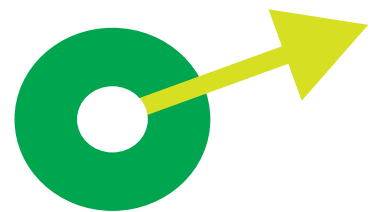


5

FOREST GENETIC RESOURCE MANA- GEMENT & TREE IMPROVEMENT



Forest Genetic Resource Management and Tree Improvement

Tree improvement and breeders in India are facing new demands to cope with the changing needs of forest industry and changing climate. To address these issues, dedicated tree breeding programme has to be reoriented towards development and deployment of productive and adaptive populations and varieties across the sites. 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, improving productivity. Therefore, selection of superior trees, their propagation, clonal testing, and establishment of production populations, clonal deployment and production of improved germplasm have been taken up in ICFRE institutes. Development of productive and drought adaptable, site matched varieties/ clones, identification of underutilized species/varieties adapted to harsh environments are some of the priorities to be taken up. Field tested genetically superior clones of Eucalypts and Poplars have revolutionized the productivity and profitability of plantations in many countries and results from Indian endeavours also quite encouraging. These plantations sustain most of the wood based pulp and paper mills and plywood/veneer factories in the country and thus saving precious foreign exchange. The same is yet to happen with regard to some other under-utilized fast growing tree species which requires proper evaluation and domestication.

Biotechnology have been an integrated tool in the ongoing tree improvement programmes and in

characterization of germplasm for their enhanced and systematic utilization. The ICFRE has initiated genomic research towards facilitating conservation and management of natural forest resources since forest genetic resources are provider of products and services for economic development.

5.1 Conservation of Forest Genetic Resources

Garcinia

Distribution of different species of *Garcinia* (Cluciaceae), its ecology, utilization in the upper Brahmaputra valley of Assam and its conservation was studied in detail. During the first year of the study, extensive survey was made in Nambar Reserve Forest and Kaziranga National Park in Golaghat District; Disoi Valley reserve Forest and Gibbon Wild Life Sanctuary in Jorhat District; Jokai and Joypore Reserve in Dibrugarh District; Panidehing Wild Life Sanctuary and Abhoipur Reserve Forest in Sivasagar District. The traditional



Fruits of *Garcinia cowa* in patch vegetation



knowledge of the people on the use of the different species of *Garcinia* was evaluated by interviewing the common villagers and the local medicine man, who were dealing with treatments of different disease by using plant products. Altogether, seven species of *Garcinia* *G. pedunculata*, *G. peniculata*, *G. xanthochymus*, *G. lanceofolia*, *G. cowa*, *G. kydia* and *G. morella* were found to be distributed throughout the study area. Except *G. lanceofolia*, all the species are evergreen trees distributed mostly in the forest areas. The species *G. lanceofolia* is a shrub, restricted to the homestead gardens as domesticated plant. All the species of *Garcinia* bears edible fruits which are eaten for its sour taste. The ripe fleshy exo-carp and mesocarp of the fruits (berry) of *G. pedunculata*, *G. cowa*, *G. kydia*, and *G. lanceolata* is edible and eaten its ripe fleshy part as sour vegetables or as prickles and also in dried form. The aril part of the seeds of *G. pedunculata*, *G. cowa* and *G. kydia* and *G. xanthochymus* has sweet taste and are eaten by the people, raw or as shorbat. It is also reported by some villagers that the juice extracted from the dry sliced fruits of *G. cowa* and *G. kydia* is used as shorbat and as the cooling agent for the patients suffering from fever and stomach pain.

Quercus leucotrichophora

Oaks (*Quercus* spp.) are among the dominant vascular plants of the Himalayas, ranging from the subtropical to the sub-alpine zones. In the Himalayan region, extensive oak forests occur between 1500 and 3300 m elevations. Banj oak (*Quercus leucotrichophora*) which 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, a project has been initiated. Eighteen populations each with 30 individual trees covering Himachal Pradesh and Uttarakhand has been sampled for DNA marker based study. DNA extraction techniques were standardized and genomic DNA isolated from 12 populations (360 samples). Polymorphic ISSR/RAPD markers were screened and used for molecular characterization work. RAPD fingerprinting of 300 samples, representing ten populations, using 10 selected primers has been completed. Their scoring work has also been completed. For SSR marker analysis, a total of 10 polymorphic microsatellite markers have been screened for population genetic analysis work.

Commiphora wightii

Commiphora wightii (Guggal, Guggul or Mukul myrrh tree) is one of the very important medicinal plants. Many commercial products have been marketed nationally and internationally, therefore, the demand of Guggal has increased and the plant is subjected to destructive tapping procedures. Thus, the Guggal has become so scarce, due to overexploitation that World Conservation Union (IUCN) has declared it as an endangered species. It was very essential to study population density and record the present status of this valuable species and assessment of its germplasm. In all the 33 districts of Rajasthan surveyed, Guggul density was found high in Sawai Madhopur ($\approx 74 \text{ ha}^{-1}$) and Jhunjhunu ($\approx 69 \text{ ha}^{-1}$) districts. The survey also revealed that Bikaner, Banswara, Churu, Shri Ganganagar, Hanumangrah and Pratapgah districts were lacking in natural guggul population. Male plants were available in only at few places



with female and male plants ratio of 99.9: 0.01. In other words, male plants were less than 0.01% in Rajasthan State. Germplasm was also collected for *ex-situ* conservation from identified trees. About 1428 vegetative cuttings with source details (GPS locations) were collected and raised in the vegetative propagation area for rooting.

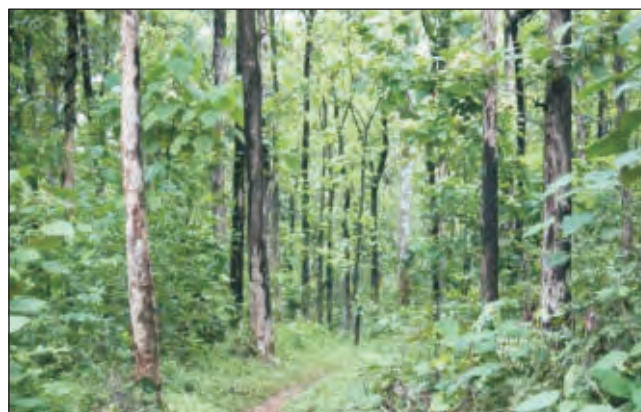
Field germplasm bank of *Grewia optiva* and *Quercus leucotrichophora*

The survey for identification of suitable land for establishing field germplasm bank was done in collaboration with Uttarakhand Forest Department. The collection of seeds from 79 selected trees of *Quercus leucotrichophora* and 107 selected trees of *Grewia optiva* was carried out from Uttarakhand, Jammu & Kashmir and Himachal Pradesh. The drying, cleaning and storage of individual tree seed was done and the morphological characterization of seed and their germination studies carried out. One day training was organized in the month of August, 2012 for the village women and farmers of Maldevta, Raipur block Dehradun to enrich their knowledge about all nursery and field plantation of fodder trees. A total of 64 women/farmers participated in the training. The *Grewia optiva* seedlings were distributed to the villagers of Maldevta and adjacent areas for planting in the bunds of the agriculture fields and panchayat lands to meet their fodder requirement.

