Technical Bulletin
CAFRI/2026/01

# Tree Genomics Research for Strengthening Agroforestry

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**Citation:**

K. Rajarajan, A. Arunachalam and A.K. Handa (2026) Tree Genomics Research for Strengthening Agroforestry. ICAR-Central Agroforestry Research Institute, Jhansi, Uttar Pradesh; 30 Pages.

**Technical Bulletin No.:** CAFRI/2026/01

**Year of Publication:** 2026

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**Published by:**

ICAR-Central Agroforestry Research Institute, Jhansi 284003, Uttar Pradesh

**Printed at:**

Classic Enterprises, Jhansi 284003
7007122381, 9415113108

त्रिलोचन महापात्र
अध्यक्ष
पौधा किस्म और कृषक अधिकार संरक्षण प्राधिकरण
(संसद के अधिनियम द्वारा निर्मित सांविधिक निकाय)
कृषि एवं किसान कल्याण मंत्रालय
भारत सरकार

सत्यमेव जयते

**Trilochan Mohapatra**
**Chairperson**
**Protection of Plant Varieties and Farmers' Rights Authority**
(A Statutory body created by an Act of Parliament)
Ministry of Agriculture and Farmers Welfare
Government of India

## FOREWORD

Agroforestry has become the cornerstone of sustainable land-use systems in India, contributing immensely to farm income diversification, security of raw material supplies for wood-based industries, and ecological stability amidst changing climatic conditions. However, traditionally, the genetic improvement of tree species has been restricted due to their long generation cycles, complex genomes, and limited molecular resources. Against this background, modern molecular biology, genomics, and transcriptomics offer all-round opportunities for accelerating tree improvement and strengthening research in agroforestry.

The present technical bulletin, "Tree Genomics Research for Strengthening Agroforestry", presents the pioneering efforts of ICAR–Central Agroforestry Research Institute, Jhansi, in generating and applying genomic knowledge for key agroforestry tree species. It puts together important studies on whole-genome sequencing, transcriptome profiling, gene expression analysis, and functional marker discovery in economically important species such as *Melia dubia, Pongamia pinnata, Azadirachta indica*, and *Tectona grandis*. The genomic and molecular information provided in this bulletin serves as a strong scientific platform for both precision breeding for stress-resilient trees and for the optimization of the entire value chain. It should contribute to sustainable and climate-resilient agroforestry and serve national agroforestry strategies in the country.

I am sure that this publication would remain a very useful resource for researchers, students, policymakers, as well as developmental institutions, and would even further enhance ICAR-CAFRI's leadership role in the area of agroforestry genomics research in India.

Date: 12th February, 2026

(T. Mohapatra)

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# Preface

The recent developments in the field of high-throughput sequencing technology have completely changed the landscape of plant biology by virtue of the ability to thoroughly analyze the genomes and gene expression networks of plants. Realizing the power of these technologies in the improvement of agroforestry, the ICAR-CAFRI, Jhansi, has started research on the analysis of the molecular determinants of the aforementioned factors in important tree species.

This technical booklet brings out the integrated view of the tree genomic studies that are being pursued at the ICAR-CAFRI. It includes the draft nuclear genome of *Melia dubia*, the transcriptome analysis of drought tolerance in *Pongamia pinnata,* the identification of functional SNPs linked to the production of azadirachtin in neem, the gene expression analysis pertaining to fast growth and maturity stage of *Melia dubia*, and the gene-specific defense reaction to defoliator attack in teak.

In each case, the importance of successfully applying molecular data to practical ends such as marker-assisted selection, precise harvest scheduling, the development of stress-resistant genotypes, and efficient plantation management is brought out. A focus on regulatory genes, pathways, and markers with direct application to tree improvement and conservation activities has been maintained.

The primary aim of this bulletin is to provide a vital source of knowledge for scientists, breeders, students, and extension personnel involved in agroforestry and forestry-related research. Additionally, it is believed that it will help to establish genome-assisted approaches in tree improvement programs and encourage more research to combine the concepts of molecular biology with field-related agroforestry systems.

**- Authors**

# Contents

# 1 Background

Agroforestry practices based on tree species are of foremost importance for improving the productivity of agricultural land, thereby ensuring the supply of raw materials for wood-based industries. The generation time of tree species, heterozygosity, and complex genome structure were some of the factors that hindered rapid improvement in the genetic backgrounds of trees using conventional breeding methods. However, the generation period *vis-à-vis* plant response is longer and slower, respectively. In recent times, tree genomic and transcriptomic analyses have been immensely useful for understanding growth processes, stress resistance, wood properties, and adaptability at the molecular level.

The ICAR-Central Agroforestry Research Institute (ICAR-CAFRI) at Jhansi has adopted novel genomic and transcriptomic technologies for some biologically important agroforestry tree species, including *Azadirachta indica, Melia dubia*, *Pongamia pinnata*, and *Tectona grandis*. The utilisation of whole genome sequencing, *de novo* assembly of the transcriptome, gene expression analysis, and validation of gene markers has helped the institute to research and develop some important genomic resources on fast-growing tree species for timber, bioenergy, and multipurpose tree species.

The focus is on key constraints in agroforestry, such as slow growth, vulnerability to abiotic and biotic stresses, suboptimal wood quality, and the lack of molecular markers for tree improvement. These studies lay a strong scientific foundation to link phenotypic traits with underlying gene regulation, thereby enabling precision breeding and informed management decisions in the systematic development of tree-based production systen

# 2 Tree Germplasm Resources for Agroforestry

ICAR-Central Agroforestry Research Institute (CAFRI), Jhansi and the All India Coordinated Research Project on Agroforestry play a pivotal role in the collection, conservation, and utilization of agroforestry tree germplasm across diverse agro-ecological regions of India. The programme maintains extensive germplasm resources of economically and ecologically important tree species through CAFRI and its network of AICRP centres. These resources support genetic improvement, climate resilience, biodiversity conservation, and sustainable land-use systems. Systematic evaluation and characterization of germplasm contribute to enhanced productivity, adaptability, and long-term conservation of agroforestry species.

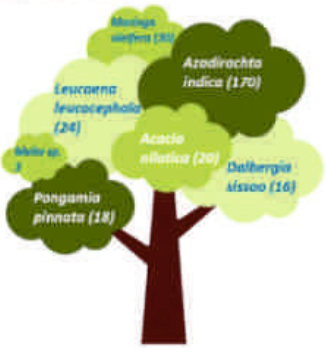
CAFRI conserves approximately 300 germplasm accessions representing diverse agroforestry tree species in its field gene banks and institute collections. Also, AICRP on Agroforestry maintains approximately 300–350 germplasm accessions across multiple agro-climatic regions of India, covering major timber, pulpwood, fruit, and industrial tree species. It is also a strategic national resource for the conservation, evaluation, and use of tree genetic diversity, systematically characterizing the germplasm for growth performance and stress tolerance for wood quality and bioactive compounds to highlight elite and climate-resilient genotypes. It will also be most useful in providing a strong backup for tree improvement programs, molecular characterization, and genomie-assisted breeding. With this background, the conservation and enrichment of diverse agroforestry tree germplasm assume prime importance, and ICAR-CAFRI is playing a lead role in strengthening sustainable agroforestry systems, safeguarding genetic resources, and ensuring long-term raw material security for farmers and wood-based industries against rapidly changing climatic conditions.

# कृषिवानिकी के लिए वृक्ष अनुवंशिक संसाधन
# Tree Genetic Resources for Agroforestry

**Active Tree Germplasm Site @ CAFRI, notified by National Bureau of Plant Genetic Resources, New Delhi**

Tree genetic resources (TGRs) are the heritable materials maintained within and among trees that are of actual or potential economic, environmental, scientific or societal value.
TGRs provide goods and services which are essential for human well-being and sustainable development. Most tree species have high levels of genetic diversity, offering great opportunities for growing, selecting and breeding trees for various purposes.

TGR @ ICAR-CAFRI




Released varieties/germplasms of different species assembled at CAFRI

Neem field gene bank @ CAFRI

PT-2 & PT-6 Shisham genotype registered as superior genetic stock at NBPGR, New Delhi

Pongamia germplasm field @ CAFRI

Acacia germplasm field @ CAFRI

Moringa germplasm field @ CAFRI

RFID to monitor neem germplasm @ CAFRI

# 3 Tree Genomics Research @ CAFRI

Tree genomics research at ICAR-CAFRI represents a significant advancement in modernizing tree improvement programmes in the Indian context.

**Development of the first draft nuclear genome of *Melia dubia* has provided:**

- A valuable resource for gene identification
- Improved resolution of phylogenetic relationships
- Development of congeneric molecular markers

**Identification of tens of thousands of SNPs and SSR markers in *Melia dubia* has enabled:**

- Genetic diversity analysis
- Clone identification
- Marker-assisted selection for enhanced growth rate and improved wood properties

**Transcriptome analyses in *Pongamia pinnata* have revealed molecular mechanisms underlying drought tolerance, including:**

- Genotype-specific transcriptome reprogramming
- Regulation of stress-responsive genes
- Involvement of transcription factors and metabolic pathways

**These findings are crucial for developing climate-resilient planting material, especially for:**

- Rainfed agroforestry systems
- Marginal lands facing increasing water scarcity

**Gene expression studies in *Melia dubia* have demonstrated:**

- Molecular relationships between growth traits, wood processing characteristics, and harvest maturity
- Regulation of genes involved in cellulose and lignin biosynthesis affecting pulp quality

**Biotic stress studies in teak (*Tectona grandis*) have identified key resistance-related genes associated with:**

- Defoliator resistance
- Enhanced understanding of tree–insect interactions
- Development of biotic stress–resistant planting stock

**Collectively, tree genomics research at ICAR-CAFRI:**

- Bridges the gap between phenotype and genotype in agroforestry tree species
- Accelerates tree improvement and molecular breeding programmes
- Supports sustainable wood production and climate adaptation strategies
- Strengthens national genomic resources for conservation and future research on functional genomics

These efforts position ICAR-CAFRI, Jhansi, as a national leader in tree genomics and molecular agroforestry research, contributing directly to resilient agroforestry systems, industrial raw material security, and sustainable forestry development in India.

