

## Appendix I – Plan for Research

### GREAT TREES RESEARCH COOPERATIVE

*July 2024-June 2029*

*Revised 14 February 2024*

Based on member feedback and evaluation of company priorities during December 2023-February 2024, our effort will be split approximately 7:2:1 between the areas of transformation improvement, modification of flowering, and regulatory/market engagement to elevate the role of science with respect to the use of gene editing and transgenic biotechnology in tree crops.

Within the area of transformation, major and similar levels of effort will be allocated to altruistic cotransformation, clean gene editing, in planta transformation, and exploration of new *Agrobacterium* strains.

Within the area of flowering, effort will be primarily focused on graft-induction of rapid flowering to speed breeding and introgression, but studies of genetic containment and male-sterility may be undertaken if members are willing to take part in in-kind field trials, including taking responsibility for the needed regulatory/market compliance, with transgenic or edited eucalypts in suitable production environments.

Specific tasks include:

1. Using transcriptome, bioinformatics, and scientific literature searches, identify specific genes and *Agrobacterium* strains whose application is likely to significantly influence the rate of *in vitro* regeneration or transformation (RT) of tree cells.
2. Create constructs using one or more genes cited above that are expected to influence RT, and which generally contain reporter genes that facilitate recognition and quantification.
3. Test the effects of these genes *in vitro* or *in vivo* using eucalypts, but also employ poplar, tobacco, or other plant species as models where it would enable more rapid or more precise testing of new genes and concepts.
4. Develop and test accessory technology to control and limit RT-modifying gene expression, such as promoters, insulators, inducers, and recombinases.
5. Develop and test accessory technology that is likely to affect RT, such as various growth media and transformation treatments.
6. Adapt and test the technology used to control or transiently express RT and gene-editing genes to enable their avoidance as transgenes in the genome, or their elimination after transformation.