



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

Over the years, IFGTB has forayed into taking up research on diverse tree species integrating tools of molecular markers and genomics. In this issue of IFGTB News, such studies on red sanders and the mangrove species of *Rhizophora* are highlighted. Programmes have been initiated for hybridization of two species of *Rhizophora* to harness the improved biomass yields and salt stress adaptation. Molecular markers have been integrated into the hybridisation programme to characterise these hybrids, and have provided better insights into the genetic structure, and population diversity. Differential gene expression analysis is being used to unravel the genetic control of salt stress adaptation mechanisms in *Rhizophora*. In red sanders, advanced SSRseq techniques have been developed and are being used for population genetics studies. The importance of taking up studies on the tamarind weevil pest, importance of x-ray radiography in seed research and IFGTB's capacity building initiative to tribals for value addition through processing of NTFPs are also highlighted.

Dr. C. Kunhikannan
Director, IFGTB

Controlled Pollination and Hybridisation in *Rhizophora*: Targeting Hypersalinity and Productivity

Utchimahali, M. and Nagarajan, B.✉

Pichavaram, a Ramsar site in Tamil Nadu, is home to *Rhizophora apiculata*, *R. mucronata* and their putative natural hybrid *R. x annamalaiyana*. Large scale drying of mangroves was observed by the Tamil Nadu Forest Department, during 2018 - 2019. Field surveys conducted by IFGTB indicated that *R. apiculata* exhibited a greater tendency towards wilting and drying. The scale of incidence was higher in narrow creeks that reached hypersalinity in a shorter span owing to differential hydrological regimes. Interestingly *R. x annamalaiyana* hybrids were found to be resilient with no signs of wilting or drying in any of the documented 156 individuals. These are also heterotic, exhibiting a higher biomass than either parent. As they are huge and darker in appearance, the local fishermen refer to them as “*peikandal*” [ghosts]. The cover photo depicts a natural hybrid, *R. x annamalaiyana*, flanked by *R. apiculata* on the left and *R. mucronata* to the right. The hybrids are however, sterile, with profuse flowering throughout the year, which is a foraging ground to several entomophilous visitors. Studies on the vegetative propagation of the natural hybrids are therefore, in progress.

Controlled pollination studies have been initiated in *Rhizophora* to improve productivity and tolerance to salinity

Based on the template of the earlier species recovery research successfully carried out in *Ceriops decandra* and

Bruguiera sexangula, controlled pollination studies were initiated in *Rhizophora* (See figure below). About 2 - 5 % of fruit setting was observed in the *R. mucronata* x *R. apiculata* crosses, while the reciprocal cross failed, indicating that natural *Rhizophora* hybrids arose only from the unimodal combination of *R. mucronata* x *R. apiculata*. DNA studies using SSR markers, however, indicated that eight of the fifteen natural hybrids sampled were proximal to *R. mucronata* genotype, and the rest closer to *R. apiculata*, indicating that both *R. mucronata* and *R. apiculata* could act as maternal parents.



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Transcriptome evaluation of the salt-treated roots of *Rhizophora mucronata* and *R. apiculata*

Manoj-Kumar, R., Vaishnavi, J., Balasubramanian, A., Shamili, K., Sandhya M.C., Sivakumar K.*, Kathiresan K.*, Nambiar-Veetil M.✉

The Mangrove species of the Rhizophoraceae, are well adapted to salinities of intertidal zones.

They survive these high salinities due to their ultrafiltration properties at the roots enabling restricted sodium uptake from the roots to the shoots. To understand the genes that confer salt tolerance in Rhizophoraceae, propagules of the salt tolerant *R. mucronata* and the relatively salt

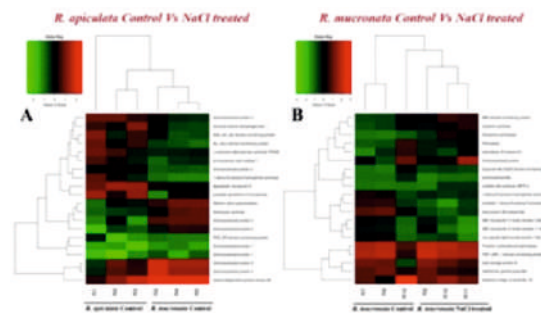
Genes differentially expressed during salt stress in the roots of the salt tolerant *R. mucronata* and the relatively salt susceptible *R. apiculata* post salt stresses were identified.

