

AGROSCAN

Screening wild Agrobacterium collections for strains and genes that promote transgene delivery and regeneration in woody plants

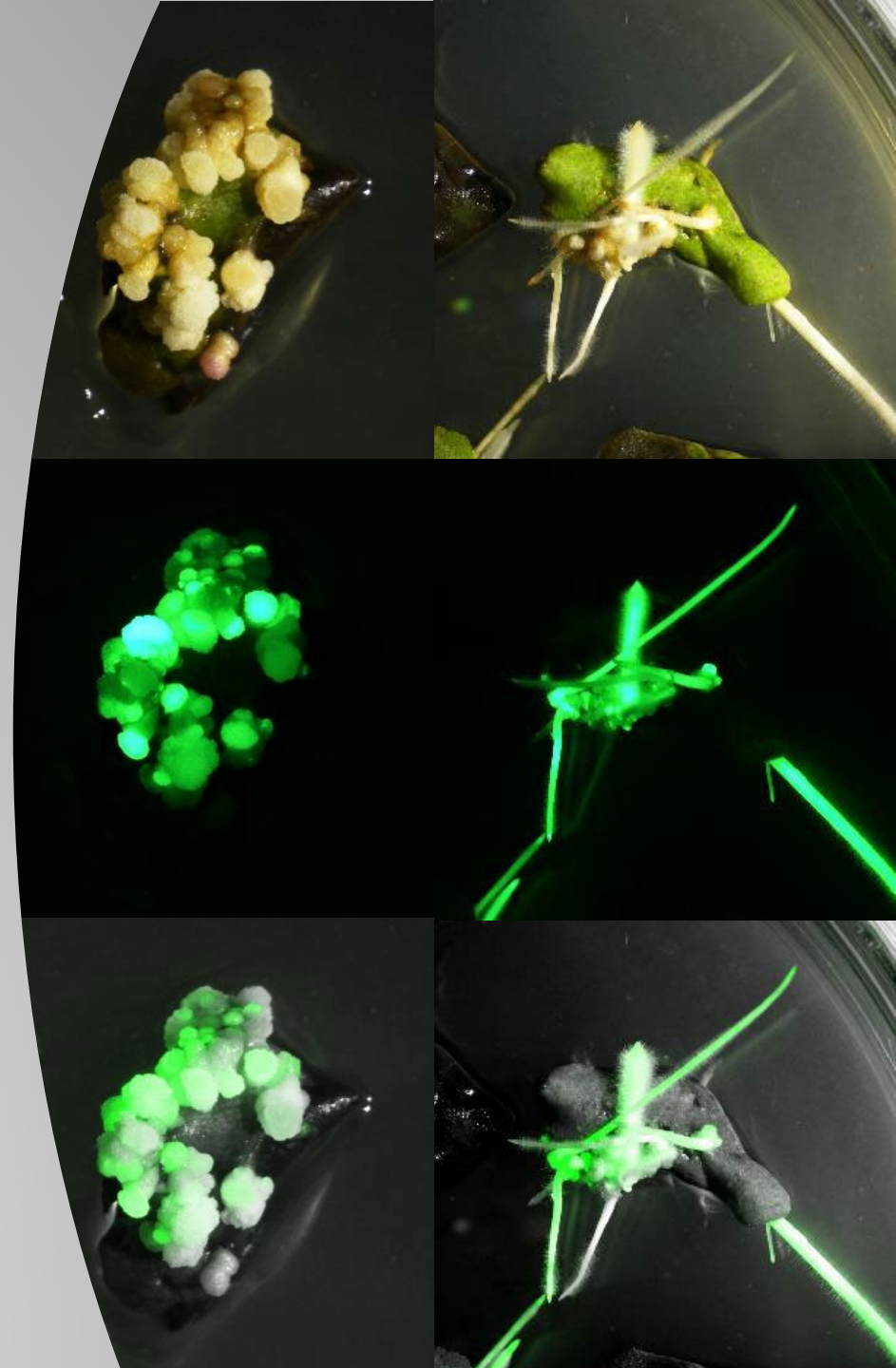
Zachary M. Heinhold

Oregon State University

Plant Biotechnology Laboratory



Oregon State
University





Plant biotechnology enabled by Agrobacterium-mediated transformation (AMT)

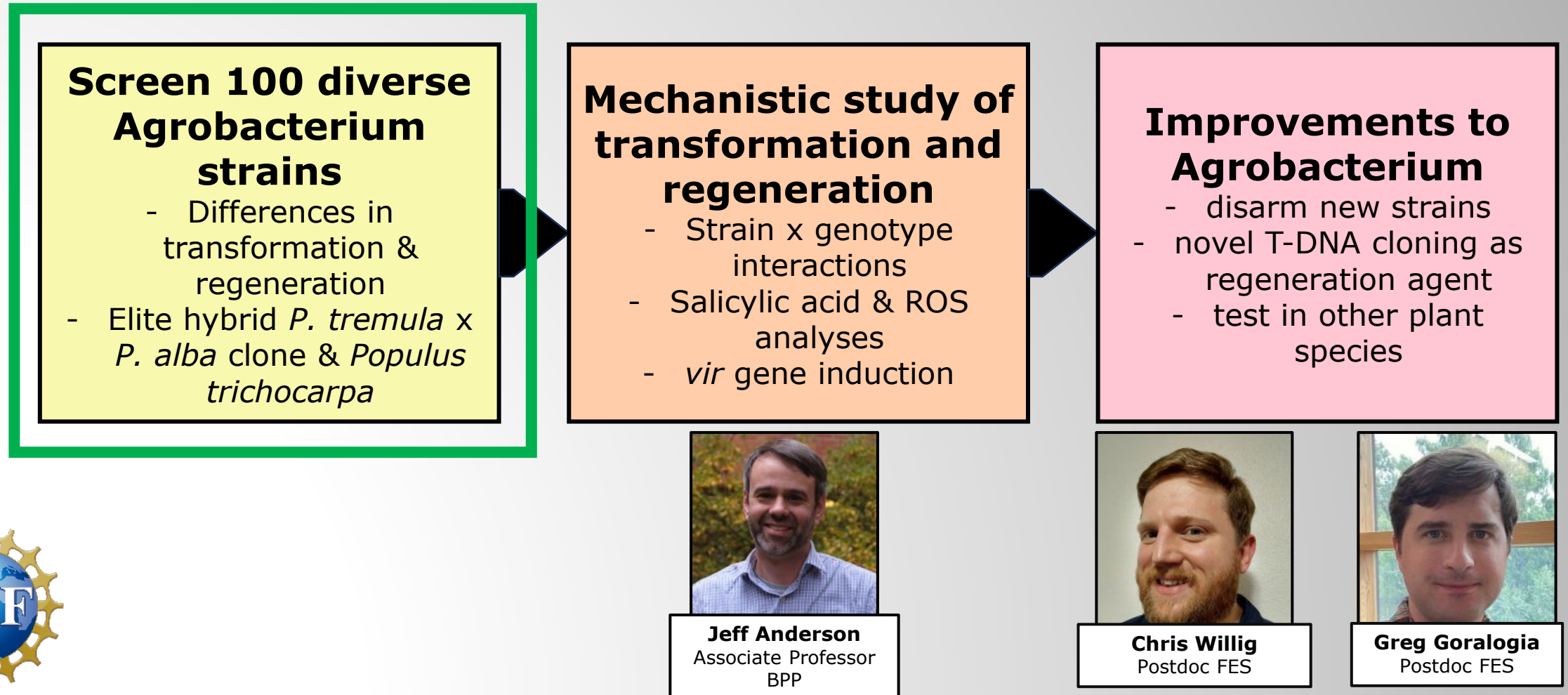
- Rapid development of plant species with desired traits
 - Introduction of traits not in breeding population
- CRISPR/Cas & nuclease systems delivery

- Limitations with efficiency of transformation and regeneration in many plant species

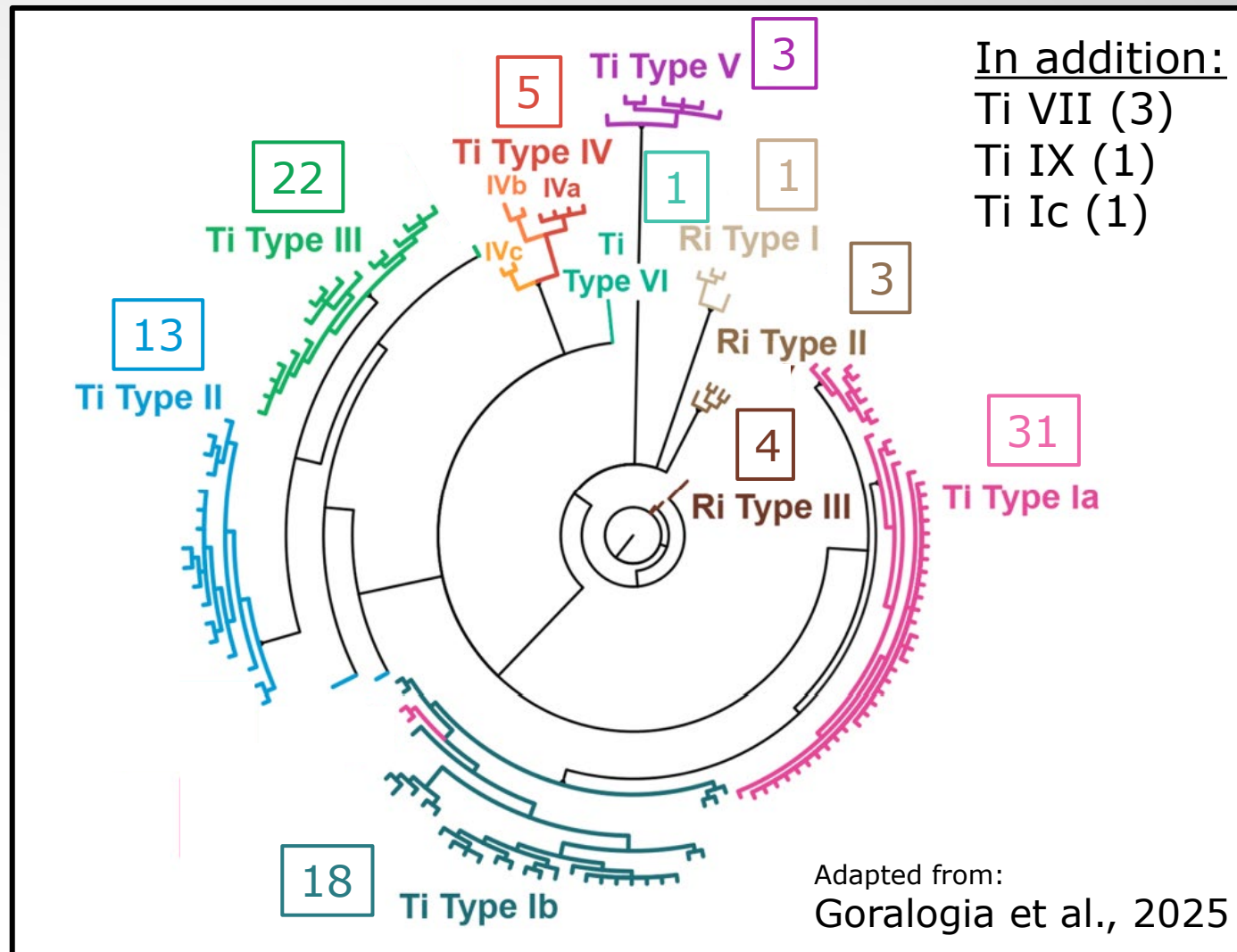


Transgenic purple poplar

Utilizing Agrobacterium diversity to overcome bottlenecks in woody plant transformation and regeneration – project overview



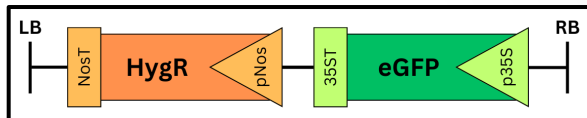
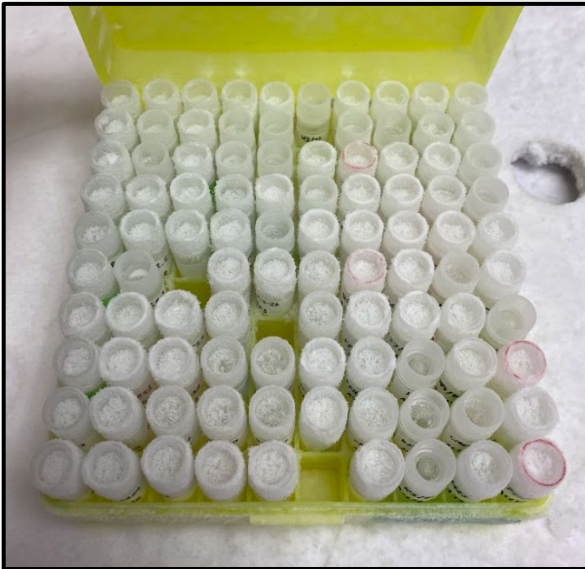
100+ wild strains selected from Oregon State University collection maximizing plasmid & chromosomal diversity



Plasmid
phylogeny of
wild strain Ti
and Ri
plasmids in
OSU collection

Using high-throughput phenomics to characterize transformation & regeneration in wild strains