Teak

For exploration of Teak genetic resources, its populations have been selected in different forest divisions of Kerala namely Thrissur, Chalakkudy, Malayattoor and Kothamangalam, on the basis of topography, soil factors, and morphological characteristics, such as tree form, branching

pattern, canopy, leaf, flowering and fruit. The details of Teak and Eucalyptus clones available with various stakeholders have been recorded and the clones will be collected for establishing National Gene Bank. In addition, a vegetative multiplication garden of teak with 56 clones and clone bank of teak are also being maintained.



View of teak population at Kannimangalam, Kerala



A teak tree showing numerous holes on trunk at Illithodu, Kerala



Variation in bark color of Teak at Kodanad and Evergreen outpost beat in Kerala.

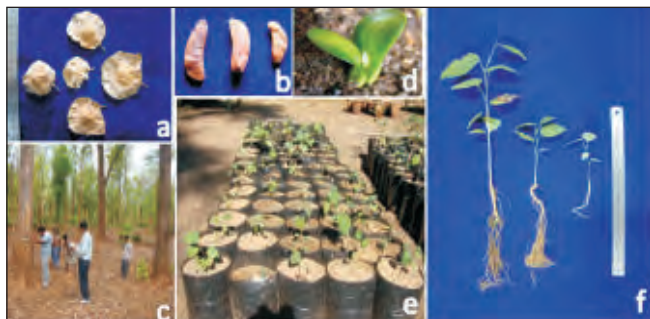


Ex-situ conservation of *Pterocarpus marsupium*

The work of germplasm collection was completed in three localities of Bastar region, namely, Bhanupratappur, Antagarh, and Narayanpur and one locality of Hati, Dhramjaygarh Range. In each locality, 20 trees were marked as superior trees on the basis of phenotypic characters. The pods and bark from a total of 60 trees were also collected.



Selection of phenotypically superior trees, collection of bark and recording of field data of *Pterocarpus marsupium*



Germination in *Pterocarpus marsupium*: (a & b) variation in pod and seed size (c) selection of phenotypically superior trees from Antagarh range in Bastar Forest Division, Chhattisgarh (d) emergence of cotyledons and (e & f) variation in germination of seeds of different size classes

NTFP species

Data were recorded on *Pongamia pinnata*, *A. marmelos*; *P. marsupium*, *Nux-vomica* and *S. suaveolens* for number of traits i.e. Plant height, girth, number of primary branches, 100-seed weight, crown diameter, clear bole height, crown height, crown volume, 100-pod weight etc. Recorded data were analysed statistically for

finding the correlation between the traits and also to find out the traits having direct effect on economic produce.

Based on the results of correlation and path coefficient analysis in *Pterocarpus marsupium*, the highest score has been awarded to the clear bole height in the rating scale, followed by girth and plant height. Correlation and path analysis revealed the importance of the clear bole height, plant height and GBH in selection of superior plants. Similarly for *Stereospermum suaveolens*, clear bole height, plant height, GBH and crown volume were found to be important for increasing timber and heartwood, used for medicinal purpose.

In *Strychnos nux-vomica*, *Aegle marmelos* and *Pongamia pinnata* fruits and/or seeds are the economically important produce. From correlation and path analysis, it became clear that crown diameter, number of branches and GBH had positive correlation with the crown volume, imparting higher direct and indirect effect on the parameter. Therefore, these traits along with crown volume deserved more weightage during selection of superior plants, where fruits/seeds are important.

A package of simple and scientific vegetative propagation method was developed for Guggul. Detailed guidelines in Hindi for vegetative propagation of Guggul plants in mist chamber have been prepared and sent to the State Forest Department for implementation in their nurseries.

For selection and improvement of natural dye yielding plants, both *M. philippensis* and *W. tinctoria* possess fairly good amount of natural variability in the present population as well as in selected genotypes. 19 CPTs were selected, based on good crown size, higher fruiting capacity, high dye yield and good colour intensity of dye powder.



The dye yielding parts- fruits in *M. philippensis*, highly varied with dye content. Sexes are separate and male plants occur rarely, thus creating physical barriers like large distance for pollination. However, in spite of this, female plants are profuse seed bearers and exhibit good amount of variation, indicating existence of alternate breeding mechanisms. Similarly, in *W. tinctoria*, most of the populations are confined to forests and that species did not acclimatized to agricultural or wastelands. A total of seven CPTs are selected, based on higher clear bole and good foliage. Leaves are source of green dye and some genotypes possess anthocyanin pigments in the leaves indicating were premature nature of genotypes and non-domestication. Laboratory protocols developed and improved dyeing techniques added to enhance colour intensity and consistent dyeing.

Population Assessment and Identification of Superior Genetic Stock of *Picrorhiza kurroa* Royle Ex. Benth and *Valeriana jatamansi* Jones by Screening Different Populations From North-Western Himalayas (Himachal Pradesh and Uttarakhand).

Identified the Superior Genetic Stock of *P. kurroa* and *V. jatamansi* from different geographical locations all along the zones of these species from the states of Himachal Pradesh and Uttarakhand. Geo-referencing along with characterization of micro-habitat has been carried out. Besides, Field Gene Banks (FGBs) in the Field Research Stations/ Nurseries of HFRI, Shimla (HP) and High Altitude Plant Physiology Research Centre (HAPPRC), Garhwal (UK) were also established. Infact, the Institute along with its network partners identified the superior genetic stock of *Picrorhiza kurroa* Royle ex Benth and *Valeriana jatamansi* Jones by screening different populations throughout from North-Western



One of the study site of *P. kurroa* in HP

Himalayas (Himachal Pradesh and Uttarakhand). Similarly, the superior chemo-types of *Podophyllum hexandrum* Royle, by screening different populations throughout from Himachal Pradesh and Jammu & Kashmir (Ladakh Valley) were also identified.

5.2 Tree Improvement

Dalbergia sissoo

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

A few selected clones were analysed for genetic variation in quantitative traits and through isozyme analysis as well. Resistance of these were also tested for stress and for their insect-pest. These clones have been found to possess genetic variation which has also been reflected in their growth performance in the field at two different sites. Advance generation orchards, raised with these selected clones are being maintained and growth data recorded periodically.

