Exploring Nature's Transformation Toolbox

Screening Wild Agrobacterium Strains to Improve Transformation and Regeneration in Woody Plants

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Gene editing and genetic engineering: big technologies for improving plants

- Plant biotechnology heavily invested in R&D
  - CRISPR/Cas systems use and research
- Insert desirable traits rapidly into plants
- Challenges with current transformation techniques

Preceon™ Smart Corn System: Bayer Crop Science; edited gibberellin 20 oxidase

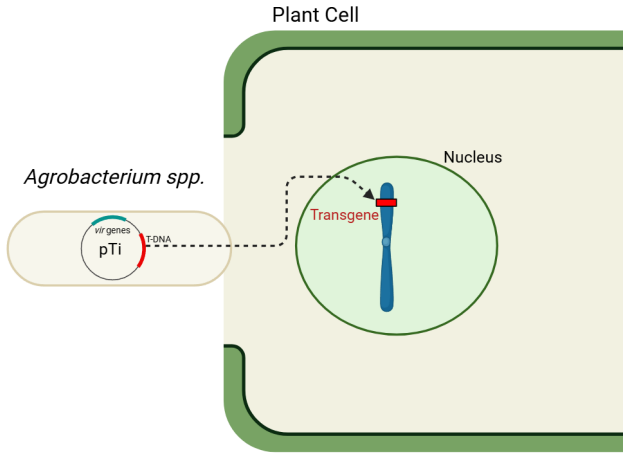
Agrobacterium Mediated Transformation (AMT): the most popular way to transform plants

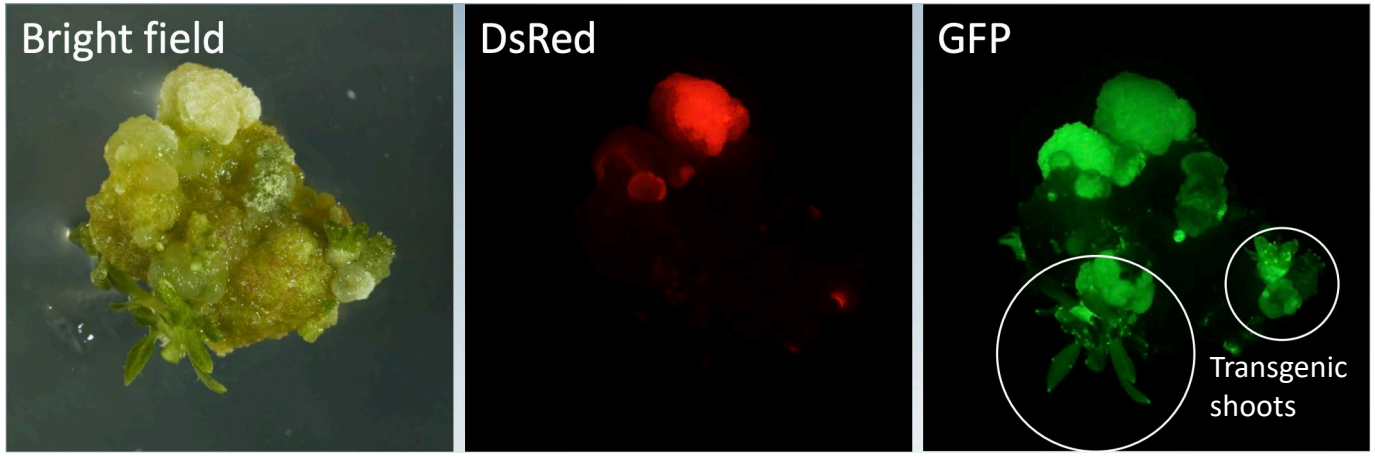
Pros:

- Ease of use
- Promotes regeneration after transformation
- Robust with many common molecular cloning techniques

Cons:

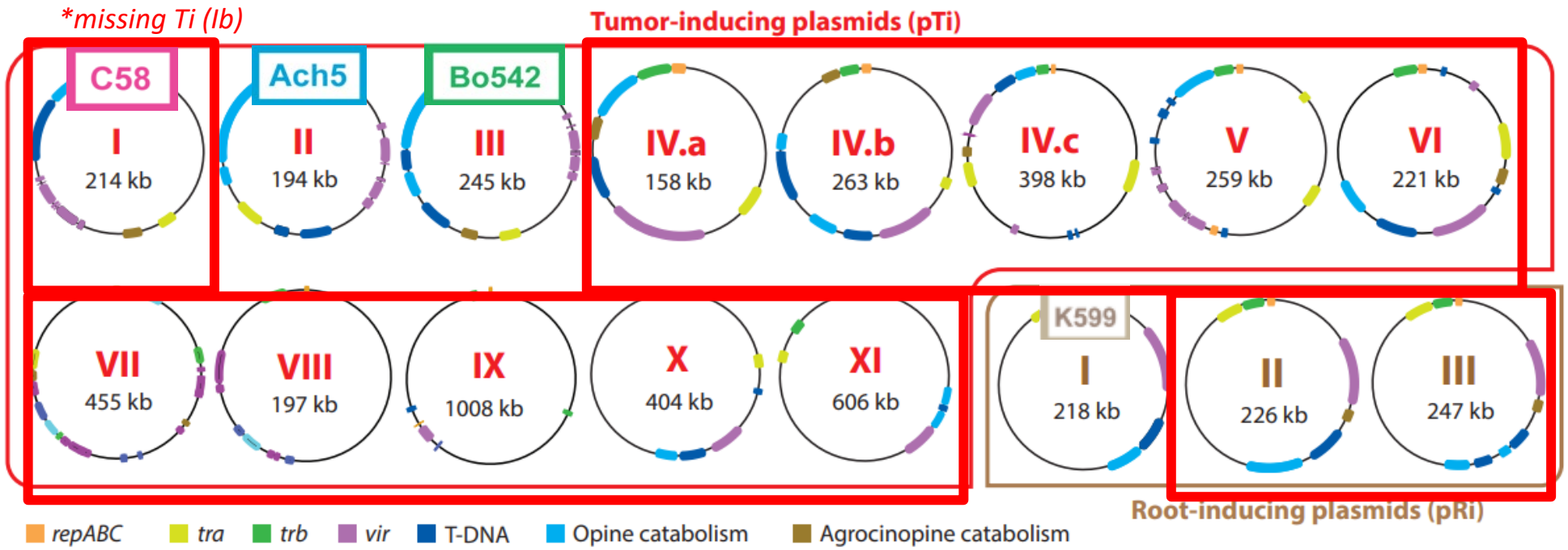
- Many species & genotypes recalcitrant to transformation
- Organogenesis after transformation limited in many species





