



IFGTB NEWS



Quarterly Newsletter on societal applications of research **Interventions in Forestry, Genetics and Tree Breeding** from the Institute of Forest Genetics and Tree Breeding, Coimbatore.

(A national institute of the Indian Council of Forestry Research and Education, Ministry of Environment, Forest & Climate Change, GOI)

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From the Director's Desk

The Institute of Forest Genetics and Tree Breeding has a mandate to characterize forestry genetic resources and develop tree varieties having desired traits. Towards these goals, IFGTB has been developing and integrating advanced biotechnological tools in forest genetic resource management and tree breeding programmes. This issue of IFGTB News highlights some of the advancements made in the application of biotechnology in these areas. Applied and basic studies on application of DNA markers in understanding genetic diversity of breeding populations, development of genetic linkage maps for localization of QTLs involved in desired traits, CRISPR/Cas9 mediated gene editing initiatives in *Eucalyptus*, DNA marker development for gender discrimination in *Ailanthus excelsa*, generation of transcriptome and genome sequence resources in teak and sandal, and genome size estimation in *Pterocarpus* spp. are highlighted. Furthermore, this issue also has a feature on Seed Cake mix developed at IFGTB. This issue thus provides insights on the technologies being developed and the applications in the fields of biotechnology and seed technology.

Dr. C. Kunhikannan
Director, IFGTB

DNA technologies in forest tree improvement

R. Yasodha

Forest biotechnology is an advancing field in forestry encompassing genetic- and biology-based technologies to enhance the yield of tree plantations. Breeding pattern of forest trees throws massive amount of genetic variations to sustain in the dynamically changing environment. Genetic improvement strategies to improve productivity slowly but surely reduce the diversity. Thus, to reap the positive benefits of tree improvement sustainably, tailoring of simple to complex biotechnological tools has become indispensable. High throughput DNA markers revolutionized breeding techniques to expedite the release of improved varieties. DNA markers such as RAPD, ISSR, AFLP, and SSR are employed to understand the diversity of breeding populations, seed orchards and clonal lines of eucalypts, casuarina, and teak. Putative hybrids generated through controlled pollination were DNA diagnosed for their genetic purity. Clonal

DNA markers are rapid and accurate tools to accelerate forest tree breeding and improvement.

lines have been tagged for their uniqueness using DNA markers. Chromosome locations controlling quantitative traits such as adventitious rooting, salinity tolerance, cellulose and lignin content in eucalypts were identified. SSRs and SNP marker based high density linkage maps were generated and utilized in localization of QTL influencing stomata, adventitious rooting and salt tolerance related traits (Cover photo).

Next generation sequencing based approaches provide huge information on genomic scans of polymorphism to precisely estimate population genetic parameters in species like teak. Presence of millions of genomic variations and structural alterations are likely to play major role in productivity, adaption and evolution. A number of challenges exist in tree genomic research, demanding concerted efforts to lay a solid foundation for marker assisted selection.

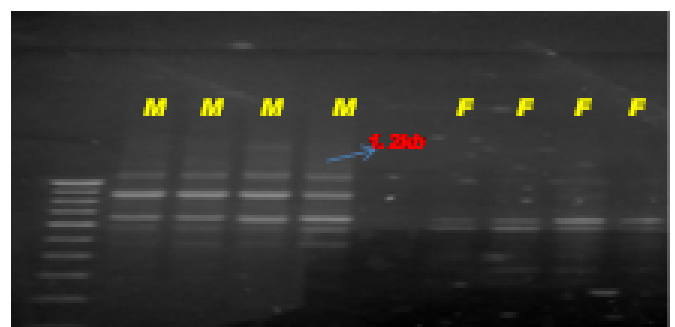
Gender identification in *Ailanthus excelsa* through RAPD markers

M. Suganthi, Poongodi Goutham, S. Haritha, B. Sathya Vani, S. Sudha, D. Rajasugunasekar and A. Shanthi

Ailanthus excelsa, commonly known as tree of heaven, is a large deciduous tree found in India and Sri Lanka. It belongs to the family Simaroubaceae. The softwood is mainly used in the matchwood industry. It is predominantly dioecious (separate male and female individuals), while bisexuals also occur. Gender identification at seedling stage can help tree breeders in designing seed orchards. A study was therefore taken up for developing DNA markers for gender discrimination using Randomly Amplified Polymorphic DNA (RAPD) markers. Genomic DNA isolated using IFGTB's Arbor Easy® DNA isolation kit, from leaves of 10 male and female trees were profiled using 15 RAPD primers. Two RAPD primers viz, OPN-03 and OPE-04

Markers developed for discriminating males and females would aid breeders establish seed orchards of *A. excelsa*

discriminated males and females with the presence of a male specific amplicon of size 1.2 kb. The OPN-03 primer was further used to identify 4 males and 2 females in nursery seedlings. These primers need to be validated in a larger population and used for SCAR marker development.