Dalbergia sissoo clones were also screened for their nitrogen utilization and biomass production. A total of 9 clones were multiplied in desired quantity from single plant and then, N assimilation, nodulation and biomass production study carried out.

Populus deltoides

Field trials of 30 clones of *Populus deltoides* were established at four sites in western Uttar Pradesh, Punjab and Uttarakhand. Cellulose content of 30 clones was estimated and wood anatomical studies made on 24 clones. Assessment of wood properties and growth of the progenies of different clones of *Populus deltoides* was also studied. Within tree, radial variations in wood properties, indicated the impact of cambial age on the wood properties of these progenies. Non-significant variations in replication for wood properties indicated that similar wood properties could be achieved from the population of the same progeny. Significant variations due to progeny for

wood properties indicated that these progenies were genetically different for wood traits.

Tecomella undulata

Survey and selection of candidate plus trees (CPTs) of *Tecomella undulata* were carried out in seven districts viz. Sikar, Churu, Jaipur, Nagaur, Bikaner, Pali and Jalore of Rajasthan. A total of 41 CPT's were identified on the basis of quantitative (height, girth, clear bole and crown diameter) and qualitative traits (straightness and health). Phenological observations like flowers initiation time, its color variation and seed setting were recorded from all the identified CPT's of *Tecomella undulata* in Rajasthan. Along with this, the growth parameters of the existing progeny trial of *Tecomella undulata* were also evaluated.



Identified Candidate plus trees of *Tecomella undulata* from Rajasthan

Ailanthus excelsa* and *Ailanthus triphysa

Study on breeding systems and reproductive biology in *Ailanthus excelsa* and *Ailanthus triphysa* was made. In *Ailanthus triphysa*, Karyotyping work was done with root tips and FAA fixed pollens. Pollen viability and male & female structural



Pollinator in *Ailanthus excelsa*

variation in *Ailanthus triphysa* have been studied. Indian Honey bee (*Apis cerana indica*) and Dammar bee (*Trigona iridipennis*) have been identified as key pollinators of *Ailanthus*.

Tamarindus indica

For evaluation and identification of optimal parameters for flowering and fruit set in different Tamarind (*Tamarindus indica* L.) orchards, the orchards located at Neyveli, Thoppur, Theni and Mullangaddu have been evaluated for flowering and fruiting. Soil of the orchards was analyzed for micro and macro nutrients status. The tamarind orchards were given 30 different treatments like ploughing, mulching, light shoot pruning, heavy shoot pruning, root pruning, girdling, notching, application of organic, inorganic, micro nutrient, spraying of KNO_3 , K_2HPO_4 , SADH, Thoiurea and Cultar in different concentration for improving flowering and fruiting. Among different treatment soil drenching of Cultar @3000 ppm and spraying of 2% KNO_3 were found giving positive implication on enhancing fruit productivity.

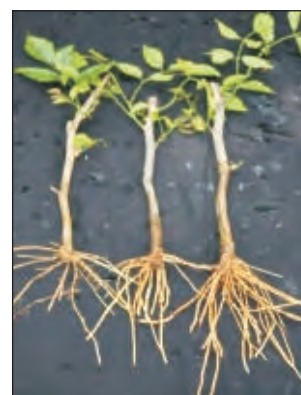
Gmelina arborea

Intensive survey conducted in the natural forest of Siruvani, Anaikatti, Anthiyur, Sathiyamangalam,

Dindugal, Kodaikanal, Sirumalai, Theni and in the Farmers' plantation in Pudhukottai. Identified natural population of *Gmelina arborea* in the above set locations and 50 CPTs selected based on growth superiority, clear bole and pest and disease resistance. Data was collected on bio-metric on phenology characters of *Gmelina arborea*. The reproductive traits like flowering phenology, pollen fertility, pollen germination on stigma and pollinator interaction of *Gmelina arborea* were studied on the selected CPTs. Wood sample (core) were also collected and wood parameters analyzed.

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



Rooted cuttings of selected CPT's of *Pongamia pinnata*

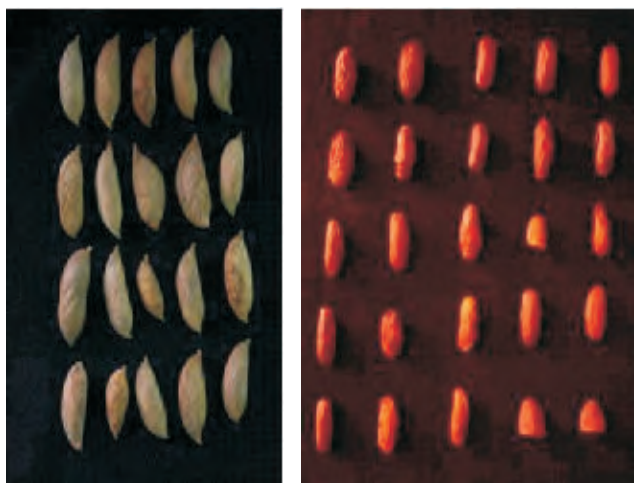


View of CPT-44 (*Pongamia pinnata*) from Velankanni, Nagapattinam district



Pantnagar (Uttarakhand) for testing stability, adaptability and growth performance.

A total of 87 high fruit yielding candidate plus trees of *Pongamia pinnata* were selected from 24 districts in different agro-climatic zones of Tamil Nadu, Puducherry and are clonally multiplied. Flowering and fruit production in the selected trees were recorded. Generally, the trees at Krishnagiri, Dharmapuri, Theni, Erode and Nagapattinam exhibited higher yield as compared to the other locations. The pod and kernel showed significant variation among the clones, which will be used for genetic improvement of the species. A Vegetative multiplication garden (VMG) with 87 CPTs is being established for mass multiplication of CPTs.



Fruits and seeds of CPT 7 (*Pongamia pinnata*) from Erode



Seeds of CPT25 (*Pongamia pinnata*) from Theni



Fruits of CPT 54 (*Pongamia pinnata*) from Pudukkottai

Hardwickia binata

To study the variability in *Hardwickia binata*, core samples were collected from natural populations and known aged plantations and bark thickness, sapwood and heartwood content and specific gravity was estimated. Seeds were collected from different populations and seed and



Single tree seed collection from selected CPTs of Red Sanders



seedling parameters recorded. To estimate the genetic variability, molecular marker studies are being carried out.

Acacia mangium

For development of advanced generation seed orchard of *A. mangium*, the progeny trial was maintained through regular weeding and fireline tracing. Significant variation in growth parameters among 126 families was observed. Heavy undergrowth of mangium seedlings in the trial was also observed. The families have been ranked and after the second year evaluation, further ranking will be done for thinning.