Altrustic transformation with "shooty" 82.139 strain

Limited plasmid type diversity for currently used lab strains



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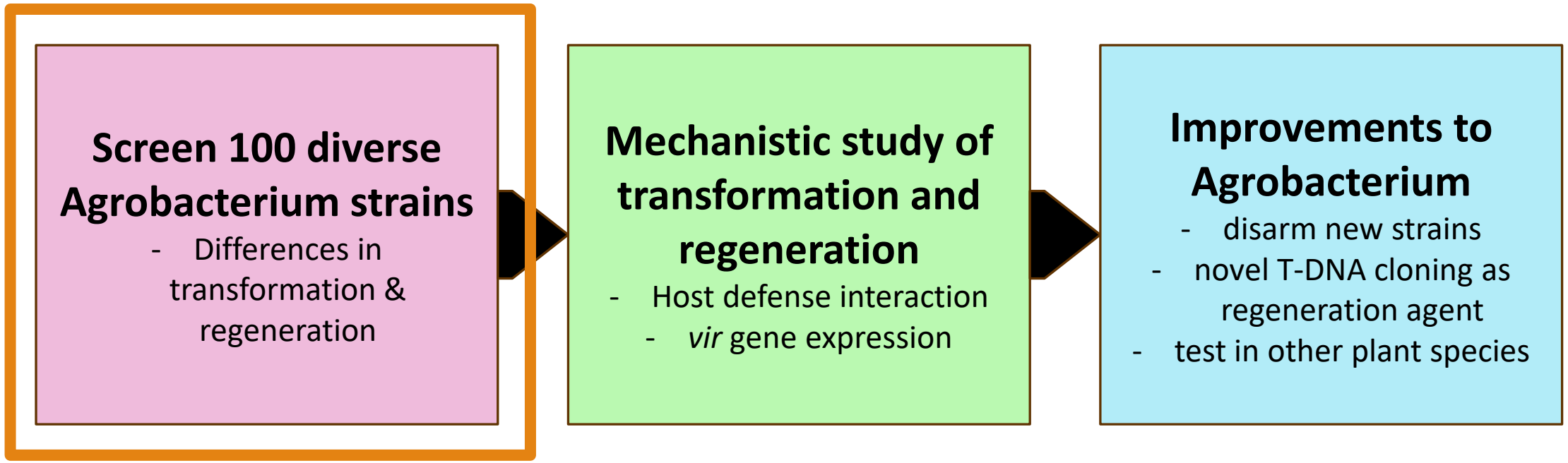
Virulence and Ecology of Agrobacteria in the Context of Evolutionary Genomics

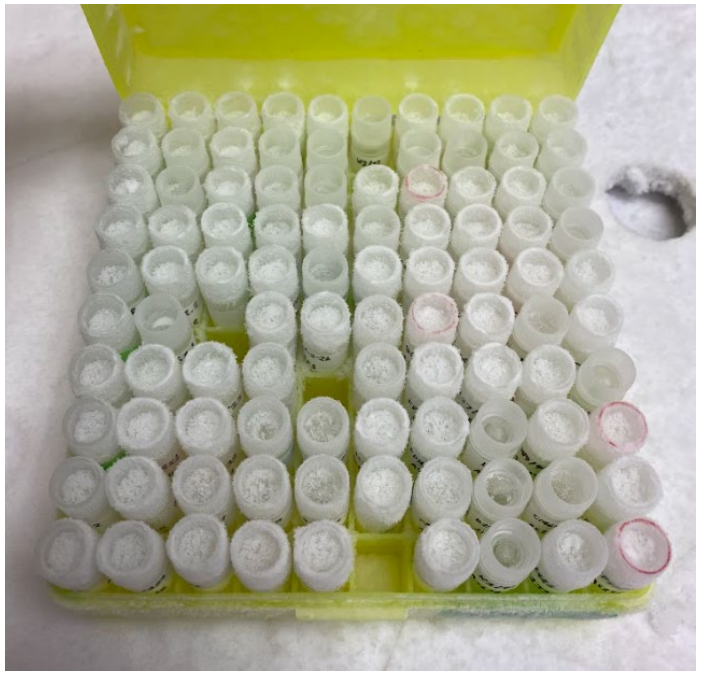
Alexandra J. Weisberg[1], Yu Wu[2,3,4], Jeff H. Chang[1], Erh-Min Lai[2,3,5], and Chih-Horng Kuo[2,3,5]

About 50 years of Agrobacterium research at OSU

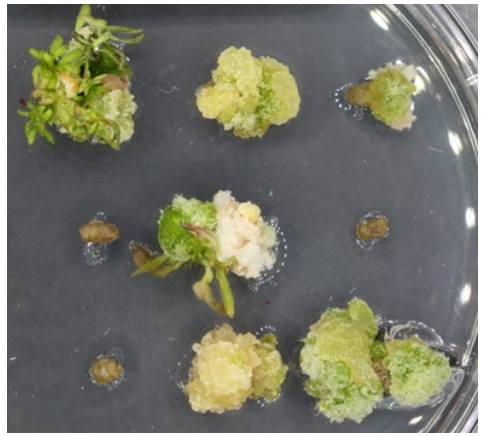
Strategy to Utilize Agrobacterium Diversity - Promoting Woody Plant Transformation & Regeneration