susceptible *R. apiculata* were treated with 250 mM NaCl for 24 hrs. The roots of the treated propagules

were then analysed for the differential expression of salt- induced genes (See figure for heat maps). Total RNA was sequenced using Illumina HiSeq-2500 platform, followed by *de novo* assembly of the expressed genes (transcripts). The transcriptome analysis identified 3253 and 23

Differentially Expressed Genes (DEGs) in salt-treated roots of *R. apiculata* and *R. mucronata*. The comparative gene expression profiles between the two species identified 9216 DEGs. Heatmap of top 20 Differentially Expressed Genes (DEGs) between A) *R. apiculata* Control Vs 250mM NaCl treated. B) *R. mucronata* Control Vs 250mM NaCl treated are shown in the figure below. The gene enrichment analysis showed key functions (ATP binding, metal ion binding, protein serine/threonine kinase activity, and water channel activity) and major pathways (Biosynthesis of secondary metabolites, Metabolic pathways, and Phenylpropanoid biosynthesis) modulated during salt stress. Thus, this study provides insights into the salt stress response in *Rhizophora* species by generating a salt-induced root transcriptomic resource. In

addition, the potential candidate genes conferring salt tolerance were identified for further characterisation through expression analysis in bacteria and model plants.



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Population Genetics

Novel Microsatellite Genotyping in Red Sanders

Sindhu, A., Maheswari, P., Nithishkumar, K., Vijayakumar A., and Yasodha, R. ✉

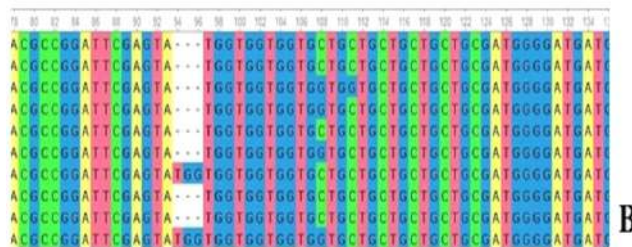
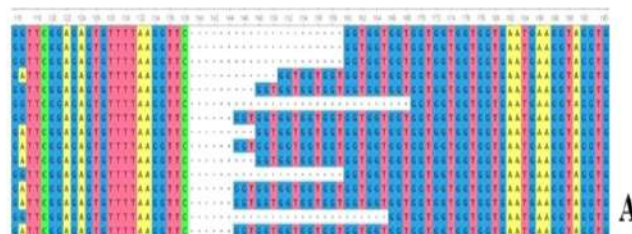
The microsatellite or simple sequence repeat (SSR) markers are widely used in forest trees for various genetic studies. These markers not only have polymorphism in repeat numbers but also encompass single nucleotide variations (SNP) and insertion/deletions (indels). Traditionally, these variations are detected through low throughput electrophoresis methods.

Currently, next generation sequencing (NGS) based microsatellite genotyping (SSRseq), a high-throughput genotyping method is being successfully applied in forest tree genetics. The SSRseq consists of simple two step multiplexed PCR based library construction and sequencing at high-depth coverage in NGS platform. The protocol supports multiplexing of samples to reduce the cost. It is also suitable for the identification of SNP and indels present in SSR motifs and flanking regions. These microsatellite motif number variation, SNP and indels offer markers specific to target populations. SSRseq clearly discriminate the size homoplasy alleles with sequence specific molecular variation and it is used for the interpretation of population structure, estimation of genetic diversity, identification of population specific alleles, identification of polyploid

SSRseq techniques developed for red sanders could be used for population genetics studies.

genotypes and prediction of sample heterogeneity across the populations.

IFGTB has developed SSRseq technique successfully for red sanders (*Pterocarpus santalinus*). Hundreds of samples are multiplex sequenced using 10-15 SSR loci. Sequence data generated is passed through dedicated bioinformatic pipelines, and SSR, SNP and indel alleles are extracted for population genetic analysis. The figures below depicts the SNP variation in red sanders populations using the SSR loci IFGTBPS4100 (A) and IFGTBPS682 (B).