Insert binary vector



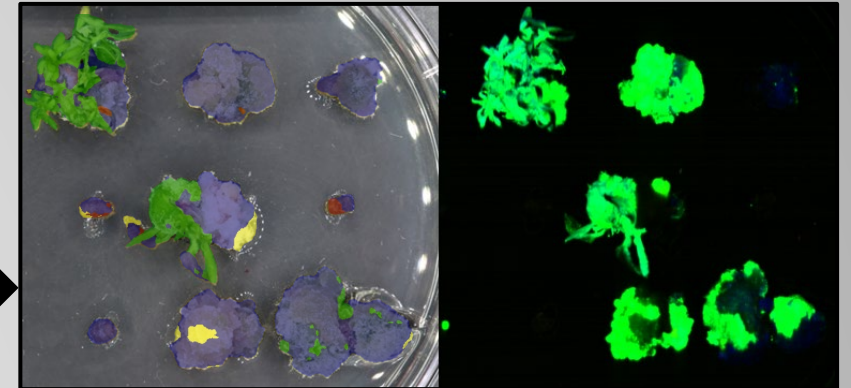
Transform plants



Modified Media

- No Acetosyringone
- Low Hygromycin (1mg/ml)
- No plant growth hormones
- 3 plates per strain

Phenomics Analysis

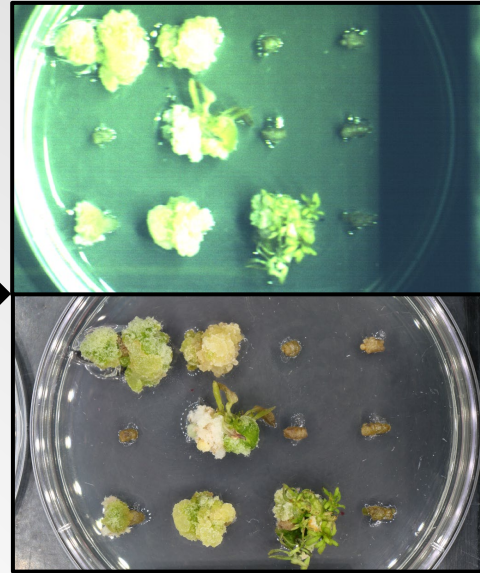


10 weeks

- Transgenic tissue
- Regeneration
- Necrosis

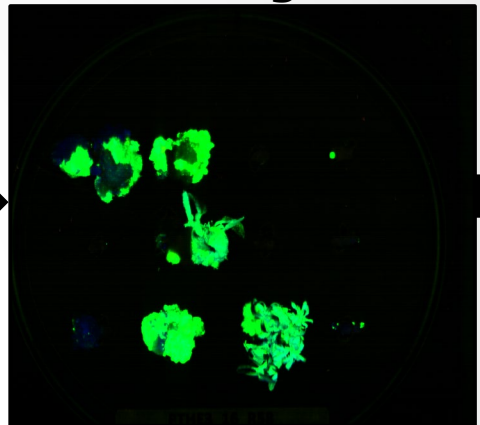
High-throughput phenomics pipeline overview

**MacroPhor Array
Plate Imager**



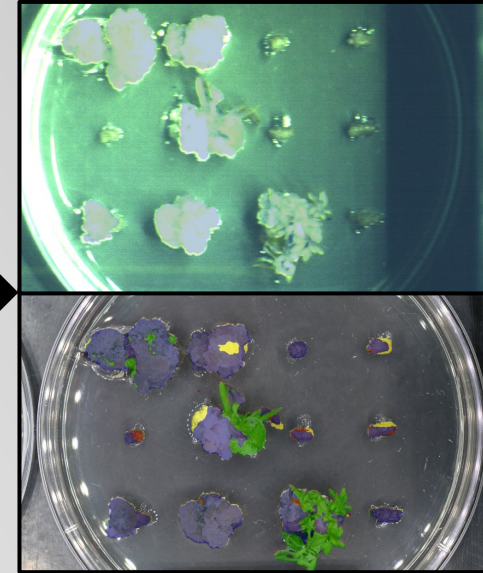
RGB/Broadband Image

**Fluorescent Hyperspectral
Image**



Deep
Segmentation

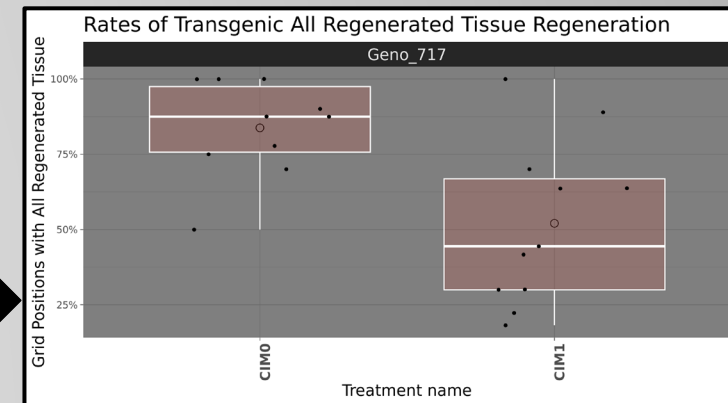
Label Studio



Segmented Image

Quantify Fluorescent Reporters

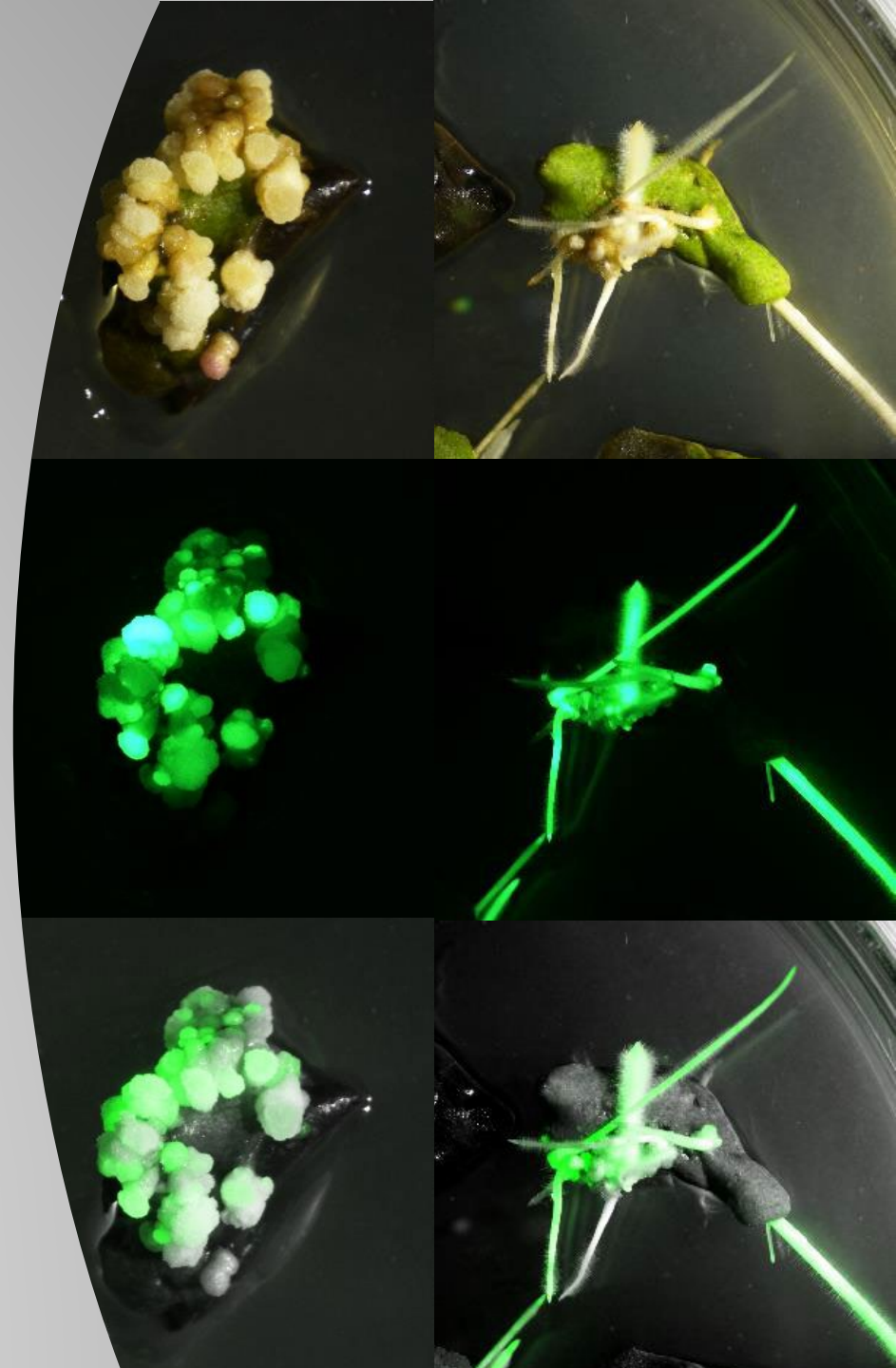
CubeGLM

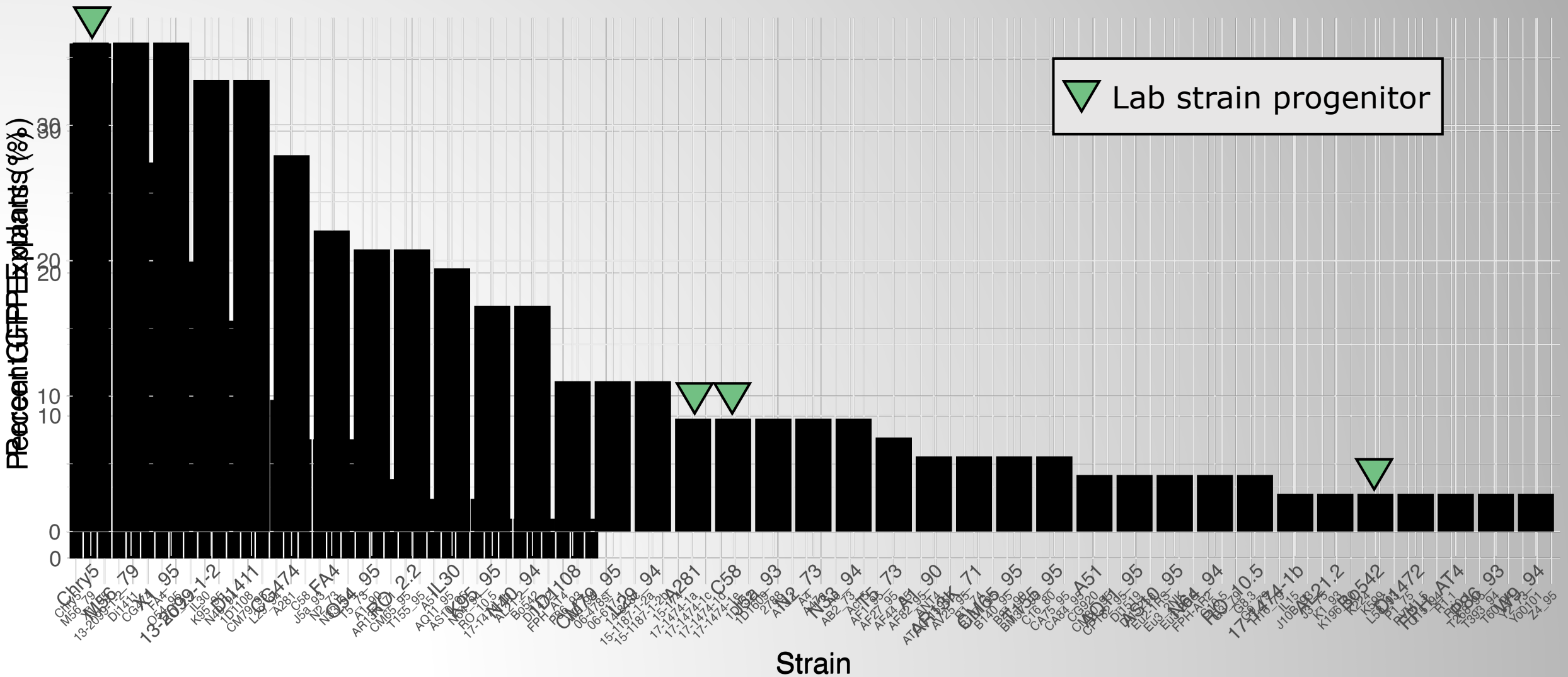
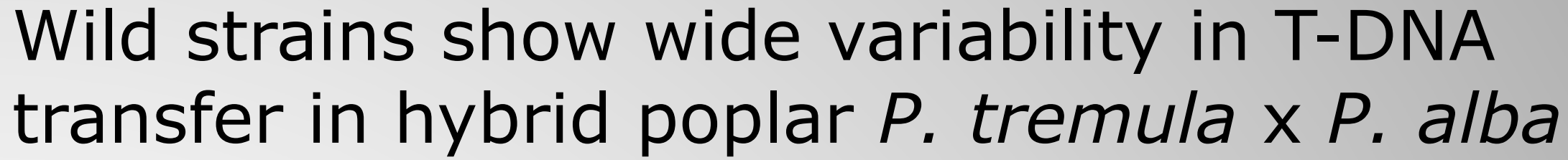