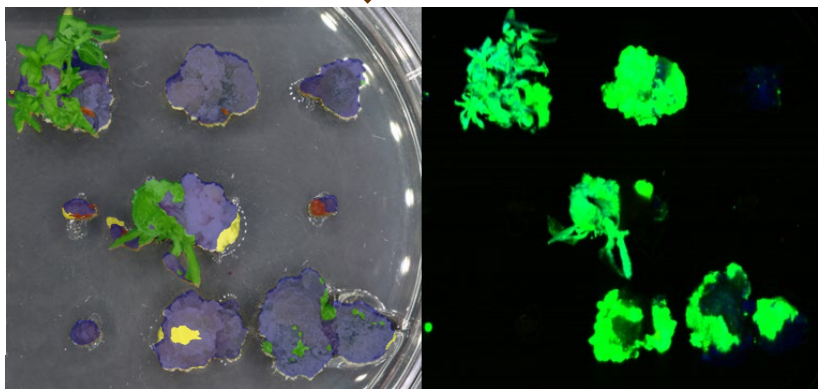
Screening 100 diverse Agrobacterium strains using high throughput phenomics



Insert GFP or RUBY reporter genes into the Agrobacterium



- Hybrid poplar (*easy*)
- Black cottonwood (*moderate/hard*)
- Eucalyptus (*hard*)

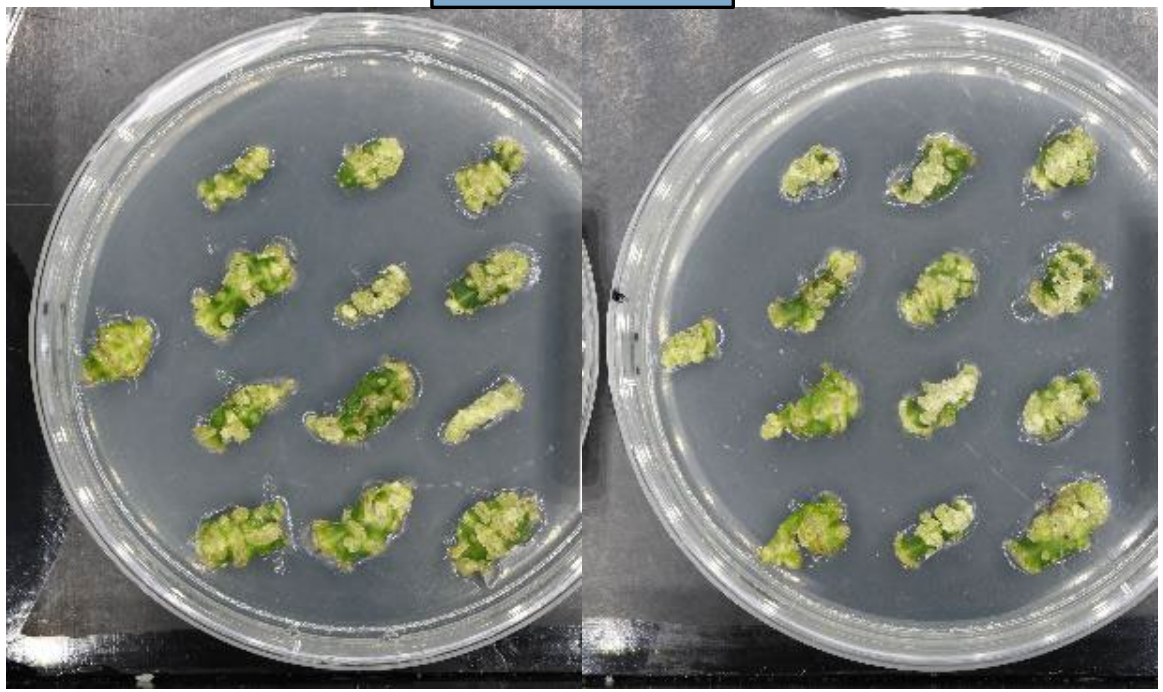


- Necrosis
- Transformation
- Regeneration

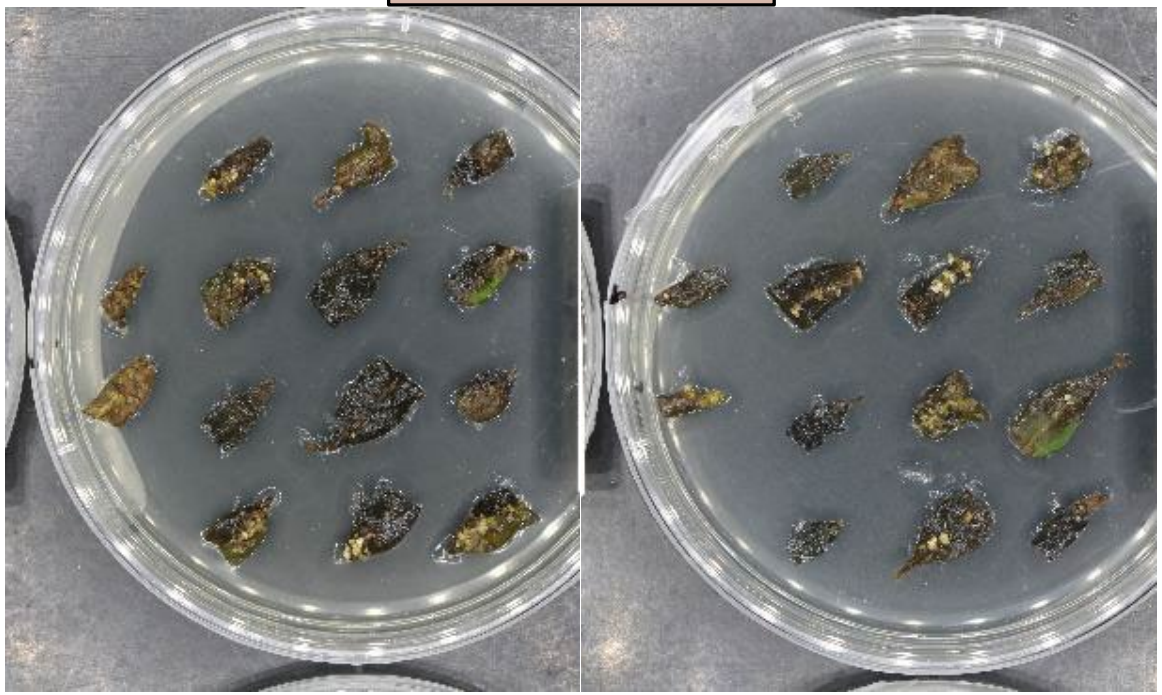
Recalcitrant species & genotypes display high rates of necrosis following AMT

