Project Profile

1. Project Title & Code	:	**Development of CRISPR-mediated gene activation tools to enhance santalol production in Sandalwood NFRP-196**
2. Name of the Principal Investigator	:	Dr. A. Balasubramanian, Scientist B
3. Date of start & duration	:	1st April, 2025, Three years
4. Total Budget	:	Rs.10.00 in lakhs

5. Main Objectives

- To develop methods for generating GFP-tagged hairy roots in *Santalum album*.
- To identify gRNA targets and develop gene activation constructs for enhancing the expression of *santalene synthase* and *cytochrome p450 monooxygenase*.
- To generate and evaluate GFP-tagged *Santalum album* roots expressing a gene activation construct for enhanced santalol content.

6. Outline of Research Programme (yearly plan of action):

Year	Activity
First	• Determination of suitable culture conditions for generating *S. album GFP* tagged hairy roots using *Agrobacterium rhizogenes* strains • Identify gRNA targets for activation of *santalene synthase* and *cytochrome P450 monooxygenase* genes
Second	• Identify gRNA targets for activation of *santalene synthase* and *cytochrome P450 monooxygenase* genes • Develop gene activation constructs for improved santalol production. • Generate hairy roots of *S. album* using gene activation constructs
Third	• Generate hairy roots of *S. album* using gene activation constructs • Quantify the expression of *Santalene synthase* and *Cytochrome P450 monooxygenase* genes in *S. album* • *Quantify santalol content in the GFP tagged roots of S. album*

7. Progress of the project in brief:

- Mobilized betaline-based Ruby vector into *Agrobacterium rhizogenes* strain LBA1334 and *A. tumefaciens* strain AGL1 and confirmed with PCR for use in non-destructive screening of transgenics.

- Optimized RNA isolation protocol for the root, shoot, and stem of *S. album*. Intron-spanning primers were designed and synthesised for quantifying the expression of santalol biosynthesis genes, Viz., santalene synthase and cytochrome P450 monooxygenase.
- Experiments were initiated to induce GFP-tagged hairy roots from *S. album.*

8. Action taken on the recommendation of RAG 2024:

NA

Progress of ongoing Externally Aided projects

1. Project Title	:	**Genomic selection for superior heartwood formation in two commercial timber species teak (*Tectona grandis*) and oak (*Quercus robur*) - FASTWOOD (DBT-17)**
2. Name of the Principal Investigator	:	Dr. R. Yasodha, Scientist G
3. Name of the funding agency	:	Department of Biotechnology, GoI
4. Date of start & end; Total duration	:	February 2022 & February 2025; 3 years
5. Total Budget	:	Rs. 130.3112 lakhs

6. Objectives

- Precise and accurate phenotyping of wood (heartwood quantity and quality) using spectroscopy for efficient GS analysis.
- Genomic characterization of teak and oak genotypes and development of customized SNP microarray sets for genotyping.
- Identification of effective trait-associated markers and implementation of GS to identify individuals with superior wood properties.

7. Outline of Research Programme (yearly plan of action):

Year	Activity
First	• Sampling of wood cores and assessment of heartwood percentage. • Spectroscopy analysis of wood cores.
Second	• DNA/RNA sampling • SNP characterization

Third	• GS modelling • Data analysis and Manuscript preparation

8. Overall progress since the implementation of the project:

- Morphometric data and leaf and wood core samples from clones across India's teak-growing regions recorded.
- Wood metabolomic features using FTIR completed.
- Genomic information generated, 1,85,712 SNP markers identified, leading to the discovery of three genetic clusters within India's germplasm.
- Genomic modelling using the GBLUP model and diverse sampling methods, is being conducted.

9. Publications made:

Adwait et al. 2024. A manual on field and laboratory data collection of teak. pp 2 (ISBN NO: 978-93-82387-27-5).