# 4 Genomic and Transcriptomic Datasets @ CAFRI

The ICAR-Central Agroforestry Research Institute (ICAR-CAFRI), Jhansi, has made significant contributions to global genome resources by generating and depositing high-quality genomic and transcriptomic datasets of major agroforestry tree species in internationally recognized public repositories.

**1. Whole-genome sequencing of *Melia dubia***

- Whole-genome sequence reads of *Melia dubia* were generated at ICAR-CAFRI, Jhansi.
- The dataset has been deposited in the NCBI Sequence Read Archive (SRA) for public access.
- BioProject ID: PRJNA1299751 (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1299751)
- This resource supports gene discovery, comparative genomics, molecular breeding, and improvement of growth and wood quality traits in agroforestry species.

**2. Drought stress–responsive transcriptome of *Pongamia pinnata***

- Transcriptome datasets capturing drought stress responses in *Pongamia pinnata* were developed to elucidate molecular mechanisms of stress tolerance.
- These datasets are publicly available in the NCBI-SRA repository.
- BioProject ID: PRJNA997581 (www.ncbi.nlm.nih.gov/bioproject/PRJNA997581)
- The data are crucial for understanding stress-regulated gene expression and serve as a core resource for genetic and combinatorial genomics aimed at developing climate-resilient tree varieties.

**3. DNA barcode dataset of *Melia composita***

- The complete DNA barcode sequence of *Melia composita* generated at ICAR-CAFRI has been deposited in the Barcode of Life Data Systems (BOLD) database.
- Repository: BOLD Systems (www.boldsystems.org)
- This barcode dataset enhances species identification, molecular taxonomy, and biodiversity assessment of agroforestry tree species.

Overall significance, taken together, these public dataset submissions highlight CAFRI's strong commitment to:

- Open science and data sharing
- Global collaboration in tree genomics
- Advancement of molecular agroforestry and conservation genomics

By depositing datasets in internationally recognized repositories, ICAR-CAFRI enhances the transparency, reproducibility, and long-term usability of its research outputs, thereby supporting future tree breeding, genetic improvement, and conservation programmes at national and global levels.

# 5 Species-level Genomics

**Draft Nuclear Genome of *Melia dubia* Cav.**

The draft nuclear genome of *Melia dubia* was assembled using the Illumina paired-end high-throughput sequencing platform. With the technology, the total amount of raw sequencing data produced was estimated to be around 7.13 Gb, resulting in a mean coverage of ~35×, which was adequate for de novo genome assembly. The estimated assembled genome size was 246.5 Mb, which was relatively small compared to other species of the Meliaceae family. The result of the analysis of the assembled genome quality revealed a high level of genome completeness, in which more than 96% of the core plants genes were found, indicating the ability to capture the functional gene space using the assembled genome. Over 37,000 protein-coding genes were predicted and annotated in the newly assembled reference genome of *Melia dubia*.

**Key Findings**

The genome characterization provided a vast amount of genetic and functional information of ultimate significance for breeding. The information obtained includes over 95,000 single nucleotide polymorphisms, together with various insertions and deletion events, suggesting a remarkably high level of genetic diversity in the genome. Furthermore, over 50,000 simple sequence repeat markers were detected, and they proved extremely useful for analyzing genetic diversity, confirming clones, and making selections using markers. The genome showed a remarkably low level of repetitive DNAs, suggesting a stable and optimized genome. The protein family study indicated the dominance of gene families linked with gene regulation, stress responses, growth regulation, and organelle functions, suggesting adaptability of the species for survival in different environments.

**Table 1. Overview of repeat elements in the Melia genome**

| | |
|---|---|
| Sequences | 50,762 |
| Total length | 2,06,44,0527 bp |
| bases masked | 6,29,7141 bp (3.05 %) |
| | Number of elements |
| SINE | 99 |
| LINE | 334 |
| LTR elements | 139 |
| DNA elements | 147 |

| | |
|---|---|
| Low_complexity | 24,638 |
| Simple_repeat | 1,12,341 |
| Satellite/centr | 29 |
| rRNA | 232 |
| snRNA | 807 |
| tRNA | 690 |
| Unclassified | 11 |

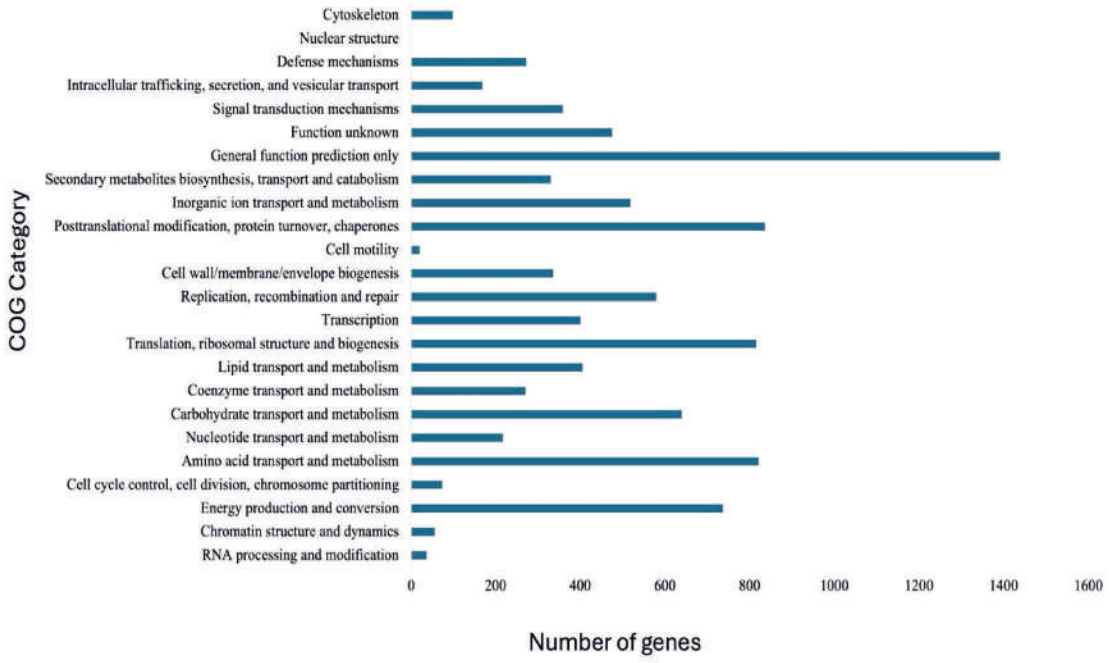



**Figure 1. Gene ontology category of the *M. dubia* genome**

## Phylogenetic Analysis

The result of the genome-based phylogeny of *Melia dubia* has been found to place *Melia dubia* in the family Meliaceae without ambiguity, and its very closest evolutionary relationship has been found to be with *Melia azedarach* and *Khaya senegalensis*. The application of high-quality genome information to draw evolutionary relationships offers much stronger evolutionary interpretations than have been possible by conventional morphological or gene similarity measures to draw evolutionary relationships of closely related species of any genera. The accurate specification of evolutionary differences and evolutionary divergences of any particular genera Melia would never be threatened by applying genome information on evolutionary

relationships of closely related species of genera Melia. The application of genome information on evolutionary relationships of closely related species of genera Melia has been found to provide very important information regarding common adaptive abilities, evolutionary relationships, and feasibility of comparisons of closely related species of genera Melia, which would be of much help in breeding lines to improve yield and evolutionary divergences.

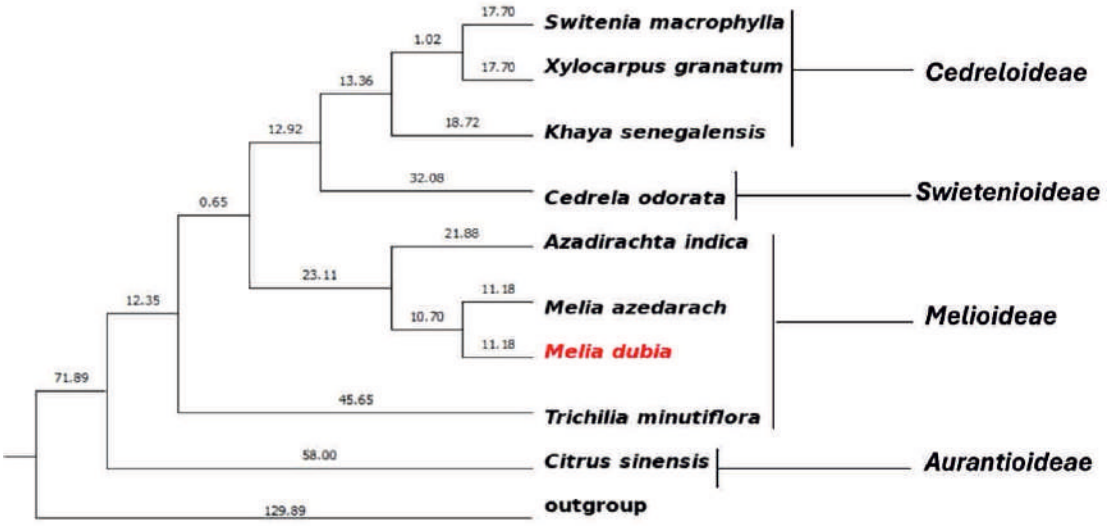



**Figure 2. Phylogenetic relationship among the species of the Meliaceae family based Neighbor-Joining alignment in MEGAX**

## Genomic Insights and Future Prospects

The production of such a quality draft genome for *Melia dubia* sheds significant light on genome composition as well as placing it along the course of evolution for the Meliaceae family of genomes. Gene annotation, repeat elements, genome-wide variation, as well as proper phylogenetic studies, play significant roles in determining such genomes for improvement. The numerous SSR markers present notable applications for genetic diversity analysis as well as facilitating marker-assisted technology for improvement. In general, it can be noted that such a genome will play significant roles in genetic improvement for agroforestry and sustainable forestry development using *Melia dubia*.