Pterocarpus santalinus

The seeds from 32 CPTs selected from plantations as well as four bulk seed lots obtained from IFB Hyderabad (total 36) were prepared and treated and sown for germination. Germination varied from 0-40 per cent among seed lots. A total of 50 CPTs selected and seeds from eighteen trees were collected. Wood samples have been collected and analysed for heartwood content.

Calophyllum inophyllum

Through intensive survey, identified populations of *C.inophyllum* in Trivandrum, Allepey, Kollam, Kazargod, Nagercoil, Kanya Kumari, Courtrallam, Chidambaram, Aliyar, Chennai, Karur, Annur, Avinashi, Anaikatti, Nagapattinam, Tenkasi, Sengottai, Puducherry, Karaikkal, Havelock, Mayabunder, Diglipur, Wandoor, Chidiyatapu, Rangat and Betapur in the states of Tamil Nadu, Kerala and Andamans. A total of 159 CPTs were identified and their passport data collected. Standardized, vegetative multiplication technique, through stem cuttings and produced rooted clones of selected CPTs. Established, Clone bank and Half-sib Progeny trial at Panampally and

maintained. Established, one multilocation trial of *Calophyllum inophyllum* in 1 ha. at Gudalur research station near Chennai. Shortlisted 40 high yielding clones having more than 55 per cent oil content.

Sapindus emarginatus

Identified 133 CPTs and established germplasm bank at Panampally. Progenies of the collected CPTs were raised and maintained. Average of 13 per cent saponin was identified as benchmark for shortlisting high saponin yielding CPTs.

Jatropha curcas

Performance of different accessions of *Jatropha curcas* in unreclaimed sodic soils was found to be unsatisfactory. At 6½ years of age, the plants exhibited poor survival and growth. None of the accessions could record growth rate comparable to plantation of this species on a good site. Seed yield has been negligible. Pruning at 30, 45 or 60 cm height resulted in production of more number of branches and greater canopy diameter. Superior germplasm of *Jatropha curcas* established in Uttarakhand as a part of multilocation trial, was evaluated. Seed production started at the age of four years. Significant differences in yield were found among accessions.

***Bauhinia* sp.**

Variability studies on seed quality parameters and seed mycoflora of *Bauhinia purpurea*, *Bauhinia semla* and *Bauhinia variegata* were conducted for their *ex-situ* conservation. Seeds stored at 5°C and -20°C maintained 100 per cent viability for 19 months storage, in comparison to seeds kept at room temperature. A rich mycoflora comprising a total of 10 fungal genera was detected on seeds of *B. purpurea* and *B. variegata* in fresh as well as in storage (6-month) condition. Among 10



genera, *Aspergillus* had highest number of colonies followed by *Penicillium*; while *Rhizopus* and *Fusarium* had minimum number of colonies.

Bombax ceiba

For evaluation of *Bombax ceiba* seed sources in northern India, growth and form of the species has been examined and criteria for selection of trees worked out. Experiment on rooting of branch cutting was established. Rooting has been found to be affected by age. Ten CPTs have been selected. Geremplasm from Assam was also collected and multiplied.

***Dipterocarpus retusus* (Hollong)**

The effect of storing Hollong fruits for different periods in different storage containers at different temperatures was studied by storing the fruits in a) paper bags at ambient condition, b) in polythene bags at 10°C, c) by treating the fruits in liquid paraffin wax and d) by storing the fruits in mud pots embedded in moist sand bed. The results indicated that seeds can be stored in mud pots for a longer period. The experiments on the effect of moisture content on storage showed that the germination percentage reduced below 25 per cent moisture content.

Aquilaria malaccensis

Germplasm banks of Agar were established at VVK, Chessa, Arunachal Pradesh and ARCBR, Aizawl, Mizoram. DNA finger printing was done using 22 RAPD primers to find out the variation existing among the accessions established in the germplasm bank.

***Shorea assamica* (Makai)**

Experiments were carried out for the effect of moisture content and storage conditions on seed germination. The fresh seeds showed 72 per cent germination. Among the various storage conditions, the seeds stored in mud pots, showed 40



Seedlings of *Shorea assamica* in nursery bed



Germinating seeds of *Shorea assamica*

per cent germination even after 25 days. Under various storage conditions with different moisture contents, the fruits showed poor germination with reduction in moisture content.

Neolamarckia cadamba

For improvement of *Neolamarckia cadamba*, selection in the natural population and existing plantation in different parts of Tamil Nadu, Kerala, A & N Islands and North Eastern State (Assam) was carried out. Standardization of vegetative propagation, mass multiplication of clones and to identify the best performing progeny/clones was also carried out. Selection of candidate plus trees has been done by scoring the selected trees in two



plantation of Tamil Nadu (Narasipuram and Devarayapuram) and in natural forest of Kerala, Andamans and Assam. Till date, about 114 Candidate Plus Trees have been identified. Coppice shoots were collected and tried for rooting with 1000, 2000 and 3000 ppm of IBA and NAA and out of three Cuttings treated with IBA/NAA 1000ppm gave good result. It was found that the cuttings treated with IBA/NAA at 500 ppm concentration have given better result as compared to all other treatment.

Thespesia

Selection and Screening of germplasm of *Thespesia populnea* was carried out for improving productivity. Under took extensive field surveys in Western Zone, North Western Zone, Cauvery Delta Zone, Southern Zone, North Eastern Zone and High Rainfall Zone of Tamil Nadu and selected 104 Candidate Plus Trees. Branch cuttings were collected from these CPTs and kept for rooting in vegetative propagation complex. Average rooting percentage obtained was 60. Established a Clonal Multiplication Area with 52 clones at Panampally Research Station, Kerala during the last week of October 2012.



Clonal multiplication area of *Thespesia* at Panampally



Vegetative propagation of *Thespesia*

Prosopis cineraria

Pods have been collected from selected CPTs, available and parameters from individual pods of these CPTs like length, width and weight recorded. The data from collected pods from individual trees were then subjected to statistical analysis.



CPTs of *Prosopis cineraria*



5.2.1 All India Coordinated Project (AICP) - *Melia composita*

An All India Coordinated Project for Genetic Improvement of *Melia composita* has now been approved by the ICFRE, whereby, all the ICFRE Institutes and other associated organizations will be carrying out the research work on defined lines. Survey was carried out in Nagaland, Meghalaya, Sikkim and in West Bengal (Cooch Behar) and Tripura to identify promising genotypes and provenances through selection and field evaluation. Fruits were collected to study their physical attributes. Seeds of 42 accessions were also procured from FRI, Dehradun and sown in nursery for rising stock to establish multi-locational trial.

