Project Profile

1. Project Title & Code	**:**	**Evaluating *AtF5H* expression in *Eucalyptus camaldulensis* for lignin modification and improved pulp yields NFRP-194**
2. Name of the Principal Investigator	**:**	Dr. A. Balasubramanian, Scientist B
3. Date of start & duration	**:**	1st April, 2024, Four years
4. Total Budget	**:**	Rs.12.40 in lakhs

5. Main Objectives

- To assess the variations in S/G ratio, pulping efficiency and F5H gene sequence in *Eucalyptus camaldulensis*.
- To identify gene editing targets for enhancing lignin S/G ratio in *Eucalyptus camaldulensis.*
- To generate gene-edited *Eucalyptus camaldulensis* and evaluate the effect of gene edits on *EcF5H* expression.
- To engineer enhanced lignin S/G ratios in *Eucalyptus camaldulensis* through heterologous expression of *AtF5H* gene.

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

Year	Activity
First	• Estimate the S/G ratio of lignin in selected clones of *E. camaldulensis*. • Assess pulping efficiency of clones with significantly contrasting S/G ratio. • Study the F5H gene sequence variation in selected clones of *E. camaldulensis* with contrasting S/G ratio. • Isolation of RNA from cambium tissues of *E. camaldulensis* and sequencing of small RNAs. • Clone *AtF5H* gene from *Arabidopsis thaliana* and develop transformation construct for expression in *E. camaldulensis.*
Second	• Identify potential miRNAs with homology to the EcF5H gene. • Quantify the expression of small RNAs and EcF5H gene using qRT-PCR. • Design guide RNA and develop gene-editing constructs for increasing S/G ratio. • Generate *AtF5H* expressing Eucalyptus plantlets by *A. tumefaciens* mediated transformation and hardening of transgenic events at the transgenic greenhouse.
Third	• Clone *EcF5H* gene and identify gene editing targets in *EcF5H.* • Generate *E. camaldulensis* plants using the developed gene editing construct. • Generate AtF5H expressing Eucalyptus plantlets by *A. tumefaciens* mediated transformation and hardening of transgenic events at the transgenic greenhouse.
Fourth	• Generate *E. camaldulensis* plants using the developed gene editing construct.

	• Generate *AtF5H* expressing Eucalyptus plantlets by *A. tumefaciens* mediated transformation and hardening of transgenic events at the transgenic greenhouse. • Evaluate the effects of gene edits on the expression of *EcF5H* gene by qRT-PCR/stem loop RT-PCR. • Quantify *AtF5H* expression and determine transgene copy number in transgenic events by qRT-PCR. • Estimate S/G ratio of wood using Py-GC-MS / NMR, pulping analysis of the transgenic events.

7. Progress of the project in brief:

- To estimate the S/G ratio of lignin, wood samples of ICFRE-IFGTB-released *E. camaldulensis* clones (11 Nos.) were collected from the Field Research Station, Kurumbampatti, Salem.
- The total RNA and genomic DNA were isolated from *Arabidopsis thaliana* leaves. The F5H gene and C4H promoter were PCR amplified for cloning into the pCAMBIA1305.2 plant expression vector.
- Obtained permission from ICFRE to procure plasmids from Addgene, USA