## How *Pongamia pinnata* Responds to Drought: A Transcriptome Perspective

### Transcriptome Assembly

For revealing the mechanism of drought tolerance in *Pongamia pinnata*, a de novo transcriptome assembly was created based on the leaves of two genotypically contrasting accessions: NRCP9 (droughttolerant) and NRCP10 (drought-sensitive)

under well-watered and water-drought treatments. RNA was isolated from both water-drought-treated and control samples. High-quality RNA-seq libraries were constructed and sequenced on Illumina paired-end platform with a read length of 2 × 150bp. The transcriptome sequencing generated a total amount of high-quality data at a remarkable 53.47 GB. The assembly of transcripts was done by the SPAdes assembler. The assembly was optimal at a k-mer value of 55. A total of 104,938 non-redundant transcripts with N50 = 1301 bp and a maximal transcript size of 15,653 bp was generated. The average size is about 200bp, which is a notable assembly.

High read alignment efficiency confirmed assembly quality, where mapping percentages were greater than 98% in both the control and drought-treated samples. These results indicate that the assembled transcriptome is a reliable representation of the expressed gene space of *Pongamia pinnata* under contrasting moisture regimes and thus forms a good basis for downstream differential expression and functional analyses.

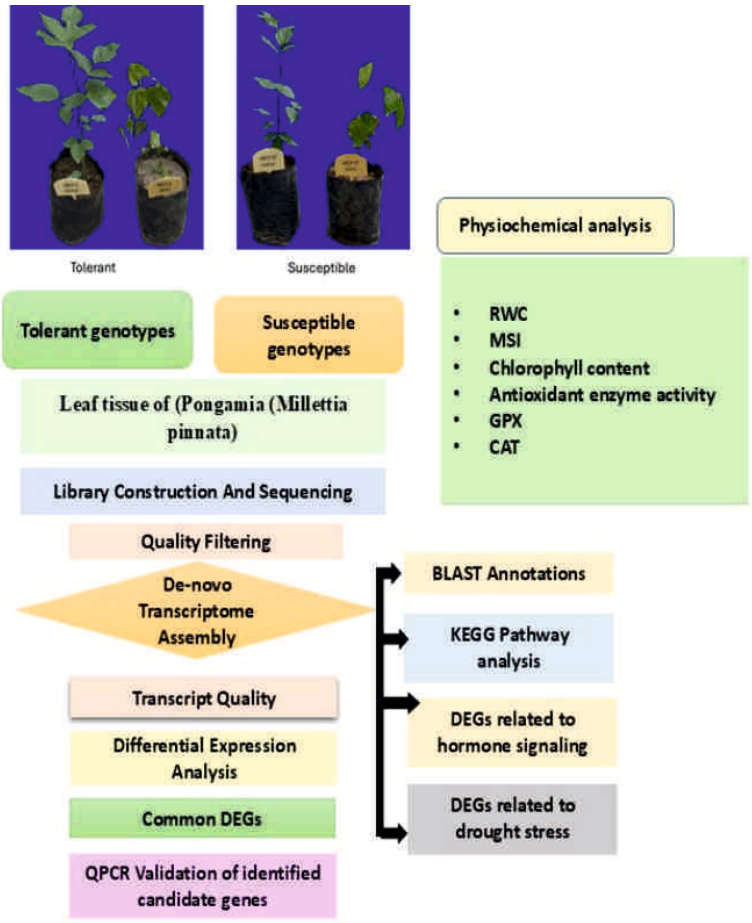
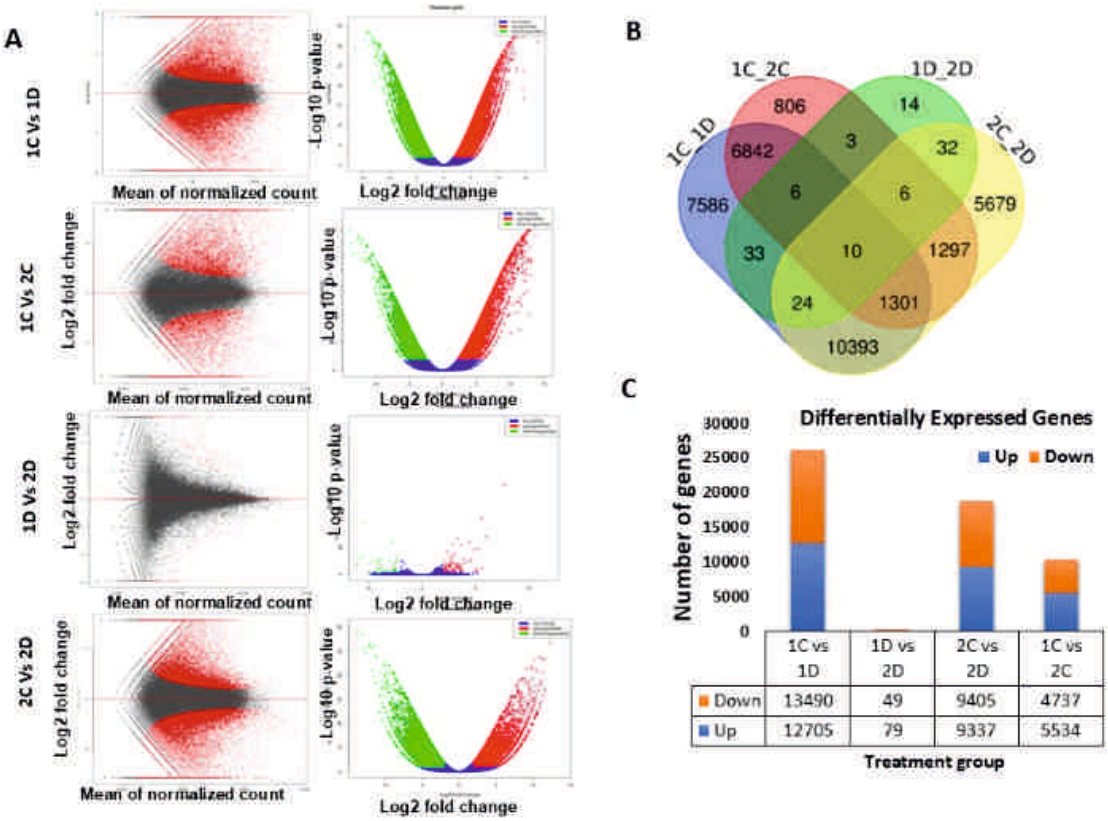



**Figure 3. Transcriptome analysis pipeline used for identification of DEGs related to drought stress in Pongamia**

## Differential Expression Analysis

The analysis of differential gene expression was conducted to explore drought-regulated genes related to tolerance and susceptibility. The reads were normalized, and differentially expressed genes (DEGs) were obtained with the help of the DESeq algorithm. The criteria used were highly stringent: |log2 fold change| ≥ 2 and the adjusted p-value ≤ 0.01. A great many differentially expressed genes were obtained among treatments and genotypes. A remarkable 26,195 and 18,742 differentially expressed genes were identified in the tolerant and susceptible genotype, respectively. Among these, 128 differentially expressed genes were shared during drought stress. The finding indicated the existence of a common set of drought-regulated genes for the genus *Pongamia*. The differentially expressed genes were more upregulated in the




| | 1C vs 1D | 1D vs 2D | 2C vs 2D | 1C vs 2C |
|---|---|---|---|---|
| Down | 13490 | 49 | 9405 | 4737 |
| Up | 12705 | 79 | 9337 | 5534 |

**Figure 4. A) MA Plot and Volcano Plot: The MA plot shows the relationship between mean expression (x-axis) and log fold change (y-axis) of genes representing p-value from the statistical test of the comparison. Blue, red and green color depict non-significant (padj > 0.01), upregulated (padj <= 0.01 and fold change >=+2) and downregulated (padj <=0.01 and fold change >=2) respectively. B) Differentially Expressed Genes (DEGs): The plot shows the total number of upregulated and downregulated genes between experimental groups. In DEGs, we choose 2 Foldchange and padjusted 0.01 as a threshold using DESeq algorithm. C) Total Number of Upregulated and Downregulated Genes: The table/plot shows the total number of upregulated and downregulated genes per group, with statistical significance based on an adjusted p-value threshold.**

tolerant genotype during drought stress for osmotic adjustment, antioxidant responses, water transport, and cell wall modification. Conversely, the differentially expressed genes were more expressed in the susceptible genotype during drought stress for stress damage. The volcano plot and MA plot provided a clear representation among the tolerant and susceptible genotypes of drought stress. The plot indicated the differences in expression between the two genotypes with regard to drought stress. These findings indicate that transcriptional plasticity and efficient stress signaling play a crucial role in drought tolerance in *Pongamia pinnata*.

**Functional Annotation**

DEG functional annotation was conducted employing the BLASTX tool with the NCBI-NR database, followed by functional annotation using the UniProt, KEGG, COG, and Plant Transcription Factor databases. Functional annotation of the DEGs identified a total of 47,889 transcripts that were divided into Gene Ontology categories such as biological processes, molecular function, and cellular components.