The natural forests and the plantations of the species surveyed in different states to select plus trees. A total of 230 candidate plus trees (CPTs) were selected from different geographical regions and analyzed for index value based on height, diameter at breast height, straightness, clear bole height, crown diameter and knots. The field trials of different progenies at various locations were established to evaluate their performance.

Survey work was carried out to identify suitable plantations of different age as well as different spacing in southern part of Karnataka. Sample plots were laid out and preliminary growth observations were recorded.

In South, 50 plus trees were selected. Girth at breast height and clear bole length were the main criteria considered for the selection of trees. Single tree collections were made and maintained the identity of the fruits. The fruits and seeds were studied for their physical characteristics. Germination studies revealed that the source of seeds and medium of germination were the major influencing factors for establishment of the plants.

Three provenance trials have been established, one each in the states of Tamil Nadu, Kerala and Karnataka.

5.2.2 All India Coordinated Project (AICP) - *Eucalyptus*

Interspecies hybridization between *E. peliita* and *E. urophylla* was carried out at FRI Dehradun and F1 hybrids were produced. The hybrids so produced were transferred in the field in the form of field trials. Data have been recorded for the field trials.

Inter-specific hybrids were also developed at IFGTB, Coimbatore and efforts are made towards generation of genetic linkage and QTL maps for traits such as salt tolerance, adventitious rooting and wood pulping characteristics were sketched. The crosses developed were *Eucalyptus camaldulensis* x *E. tereticornis* and *Eucalyptus tereticornis* x *E. grandis*. These inter-specific hybrids were genotyped and hybrid purity index was calculated to confirm the interspecific parentage. Phenotyping of *Eucalyptus camaldulensis* x *E. tereticornis* mapping population was carried out for salinity tolerance traits and putative QTLs were identified.

Control pollination programmes to produce Inter-Specific hybrids in *Corymbia* and intra specific hybrids in *Eucalyptus* were developed in the projects supported by the industries. Messrs ITC Ltd. and Messrs TNPL Ltd. Using a fullsib diallel mating design, the clones namely IFGTB-1, IFGTB-2 and IFGTB-3 were combined and eleven seed lots were released to TNPL during June, 2012.

The first generation *Eucalyptus* provenance trials were assessed and trees for collection of seeds for establishment of second generation seed orchards shortlisted. Further, superior trees in the



Clonal seed orchard of Eucalyptus at Salem, TN



Progeny trial of Eucalyptus at Chennai, TN

first generation progeny trials were also shortlisted for making clones.

Progeny trials for selected eucalyptus clones were established during 2009 to 2011 at Chennai, Coimbatore, Puthukottai and Hyderabad. These trials were assessed for their growth. Annual maintenance works were also carried out in these trials.

IFGTB screened the clones of *Eucalyptus tereticornis* and *E. camaldulensis* in multilocation trials in south India. From the observations recorded in Andhra Pradesh at Hyderabad, Tirupathi, Warangal, Rajamundhry and (PAJANCOA) Karaikal, few promising clones have been identified for their release.

For evaluation of wood properties and growth performance of *Eucalyptus* hybrids raised in multilocation trials, hybrids of *Eucalyptus* were evaluated for wood traits. Vertical variations were recorded for all the wood traits while horizontal radial variations were recorded for all the wood traits except for fibre diameter.

5.2.3 All India Coordinated Project (AICP) - *Casuarina*

Developing Genetic Improvement of *Casuarina* Species through Second Generation Orchards

This component aims at moving the ongoing breeding programme of *Casuarina equisetifolia* and *C. junghuhniana* from the first to second generation. The most outstanding entries of the first generation orchards were designated as mother trees for sourcing families to establish large breeding populations as second generation progeny trials. Over 16 ha of breeding populations established were continued to be assessed for growth, form and wood properties. Ranking of families and individual trees was completed for all trials aged 3 years and above. Culling of inferior trees has been started to convert the progeny tests



Outstanding growth of a *Casuarina junghuhniana* clone short listed for public release (age: 4 years; Erode, Tamil Nadu)



into second generation orchards for producing genetically improved seeds.

Improving the accessibility and affordability of improved seeds

Three Community Seed Orchards established during the previous years have been intensively managed by involving the farming and nursery operator communities. One of them (Valluvamedu, U.T. of Puducherry) started yielding seeds this year. The stakeholder community was trained in seed collection and modern nursery techniques for using the orchard-produced seeds in their nursery. A short film entitled Casuarina Improvement for Rural Livelihood Support was produced to document the socio-economic importance of Casuarina and its genetic improvement programme.

Screening for blister bark disease resistance in clones

C. equisetifolia often infected by a fungus called *Subramanianospora vesiculosa* (= *Trichosporium vesiculosum*) that causes blister bark or stem wilt disease. Identifying resistant phenotypes of *C. equisetifolia* is the long term solution for this disease. In this project, pathogenic cultures of *S. vesiculosa* was inoculated to *C. equisetifolia* clones and assessed for disease resistance through disease severity score and phenol contents in different clones. 250 clones (15 replicates each) were vegetatively propagated and inoculated with the pathogen *S. vesiculosa*. The inoculated clones were screened for disease resistance through disease severity score. The clones TNPP -4, TNKP -1, TNIPT -5, TNCS -3, TNIPT 12 showed severe



Root nodules

infection. Totally, 36 clones are showing resistant and 55 clones showed moderately resistant. Rest of the other clones showed moderate symptoms. The resistant clones have been planted at Puducherry in RBD to test the resistance against blister bark disease under field conditions. Under field conditions, the clones TNIPT 1 and TNIPT 7 showed resistance against the blister bark or stem wilt disease.

Identification of superior growth promoting strains of *Frankia* in *Casuarina equisetifolia* and *C. jughuhniana*

Casuarina species were associated with a nitrogen fixing bacteria called *Frankia*. To achieve *Frankia* inoculation in seedlings, the root nodules from matured trees were collected and used conventionally for inoculation. Site specific strains of *Frankia* were collected, cultured and inoculated in seedlings and cuttings of *Casuarina* spp. Simultaneously, in nursery conditions, the *Frankia* inoculated seedlings and cuttings were observed for the growth improvement in *C. equisetifolia* and *C. junghuhniana*. This method will give active *Frankia* for nitrogen fixation than the existing method which is directly uses root nodules for *Frankia* inoculation. Collected totally 10 strains of *Frankia* associated with *C. junghuhniana* and *C. equisetifolia* and cultured in P media and maintained in the laboratory. 93 clones of *C. equisetifolia* and seedlings of *C. junghuhniana* were inoculated with *Frankia* strains and assessed the growth parameters. Increased shoot length, root length, collar diameter and biomass were found in *C.*



Nodulated trees



equisetifolia and *C. junghuhniana* due to inoculation of *Frankia*. The field performances of *Frankia* inoculated trees of Casuarinas showed three times higher growth and biomass than that of un inoculated controls. Profuse growth of root nodules was also observed in the planted Casuarinas due to inoculation of *Frankia*.