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NTFP Value Addition

Scientific Validation of Value Added NTFPs

Rekha R. W.✉

Non-timber forest products (NTFP) play an important role in the local population's daily lives and well-being. NTFPs are also a source of food, fodder, medicines, gums, resins, and construction material. Besides local consumption, they are traded on local, regional, national, and international markets. Internationally they achieve high prices and contribute to economic development.

IFGTB, Coimbatore has been recognised as a Centre of Excellence by the Ministry of Tribal Affairs for developing processing and value-addition technologies for NTFPs. The main goal is to develop harvesting and processing methods for Terminalias and confirm quality enhancement and commercialisation. As a first step, sensitising the tribals on the need for value addition, and its impact on enhanced income was undertaken. Parallely, scientific validation of the products following value addition was carried out. The

Quality control with value addition enhances the market potential of NTFPs

chemical composition of the dried pericarp varied with stages of fruit maturity, affecting potency. This emphasises the importance of the time of collection. The project addressed the research gaps through a practical and result-oriented approach. It paved the way for devising appropriate strategies for tribal development and enhanced the institution's capacity. Through capacity building, the generated information was disseminated to tribal folk, the main stakeholders.



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Research Gaps

Infestation and Damage by *Sitophilus linearis* (Herbst) Weevil on *Tamarindus indica* L. fruits and seeds

Ashrith K.N.✉ and Jacob J. P.

Tamarind is an important multipurpose tree. Almost every part of it has industrial and economic importance. The tamarind seed weevil is known to occur in tamarind growing areas and can cause damage to seeds and fruits (see figures below), affecting the quality and market value. In general, tamarind weevil is

Studies on the biology of the tamarind weevil pest would enable the development of control measures.

observed during seed storage. Recently it has been observed in seeds of standing trees also.

Studies on the infestation pattern and damage by weevil on tamarind fruits and seeds in IFGTB campus showed that infestation of weevil on standing trees was found maximum during the



month of June, with upto 36 per cent damage observed in both fruits and seeds. The infestation of *S. linearis* begins from field after fruit ripening and damage continues when the fruits and seeds are harvested and stored. The life cycle of tamarind weevil is completed in 30 days. Females are relatively larger than males. Both the adult and grubs feed on cotyledons of tamarind seed. Feeding and movement of adult through pulp also

invites mould growth. Both fruit pulp and seeds are consumed by humans and cattle. The insect is also known to attack other seeds or grains. It is therefore, pertinent to investigate the developmental biology of this pest in local environmental conditions for effective management of the pest.

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New Facility

X-Ray Radiography Facility for Seed Quality Assessment at IFGTB

Anandalakshmi, R.✉, Sivakumar, V., Vineetha, M.V., and Peryanayaki, S.

