Overexpression of the GRF-GIF transcription factor chimera modifies transformation and regeneration efficiency in *Populus* and *Eucalyptus*

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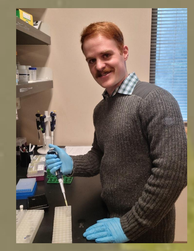

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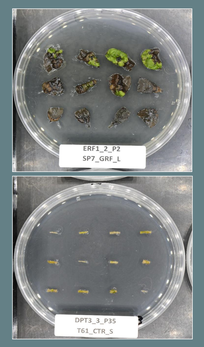
Low transformation and regeneration efficiency limit forest biotechnology

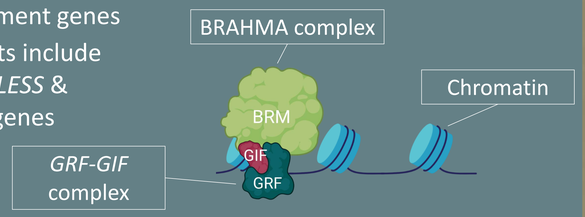
- Genetic engineering and gene editing are limited by ability to produce and regenerate transgenic plants
- We are testing the transcription factor-protein chimera consisting of *GROWTH REGULATING FACTOR 4 (GRF4) & GRF-INTERACTING FACTOR1 (GIF1),* which increased regeneration in citrus and grape (Debernardi et al., *Nature Biotechnology* 2020).
- Our experiments show a complex interaction between *GRF* transcript stability, genotype, and expression level



Eucalyptus (top) and *Populus* (bottom) are typically recalcitrant to *in vitro* regeneration

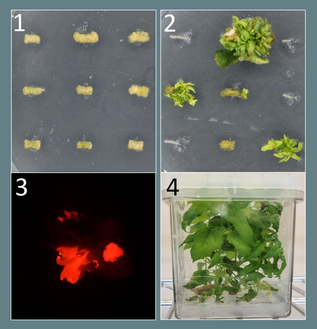
GRF & GIF proteins reprogram cells and help specify new meristems

- *GRF* and *GIF* proteins interact with BRAHMA (BRM) chromatin remodeling machinery to modify the transcription of important meristem development genes
- Downstream targets include *SHOOT MERISTEMLESS* & *PLETHORA*-family genes



Steps in *Eucalyptus and Populus* transformation

- Produce *GRF-GIF* construct with antibiotic resistance and fluorescent protein markers
- Transform *Agrobacterium* with plasmid
- Co-cultivate explants with *Agro*
- Induce callus then shoot regeneration under antibiotic selection
- Micropropagate transgenic, dsRed-expressing transgenic shoots



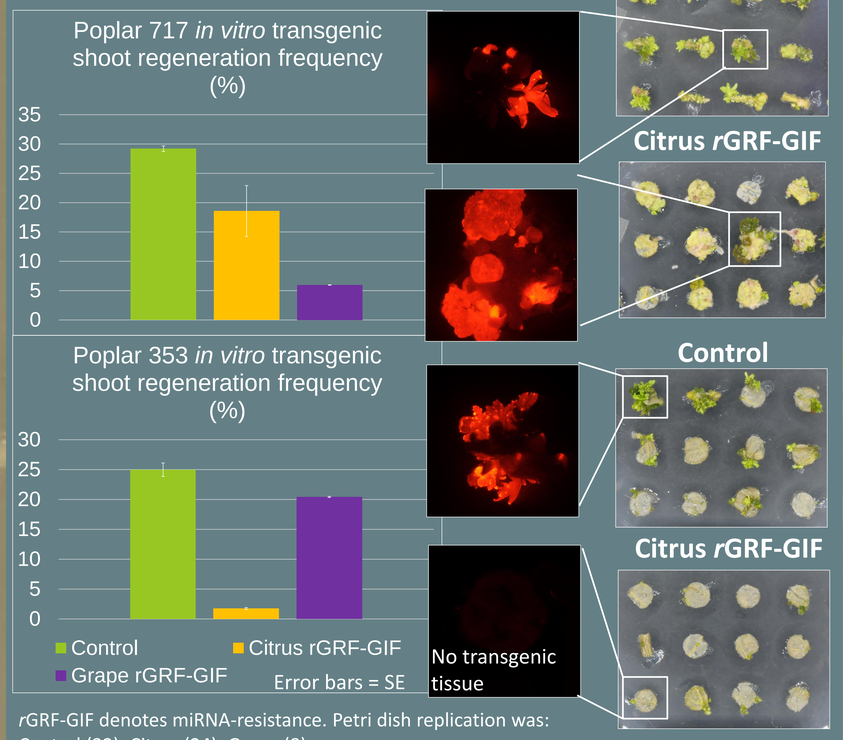
1. Callus formation, 2. Shoot regeneration, 3. dsRed shoot, 4. Micropropagation