5.2.4 All India Coordinated Project of Teak

Realizing genetic gain from teak seed orchards

Assessment of flowering, fruiting and seed production from two clonal seed orchards (CSO) and a seed production area has been continued for the tenth consecutive year. Orchards and clones showed marked differences in these attributes across years and locations. Clone- and ramet-wise seed collection was from the orchards and subjected X-radiography and germination tests. Orchard seeds recorded lower filling and germination as compared to those from the seed production area. Estimation of outcrossing rate using DNA derived from seeds is in progress.

Developing breeding populations of Teak

A survey was conducted in Seed Production Area of Teak at Topslip, Parambikulam, Walayar, Kulathu puzha, Konni, Ariyankavu and Nilambur. Identified and selected 200 CPTs of Teak based on superiority in growth, stem straightness, pest free nature and flowering. Collected open-pollinated seeds from selected CPTs, ready for sowing in nursery beds.

Evaluation of progeny trials

Progeny trials established with 18, 16, 28 and nine half-sib families of teak at Rajpipla Shivrajpur, Sajjangarh and Jodhpur respectively were evaluated. Analysis of variance of these trials revealed that variation due to families was

significant for most of the traits, indicating scope for family selection except for basal area in Shivrajpur and height, girth in case of Sajjangarh materials. In all the trials, narrow sense heritability ranges from 2 to 97 per cent for various traits. Height and collar girth exhibited low 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 per cent. General combining ability (gca) analysis revealed that in all, 26 parents exhibited positive gca values. These parents are expected to harbour desired combination of alleles. In addition to this, seven new phenotypically superior trees were selected from different locations.

5.2.5 Development of DUS descriptors

Three populations each of *Pinus roxburghii* and *Cedrus deodara* were surveyed for distinct traits. The observations with regard to needle length and colour were found to vary considerably. The distinctness in traits for bark pattern and crown form and cone size in both the species were studied to identify distinct genotypes. Variants of the species with distinctness for morphomeric traits have also been identified.



Needle Colour variations in *Cedrus deodara*



Extensive field surveys were carried out to ascertain the variability of *B. balcooa* and *D. hamiltonii* in different parts of North East India. The selected clumps were also raised in the nursery for plantation programme to evaluate the DUS characters. The raised propagules have been planted in the field for the evaluation of DUS characters.



Ligule variation in *D. hamiltonii*



Variation of Cilia (presence or absence) in *B. balcooa*



Groove and Bud variation in *D. hamiltonii*

5.2.6 Seed Technology

Seed handling technique is under progress for *Neolamarckia cadamba* and based on the germination studies, it is found that it has 60- 70 per cent germination.

As per the demand of Punjab SFD, quality seeds of more than 30 species have been supplied. The required quantity of seeds of *Anthocephalus chinensis*, *Cinnamomum camphora*, *Eucalyptus* hybrid, *Dendrocalamus strictus*, *Melia burmanica*, Cassias, *Terminalia bellerica*, *T. chebula*, etc. were supplied to Punjab SFD. The seeds were cleaned, tested for germinability, fumigated and treated with bavistin before packing to prevent any fungal/pest infestation. In addition to it, bamboos plants (*Bambusa bambos* and *Dendrocalamus strictus*) have also been supplied to the department, for planting and creation of a rhizome bank.



Rooted nodal cutting of *Myrica esculenta*

5.3 Vegetative Propagation

In *Myrica esculenta* 10 per cent nodal cuttings, treated with 400ppm IBA showed rooting response. Air layering along with IBA treatment (4000 ppm) was successful in root induction. The air layered rooted branches were planted in the polythene bags for field planting.

Effect of N fertilizer on growth, nodulation and N fixation activity in *M. esculenta* was studied and preliminary observations showed that N fertilizer @ 80kg/h showed better performance than other treatments. For N assimilation study, necessary



Air layered rooted branches of *Myrica esculenta*

buffer and substrate solution has been standardized. It was observed that buffer solution ($0.2M\text{ KH}_2\text{PO}_4$) at pH 8.1 and substrate solution $0.15M\text{ KNO}_3$ is suitable for the N assimilation study in the leaves of *M. esculenta*.

Effect of growth hormones and their concentrations, size and type of cuttings and season on rooting for *Melia dubia*, *Ailanthus excelsa*, *Grevillea robusta*, and *Anthocephalus chinensis* was studied. In *M. dubia*, sprouting and rooting has been observed in experiments laid in February and March. Maximum rooting was



Rooting of *Acacia auriculiformis* CPTs under poly tunnels in root trainers

observed in lignified cuttings, treated with 1500 ppm of IBA.

61 superior trees of *Acacia auriculiformis*, selected in various progeny trials based on stem form and growth, have been multiplied vegetatively and 7000 rooted cuttings being maintained in the Nursery. The cuttings were initially rooted in root trainers and, later, transferred to polythene bags. A Vegetative Multiplication Garden (VMG)/clone bank has been established at Panampally Research Station and is being maintained.

A grafting technique for raising clonal plants of male and female *Ailanthus excelsa* trees has been developed by AFRI Jodhpur. This technique can easily be adopted by farmers and field staff of Forest Departments. It is equally beneficial to the firms which are exploiting the tree for fodder, timber or biomass.

As per the demand of State Forest Department of Rajasthan, a package of simple and scientific vegetative propagation method was developed for Guggul so that field officials can follow these guidelines in their nurseries. Detailed guidelines in Hindi for vegetative propagation of Guggul plants in mist chamber are prepared and submitted to State Forest Department for implementation in their nurseries.

Vegetative propagation of *Dalbergia sissoo* and Eucalyptus clones carried out at FRI. In *D. sissoo*, 75 clones were multiplied and about 15000 plantlets 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.