**Transformation &
Regeneration Data**



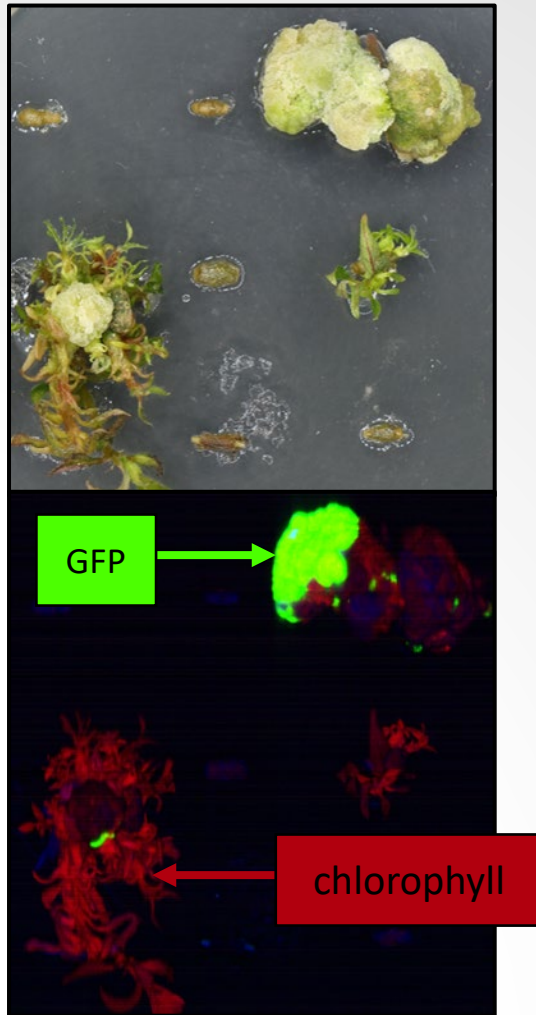
Results







Transgenic index incorporates both area of tissue type containing GFP and the number of explants which are transgenic

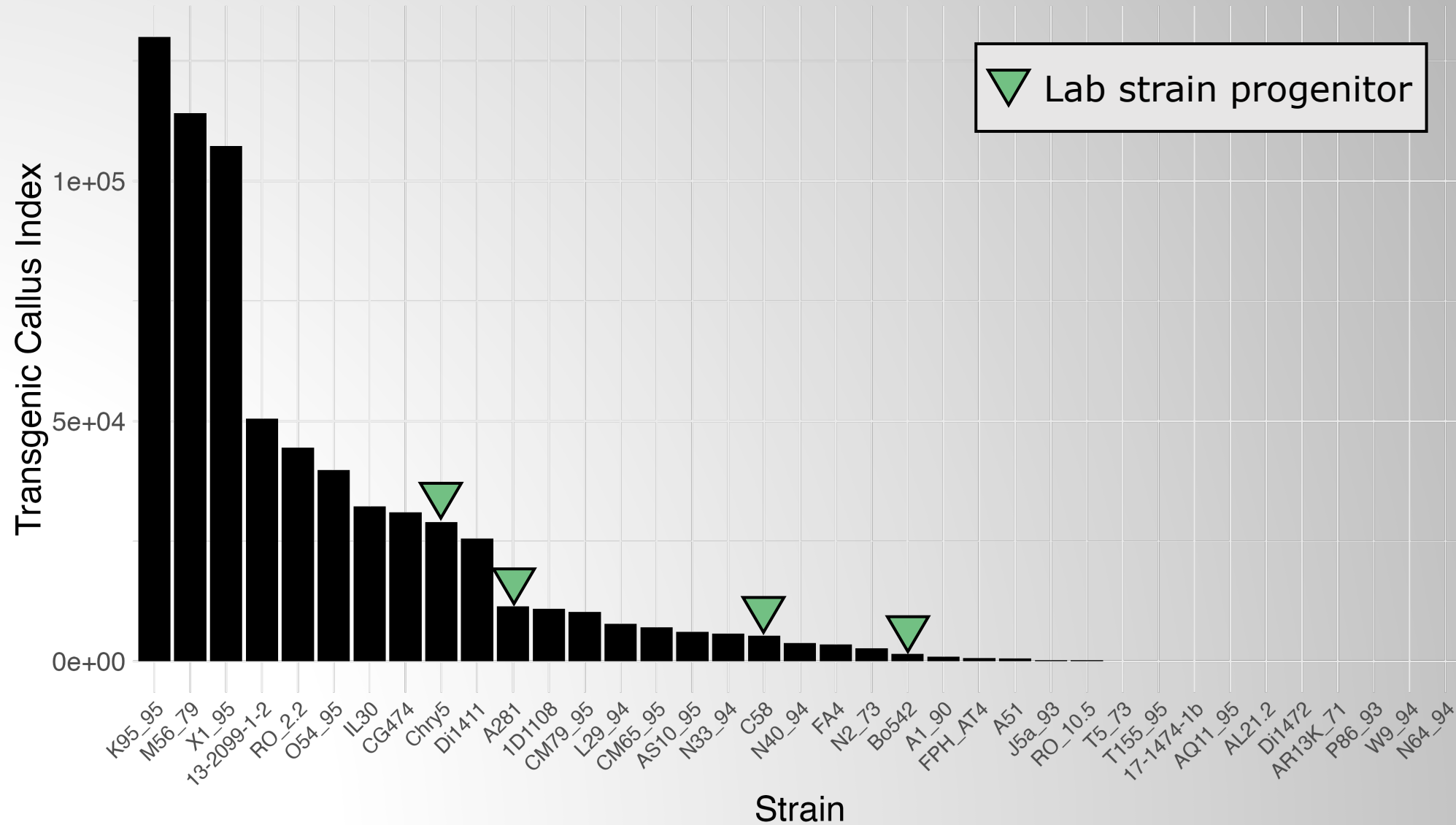


Transgenic index formula:

$$\frac{\text{Total GFP pixels} \times \text{Total number of transgenic explants}}{\text{Number of plates}}$$



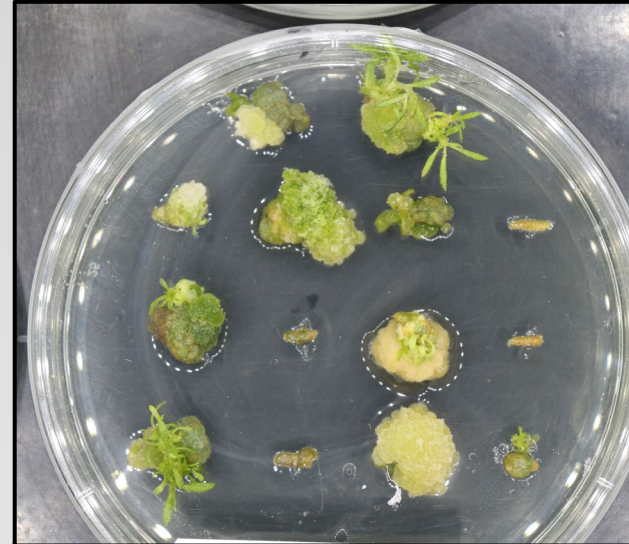
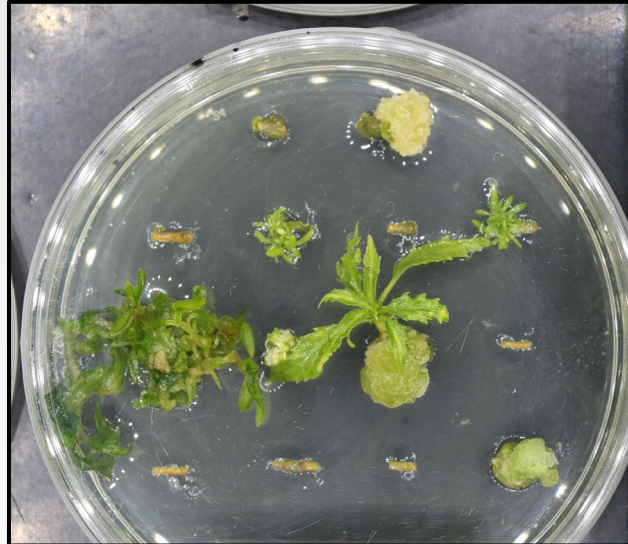
Transgenic callus index shows overall GFP callus development in absence of plant growth hormones



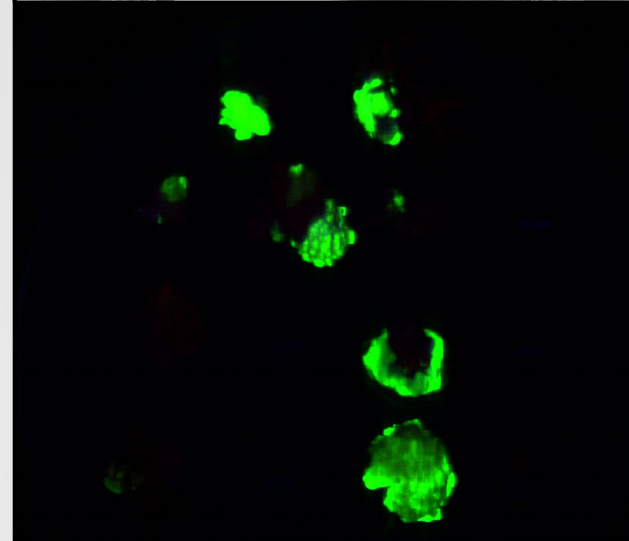
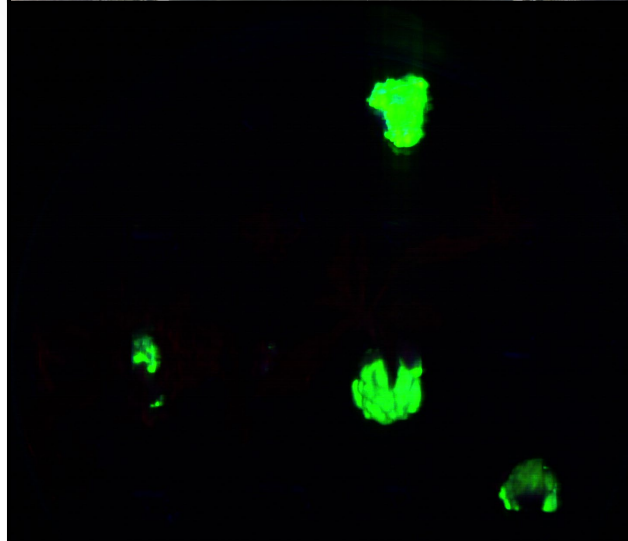


High rates of GFP callus formation from wild strains in absence of plant growth hormones

