1. Project Title & Code	**:**	**Evaluation of genome engineering approaches for enhanced gibberellin accumulation to improve biomass in Eucalyptus (NFRP 195)**
2. Name of the Principal Investigator	**:**	Dr. Mathish Nambiar-Veetil, Scientist-G
3. Date of start & end; Total duration	**:**	2025, Four years
4. Total Budget	**:**	Rs. 12.80 Lakhs

5. Main Objectives

- To develop fast-growing Eucalyptus trees by enhancing gibberellin (GA) accumulation through advanced gene-editing technologies

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

First	● Identify candidate homologues of Gibberellin 2-oxidase (EcGA2ox) genes in Eucalyptus and validate with RT-qPCR. ● Design guide RNA (gRNA) sequences targeting EcGA2ox genes for gene editing. ● To generate the AtGA20ox1 gene construct directed by the MsPRP2 promoter into binary expression vectors.
Second	● Generate EcGA2ox gRNA construct and clone it into binary vector ● Perform *Agrobacterium tumefaciens*-mediated transformation of Eucalyptus explants with EcGA2ox knockout constructs and MsPRP2::AtGA20ox1 expression constructs
Third	● Screen putative transgenic lines by PCR and sequencing for successful gene editing and transgene integration. ● Evaluate expression levels of EcGA2ox and AtGA20ox1 transcripts in transgenic plants through RT-qPCR.
Fourth	● Quantify GA content in roots and shoots to assess the impact of gene editing and overexpression on GA accumulation. ● Analyze physiologic traits such as root and shoot growth, biomass accumulation of transgenic lines.

7. Progress of the Project in brief

- **T**he candidate homologues of Gibberellin 2-oxidase (GA2ox) genes in *Eucalyptus camaldulensis* were identified by using *Eucalyptus grandis x urophylla* genes as references. Both NCBI sequence databases and the AUGUSTUS gene prediction tool were employed to predict and confirm gene structures and homologs.
- Guide RNAs (gRNAs) specific for each EcGA2ox gene were designed using the CRISPR Direct tool to enable precision gene editing of target loci. Off-target analysis was performed using Cas-OFFinder and the CRISPRscore package in R Studio to ensure specificity and minimize unintended genome edits.
- Primers for RT-qPCR were designed for quantification of EcGA2ox5,6,7,8,9,10,11 expression, and gradient PCR has been performed to optimise the annealing temperature.