5.4 Biotechnology

Salt tolerant trees are an unexplored resource for genes conferring salt tolerance. The sodium-

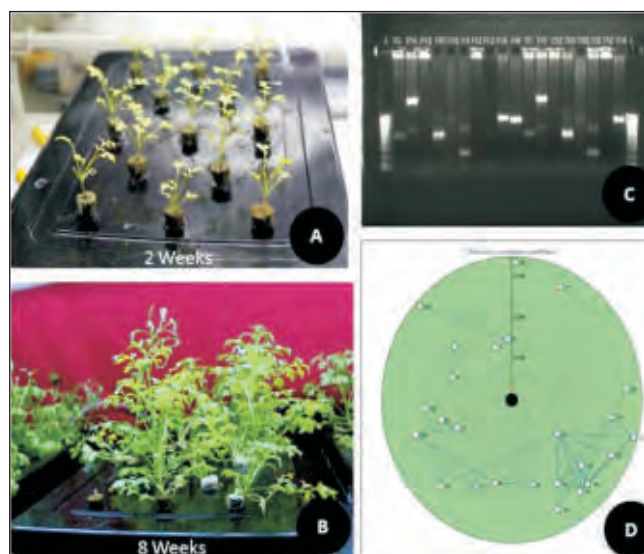


hydrogen antiporter genes (*NHX*) from *Bruguiera gymnorhiza*, (355 bp), *B. cylindrica* (445 bp), *B. sexangula* (351 bp), *HKT1* gene from *Prosopis juliflora* (219 bp), *AKT1* genes from *Casuarina equisetifolia* (236 bp), *Eucalyptus camaldulensis* (309 bp), *Prosopis juliflora* (300 bp), *B. sexangula* (357 bp), *B. cylindrica* (409 bp), *K. candel* (273 bp), *P. juliflora* (291 bp) and *Acacia nilotica* (261 bp), and the Actin genes from *B. cylindrica* (203 bp), *B. gymnorhiza* (209 bp), *B. sexangula* (221 bp), *Kandelia candel* (204 bp), were sequenced and published with accession Numbers, JX679717, JX679718, JX679719, JX679724, JX840853, JX840854, JX840855, JX840856, JX840857, JX840858, JX840859, JX840860, JX679720, JX679721, JX679722, and JX679723 at the Gene Bank Database of the National Centre for Biotechnology Information (NCBI), National Library of Medicine, National Institute of Health, USA. These represent the first sequence information for the respective genes for these tree species.

Study of salt tolerance through gene expression:

With the aim of analyzing salt-induced genes through a comparative genomics approach, establishment of fresh set of *Lepidium sativum* (selected halophyte) was done hydroponically. Electrophoresis of RT-PCR products was carried out to analyze the gene expression levels under different time intervals. Analysis of expression of gene product formation at different exposure intervals of salt solution was carried out. Nine putative genes were identified that have been found to be expressing under high salt conditions (100 and 140 mM NaCl). These genes have possible role in abiotic stress physiology.

Determination of sequence information of insect- genes is crucial for application of transgenic RNAi technology for control of the insects. In this direction, the partial gene sequence information for



Study of gene expression pattern in *L. sativum*: A. *Lepidium sativum* plants growing hydroponically at 2nd Week; B. plants growing hydroponically at 8th Week; C. A gel image showing bands generated from different genes under salt stress using gene specific primers D. Circular plot predicting closely correlated genes (including putative genes with unknown function) using Pearson's correlation coefficient for *NHX1* (showing genes with correlation above 0.957).

the teak insect pest *Hyblaea puera* Chitinase gene (480 bp), Ecdysone receptor gene (751 bp), and Chitin synthase gene (204 bp and 741 bp) were sequenced and published with accession Numbers, JX101956.1, JX644041.1, KC121027.1 and KC121028.1 at the Gen Bank Database of the National Centre for Biotechnology Information (NCBI), National Library of Medicine, National Institute of Health, USA.

Production of recombinant antifungal / antipest lectin from *Withania somnifera*

A mannose binding lectin was isolated from the leaves of *Withania somnifera* and designated as *WsMBPI*. The gene was expressed in bacteria and the recombinant lectin was purified and tested for antifungal/ antipest activity. The recombinant lectin showed limited antifungal activity but had a significant toxic effect on the food utilization, growth rate and metamorphosis of insect pest like



Hyblaea puera. In *Withania somnifera*, transcriptome analysis of leaf was conducted to identify genes involved in pathogenesis. A total of 71,062 transcript contigs were annotated and transcripts with metabolic and cellular function amounted to 70 per cent while transcripts with catalytic activity were 77 per cent.

Development of Tree DNA Fingerprint database: DNA Fingerprint information was collected from researchers, based on the experiments conducted at IFGTB in Eucalyptus, Casuarinas species using ISSR/FISSR, RAPD, and AFLP markers. ADNA fingerprint database has also been developed at IFGTB Coimbatore.

Allelic diversity of Cinnamoyl CoA Reductase (CCR) Gene in *Casuarina equisetifolia*: Allelic diversity of Cinnamoyl CoA Reductase (CCR) Gene in *Casuarina equisetifolia* clones, assembled at IFGTB was studied, using the partial CCR gene sequence. The DNA sequence information was uploaded in NCBI. Efforts are being made to isolate the whole gene sequence.

5.4.1 Use of Molecular Markers in Breeding Programmes

For the assessment of variability and genetic fingerprinting in *Pongamia pinnata*, microsatellite markers were used for the genotyping of the collected accessions from West Bengal and Odisha. DNA extraction protocol was also standardized using different plant materials. More than 20 SSR primers have been designed, synthesized and being evaluated for molecular studies. SSR's from related forestry species also evaluated in *P. pinnata*.

For standardization of molecular based technique for timber tracing back to the forest area, screened 14 SSR primers, found working with DNA isolated from the woody tissues and three SSR loci

were found showing allelic variations across the populations.

For the molecular based characterization of the twisted pines populations, DNA isolation and quantification of 144 twisted pine accessions and 20 normal pine accessions has been completed. The samples have been characterized using 10 ISSR primers.

Validation of chemical markers conferring *Cylindrocladium* leaf and seedling blight resistance in Eucalyptus was carried out at FRI Dehradun. A correlation of marker constituents with susceptibility / resistance was observed.

For assessment of genetic diversity and structure of *Boswellia serrata* populations, RAPD and ISSR molecular markers were used. The leaves and wood core samples of 20 individuals from the 12 different populations were collected along with the observation of leaf morphology, height and girth, crown shape. Genomic DNAs was extracted from the nine populations and used for fingerprinting.

In *Acacia auriculiformis*, efforts are made to develop microsatellite markers and genotype seed orchard population for the assessment of out-crossing rates.

In order to study variation in natural populations of *Pinus gerardiana*, isoenzyme markers were used. Six populations were assayed with stable enzyme systems.



Isozyme Variation Studies and Plus Tree of *Pinus gerardiana*



5.4.2 Development of Micropropagation techniques

For optimization for *in-vitro* propagation and conservation of *Embelia ribes*, a vulnerable medicinal plant, cultures have been established for both the species i.e., *Embelia ribes* and *E. tsjeriamcottam*. Rooted plantlets were hardened and planted in field. Cultures have been put into conservation media and being tested periodically for its multiplication/ viability after storage.

Tissue culture technology was developed for *Podophyllum hexandrum* through leaf explants.



