus* species. In this approach, PCR amplification was carried out using locus-specific forward primer with M13 tail (TGTAACCGA CCGCCAGT) at its 5'end, a locus-specific reverse primer, and the fluorescent-labeled M13 primer, the same sequence added in the locus-specific forward primer. The M13 primer can be labeled with various fluorescent dyes such as FAM (blue), VIC (green), NED (yellow) and PET (red) and this enables multiloading, which further reduces the cost per sample. The three primer M13 strategy is presently used for characterizing the mapping populations, *E.camaldulensis* x *E. tereticornis* and *E.tereticornis* x *E. grandis*. Concurrently, identification of DNA markers using association mapping strategy was also initiated in *E. tereticornis*. In this study, three developing xylem specific cellulose synthase (*CesA*) genes were isolated and characterized. Alternately, another gene HD-Zip class II transcription factor responsible for fiber related traits was also isolated partially. These genes can be used as one of the candidate gene for identifying the marker for pulping traits. This study will play an essential role in isolating the differentially expressed

genes involved in xylogenesis from commercially important species of *Eucalyptus*.

Gene Isolation and Functional Analysis

- In *Casuarina equisetifolia*, a truncated gene sequence of Cinnamyl Co A reductase (CCR) was characterized and the 850 bp sequence was deposited in NCBI. This would provide a lead to identify alleles targeting lignin deposition in wood.
- Further, studies are in progress to identify genes conferring biotic and abiotic tolerance in various forest tree species and medicinal plants. In this effort, a total of fifteen new gene sequences pertaining to sodium transport (HKT1, NHX1), housekeeping genes (Actin) from *Eucalyptus tereticornis*/ *Eucalyptus camaldulensis*/ *Casuarina equisetifolia*/ *Prosopis juliflora*/ *Millettia pinnata*, and insect growth and development (chitin synthase), xenobiotic responsive gene (SXR-like protein), housekeeping gene (Actin, elongation factor 1-alpha mRNA), from *Leptocybe invasa*/ *Hyblea purea* were identified and published in NCBI. The database (*In silico* Gene Bank for Adaptation to Abiotic Stresses) was hosted in the web (www.igpaas.co.cc) and is, now, in the test phase. In *C. equisetifolia*, salt tolerance was found to be associated with higher proline levels in the salt tolerant clone TNIPT4, when compared to the susceptible clone, PYN.
- Full length lectin gene with approximate size of 894bp was cloned and sequenced from salicylic acid treated leaves of *Withania somnifera*. The gene designated as *WsMBP1* showed significant similarity to *CaMBL1*, a mannose binding lectin from *Capsicum annum* (e=0.0, maximum identity = 88%). Presently, study is in progress to characterize the recombinant lectin for its antifungal and antipest properties.

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. Plant samples were tested for morphological, physiological and



biochemical responses. A demonstration trial to test the clonal performance on saline soil was established. Identified biochemical markers and grouped the clones based on saline tolerance

Pretreatment of Bamboo chips

On the basis of chemical analysis and SEM, pretreatment of Bamboo chips and mechanically processed fibers with best identified fungus *Trametes versicolor* initiated for conducting pulping experiments in bulk. Kraft pulping of treated and untreated was carried out. Black liquor generated was analyzed for various parameters with respect to chemical recovery. Pulp yield and kappa number of unbleached pulp in both the cases were determined. Bleaching by conventional CEHH sequence was carried out on the basis individual kappa number of control and treated chips's pulp. Chlorine was applied on the basis of kappa number. Physical strength properties and optical properties were determined. 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.

Development of Micro propagation Technique

- Studies were taken to scale up the protocols for *in vitro* propagation, hardening, production of cloned plants and establishment of field trials of Sandalwood (*Santalum album* L). For micro propagation through axillary shoots proliferation, the best medium for *in vitro* rooting was standardized as MS basal medium consisting of Glucose 3% and Agar 0.58% when shoots were inoculated after pulse treated with IAA 2500ppm + IBA 2500ppm (pH-7.0) for 30 min. For hardening, it was found that primary hardening in sand followed by secondary hardening in potting medium consisting of sand : compost: soil (35:50:15) is ideal for hardening of *in-vitro* raised plants of sandal. Field trial of *in-vitro* raised plants of sandal was carried out. 73.68 % survival was recorded after five months.