CRISPR/Cas mediated gene editing for imparting salt stress tolerance in *Eucalyptus*

Balasubramanian Aiyar, Sivakumar V. and Mathish Nambiar-Veetil

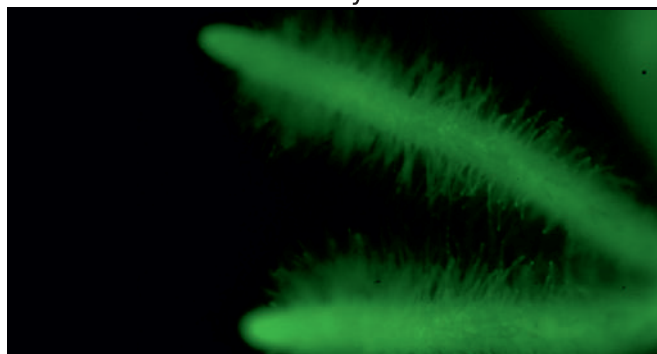
The technology to edit DNA using CRISPR-Cas9 tools has been one of the recent scientific breakthroughs. The tools permit breeders to edit DNA sequences, study gene function, generate genetic variations and genetically modify for traits like high biomass, salt tolerance and better pulping efficiency.

To apply this technology for breeding better trees, IFGTB has initiated a project on "Development of a genome editing platform for functional characterization of genes". Composite transgenic strategy in *Eucalyptus* developed earlier at IFGTB, in which transgenic roots (see photo) are generated on non-transgenic shoots provides a rapid system for evaluating genes conferring salt tolerance. Gene-editing vectors targeting the *ECHK1;1* gene have been developed, and are being tested for imparting salt tolerance in the composite transgenic system.

Gene editing methods being developed in *Eucalyptus* would open up new avenues for understanding gene function, increasing genetic variation and breeding better trees.

Gene editing involves integration of transgenes encoding Cas9 and guide RNAs, the removal of which is time consuming in

trees. To minimize regulatory compliance requirements, IFGTB has initiated another project on "Evaluation of transgene-free genome engineering methods in *eucalyptus*". Methods are being developed for delivering Cas9 protein-gRNA ribonucleoproteins. These approaches would enable developing varieties that are indistinguishable from the conventionally bred varieties that have a history of safe use.



Transcriptomics for unravelling adaptive genetic diversity in Teak

Maheswari P., Nithish Kumar K., Suma Arun Dev¹, Ulaganathan K.,² and Yasodha R.

Teak is one of the most popular and important timber tree cultivated around the world. Natural populations have a variety of traits that influence their economic, ecological, and environmental significance. Since 2015, genomic methods have assisted in the identification of genes, proteins, and other regulatory elements that could be manipulated to improve teak tree productivity. The first draft genome sequence of teak was published in 2018, followed by chromosome-scale genome assembly using advanced long read sequencing technologies in the year 2019. Efforts are being made to investigate the transcriptomic variations across various populations of teak in an ongoing

Differentially expressed genes (DEGs) associated with drought stress response were identified from leaves of teak growing in high and low rainfall zones.

project supported by the Department of Biotechnology, Government of India in collaboration with

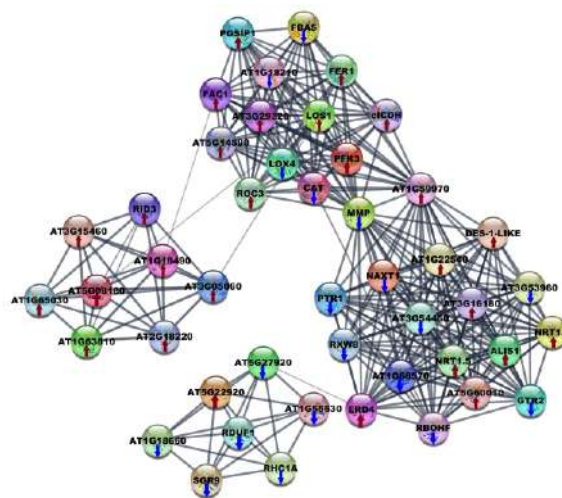
Kerala Forest Research Institute¹, Thrissur, and Osmania University², Hyderabad. We developed RNA-seq libraries from leaf samples of three individuals each from high rainfall (HR) and low rainfall (LR) zones as part of the project to examine the molecular mechanisms underlying adaptive genetic diversity. The high-quality clean reads obtained from Illumina sequencing platform were assembled into 51,596 genes. Totally 2,122 differentially expressed genes (DEGs) were obtained with the fold change value ≥ 2 and false discovery rate 0.05, out of which 1,057 were up-



regulated and 1,065 were down-regulated. Enrichment analysis of DEGs using the Kyoto Encyclopedia of Genes and Genomes (KEGG) showed that the enriched pathways were involved in abiotic stress related pathways.