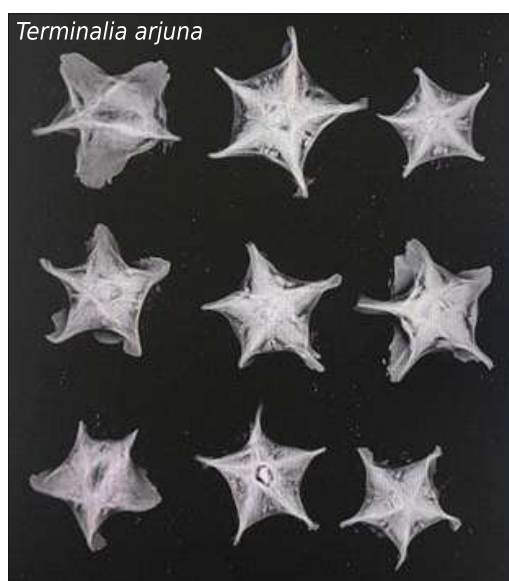
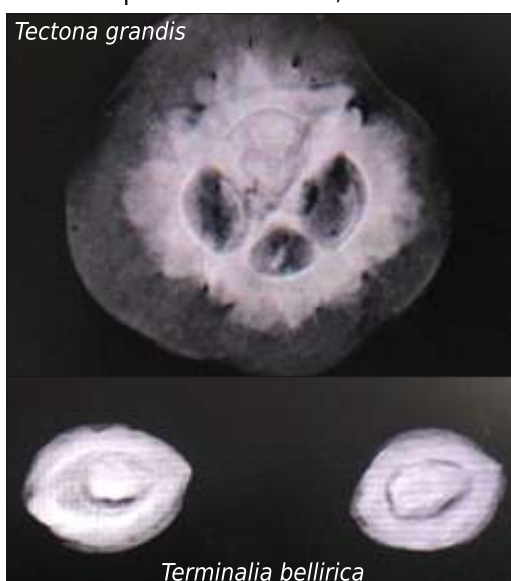
ICFRE - IFGTB, Coimbatore, has recently installed a KUBTEC-Xpert 80 X-ray system with funding support from CAMPA-MoEF & CC. Since the 1950s, X-rays have been used to determine various aspects of seed quality. X-ray imaging of seeds enables visualization of seminal structures, differentiation into fully developed, underdeveloped, or empty seeds, physically damaged seeds, and the presence of insects in seeds. KUBTEC- Xpert 80 X-ray system is being used for studies on seed filling in tree species that produce hard seed coats, such as *Tectona grandis*, *Terminalia bellirica*, and *Terminalia arjuna* (See photo below). Seed accessions are placed in the x-ray imaging platform to capture the digital images that are visualized on a monitor. The x-ray imaging system has a platform area of 4.5 to 5.5 inches for placing the seeds. Depending on the density of the seeds, the instrument optimizes the exposure duration,

X-Ray analysis facilitates rapid monitoring of the physical quality of seeds with a high level of reliability.

tube voltage, and current for a better exposure. The soft X-rays exposed on

the seeds help to display the structure of the seed, embryo, and endosperm.

X-ray imaging, being a non-destructive technology, has applications in research studies involving non-destructive sampling. The instrument can be used to identify problems related to seed orchard management, where seed filling is a major issue. Digital images can be used for measuring the sizes of the interior structures and assessing variations in various plant parts, like wood ring density. It thus facilitates tree improvement and the production of quality planting stock. It also has applications as a rapid screening tool in plant quarantine. The facility is available for use by scientific organizations, researchers, state forest departments, and students.



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Events : Apr - Jun 2022

- ◆ **TRAINING** : Women empowerment through capacity building on development of Tree Rich Biobooster (22th Apr), Agriculture inputs and organic farming (27th Apr), Increasing pulpwood production (30th Apr), Summer internship training on Bioprospecting (17th May - 30th May), Basics of remote sensing and GIS; Practical application of GPS and demonstration of application of QGIS (19th - 20th May).
- ◆ **MEETINGS / SEMINARS/ CONFERENCE** : License agreement between IFGTB and VETRY NGO, Tiruppur, for production and sale of windbreak clones (24th May), National workshop on forestry research prioritization with special emphasis on agroforestry (14th Jun), Glue Grant R&D projects review meeting (02nd Jun), Stakeholders meet with TNPL field functionaries (06th Jun), Seminar on population genetics and its implications in FGR conservation (17th Jun)
- ◆ **OTHER EVENTS**: Observance of 131st birth anniversary of Dr. B. R. Ambedkar (15th Apr), Earth day (22nd Apr), International day of Biological Diversity (22nd May) & Talk on "Importance of biodiversity awareness and conservation" in Rathinavani 90.8 Community Radio (22nd May), International Museum Day & award of Rotary Excellence Award for Gass Forest Museum (18th May), World Environment Day (05th Jun), International Day of Yoga (21st Jun); National campaign on balanced use of fertilizers and region-specific agro forestry (21st Jun); PRAKRITI programme for school and college students: Nature conservation (May), Conservation of genetic resources (10th Jun), Medicinal products from plants around us (17th Jun).
- ◆ **SUPERANNUATION**: Sh. T. Dhanapal, MTS (May), Sh. R. Natarajan, MTS (June).



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 body 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 : *Rhizophora* species and hybrids by
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