Brightfield

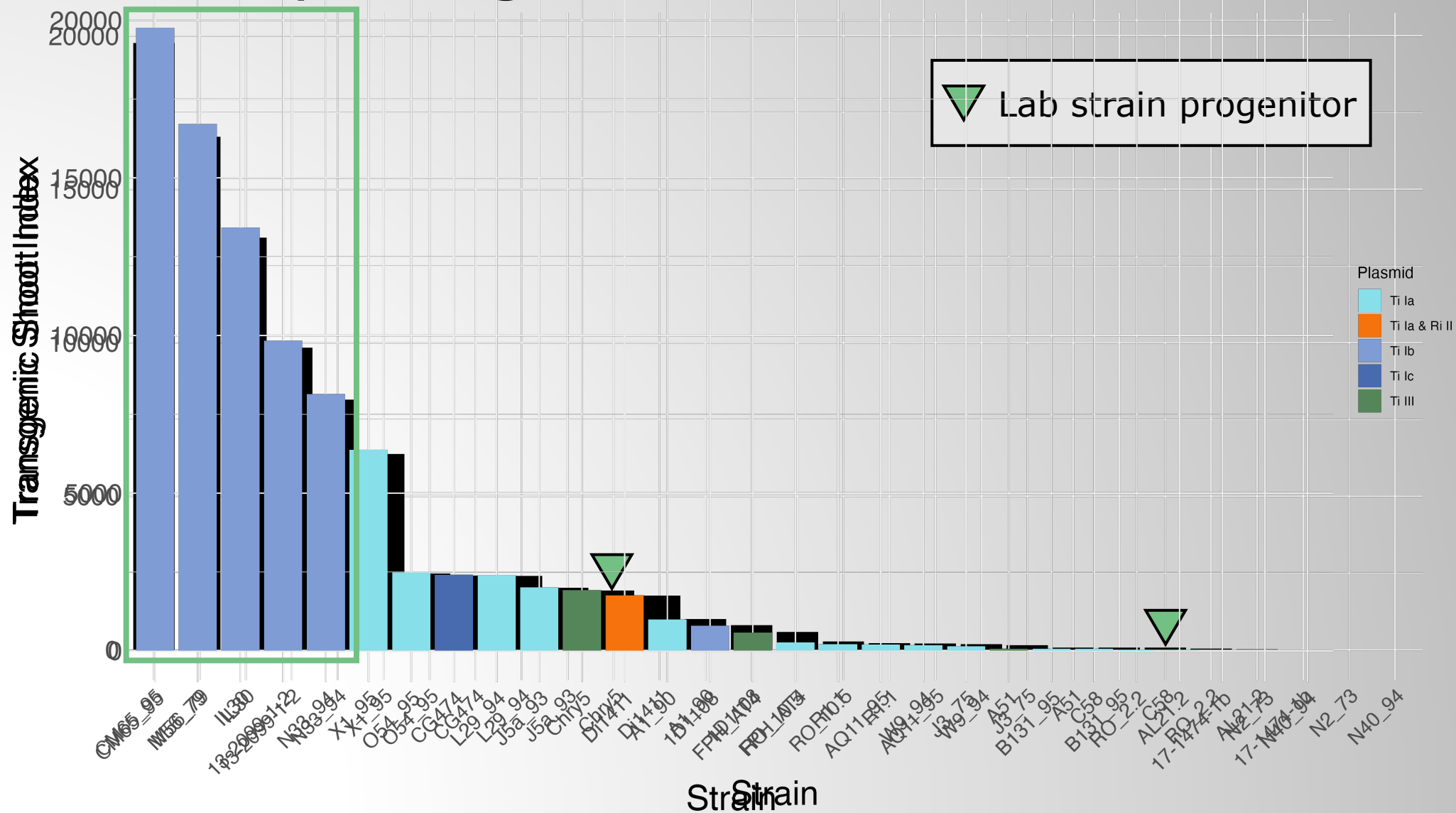


GFP





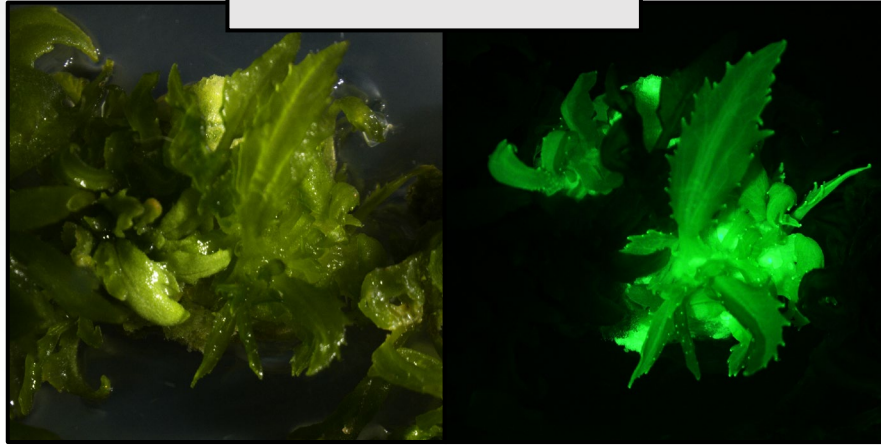
Wild strains show transgenic shoot regeneration in absence of plant growth hormones



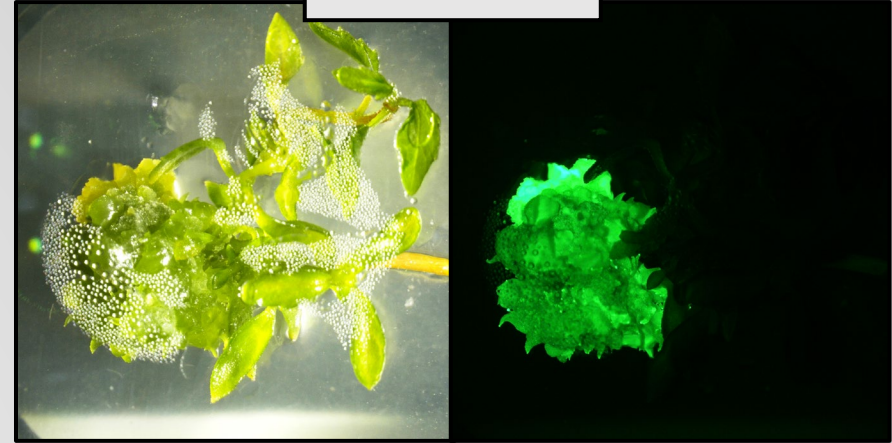


High rates of GFP shoot formation from plasmid type Ib strains in hybrid poplar without plant growth hormones

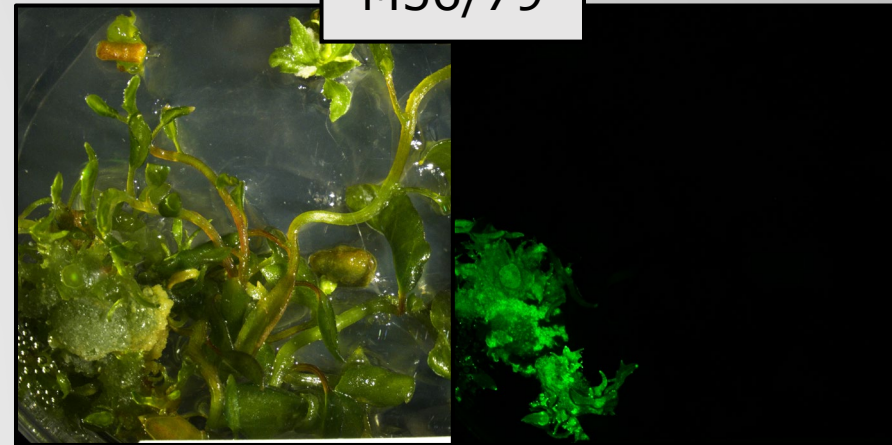
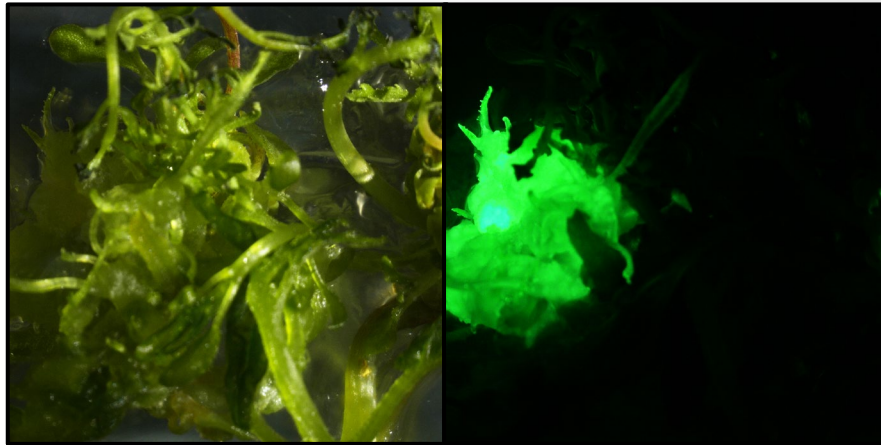
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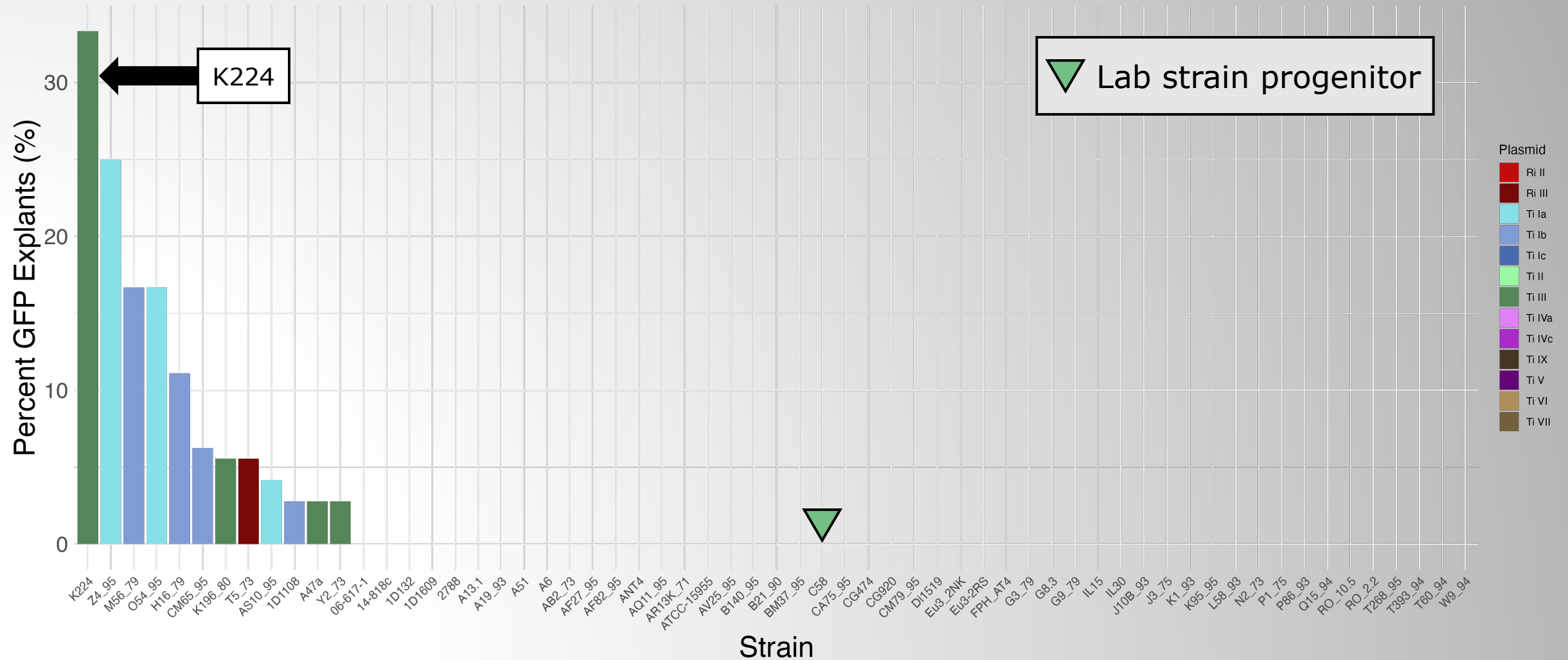
CM65/95



M56/79

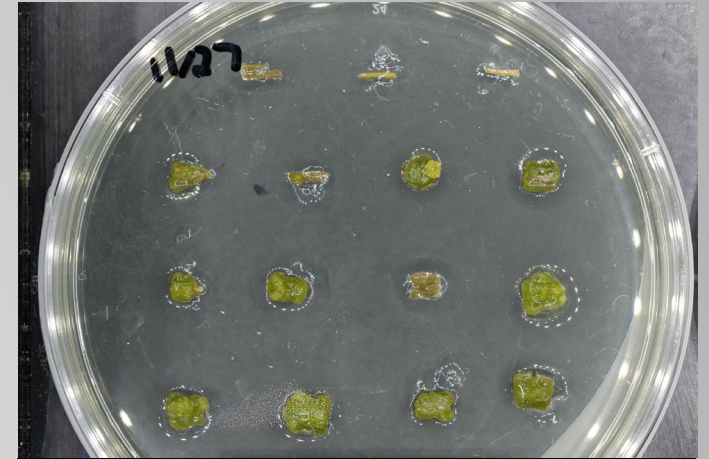
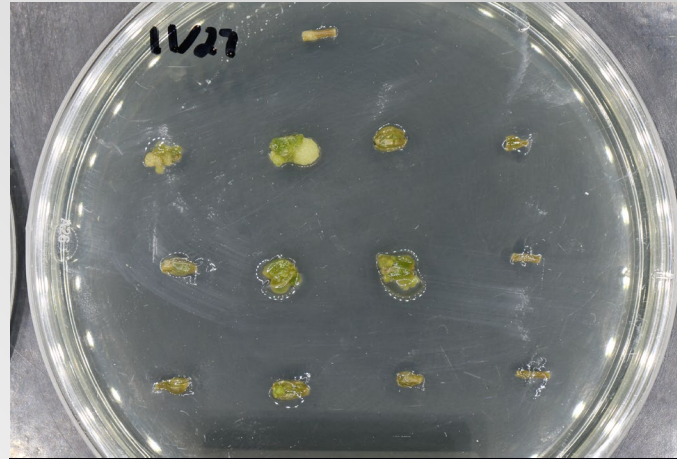
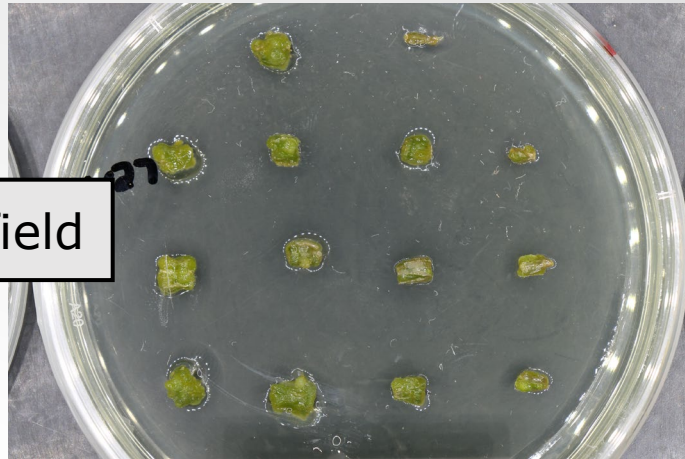