To understand the interaction of differentially expressed proteins related to environmental stress a Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database was used (confidence score ≥ 0.9 ; see figure). Nodes in the protein network represent proteins and the edges between the nodes indicate the types of evidence supporting the association. Several proteins related to abiotic stress and other related enzymes (Catalase, Matrix metalloproteinases) and transcription factors were found to be interacting partners with key enzymatic subunits of reactive oxygen species (ROS) production pathways, and

play crucial role in plant signalling, development and stress responses. Our results thus provide a useful insight into rainfall pattern influence on teak, and offer a platform for evaluating their role on secondary wood related traits.

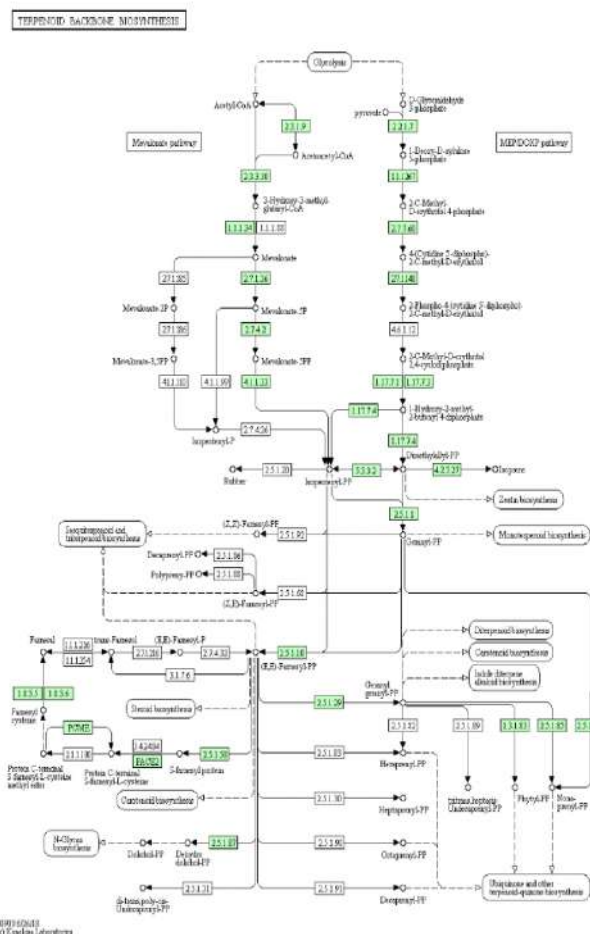


Genome sequence of Indian Sandalwood, *Santalum album* L.

Modhumita Ghosh Dasgupta, Kandasamy Ulaganathan¹, Suma Arun Dev² and Swathi Balakrishnan²

Genome and transcriptome sequence resources of Indian sandalwood would facilitate identification of adaptive potential of populations and accelerate trait based breeding.

Santalwood is a major commodity in international trade, due to its use in fragrance and essential oil industries. The oil content of Indian sandalwood (*Santalum album*) is the highest (6-7%) when compared to other species. IFGTB, through a collaborative effort with scientists from Osmania University¹, Hyderabad and Kerala Forest Research Institute², Peechi, reported the draft genome of this species. A total of 37,500 genes were predicted including 30 terpene synthases which are involved in santalol production. A total of 2,68,998 SSRs were predicted and six primer pairs flanking dinucleotide repeat motifs were amplified in 10 genotypes. The study will facilitate understanding the unique santalol biosynthetic pathway, estimating genetic diversity and population structure, accelerating breeding programs targeting heartwood formation and santalol content, and developing extended next generation DNA barcodes.



Genome size estimation in the genus *Pterocarpus*

Rekha R. Warriar and R. Yasodha

The genus *Pterocarpus*, comprises many endemic and valuable timber yielding species, making them globally important. DNA content of three different species of *Pterocarpus*, hitherto unavailable was investigated under the project supported by National Biodiversity Authority, Government of India. DNA content is an essential measure for genome size and genetic variation studies. Using propidium iodide - flow cytometry analysis, the DNA content (2C) was determined for three species - *P. marsupium*, *P. santalinus* and *P. indicus*

The first study providing genome size estimates in *Pterocarpus* would help undertake advanced studies in genetics and breeding.

following standard protocols. Replicated measurements revealed that the DNA content of *P. santalinus*, a near-threatened endemic found only in the Eastern

Ghats of India, was estimated to have a $2C=0.787$ pg, which is equal to a genome size of about 780 million base pairs (Mb). *P. marsupium*, having a wide distribution, had a $2C=0.924$ pg, and a genome size of about 920 Mb. *P. indicus*, an endangered species native to south and Southeast Asia, had a genome size of 2410 Mb and a $2C$ value = 2.426 pg.