GREAT Trees Cooperative

Water only

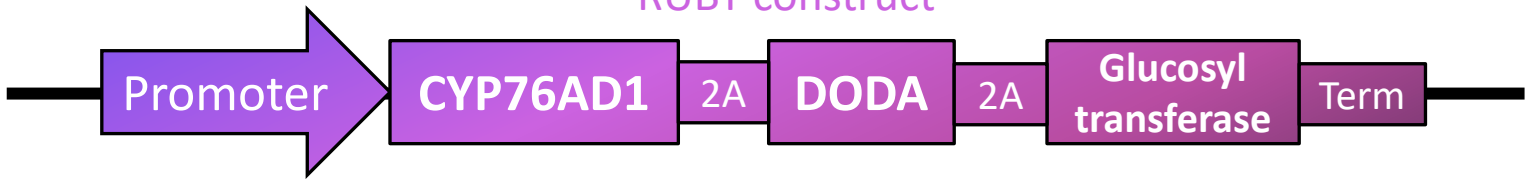


Agrobacterium



Using RUBY reporter to visually screen transformation rates

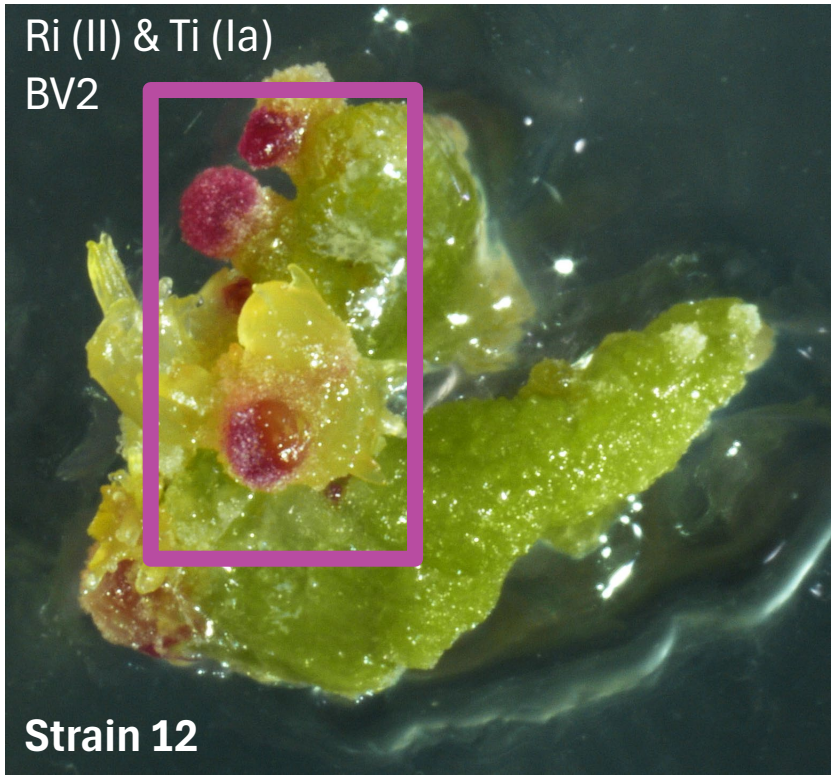
RUBY construct

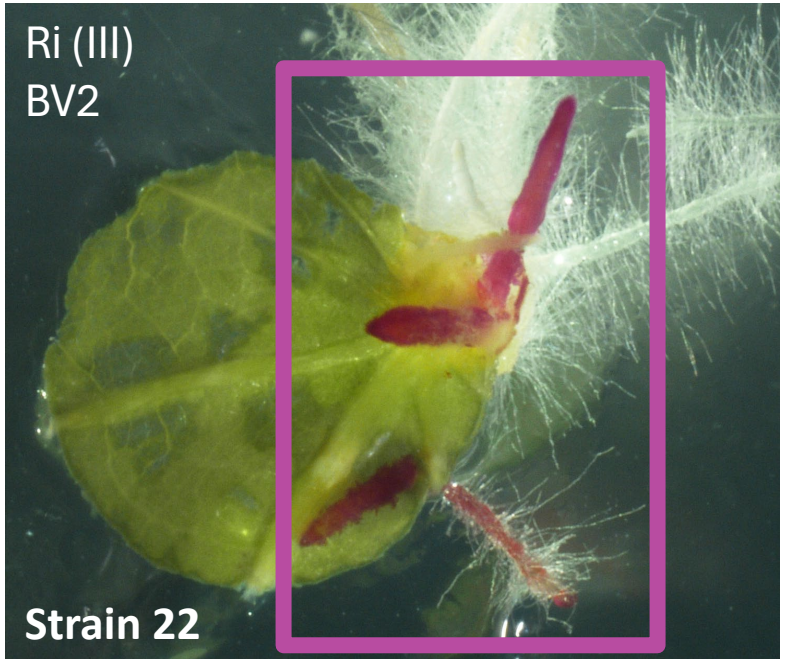


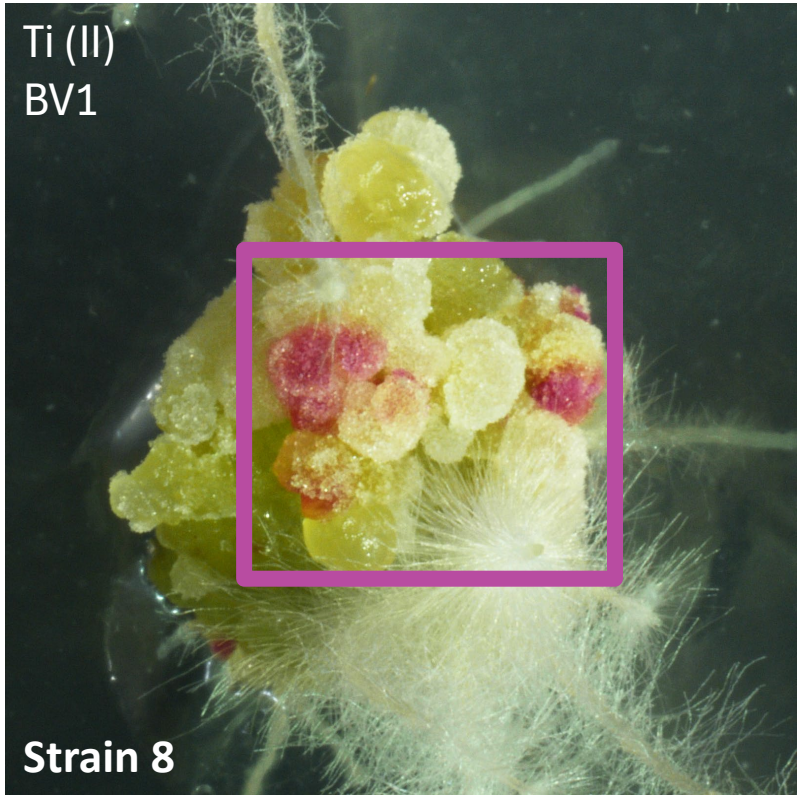
A reporter for noninvasively monitoring gene expression and plant transformation