Some wild strains show promise in *P. trichocarpa* transformation

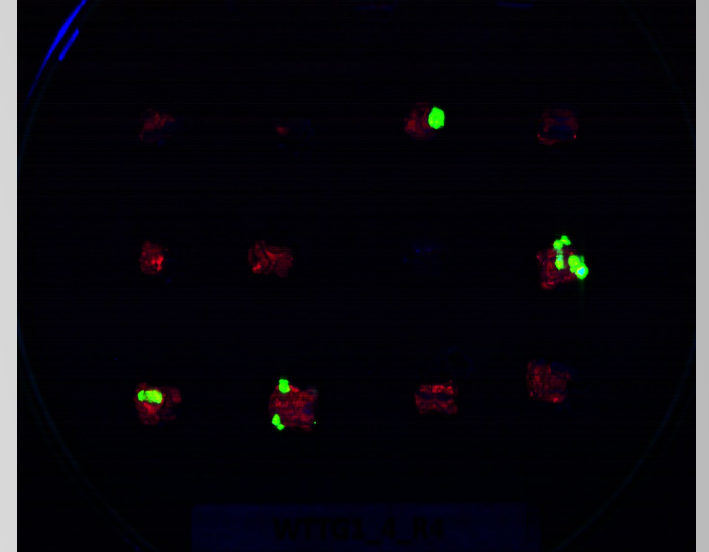
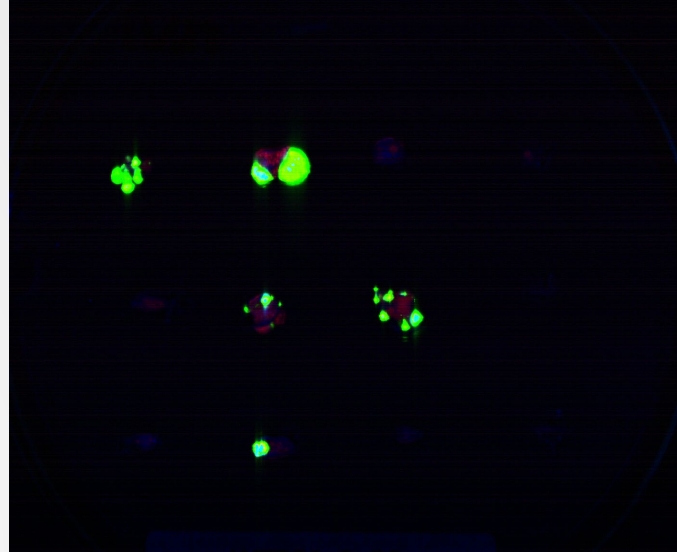
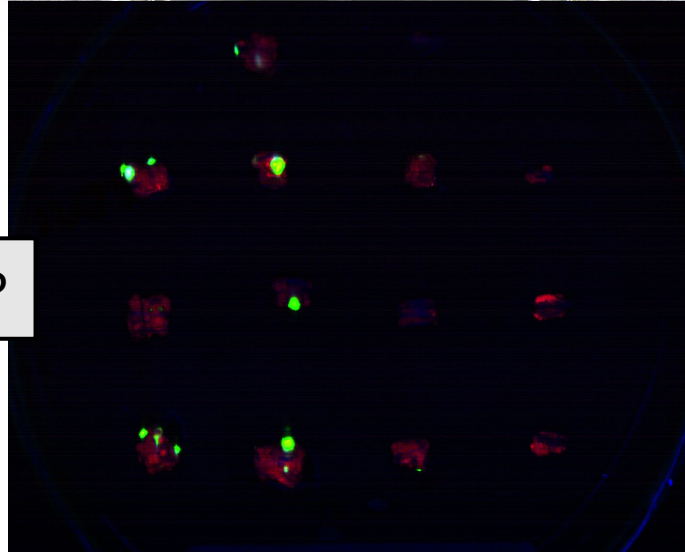


Strain K224 shows high rates of T-DNA transfer in recalcitrant *P. trichocarpa*

Brightfield



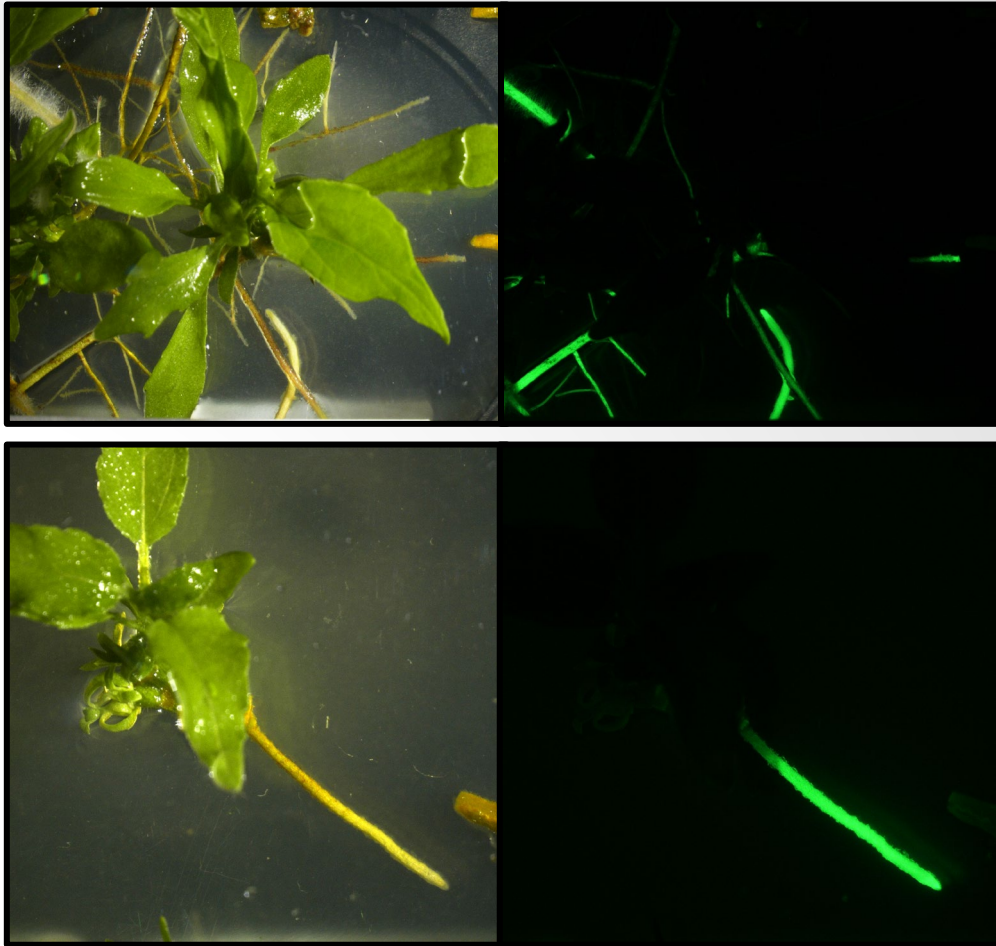
GFP



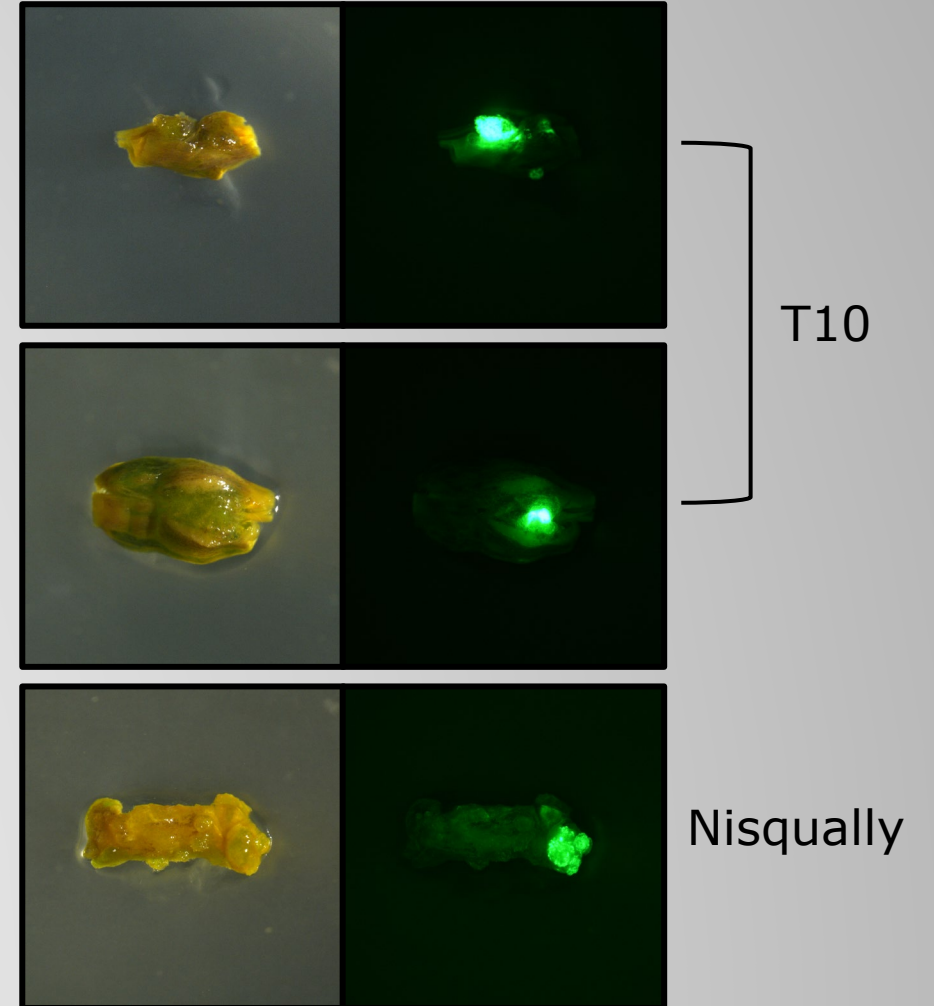


Some strains show improved transformation rates in multiple species and genotypes – FA4