GO terms showed that drought-responsive genes were mainly categorized into stress response, metabolic processes, catalytic activity, binding, and cell organization. The

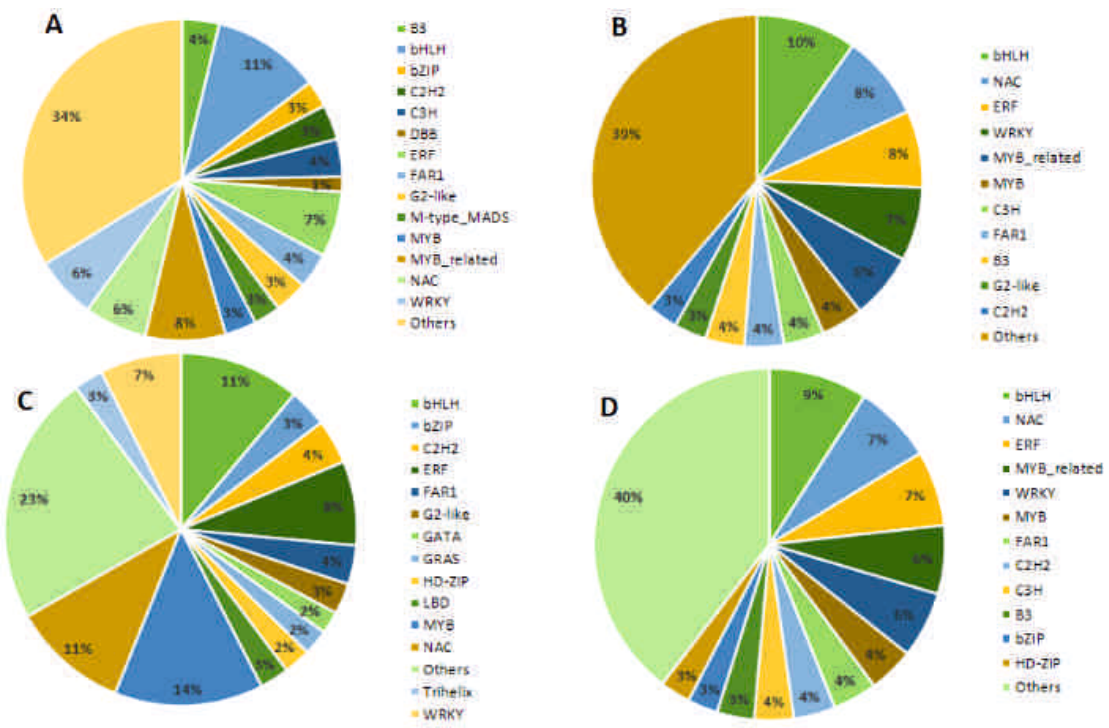



**Figure 5. Distribution of TF families in comparison between the genotypes under the drought stress. A. up-regulated in the NRCP9, B. down-regulated in the NRCP10, C. down-regulated in the NRCP9 and D. down-regulated in the NRCP10**

tolerant genotype, NRCP9, had stronger associations between its drought-responsive genes and functions like antioxidant activity, membrane stabilization, and metabolic regulation, while the susceptible genotype had fewer drought-responsive gene associations with stress-related functions.

**Pathway Analysis**

KEGG pathway analysis revealed 157 metabolic and signaling pathways enriched with 2146 drought-responsive genes. The most important pathways associated with drought tolerance included secondary metabolite biosynthesis, hormone signaling, MAPK signaling, carbon metabolism, and cofactor metabolism. More interestingly, secondary metabolite biosynthesis was among the major enriched pathways in response to drought; most of its 124 constituent genes were upregulated, indicating that it is a relevant path participating in the drought stress of *Pongamia pinnata*. This supports the hypothesis that increased antioxidant capacity contributes to protection against ROSmediated oxidative damage. These metabolites are involved in scavenging ROS, protection of cell membranes, and intra- and intercellular signaling under water

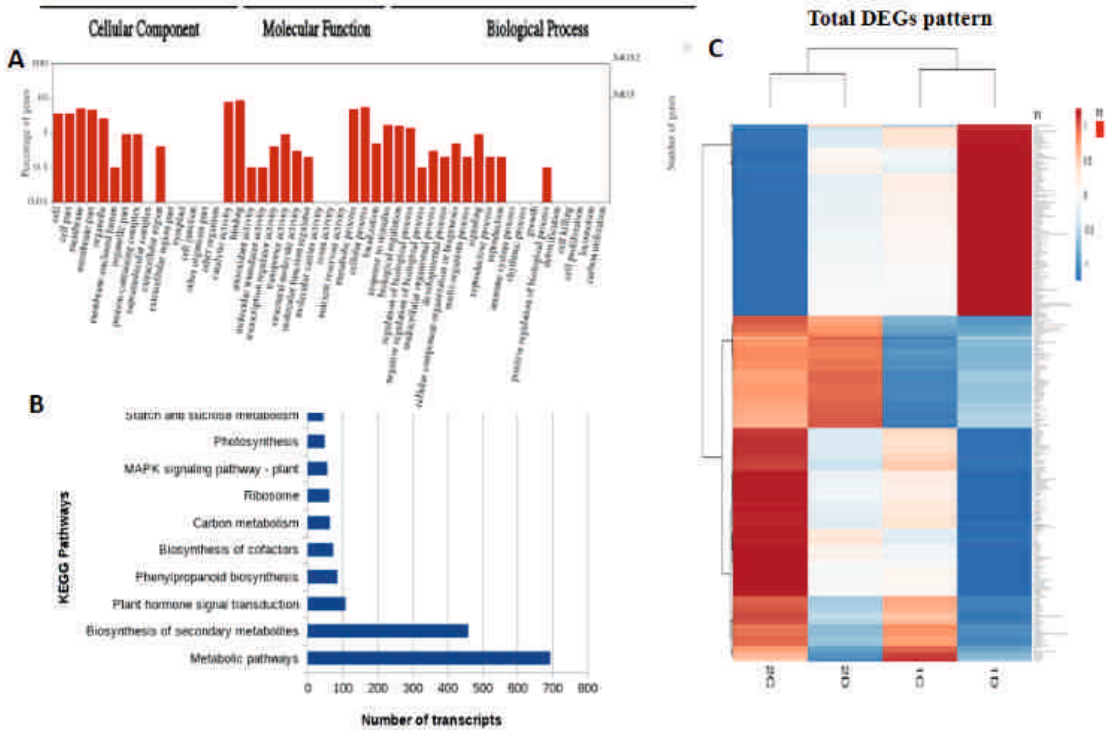



**Figure 6. (A) Gene Ontology (GO) enrichment analysis of differentially expressed genes (DEGs), highlighting the most significantly enriched biological processes, molecular functions, and cellular components. (B) KEGG pathway enrichment analysis of DEGs, showing the top enriched metabolic and signalling pathways. (C) Heat map depicting the expression profiles of DEGs between drought-tolerant (NRCP 1C & 1D) and drought-sensitive (NRCP10 2C & 2D) genotypes, with upregulated and downregulated genes represented by distinct color gradients.**

deficiency conditions. Aquaporin (PIP family), magnesium transporter genes (MGT), UDP-glycosyltransferases (UGT), heat shock proteins, and photosynthesis-related protein genes were differentially expressed, thus reflecting the coordinated regulation of water transport, ion homeostasis, protein stabilization, and energy metabolism. At the same time, ABA-dependent and independent signaling pathways were implicated, pointing out a complex and integrated network operating in *Pongamia pinnata* under drought stress conditions.

**Key Takeaway Message**

*Pongamia pinnata* shows drought stress adaptation through strong transcriptional reprogramming. The drought-tolerant genotype, NRCP9, switches on genes related to transporters of water, antioxidant defense, osmotic adjustment, secondary metabolite biosynthesis, and abiotic stress responsive signalling, whereas the susceptible genotype showed limited adaptive gene regulation. This integrated transcriptome analysis depicts that efficient regulation of transcription factors, metabolic pathways, and hormone-mediated signalling underpins drought tolerance in Pongamia, thereby serving as a valuable molecular target for climate resilient plantation development and future tree improvement programmes.

# 6 Consolidated Review of Genetic and Genomic Advances in Selected Multipurpose Trees

**Genomic Advances in Indian Sandalwood (*Santalum album* L.)**

ICAR–Central Agroforestry Research Institute (CAFRI), Jhansi, has undertaken a comprehensive and systematic review of the genome status of Indian sandalwood (*Santalum album* L.), synthesizing global advances in genome sequencing, molecular markers, transcriptomics, and functional genomics to support sustainable improvement of this high-value species.

The review consolidates evidence from draft and chromosome-level genome assemblies (~207–230 Mb), reporting more than 23,000–38,000 predicted protein-coding genes with high completeness (>94% BUSCO). These genomic resources have clarified key biosynthetic pathways governing heartwood formation and essential oil production, particularly the role of terpene synthase (TPS), cytochrome P450 (CYP736A167), MYB, WRKY, NAC, and SAUR gene families. Comparative transcriptomic studies between high- and low-oil yielding genotypes were critically examined, highlighting differentially expressed genes associated with α- and β-santalol biosynthesis and stress resilience.

The review further evaluated molecular marker-based diversity assessments, including SSRs, SNPs, RAPD, and RFLP markers, demonstrating substantial within-population variability and strong geographic differentiation across native and introduced populations. CAFRI emphasized the importance of integrating SNP-based high-throughput genotyping, Genotyping-by-Sequencing (GBS), GWAS, and genomic selection approaches to accelerate breeding cycles and enhance genetic gain.

Importantly, the review identified critical research gaps: limited functional validation of candidate genes, absence of CRISPR-based genome editing studies, inadequate early biomarkers for oil yield prediction, and restricted translation of genomic tools into operational breeding programs.

Based on this synthesis, ICAR–CAFRI recommends an integrated genomie-assisted breeding framework combining genome-wide markers, transcriptomic profiling, host–parasite interaction studies, stress physiology, and silvicultural optimization. Such a multidisciplinary strategy will enhance oil productivity, climate resilience, and conservation of genetic diversity, thereby strengthening sandalwood-based agroforestry systems in India.