Yubing He, Tao Zhang, Hui Sun, Huadong Zhan & Yunde Zhao

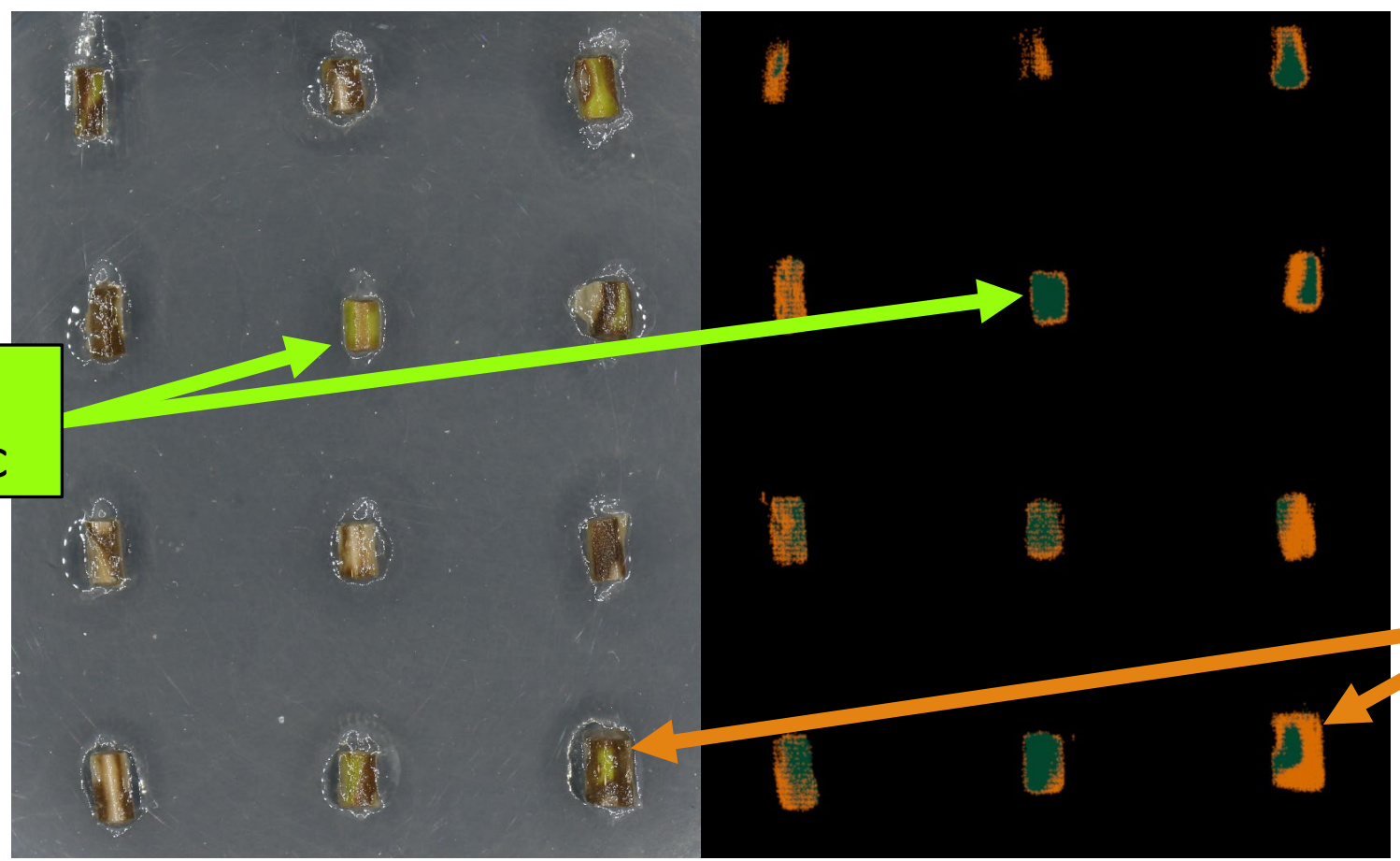
Horticulture Research **7**, Article number: 152 (2020)



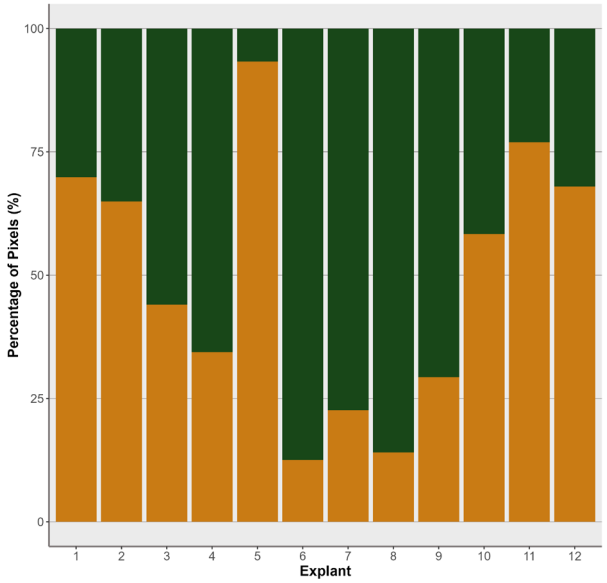




Quantifying necrosis - machine learning in our high throughput phenomics pipeline