Multiplication of culture



In- vitro rooting

A micropropagation protocol for mature superior recombinants emanating from F2 generations of *Eucalyptus* hybrid (*E. citrodora* × *E. torelliana*) was developed.



Ex- vitro hardening of E1 in vermiculite



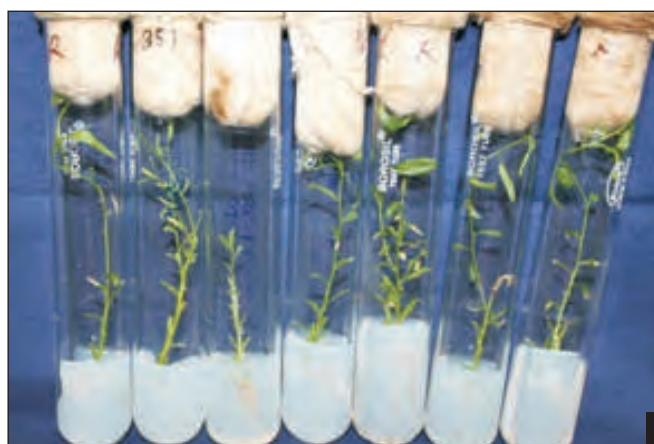
100 rooting % obtained in 2.85 μM and 1.42 μM of Indole Acetic Acid

For multiplication of economically important desert plant *Capparis deciduas*, experiments for rooting of stem cutting were conducted. Cutting harvested during the month of March-April and August-September-October were found to be suitable for rooting. Effect of various rooting medium was studied and Sand: Soil (2:1) was found good for rooting of stem cutting. For *in-vitro* propagation, amongst various auxins tried, IBA supplemented MS medium produced maximum number of roots as compared to NAA and IAA. It was observed that 1/4x MS medium found the best for *in-vitro* rooting. MS medium found to the best for *in- vitro* rooting experiment.

For *in- vitro* rooting of *Rauvolfia serpentina*, experiments were conducted to screen suitable basal media and their strength. Among different basal media and hormones, the maximum of 81.67 per cent rooting was obtained on $\frac{1}{2}$ B5 medium. Among different hardening substratum, soilrite emerged as the best medium for hardening with 100 per cent survival. Varied response of organ formation was noticed in different genotypes



A



B



Micropropagation of *Capparis decidua*: (A) Shoot multiplication and (B) *in-vitro* rooted shoots

depending upon different concentration of growth hormones. In the interaction study, the maximum shoot formation was obtained in 5 mg/l BA and 4.5 mg/l NAA. Maximum rooting (66%) was obtained in 2.5mg/l BA and 8.5mg/l NAA. The ready hardened plantlets were transferred to the field.

For development of tissue culture technology for multiplication of economically important desert plant *Salvadora persica*, studies were conducted on the effect of media, growth hormones and incubation conditions (temperature, light, humidity) for high frequency multiple shoot induction and growth. MS medium supplemented with BAP (7.5 mg/l) proved best and favoured multiple shoot induction (2-3 shoots per explants) in four weeks at the 28°C temperature with 2500 lux

intensity of light.

For micropropagation of Sea Buckthorn (*Hippophae salicifolia*), healthy and mature seeds were surface sterilized with mercuric chloride and sodium hypochlorite and technique standardize. Surface sterilized seeds were transferred to MS solid medium for germination. Stem cuttings of *Hippophae salicifolia* were implanted after treating with different concentration of IBA, IAA and NAA at Khirsu for establishment of VMG.

Complete micropropagation protocol, using axillary shoot derived and somatic embryo pathway have been developed for *Commiphora wightii* and plants successfully out planted in field condition with cent percent survival. Revival of



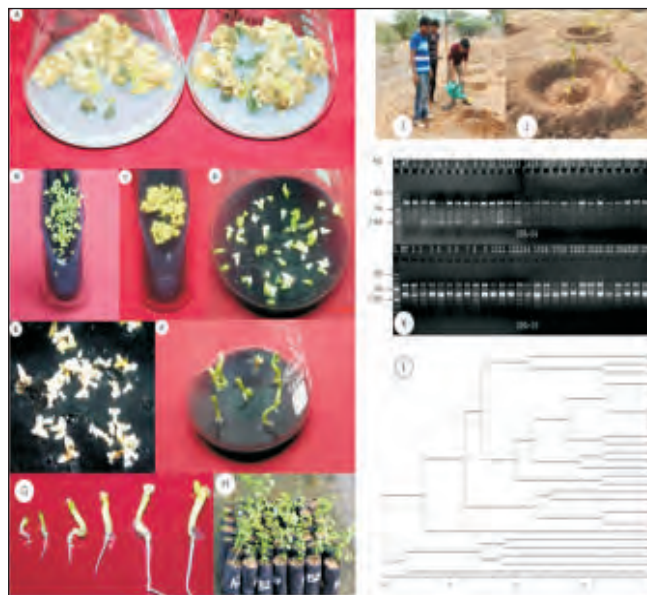
Plantlets raised through micro- propagation and seedlings are in the field condition



Maximum rooting (81.67%) of *R. serpentina* obtained on $\frac{1}{2}$ B5 medium in GO-MN genotype

embryogenecity in long term maintained cultures was achieved enabling cyclic embryogenesis for over five year old cultures. Genetic fidelity test of *in-vitro* raised *C. wightii* plants, using DNA fingerprinting, involving RAPD markers has been done. The cost of single plant produced through somatic embryogenesis pathway, came out to be ₹ 19, while that produced through axillary shoot proliferation protocols ₹27.

In-vitro propagation protocols for two bamboo species of *Bambusa balcooa* and *Thyrsostachys*



In-vitro propagation and evaluation of *Commiphora wightii* and genetic fidelity plants: A. Embryogenic callus of *Commiphora wightii* (guggal); B. Somatic embryo (SE) development; C. SE multiplication; D. cotyledonary stage SE conversion; E. mature SEs.; F. Germination of SEs; G. various stages of germination of SEs; H. SE derived hardened plantlets; I & J. field trial of SE derived plants; K. DNA fingerprinting using RAPD markers and L. Dendrogram showing genetic fidelity of regenerated plants.

oliveri were refined. Among the various treatments tested with different PGRs and varying concentrations, MS liquid medium + NAA (0.25mg/l) + BAP (2.5mg/l) at pH 6.2 was found as best treatment for shoot multiplication of *Thyrsostachys oliveri* with subculture period of 8-10 days. This treatment resulted in approximately 2 fold multiplication of shoots (15.08 ± 0.78). In *Bambusa balcooa* MS liquid medium + NAA (0.25mg/l) + BAP (2.0mg/l) at pH 6.2 was best for multiplication. March and February were found the ideal time, for initiating cultures and for establishing cultures of both species.