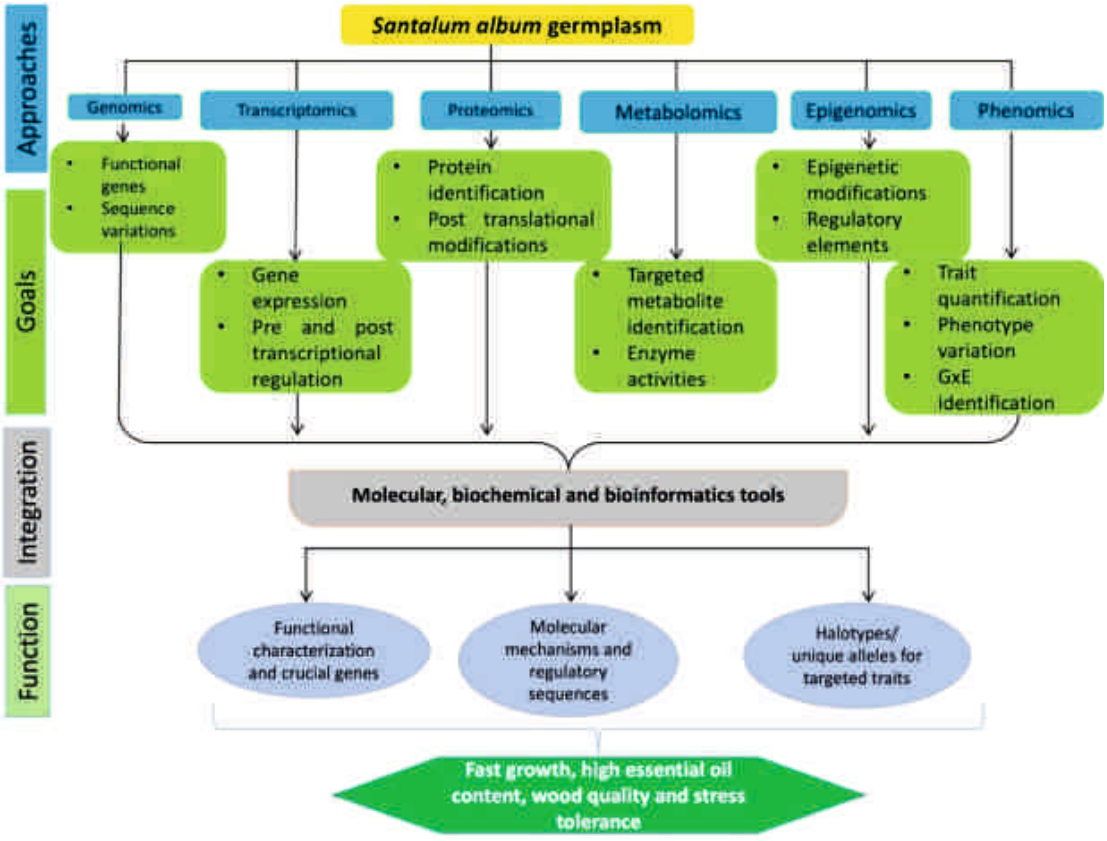



**Figure 7. Integrated genomics approach for trait improvement in *S. album***

## Integrated Genomics Research for Tree Improvement

ICAR–Central Agroforestry Research Institute (CAFRI), Jhansi, has systematically reviewed recent advances in genetic and genomics research in tree species with relevance to forestry and agroforestry systems. This synthesis integrates progress in molecular markers, population genomics, transcriptomics, and emerging breeding tools, offering a comprehensive understanding of how genomics is reshaping tree improvement programs.

The review highlights the transition from classical marker systems (RAPD, AFLP, ISSR) to robust, high-resolution SSR and SNP markers, enabling precise assessment of genetic diversity, population structure, and provenance differentiation. Across long-rotation tree species, molecular studies consistently revealed high within-population variability and strong genotype–environment interactions, underscoring the importance of region-specific selection and conservation strategies.

Genomic and transcriptomic resources have significantly enhanced insights into

complex traits such as growth rate, wood quality, oil biosynthesis, stress tolerance, and disease resistance. The review critically examined the role of next-generation sequencing (NGS) approaches—RNA-Seq, de novo genome assembly, and comparative genomics—in identifying candidate genes, regulatory networks, and metabolic pathways governing economically important traits. Emphasis was placed on transcription factor families (MYB, NAC, WRKY, bHLH) and stress-responsive genes that serve as potential molecular targets for genetic improvement.

Importantly, CAFRI's analysis identified major bottlenecks limiting field-level application of genomics: limited functional validation of candidate genes, poor integration of genomic data with phenotypic trials, and minimal adoption of genomic selection in tree breeding pipelines. The review stressed that without structured phenomics, long-term field validation, and breeder-friendly decision tools, genomic resources remain underutilized.

Based on this systematic evaluation, ICAR–CAFRI advocates an integrated genome-assisted tree improvement framework combining SNP-based selection, transcriptome-guided trait discovery, early-stage molecular screening, and climate-resilient breeding objectives. Such an approach is critical for accelerating genetic gain, enhancing productivity, and ensuring sustainability of agroforestry and forestry systems under changing climatic conditions.

**Table 2. Genome sequencing efforts of the selected important tree species**

| Tree | NGS platform used for whole-genome sequencing | Genome size (Mb) | Contigs | Scaffolds | Genes | ESTs/ SSRs |
|---|---|---|---|---|---|---|
| *Populus trichocarpa* | Short gun sanger sequencing | 434 | 1446 | 1446 | 44976 | 19989 |
| *Tectona grandis* | Illumina Hiseq 2000 and Oxford nanopore MinION | 317 | 3500 | 3004 | 36172 | 182712 (SSRs) |
| *Azadirachta indica* | Illumina TruSeq and IonTorrent Personal Genome Machine | 364 | 9714 | 9714 | 30236 | 552 for terpenoid biosynthesis |
| *Eucalyptus grandis* | Short gun sanger sequencing | 691 | 4952 | 4943 | 36376 | 19996 |
| *Casuarina equisetifolia* | PacBio RSII Single Molecule Real Time (SMRT) sequencer and Illumina HiSeq | 2000 | 300 | 6611 (>100bp) | 2936 (>100bp) | 31818 n/a |
| Salix sp. | Roche 454 and Illumina Hiseq 2000 | 450-485Mb | - | - | 26599 | - |

# 7 Trait-specific Genomic Analysis

**Functional SNPs Controlling Azadirachtin Biosynthesis in Neem**

The present investigation explains the molecular mechanism underlying the differences in azadirachtin accumulation in neem (*Azadirachta indica*) using the identification of functional single nucleotide polymorphisms (SNPs) in the candidate genes of the azadirachtin biosynthetic pathway. A comparison between the high azadirachtin-yielding genotype (VKAF-75) and the low azadirachtin-yielding genotype (VKAF-99) indicated a significantly higher frequency of SNPs in the low azadirachtin-yielding genotype, specifically in the genes directly involved in the biosynthesis of triterpenoids. The genes encoding terpene cyclase (oxidisqualene cyclase), squalene monooxygenase, cytochrome P450 monooxygenases, and NADPH-cytochrome P450 reductase showed a prominent presence of nonsynonymous, missense, stop-gain, and splicing mutations in the form of SNPs in the VKAF-99 genotype, which were found to possess significantly higher numbers of synonymous and functionally silent SNPs in the VKAF-75 genotype. These functional SNPs would supposedly affect the structures of these enzymes and decrease the catalytic activities, thus resulting in the reduced accumulation of azadirachtin. In contrast, the genetic integrity of these biosynthetic genes in VKAF-75 supports efficient metabolite synthesis. Overall, the study provides the first clear gene-level evidence linking functional SNP variation with azadirachtin yield in neem. The identified SNPs constitute valuable molecular markers for genetic diversity assessment, marker-assisted selection, and improvement of high-azadirachtin neem genotypes, thereby strengthening molecular breeding and conservation strategies for this economically important tree species.

This study offers the first evidence of a relationship between morphological traits related to plant growth and gene expression patterns of key candidate genes involved in fast-growing processes in *Melia dubia* plantations. Measurements of tree height, breast height diameter (DBH), and tree volumes in three different ages of tree stand have shown an apparent variation in tree growth, with a pronounced increase in tree height during initial phases and greater increments in tree diameter and volumes during later phases of tree life. Targeted gene expression analyses involving four candidate genes involved in plant growth, namely PXY, CLE41, ACS-1, and Hb1, have shown age-related gene transcription related to cambial zone activity and wood development in plants. Reduction and suppression of PXY gene transcription together with a steady activation of CLE41, ACS-1, and Hb1 gene transcripts indicate a

synergism involved in regulating vascular tissue division, ethylene-induced secondary growth processes, and seedling vitality. Overall, the integration of growth trait analysis with gene expression profiling establishes a molecular framework for fast-growth regulation in *Melia dubia*. These findings provide a valuable foundation for genetic improvement, selection of superior planting material, and development of short-rotation cultivars, thereby strengthening *Melia dubia*–based agroforestry and wood-based industrial systems under changing climatic conditions.