IFGTB technologies

IFGTB Seed Cake mix - An innovative seed ball technology

R. Anandalakshmi, C. Rajesh, S. Geetha, E. Murugan, K. Suresh Kumar and A. Sathish

Seed cakes are improvised form of seed pellets that enable better

A user-friendly ready-mix that could be deployed for aerial seeding in inaccessible areas.

seedling establishment and growth. Seeds are dressed with suitable inert materials, fertilizers, plant growth regulators, fungicides, insecticides, pesticides, etc., using appropriate binders.

Species suitable to the location need to be used. Good quality seeds need to be subjected to suitable pre-treatment methods for breaking dormancy, if any, or soaked in water and dried back to initial seed weight before pelleting.

IFGTB Seed Cake ingredients: Binder - 100 g of gelatin. Filler - 1.2 Kg of coir pith, Indian clay, leaf powders of *Albizia amara* and neem, vermicompost, *Trichoderma viridae*, *Rhizobium*.

Preparation of seed cakes: Dissolve 100 g gelatin powder in 1 L of boiled water. After cooling for 5 min, add the solution into a tray containing 1.2 kg of filler and mix well. Place a layer of this into a plastic ice-cube tray with a cavity size of 1.6"x1"x1", and keep 2 pretreated seeds. Fill another layer of filler mix, then place two more seeds and finally top it with the filler mix. Manually press the filler moulds to compress the cake. Allow it to stand for a day, and carefully tap the tray to expel the compact seed cakes. Allow it to dry for 3 to 4 days at room temperature. The seed cakes can be used within one month. One packet can be used

for preparing 200 seed cakes. For 1 ha, 2 packets are required. Cost of one IFGTB

seed cake mix is Rs.450/-.

The IFGTB seed cakes have been designed for direct dibbling, broadcasting and aerial seeding. Raking of the soil to loosen the earth before dibbling the seed cake gives good results. When broadcasted or aerial seeding is carried out, lodging of the seed cakes in sites having sufficient soil depth is essential for root penetration and establishment of the germinated seedlings. Dibbling or broadcasting few days or one week before onset of rains/ monsoons is essential for success of the seed cake technology. Heavy rains may wash off the broadcasted seeds especially on slopes. Cattle browsing, foraging by birds, insects damage and forest fire could affect seedling establishment.



Events section : Oct - Dec 2020

- ◆ **Meetings / Seminars** : Research Advisory Group (RAG) meeting (15th Oct), Stakeholders consultation on draft safeguards information system for REDD++ implementation in India (16th Dec), Role of Scientists and science endeavours in finding solutions to combat COVID-19 pandemic (25th Nov), “Online Knowledge Series – Talk to Scientist Program” -PRAKRITI awareness program for college and school students (Oct – Dec).
- ◆ **Trainings** : Basic statistical methods in Forestry (05th- 06th Oct), Environmental leadership and life skills (for women scientists) (26th – 30th Oct), Climate change: Challenges and response (for women scientists) (05th Oct- 09th Nov), Development of Tree Rich Biobooster using wood biomass and municipal wastes involving tribes for their livelihood support (see photo) (23rd Dec), Webinar on e-Procurement - Procurement of goods and services, and related GOI financial Rules (16th Dec)
- ◆ **Other Events** : Swachh Bharat Abhiyan (06th Oct), Wildlife week 2020 (07th Oct), Vigilance Awareness week (27th Oct – 02nd Nov), Green Deepavali (13th Nov), Samvidhan Divas (26th Nov), World Soil Day (05th Dec), International Mountain Day (11th Dec), Dr. S. Kedharnath Memorial Lecture (KML-2020) (18th Dec).



About IFGTB

The Institute of Forest Genetics and Tree Breeding (IFGTB), Coimbatore, is a national institution of the Indian Council of Forestry Research and Education (ICFRE), an autonomous council under the Ministry of Environment, Forest and Climate Change, Government of India. IFGTB has a mandate to develop new varieties, management and silvicultural techniques to maximize productivity of natural and planted forests under different ecological considerations and changing environment.

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Cover Photo : Genetic linkage map of
Eucalyptus by **Dr. R. Yasodha**, Scientist G.

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