Hybrid Poplar



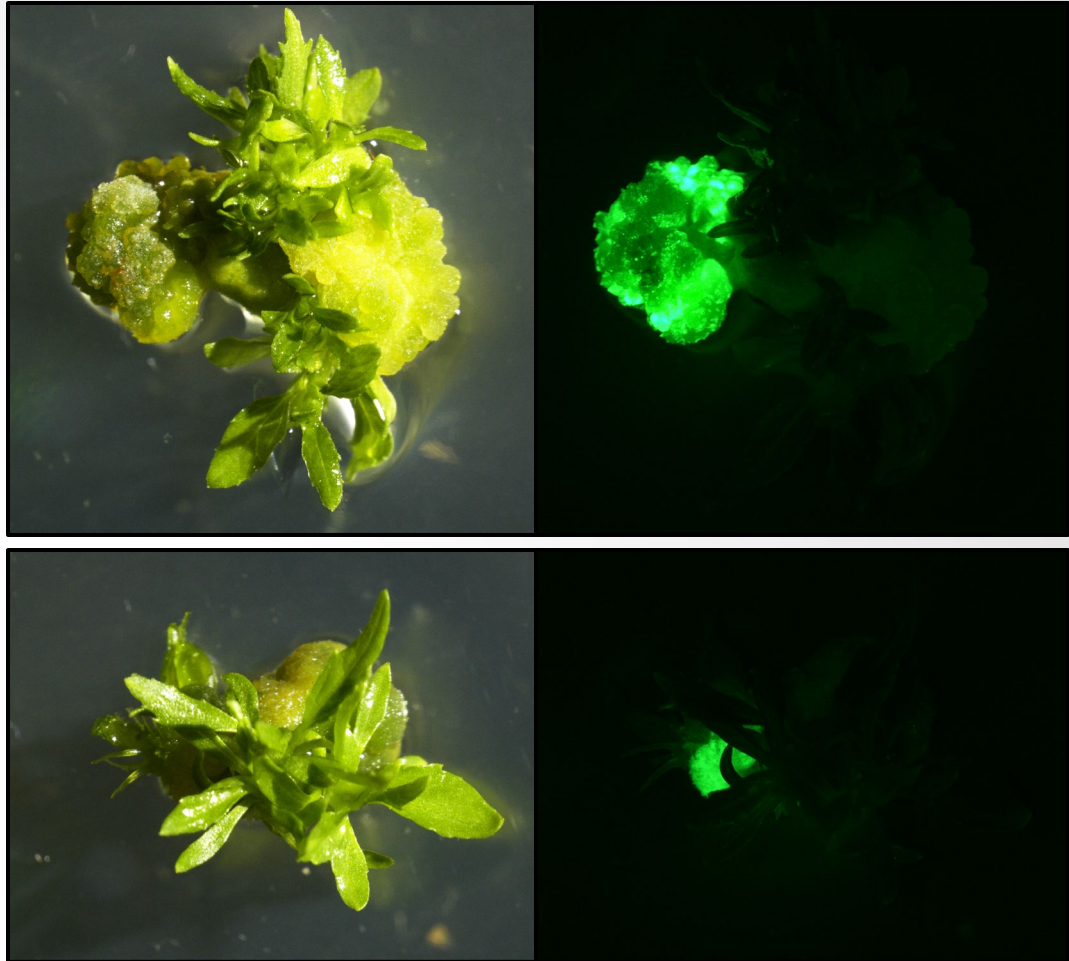
P. trichocarpa



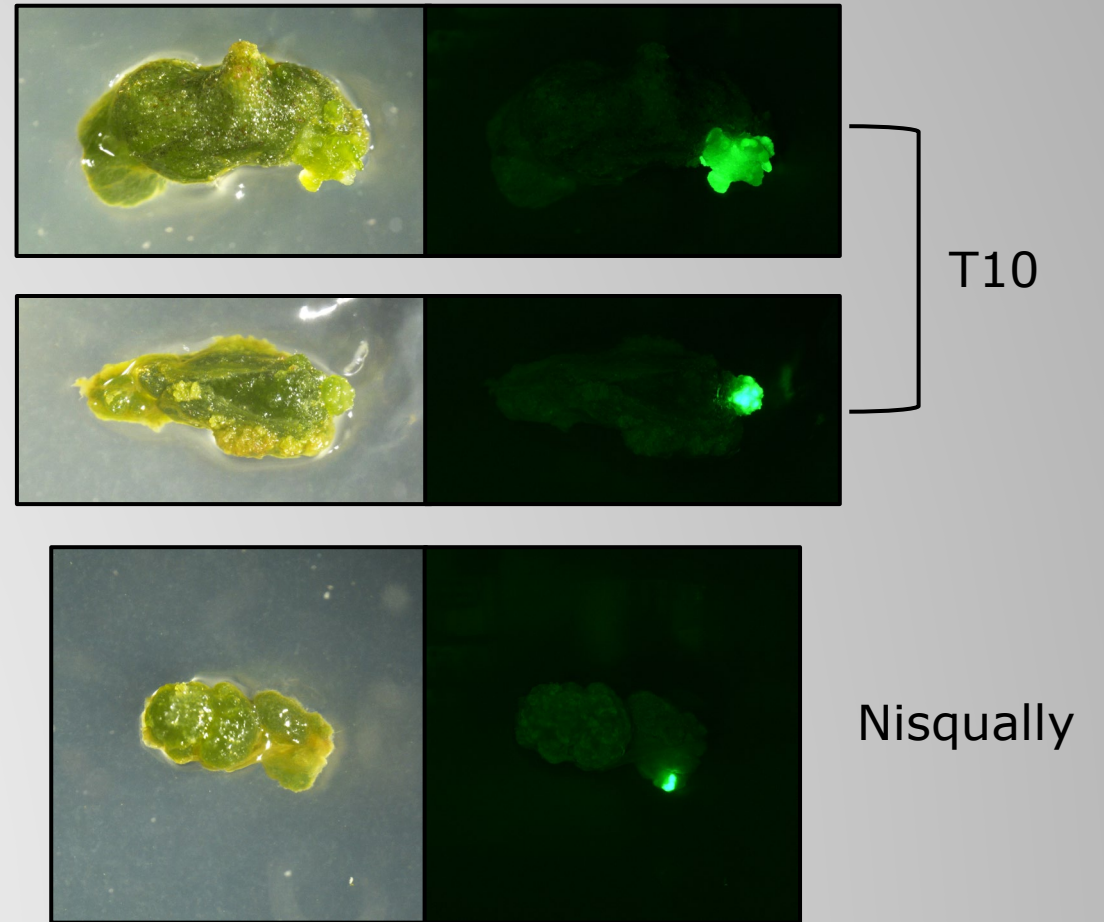


Some strains show improved transformation rates in multiple species and genotypes – Di1411

Hybrid Poplar

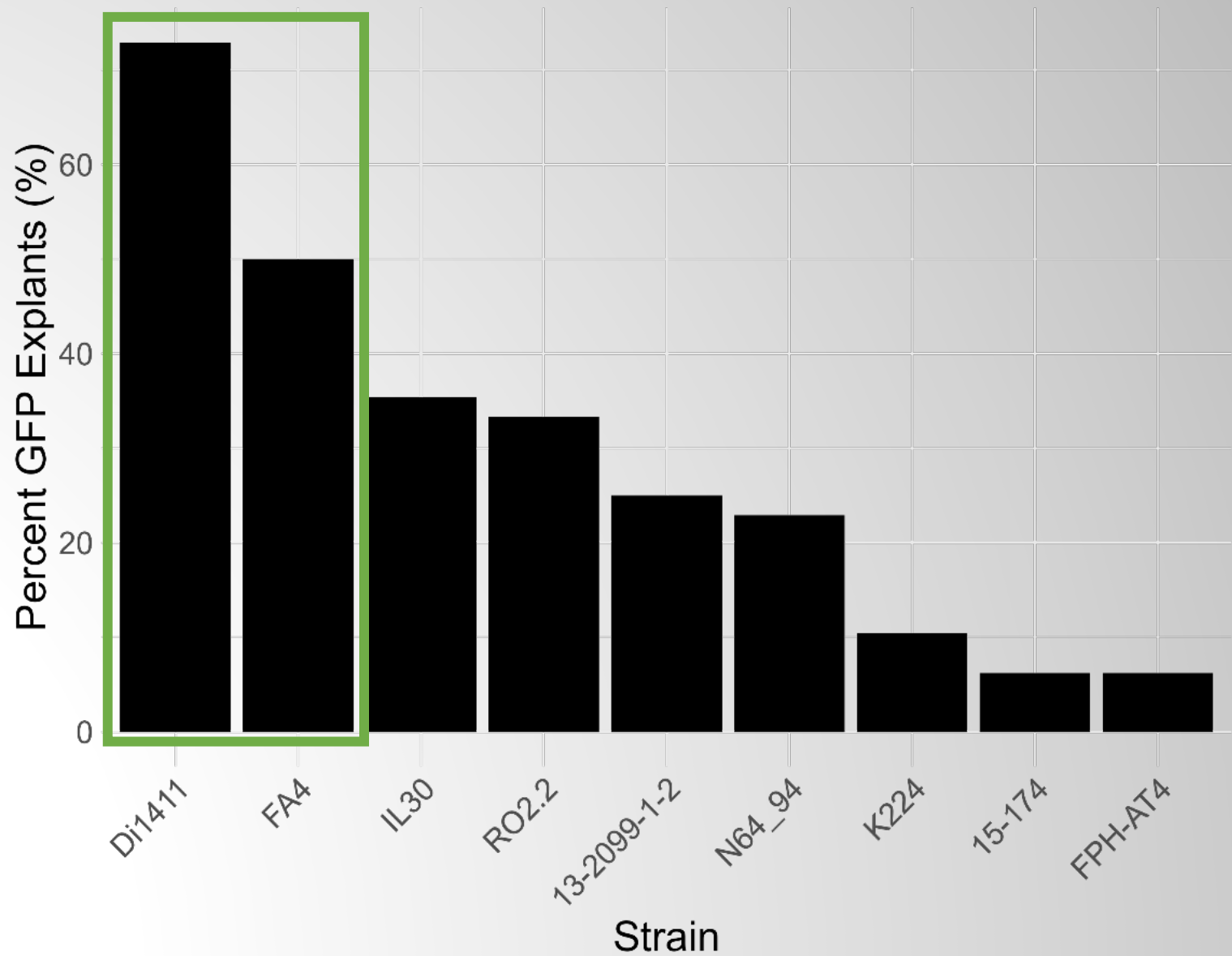


P. trichocarpa





Wild strains show potential for transgenic tissue development in other recalcitrant species including Eucalyptus

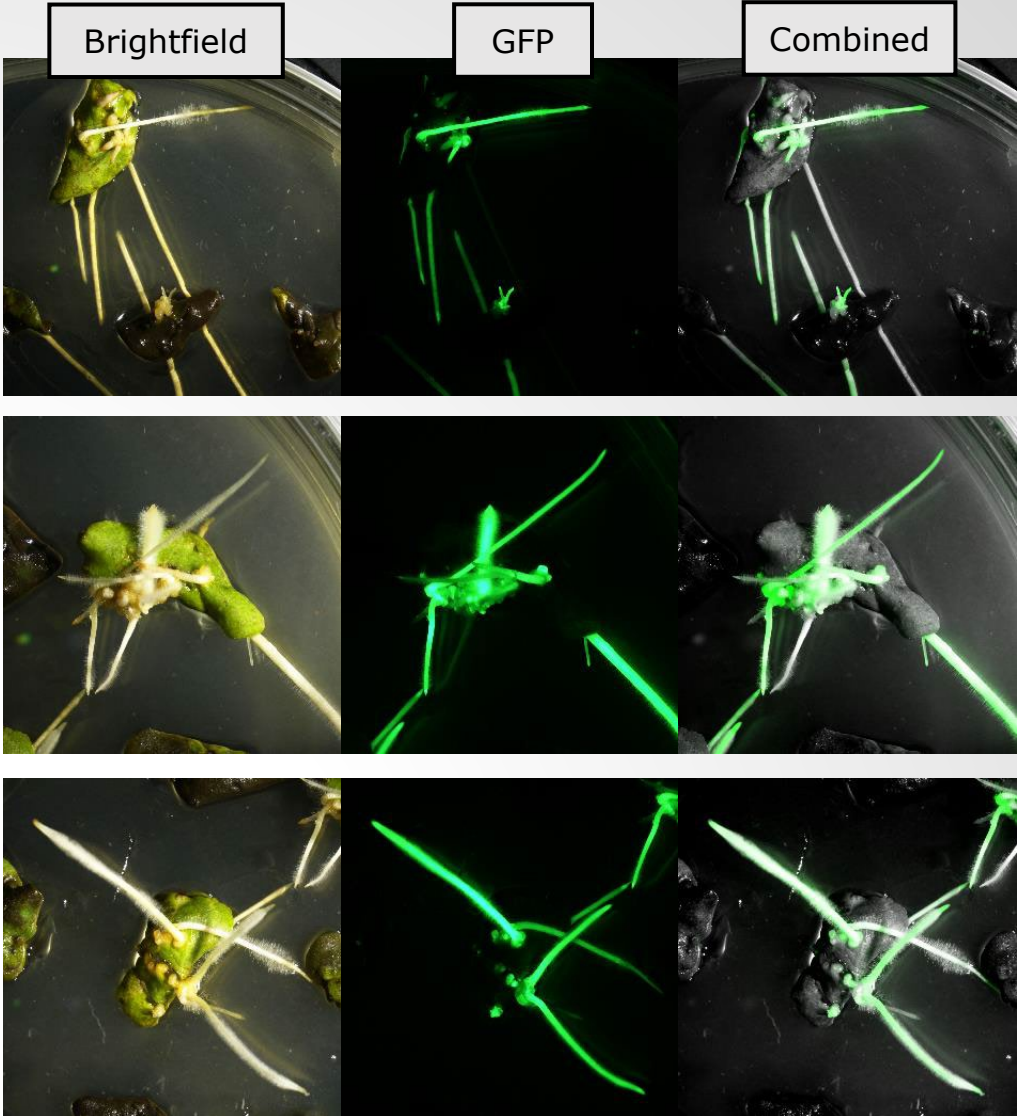




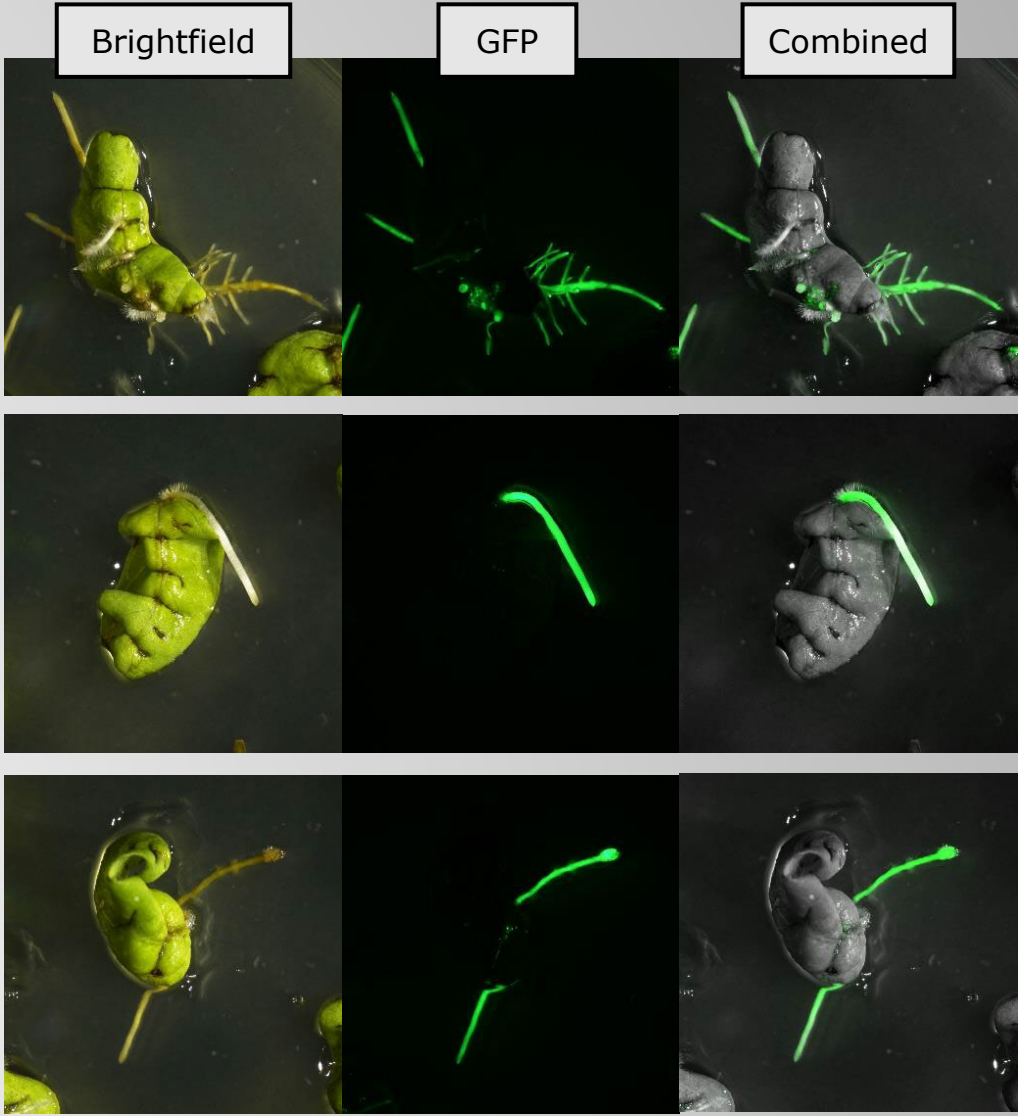
FA4 displays high transformation rates in different Eucalyptus genotypes