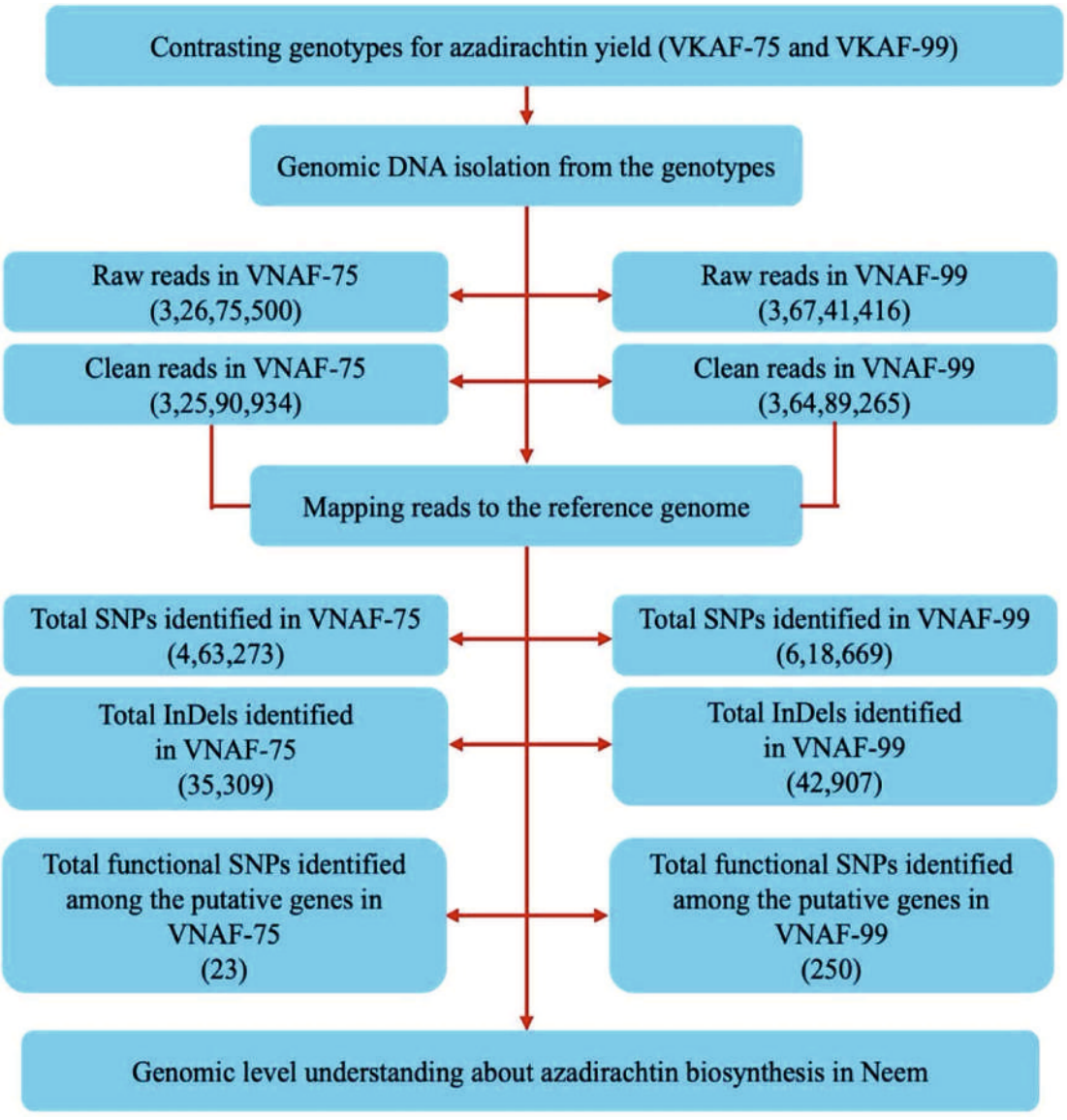



**Figure 8. Flowchart of SNPs identification among the contrasting genotypes for azadirachtin yield**
**Molecular Basis of Fast Growth in *Melia dubia***

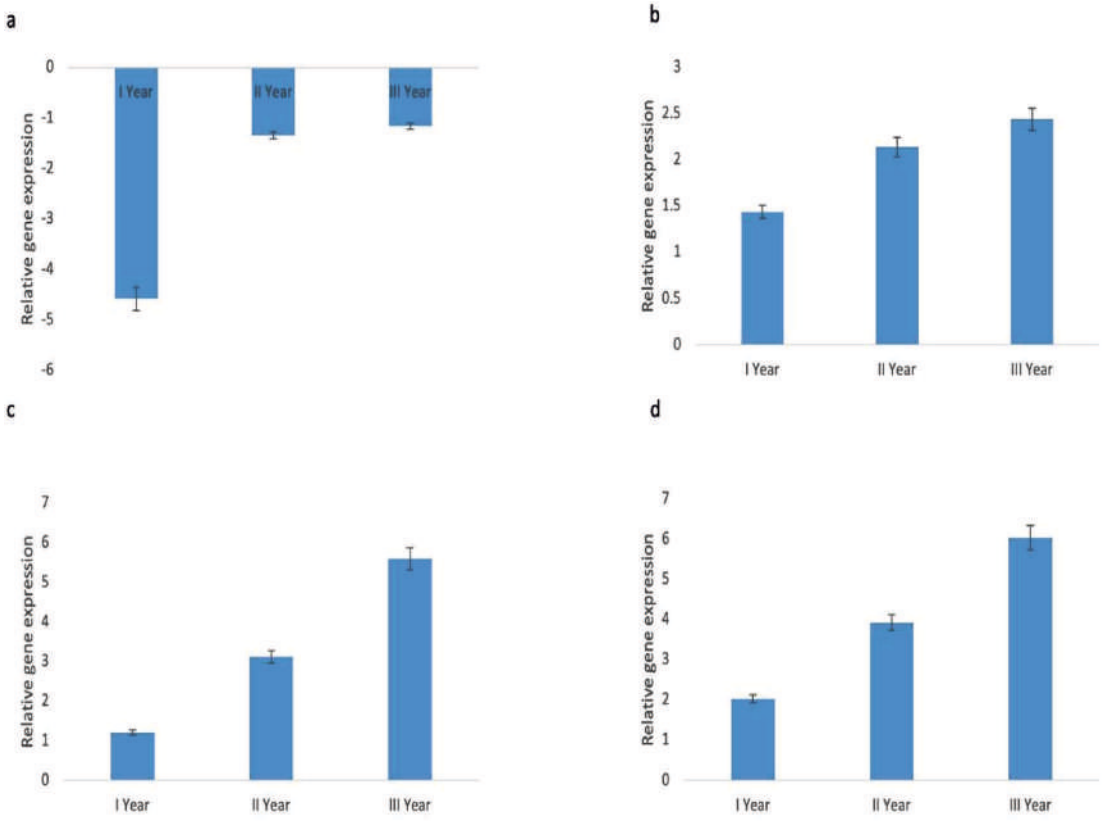



**Figure 9. Relative gene expression pattern of selected candidate genes in different age of *Melia dubia*. 2a. Differential expression of PXY (Phloem intercalated with xylem) gene, 2b. Differential expression of CLE41 (Clavata3/embryo surrounding related 41) gene, 2c. Differential expression of ACS-1 (1-aminocyclopropane-1-carboxylic acid synthase) gene and 2d. Differential expression of HB1 (Haemoglobin 1 like protein) gene.**

## Gene-Regulated Determination of Optimal Harvesting Stage in *Melia dubia*

This study clearly proves that diameter at breast height-dependent regulation of cellulose and lignin biosynthesis genes controls pulpwood quality in *Melia dubia*. Within the DBH classes tested, which were 10 cm, 15.0 cm, and 20 cm, the 15.0 cm DBH class showed the best balance between cellulose accumulation and lignification, presenting superior pulp and paper properties. Gene expression analysis showed a strong upregulation of cellulose synthase genes Ces2 and Ces4 in the 15.0 cm DBH class, with Ces2 showing a 4.9-fold increase and Ces4 showing a 15.6-fold increase, which correlated well with the highest cellulose content of 69.6% and maximum tear index of 4.9. Thus, improved cellulose biosynthesis during this growth phase directly influences the better strength and yield of pulp.

On the other hand, lignin biosynthesis genes CAD2 and LAC8 showed highest expression in the 20.0 cm DBH class, with CAD2 (7.1-fold) and LAC8 (3.5-fold)

expression closely linked to higher lignin content (24.8%), increased kappa number, and reduced pulping efficiency. These findings were further confirmed by correlation analysis that Ces2 and Ces4 expressions were positively associated with cellulose content, while CAD2 and LAC8 showed strong positive correlations with DBH and lignin accumulation.

Collectively, the differential expression profiles of Ces2, Ces4, CAD2, and LAC8 provide a molecular basis for DBH-driven harvesting decisions in *Melia dubia*. Indeed, the present results clearly determine the 15.0 cm DBH class as the key stage at which harvesting would be optimal for pulpwood, meeting the expectation of high cellulose synthesis with reduced lignification. These gene-based findings represent a sound scientific basis on which precision harvesting, marker-based selection, and improvement of *Melia dubia*–based pulpwood agroforestry systems could be well conceptualized.

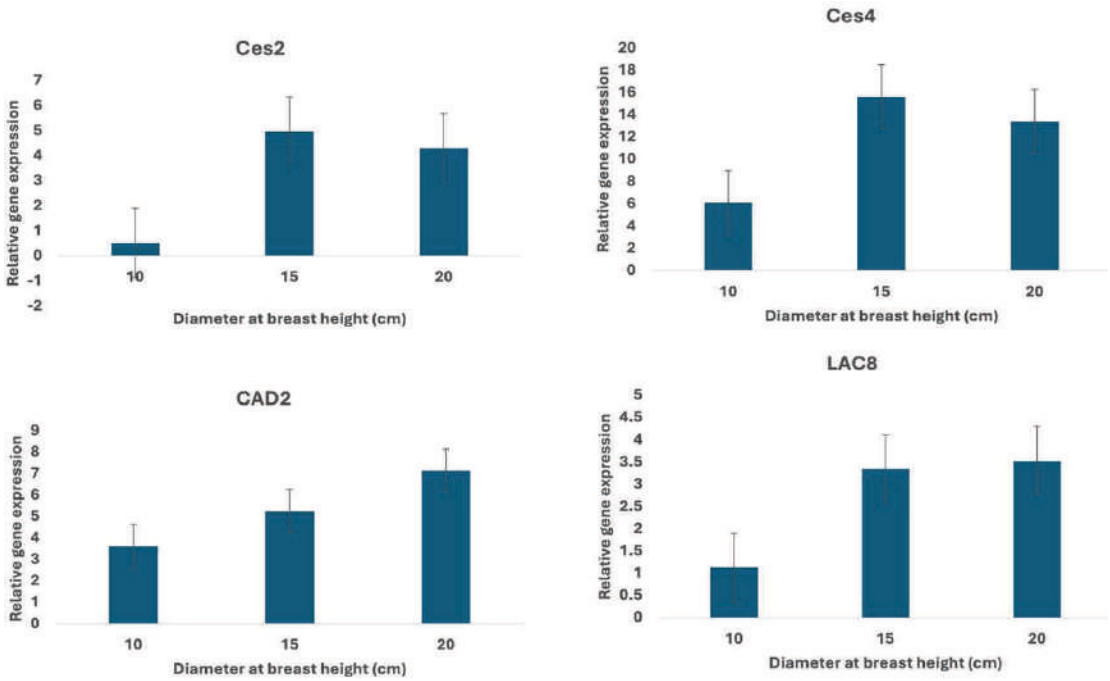



**Figure 10. Differential gene expression of selected genes under different DBH**

## Defense Responses of Teak to Defoliator Infestation

This study describes the molecular defense mechanisms of *Tectona grandis* against the infestation of defoliator insects by profiling the expression of some key defense-related genes and enzymes. Defoliator damage resulted in a significant induction of genes associated with plant defense signaling, secondary metabolite biosynthesis, and

oxidative stress regulation, reflecting an active biochemical and molecular response to herbivory. Enhanced expression of the gene encoding phenylalanine ammonia-lyase (PAL) was reported, reflecting the activation of the phenylpropanoid pathway, which plays a central role in the biosynthesis of lignin and phenolics involved in insect deterrence. Increased activities of peroxidase (POD) and polyphenol oxidase (PPO) genes further suggest reinforcement of cell walls and production of toxic quinones, which limit insect feeding and tissue damage.