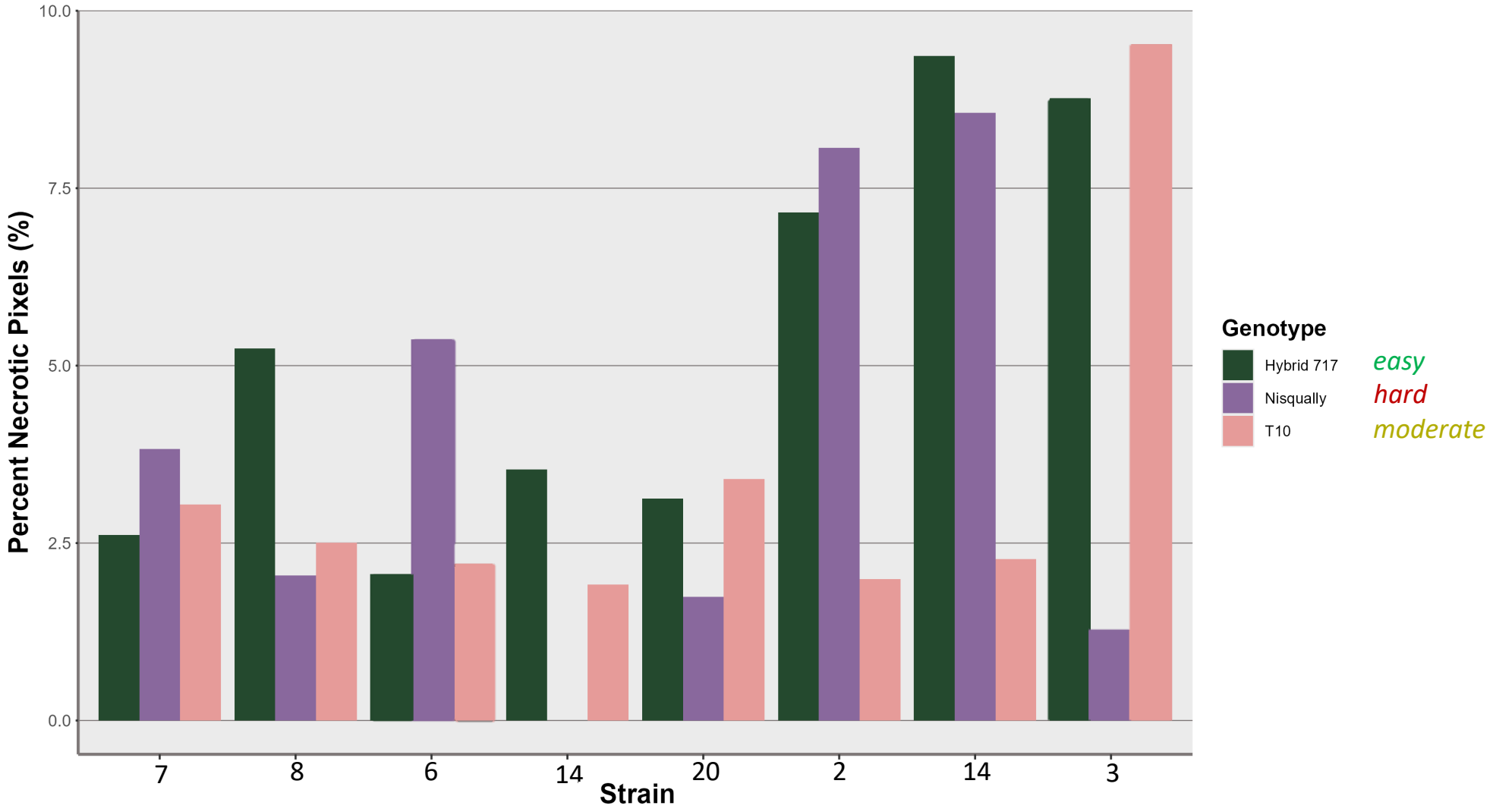
Non-Necrotic



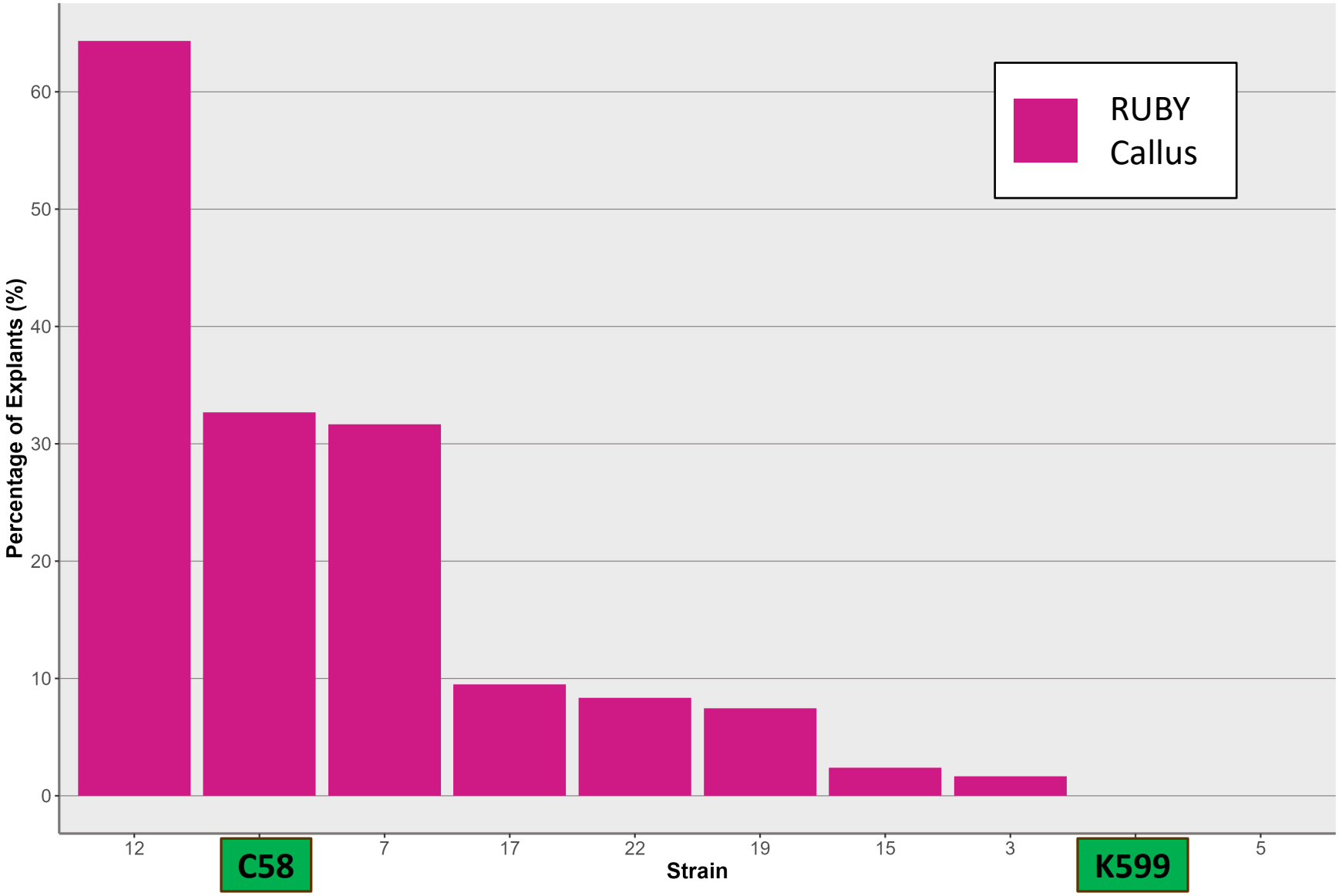
Necrotic