GRF-GIF chimeras of varying miRNA-resistance levels were tested in *Populus and Eucalyptus*

- *GRF* activity is down-regulated by *miRNA396*
- A fully miRNA-resistant chimera from *Vitis,* as well as fully-, partially-, and non-miRNA-resistant *Citrus* chimeras (Debernardi et al, Nature Biotechnology, 2020) were transformed into two genotypes of *Populus* and four genotypes of *Eucalyptus*
- The T-DNA also included a hygromycin resistance marker and a red fluorescent protein (dsRed) for selection and detection of transgenic tissue. The control vector contained only dsRed and hyg resistance.

miRNA-resistant *GRF-GIF* chimeras inhibited poplar regeneration in genotype-dependent manner

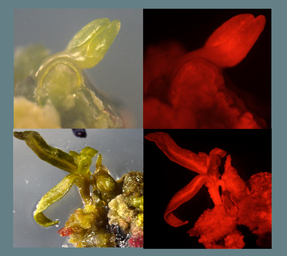
miRNA396-resistant *GRF-GIF* from *Citrus* and *Vitis* decreased the frequency of transgenic shoots in two hybrid poplar genotypes



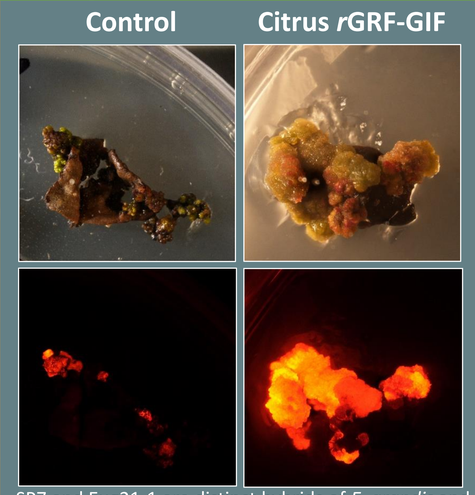
*r*GRF-GIF denotes miRNA-resistance. Petri dish replication was: Control (32), Citrus (24), Grape (8)

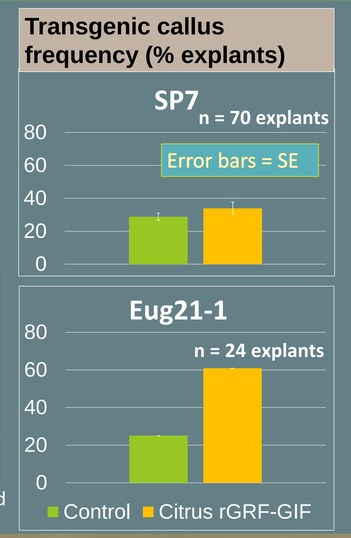
A miRNA-resistant *GRF-GIF* chimera promoted transgenic shoot formation in a recalcitrant eucalypt

- Recalcitrant *E. grandis* genotype Eg18-1 regenerated transgenic shoots at a rate of 12.5% when overexpressing the *Citrus miRNA-resistant GRF-GIF*
- This is the first report of transgenic shoot formation in this genotype, where similar treatments without GRF-GIF had failed



A Citrus miRNA-resistant *GRF-GIF* increased transgenic callus size and number in eucalypts





SP7 and Eug21-1 are distinct hybrids of *E. grandis* and *E. urophylla*. *r* = miRNA-resistant *GRF-GIF*

Conclusions

- Overexpression of *GRF-GIF* improved transgenic callus and shoot regeneration in *Eucalyptus*
- miRNA-resistant *GRF-GIF* chimeras reduced the rate of transgenic shoot regeneration in two highly-regenerable poplar genotypes and displayed a strong genotype x construct interaction
- Matching of construct type and expression level to plant genotype appears to be important for enhancing transformation rate

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