Project Profile

1. Project Title	**:**	**Engineering base edits in *EcHKT1;1* gene to enhance potassium uptake and salt stress tolerance in Eucalyptus (DBT-18)**
2. Name of the Principal Investigator	**:**	Dr. Mathish Nambiar-Veetil, Scientist-G
3. Funding Agency	**:**	Department of Biotechnology, GoI
4. Date of start & duration	**:**	January 2023; Three years
5. Total Budget	**:**	Rs. 33.58 lakhs

6. Main Objectives

- Evaluate the feasibility of mutating Ser-95 and Asn-381 to Gly in *EcHKT1;1* using ABE for enhancing K^+ uptake, limiting Na^+ influx and enhancing salt tolerance in Eucalyptus.
- Generate and evaluate *EcHKT1:1* knockout plants by deletion of Ser 95 containing extracellular loop region for enhancing K^+/Na^+ selectivity and salt tolerance in Eucalyptus.

7. Yearly plan of action

1st year	Generation of ecTadA-ecTadA*7.10-nSpCas9 (D10A) fusion based transformation vector harbouring the *EcHKT1;1* sgRNA cassette targeting Ser-95 and Asn-381. Generation of Cas9 based transformation vector harbouring the EcHKT1;1 sgRNA cassette targeting the extracellular loop region in EcHKT1;1 containing Ser-95.
2nd year	Generation of base edited *EcHKT1;1* and knockout transgenic events of Eucalyptus by *Agrobacterium tumefaciens* mediated transformation.
3rd year	Molecular and phenotypic characterization of *EcHKT1;1* base edited and knockout transgenic events.

8. Progress (in brief)

- Guide RNAs targeting base edits for mutation of Ser-95 and Asn-381 to glycine, and guide RNAs for 85 bp deletion of loop domain in EcHKT1;1 were synthesized in a tRNA-gRNA system.
- The adenine base editor, ecTadA*7.10-nSpCas9 (D10A), with NAG/NGG PAM requirements was cloned into CaMV 35S promoter driven expression cassette for use in dicots.
- Through a series of cloning, the final base editing vector "pCAMBIA1305.1::CaMV:nCas9:CaMV:SNG-sgRNA:HSP" for generation of Ser-95 and Asn-381 mutations in *EcHKT1;1* gene, and the final gene editing vector "pAtCas9_1::CaMV:LD-sgRNA:HSP" for generation of *EcHKT1;1* SDN-1 knockouts were generated.
- These vectors and control vectors were mobilized into *Agrobacterium tumefaciens* and used for genetic transformation of Eucalyptus camaldulensis. A total of six independent *A. tumefaciens-mediated* transformation experiments were initiated using AGL1, AGL1-pAtCas9, AGL1- pAtCas9::CaMV:LD-sgRNA:HSP, AGL1- pCAMBIA1305.1::nCas9 and AGL1-pCAMBIA1305.1::nCas9:CaMV:SNG-sgRNA:HSP strains. Transformed explants were selected under hygromycin selection. The transformed callus has been shifted to hygromycin-free media for regeneration.