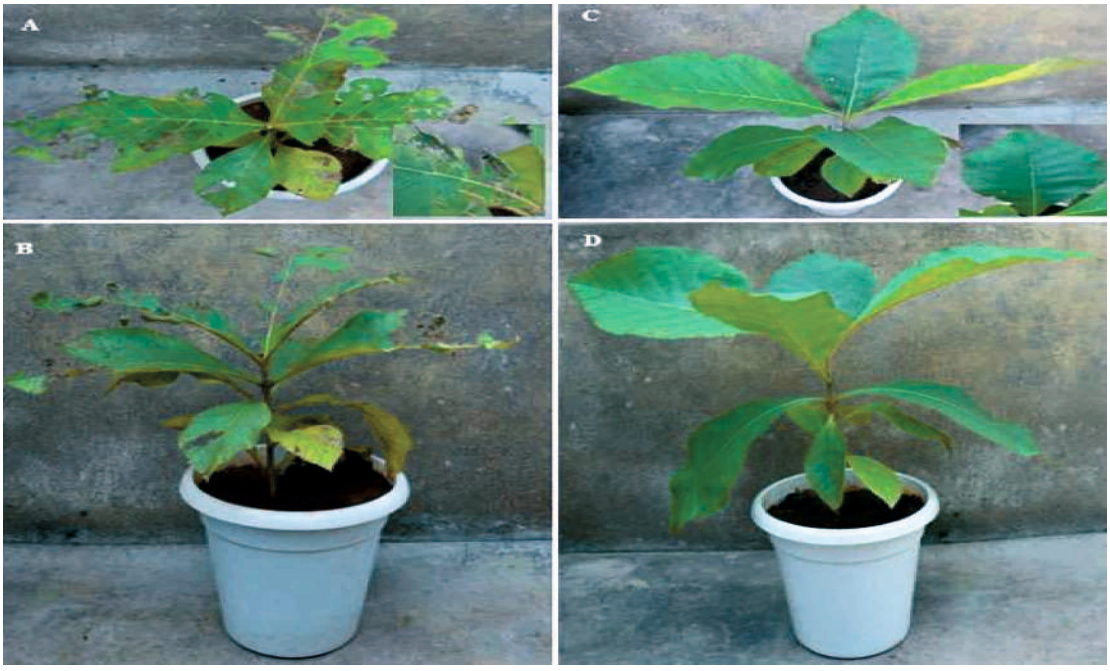



**Figure 11. Teak seedlings infested by defoliator. A. T3 (9 days), B. T2 (6 days), C. T1 (3 days) and uninfested D. Control plants in pots**

Furthermore, the expression of the gene encoding lipoxygenase (LOX) was also highly induced, underpinning the role of the jasmonic acid-mediated defense response, a major signaling molecular event that occurs in plant-insect interactions. High expression of the genes encoding superoxide dismutase (SOD) and catalase (CAT) revealed the effective elimination of the toxic effects of reactive oxygen species due to defoliator stress.

Together, the coordinated induction of PAL, PPO, POD, LOX, SOD, and CAT provides a strong molecular defensive mechanism in teak against insect defoliators. These molecular findings at the gene level form a sound scientific framework for the search for tolerant genotypes, the establishment of physiological and molecular markers, and the strengthening of teak defensive and improvement programs with increased biotic stress imposition.

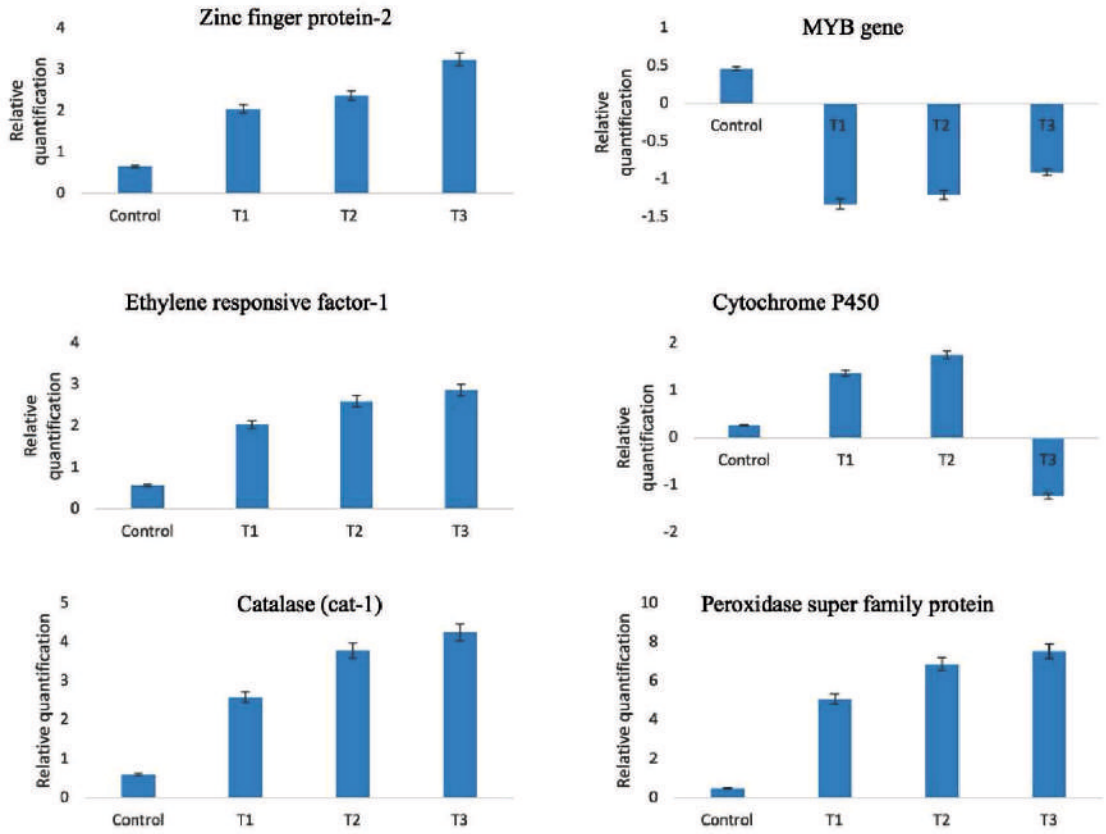



**Figure 12. Relative fold change of selected candidate genes by teak defoliator infestation at different stress intensity.**

# 8 New Knowledge to Tree Genomics

**Reference Genome Resource for *Melia dubia***

**Whole-Genome Sequencing Initiative**

ICAR-CAFRI successfully generated whole-genome sequence reads of *Melia dubia*, a fast-growing multipurpose timber species widely used in agroforestry and pulpwood systems.

Public Repository: NCBI Sequence Read Archive

BioProject ID: PRJNA1299751 (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1299751)

**Scientific Value**

- Gene discovery and functional annotation
- Comparative genomics among Meliaceae members
- Marker development for molecular breeding
- Genomic selection for growth, wood quality, and biomass traits

This represents one of the important genomic resource developments for an emerging Indian agroforestry tree species.

**Drought-Responsive Transcriptome of Pongamia pinnata**

**Climate-Resilience Genomics**

To decode molecular adaptation under water deficit conditions, CAFRI developed transcriptome datasets capturing drought stress–responsive gene expression in Pongamia pinnata, a biofuel and multipurpose tree species.

Public Repository: NCBI Sequence Read Archive

BioProject ID: PRJNA997581 (www.ncbi.nlm.nih.gov/bioproject/PRJNA997581)

**Research Implications**

- Identification of stress-responsive genes and regulatory pathways
- Network analysis of drought tolerance mechanisms
- Discovery of candidate genes for climate-resilient breeding
- Integration with combinatorial and systems genomics approaches

This resource supports the development of next-generation, stress-resilient agroforestry genotypes under changing climatic scenarios.

**DNA Barcode Resource for *Melia composita***

**Molecular Taxonomy and Species Authentication**

ICAR-CAFRI generated and deposited a complete DNA barcode dataset for Melia composita, strengthening molecular identification frameworks for agroforestry species.

Repository: Barcode of Life Data Systems (www.boldsystems.org)

**Strategic Importance**

- Accurate species authentication
- Molecular taxonomy and phylogenetic studies
- Germplasm characterization
- Biodiversity assessment and conservation planning

Collectively, these datasets signify a major step toward strengthening genomic resources for Indian agroforestry species.

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# नीम के उपयोग और महत्व

भाकृअनुप-केन्द्रीय कृषिवानिकी अनुसंधान संस्थान, झाँसी उ.प्र.