Genotype 45-1



Genotype SP7

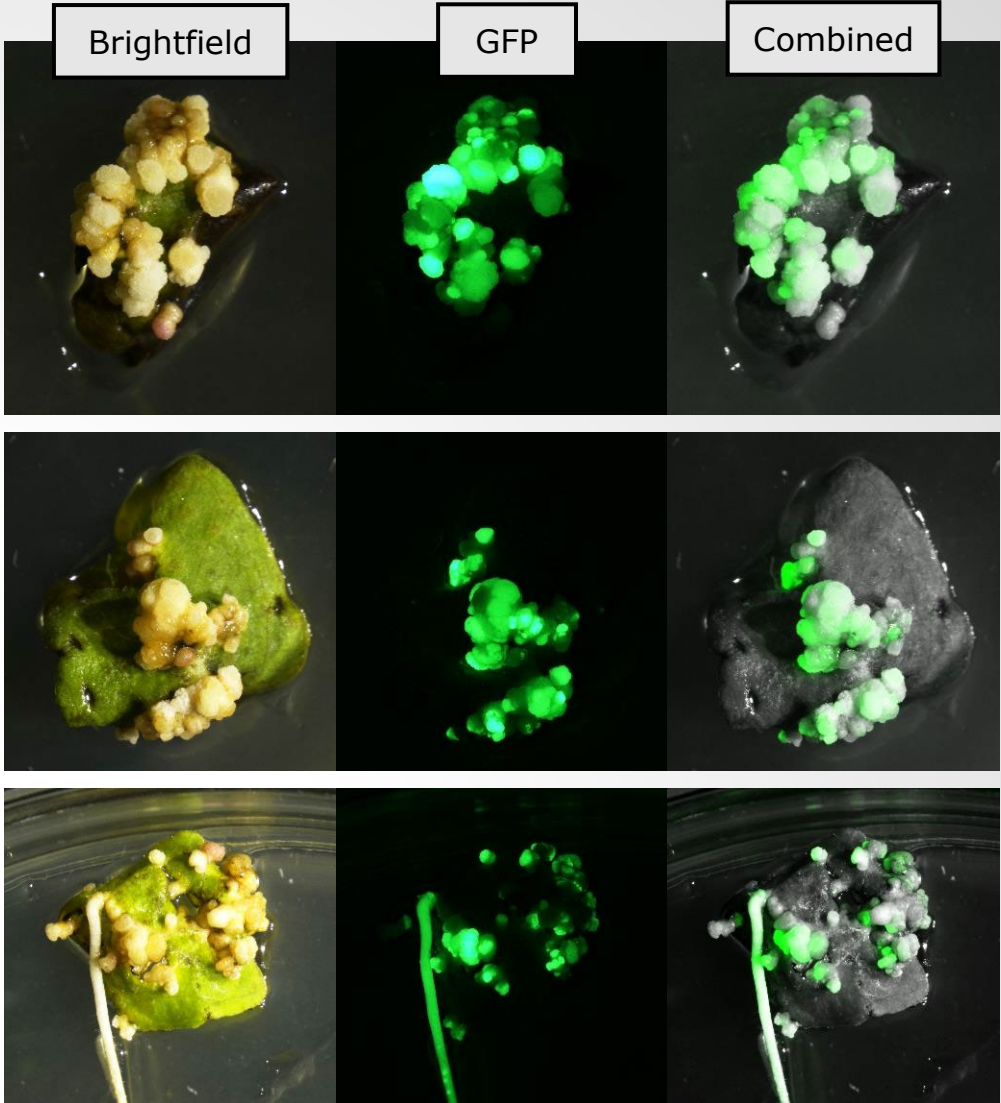




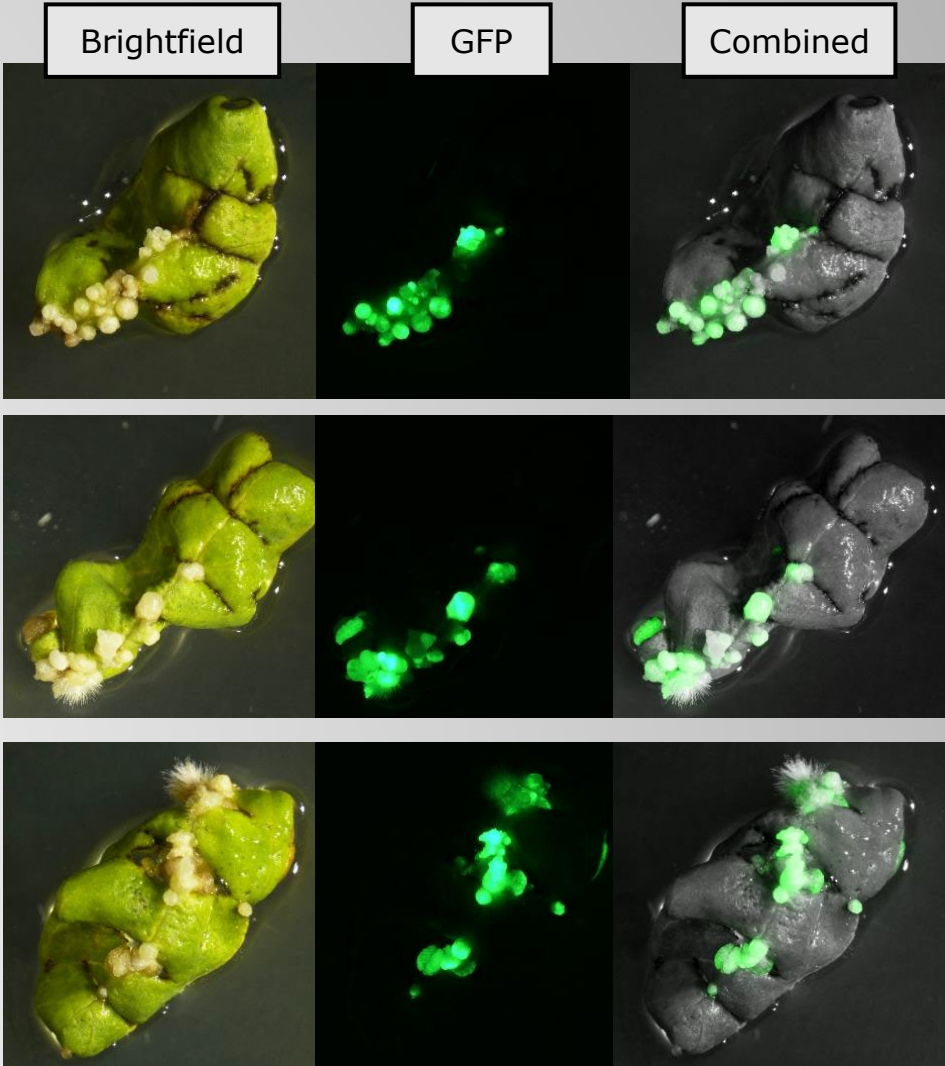
Di1411 displays high transformation rates in different Eucalyptus genotypes



Genotype 45-1



Genotype SP7





Summary and next steps

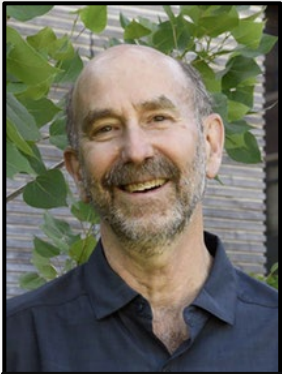
Summary

- Screening a diverse collection has identified strains that outperform current tools in several Poplar species & genotypes
- Some strains contain oncogenes capable of improved organogenesis in absence of plant growth hormones
- Certain wild strains appear to be better suited to certain species while others perform well across species

Next Steps

- Screen a second elite hybrid poplar clone with wild strains
- Disarmament of top performers & testing against current lab strains
- Utilizing novel T-DNA oncogenes for regeneration of whole plant tissues following transformation
- Testing wild & disarmed strains in more plant species (collaborations ongoing)

Acknowledgements



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Chris Willig
Postdoc FES



Jeff Anderson
Associate Professor
BPP



Cathleen Ma
Transformation &
Greenhouse
Experiments



Kate Peremyslova
Transformation
Experiments



Parker Wheeler
Transformation
Experiments



Colette Richter
Transformation
Experiments

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Dana Howe (Lab Manager)

Victoria Conrad (Undergraduate)

Abigail Lawrence (Undergraduate)

Alexandra Weisberg (Assistant Professor BPP)

This work is supported by the U.S. National Science Foundation - Plant Genome Research Project (award no. 2424938), the GREAT Trees Cooperative & by the Wessela Graduate Fellowship at Oregon State University





Thank you!

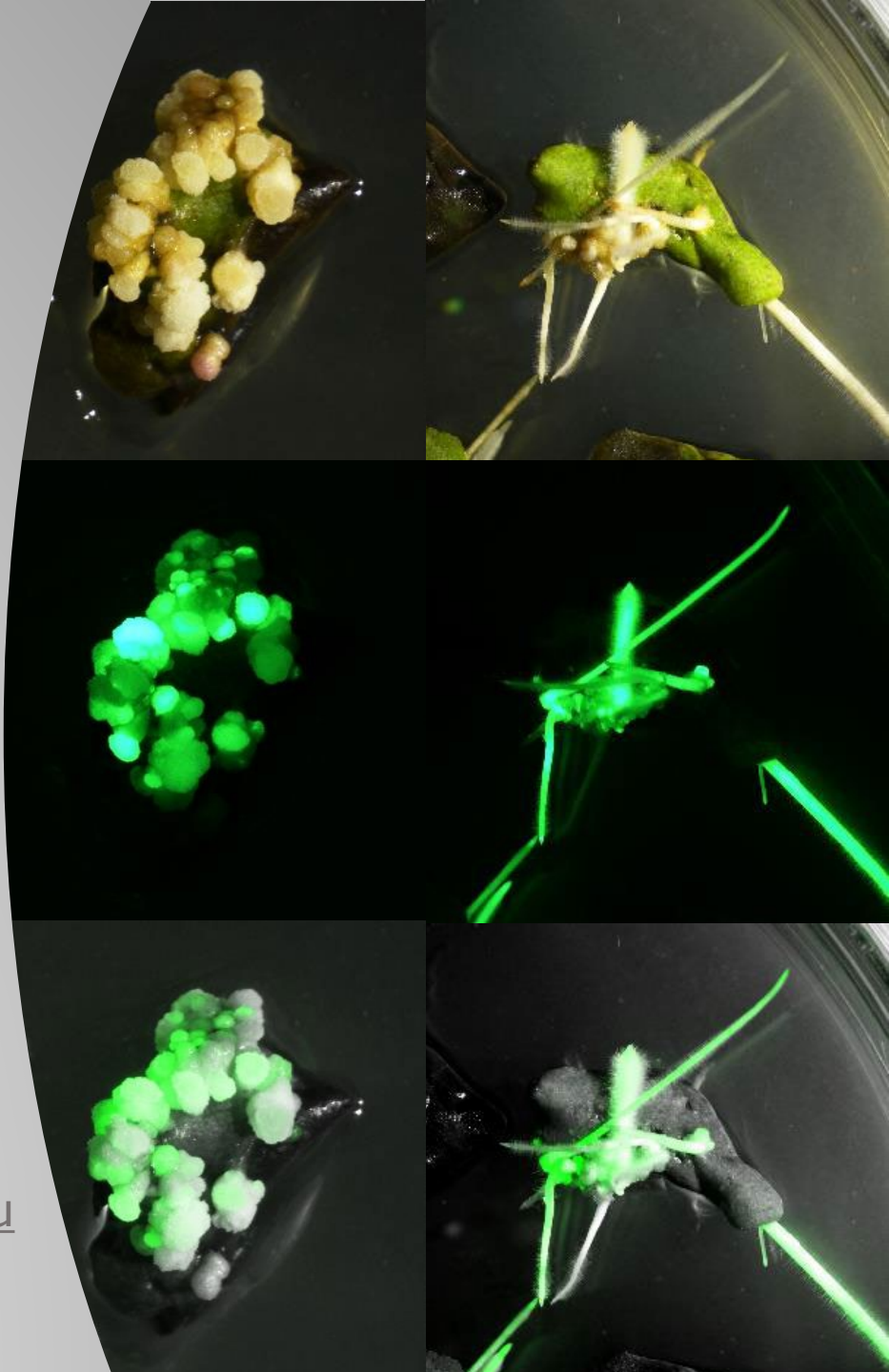
Questions?