Preliminary Results

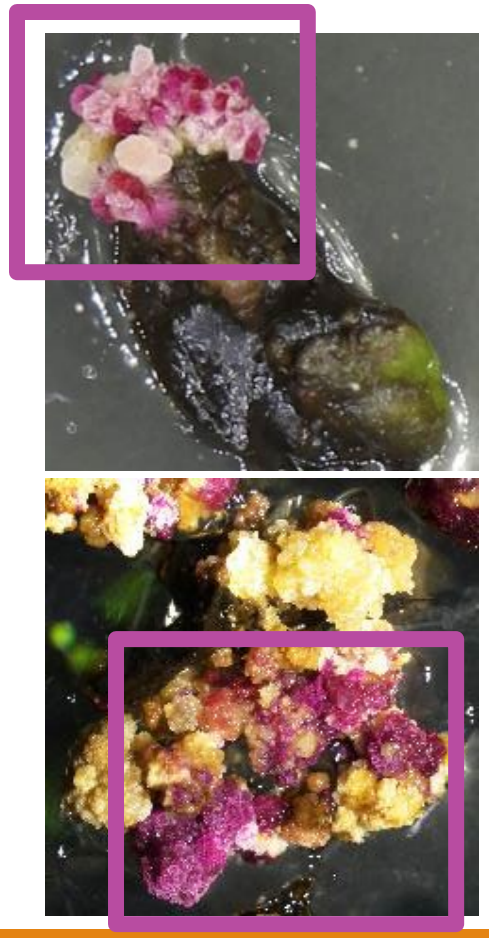
Necrosis shows strong strain to genotype interaction