- Development of micropropagation protocols for production of superior germplasm of *Dalbergia latifolia* Roxb. and *Pterocarpus santalinus* was done. Two different explant types were compared for multiple shoot induction. Nodal segment performed better in MS medium supplemented with 1.0 mg/l BAP. Among the several media combinations tested, MS medium supplemented with NAA (0.1mg/l) and BAP (1.0-2.5 mg/l) proved best 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.
- Studies were conducted to develop an efficient micropropagation protocol for clonal multiplication of *Dendrocalamus hamiltonii*. Methodologies have been developed for shoot multiplication and root induction.
- Effects of different types of medium; MS, WPM, and B₅ were studied for *in vitro* shoot multiplication in *Salvadora persica*. Amongst the media, MS medium was found to be the best for shoot multiplication. Among the different cytokinins (BAP, Kn, TDZ and Zeatin), used for *in vitro* shoot multiplication, MS medium with BAP was found to be the best. *In vitro* shoot multiplication cultures were maintained on MS medium supplemented with 0.5 mg/l BAP + 0.1 mg/l IAA + additives (ascorbic acid, citric acid, L- arginine, L- asparagine, adenine sulphate).
- For micropropagation of *Capparis decidua*, two types of basal media viz, MS and WPM were tried to optimize multiplication rate of *in vitro* raised shoots. Maximum shoot multiplication rate and 3-4



Macropropagated Plant
of *Salvadora persica*



In-Vitro Shoot Multiplication
of *Salvadora persica*

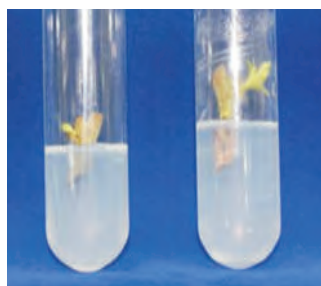


folds were obtained on MS medium supplemented with 2mg/l BAP + 0.5 mg/l IAA + additives.

- Studies have been undertaken on development of micropropagation protocol for mature superior recombinants emanating from F2 generations of *Eucalyptus* hybrid *E. citriodora* × *E. torelliana*. Experiments on multiplication resulted best in MS supplemented with 2 BAP + 1 NAA, Sterilents used: Sodium hypochlorite. Standardization of rooting is still going on.
- A network research project on guggal *Commiphora wightii* was executed. More than four years old embryogenic callus cultures were maintained



In vitro Multiplication of *Capparis decidua*



Bud Break in Nodal Explants of *Eucalyptus* hybrid

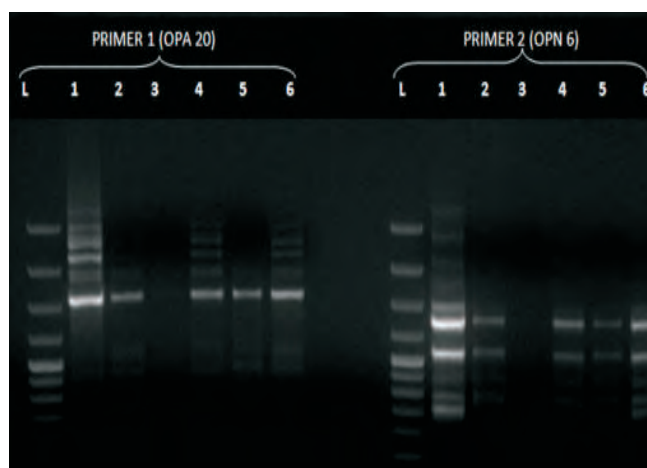


Multiplication (one to many) of *Eucalyptus* hybrid



Rooted Plantlets of *Eucalyptus* hybrid

continuously on modified MS medium supplemented with hormones and without hormone free medium with alternate sub-culturing. Secondary and tertiary somatic embryos (SEs) were also obtained. Cyclic embryogenesis was established and stabilized. White long matured SEs were harvested for the germination on hormone-free MS medium. Germinated SEs were used for *in vitro* hardening. 40 plants in *in vitro* hardening stage and 70 plants in poly-bags, which are ready for out planting. Growth data (height, collar diameter, primary and secondary branches and number of leaves) were collected at regular intervals. Plants are growing well in the field condition for the last one year with 100% survival. The plants exhibited normal growth and no morphological abnormality noticed. Isabgol, sago powder and guar gum were used along with agar as a control for alternative low cost gelling agent experiments. DNA isolation and purification from fresh leaves of tissue culture raised *C. wightii* plants growing in field were carried out by using the protocol given by Sanghamitra *et al*, (2009). Six RAPD primers were tested to check the genetic fidelity of *C. wightii* tissue culture plants at preliminary level. It was observed that all bends were found monomorphic, as such, no variation was observed.



DNA Fingerprint Generated Using two RAPD Primers (OPA 20 and OPN 6) of Tissue Cultured Plants of *Commiphora wightii* Growing under Field Condition