## परिचय

- स्थानीय नाम : नीम , निम, निंब, बाल-निंबा।
- नीम की उत्पत्ति भारत और वर्मा से हुई है।
- 3500 ऊचाई से नीचे किसी भी मिट्टी और जलवायु में उगाया जाता है।
- नीम के उत्पादन के लिए महत्वपूर्ण राज्य उत्तर प्रदेश, तमिलनाडु, कर्नाटक, मध्य प्रदेश महाराष्ट्र, आंध्र प्रदेश, तेलंगाना और गुजरात है।
- देश में लगभग 14 मिलियन से ज्यादा नीम के पेड़ हैं।
- नीम का पेड़ औसतन 8 मीटर ऊंचा होता है और लगभग 350 किलो सूखे पत्ते देता है।
- एक पेड़ प्रति वर्ष 31 से 55 किलोग्राम तक निंबोली की उपज दे सकता है।
- नीम के गिरी में लगभग 40% तेल की मात्रा होती है।

## उपयोग

- नीम का उपयोग स्वास्थ्य देखभाल, कृषि और सौंदर्य प्रसाधन में किया जाता है।
- स्वास्थ्य देखभाल में नीम का उपयोग विभिन्न बीमारियों (मलेरिया, पेट खराब, पायरिया आदि) के इलाज के लिए रोगाणु रोधी गुणों के रूप में किया जाता है।
- कृषि में इसका उपयोग विभिन्न कीटों को नियंत्रित करने के लिए जैव कीट के रूप में किया जाता है तथा नीम के बीज का तेल उर्वरक उद्योग में नीम लेपित यूरिया के रूप में उपयोग किया जाता है।
- नीम की लकड़ी का उपयोग कृषि उपकरणों और विभिन्न कॉस्मैटिक के उत्पादन में किया जाता है।

## महत्व

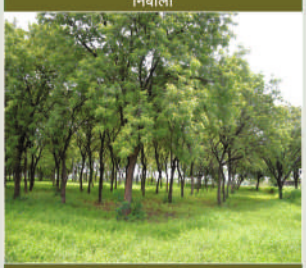
- नीम के पेड के सामाजिक और सांस्कृतिक मूल्य है।
- नीम के पेड़ के सभी भाग उपयोगी है जैसे लकड़ी, पत्ता, नीम का तेल, गोंद, शाखाएं, फूल आदि।
- नीम का पेड़ मुख्य रूप से बीज और लकड़ी के लिए उगाया जाता है। 10 साल का पेड़ 22 से 25 किलो फल दे सकता है। नीम के बीजों का काफी आर्थिक महत्व है। एक टन नीम के बीज को संसाधित करके 1.5 किलोग्राम अज़ाडिरेक्टिन और 780 किलो नीम की खली मिलती है।
- नीम की प्रति ग्राम गुठली में औसतन 2 से 3 मिलीग्राम अज़ाडिरेक्टिन होता है।

## के.कृवा.अ.सं. में अनुसंधान कार्य

- कृषिवानिकी अनुसंधान संस्था में 170 नीम जर्मप्लास्म भारत के विभिन्न राज्यों से एकत्रित करके फील्ड जीन बैंक के रूप में अनुरक्षित किये गये हैं, इनमें से 139 जर्मप्लास्म को नेशनल ब्यूरो ऑफ प्लांट जेनेटिक रिसोर्स (NBPGR), नई दिल्ली के साथ स्वदेशी संग्रह के रूप में पंजीकृत किया गया है।
- कृषिवानिकी अनुसंधान संस्थान में सफलतापूर्वक विभिन्न उद्गम, संतति मार्ग और बीज बाग स्थापित किये जा चुके हैं। नीम रिप्रोडक्टिव बायोलॉजी का भी सफलतापूर्वक दस्तावेजीकरण किया जा चुका है। नीम के आनुवंशिक सुधार को नीम कैटलॉग के रूप में सफलतापूर्वक प्रलेखित किया गया है।
- कृषिवानिकी ने उच्च तेल और अज़ाडिरेक्टिन उपज देने वाले रोगाणु की पहचान की है।
- कृषिवानिकी ने किसानों के लिए नीम आधारित कृषिवानिकी मॉडल विकसित किए है।

नीम का पेड़

निंबोली



नीम का फील्ड जीन बैंक

नीम का बीज और गुठली

नीम का तेल

# सहजन-आधारित बहुआयामी कृषिवानिकी

## भाकृअनुप-केन्द्रीय कृषिवानिकी अनुसंधान संस्थान, झाँसी उ.प्र.

### सहजन : एक परिचय

- वानस्पतिक नाम : *मोरिंगा ओलीफेरा*
- परिवार (कुल) : मोरिंगेसी
- साधारण नाम : सहजन, सुजना और मुनगा
- उत्पत्ति : भारतीय मूल
- कुल प्रयाजियाँ : 13
- अत्यधिक तेजी से बढ़ने वाला, सूखा प्रतिरोधी, बहु उपयोगी पेड़
- अच्छी जल निकासी वाली विविध प्रकार की मिट्टी एवं जलवायु में उगाया जा सकता है।

### सहजन : महत्व

- सहजन खाद्य और पोषण सुरक्षा के लिए अत्यंत महत्वपूर्ण है।
- पेड़ के सभी भाग पत्ती, फूल, फल, बीज, खली, छाल, जई, बीज खाद्य है जो औषधीय गुणों से भरपूर है और कुपोषण दूर करने में महत्वपूर्ण भूमिका निभाता है।
- पोषक तत्व :
  - 300 से अधिक रोगों के रोकथाम के गुण
  - 90 तरह के मल्टीविटामिन्स
  - 46 तरह के एंटीआक्सीडेंट
  - 35 तरह के दर्द निवारक गुण
  - 17 तरह के एमिनो एसिड
  - 45% (बीज) अच्छी क्वालिटी का तेल
- सहजन की पत्तियां, छाल, फलियां, मुधुमेह, गैस्ट्रिक, मलेरिया, अस्थमा, मिरगी, यौन संचारी रोग, अल्सर, मूत्र रोग, फंगल इंफैक्शन सहित सैकड़ों रोगों की रोकथाम में उपयोगी है।
- सहजन न केवल इन्सानों के लिए बल्कि दुधारू पशुओं के लिए भी फायदेमंद है और दूध बढ़ाने वाला है।
- पश्चिम देशों में इसे न्यूट्रीशन ऊइनामाइट के नाम से भी जाना जाता है।
- तेजी से बढ़ना और सूखा सहिष्णुता के गुण इसे जलवायु परिवर्तन के अनुकूल बनाता है।
- इसकी पत्तियां मृदा स्वास्थ्य बढ़ाता है और पारिस्थितिकी तंत्र की बहाली में मदद करता है।
- जलवायु परिवर्तन के प्रभावों को कम करने के लिये सहजन को दोहरे समाधान के रूप मे बढ़ावा दिया जा रहा है, साथ ही परिवारों के लिए दोगुनी आय और समग्र विकास का एक वैकल्पिक स्त्रोत भी प्रदान करता है।



### सहजन प्रबर्द्धन

- सहजन में बीज और शाखा के टुकड़ों दोनो से ही प्रबर्द्धन होता है।
- ट्राइकोर्ड्मा या कार्बेंडजिम उपचारित बीज पॉलीथीन बैग (हलकी दोमट मिट्टी + कम्पोस्ट) में तैयार कर सकते है।
- पॉलीथीन बैग में पौध 1 से 1.5 महीना में लगाने योग्य तैयार हो जाता है।

### सहजन - आधारित कृषिवानिकी

- सहजन कृषिवानिकी के लिए एक आदर्श पेड़ है इसको ब्लॉक प्लांटेशन (3x3 मी. या उससे ज्यादा जरूरत के हिसाब से) बाउंड्री प्लांटेशन या मेड़ पर लगाया जा सकता है।
- गड्ढा तैयार करे, पानी डालें और पौधारोपण से पहले कम्पोस्ट या खाद से मिश्रित उपरी मिट्टी को गड्ढे में डाल दें और जुलाई सितम्बर में पौधारोपण करें।
- एक एकड़ में 500-700 पौध लगाए जाते हैं।
- सहजन को सिर्फ प्रारंभिक देखभाल की जरूरत होती है और वार्षिक सहजन पहले वर्ष से ही फलियां देना शुरू कर देता है।
- मोरिंगा को किसी विशिष्ट कृषि-पद्धतियों और तकनीकों की आवश्यकता नहीं होती है।
- सहजन के साथ रबी और खरीफ ऋतु की कोई भी फसल आसानी से ली जा सकती है।
- सहजन के फलियाँ तोड़ने के समय पौधे की कटाई- छटाई करनी चाहिए।
- उत्कृष्ट किस्में : पी के एम-1, पी के एम-2, ओ डी सी-3 इत्यादि।
- उपज : प्रति एकड़ में लगभग 15 से 20 टन फली और 20 से 25 टन पत्तियां की उपज प्राप्त होती है।
- सहजन आधारित कृषिवानिकी खाद्य, पोषण, रोजगार, आजीविका सुरक्षा के साथ साथ पर्यावरण बहाली और जैव विविधता संरक्षण सुनिश्चित करने के लिए एक अत्यंत महत्वपूर्ण विकल्प है।
- सहजन आधारित कृषिवानिकी से हर वर्ग के किसान की आय दोगुनी और समग्र विकास आसानी से संभव है।

### के.कृवा.अ.सं., झाँसी

- सहजन का जर्मप्लास्म कलेक्शन।
- बुदेलखंड क्षेत्र के लिए साल भर उत्पादन देने वाली और कृषिवानिकी हेतु उन्नत प्रजाति विकसित करना।
- सहजन के बीज, पौध और समस्त जानकारी उपलब्ध करवाना।

# Notes

# Notes

Agrisearch with a human touch

केन्द्रीय कृषिवानिकी अनुसंधान संस्थान

''लकड़ी, चारा, फल और अन्न - कृषिवानिकी है जीवन''

Swachh Bharat Abhiyan

स्वच्छ भारत

एक कदम स्वच्छता की ओर

UNITED NATIONS DECADE ON ECOSYSTEM RESTORATION 2021-2030


प्रकाशकः

**निदेशक**

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कृषिवानिकी विहार, झाँसी 284003 (उ.प्र.)

Published by

**Director**

**ICAR-Central Agroforestry Research Institute**

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