Connect with the lab

My email: heinholz@oregonstate.edu

Steve Strauss: steve.strauss@oregonstate.edu

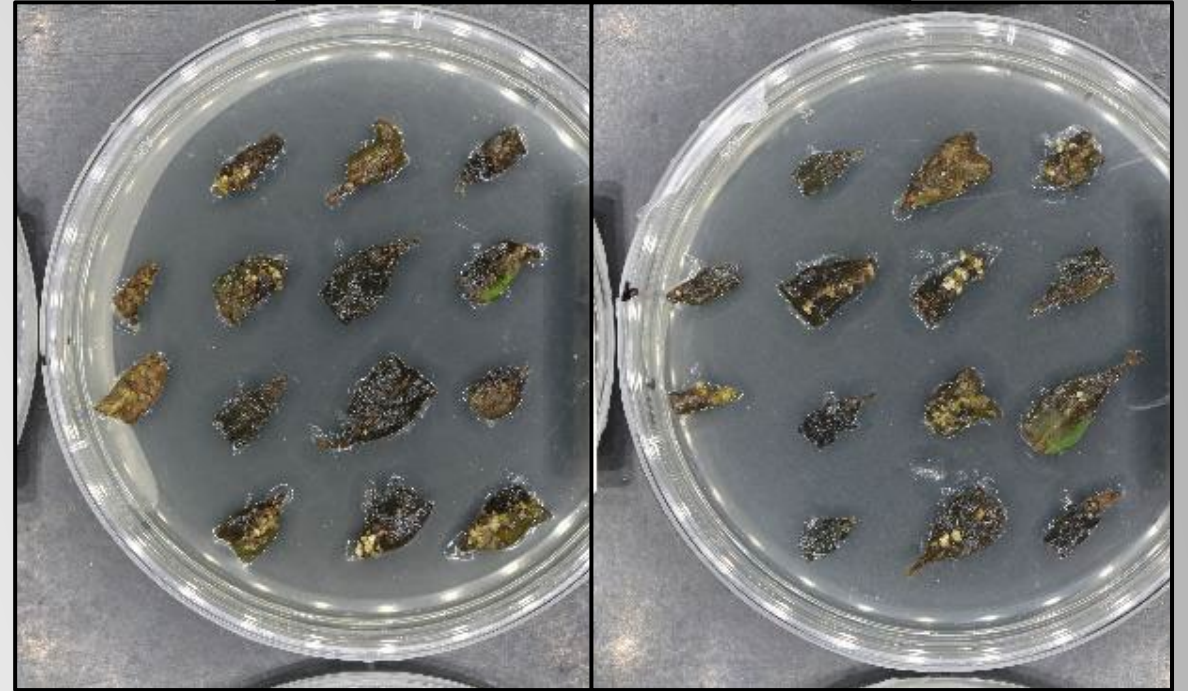


Eucalyptus displays high levels of necrosis following AMT

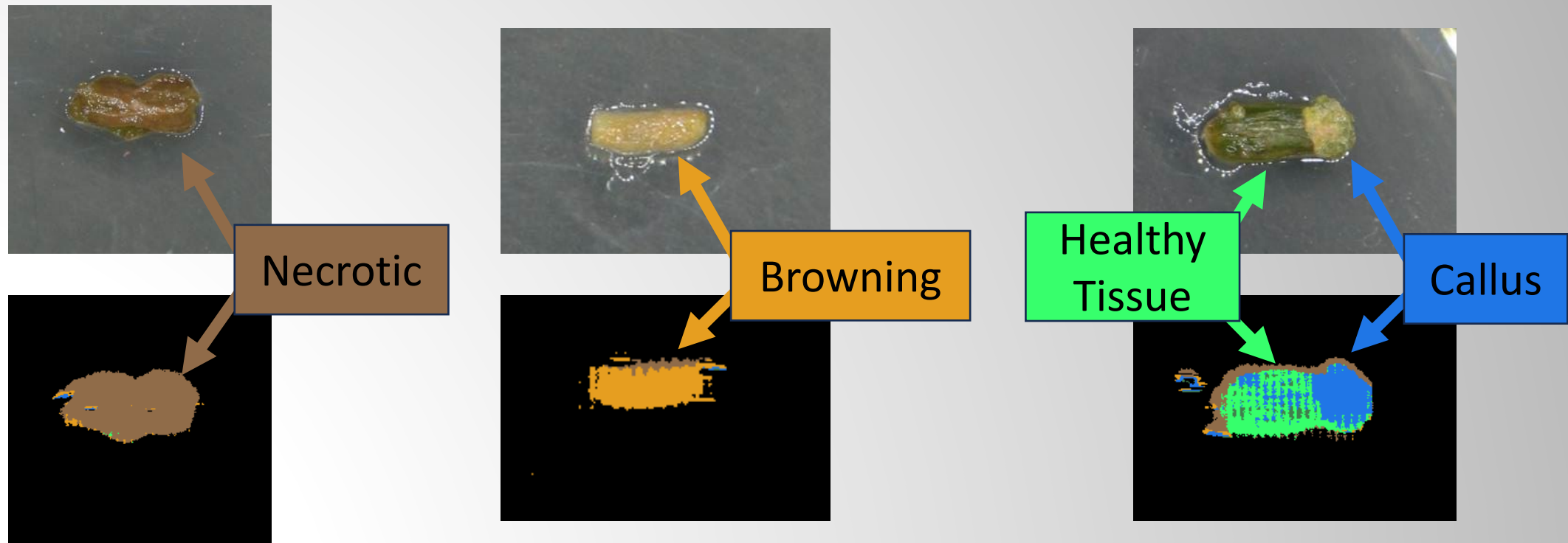
**Water
Treatment**



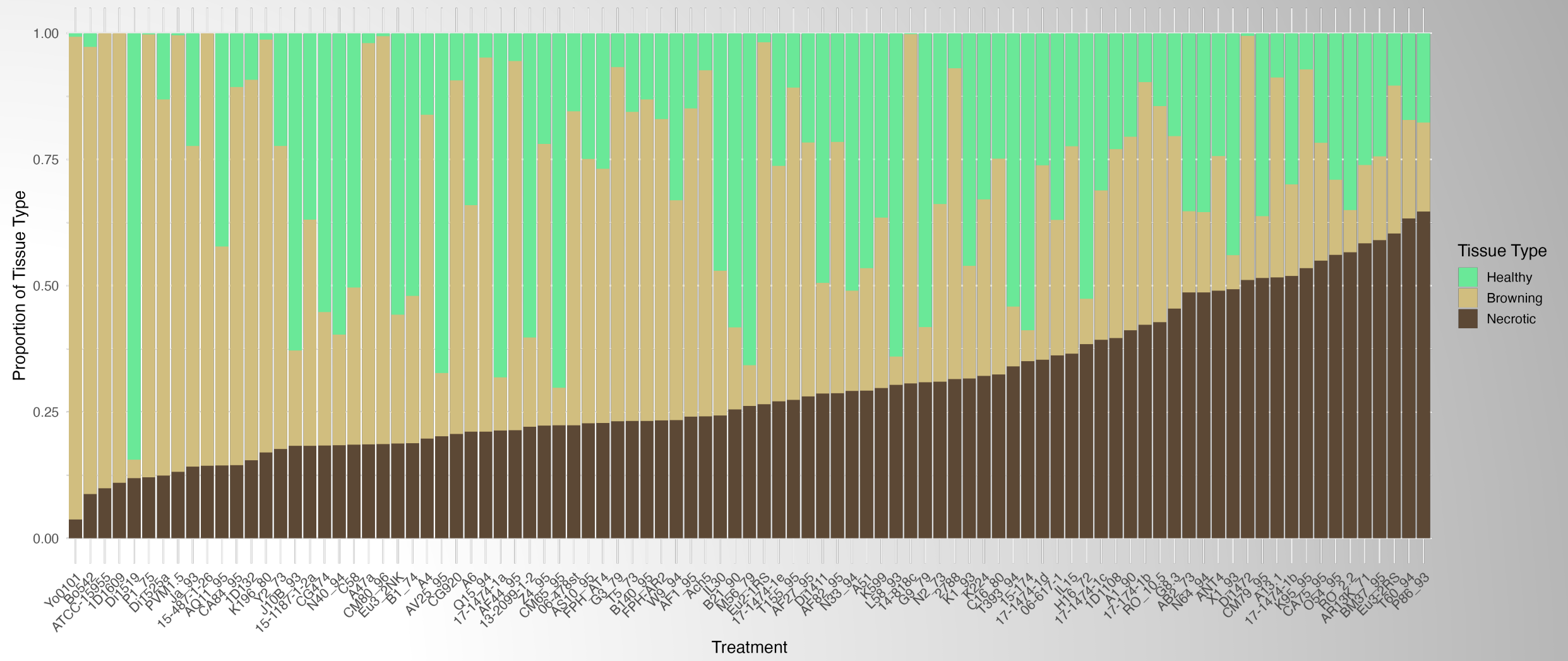
**Agrobacterium
Treatment**



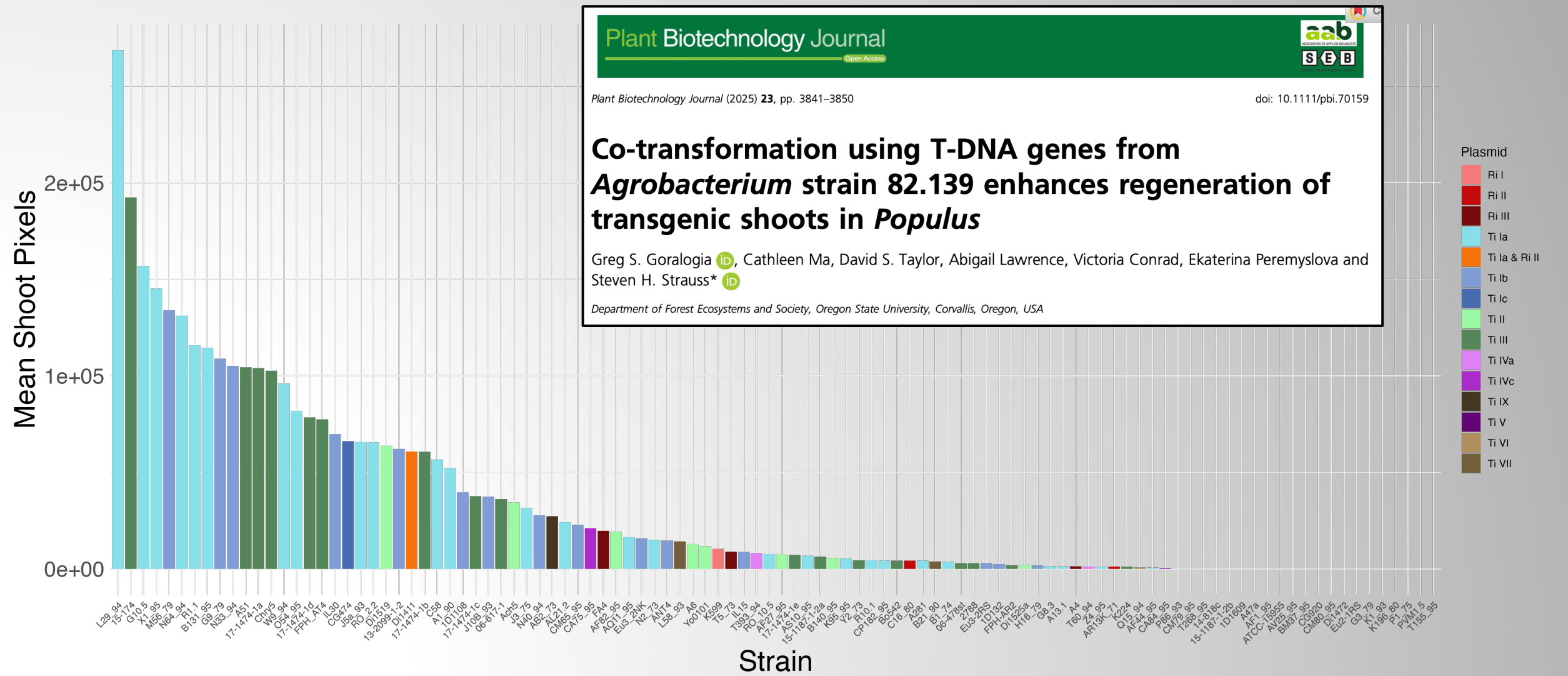
Machine learning models for quantifying different degrees of immune/necrotic response after Agrobacterium treatment



Varying levels of necrosis development amongst wild strains tested in hybrid poplar



Average total shoot production gives indication of strains capable of promoting non-transgenic, distal shoot production



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Co-transformation using T-DNA genes from *Agrobacterium* strain 82.139 enhances regeneration of transgenic shoots in *Populus*

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