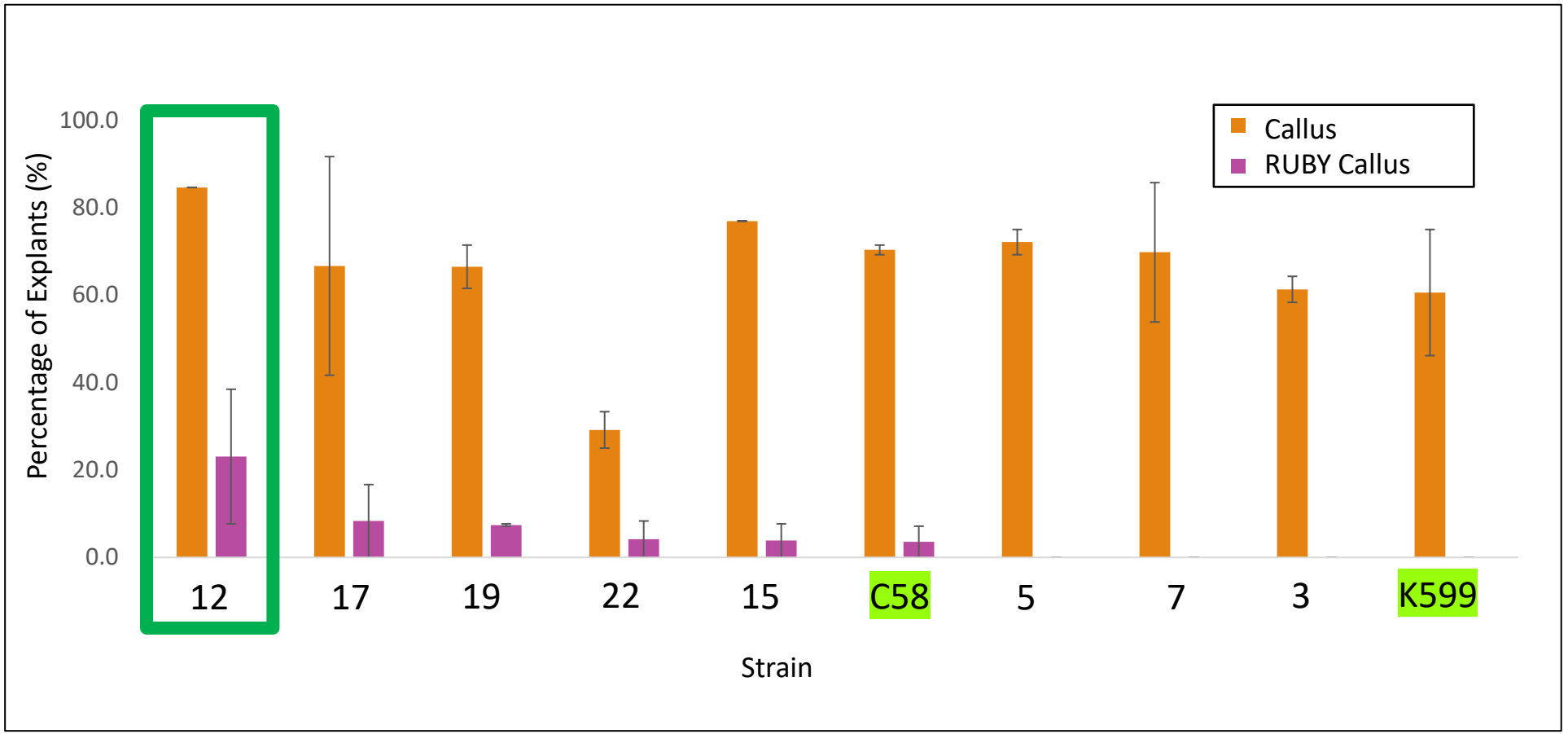


Strain 12 outperforms C58 in hybrid poplar

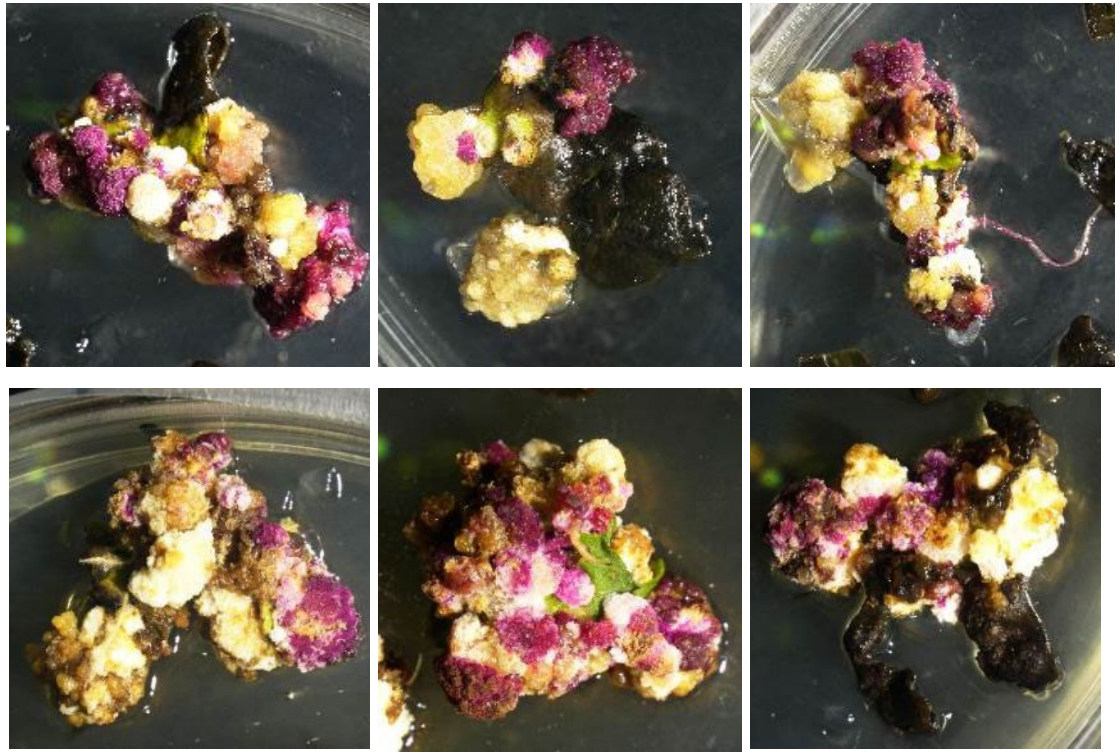


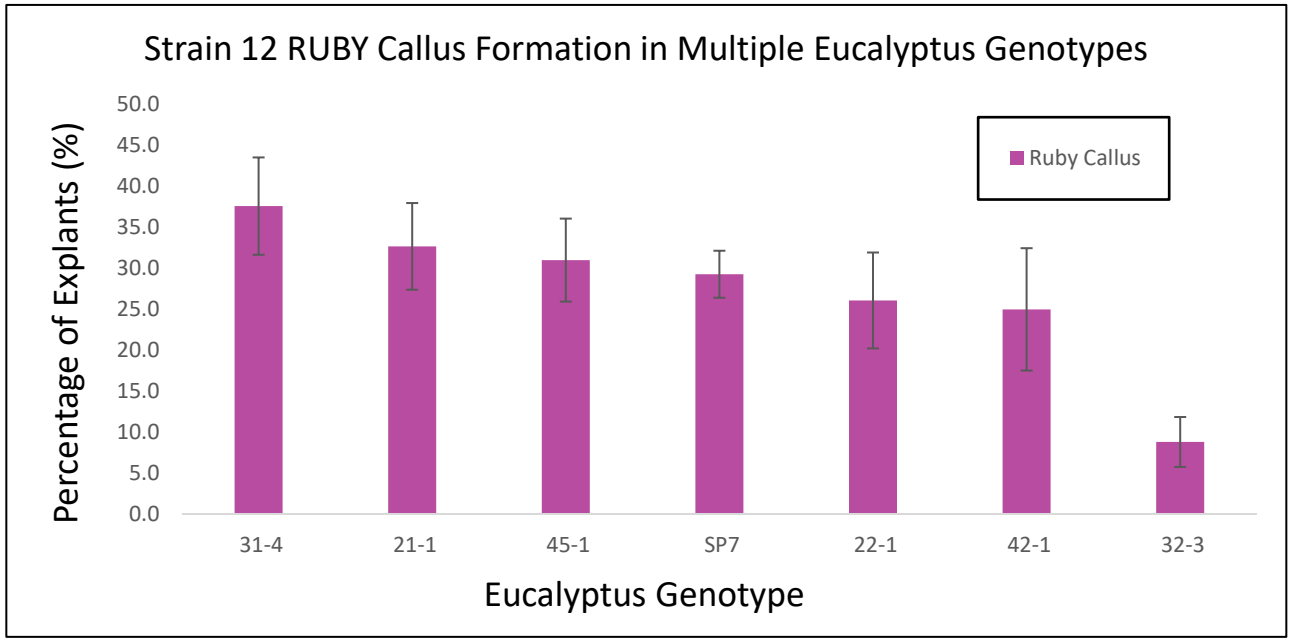
Several wild strains outperform C58 in Eucalyptus





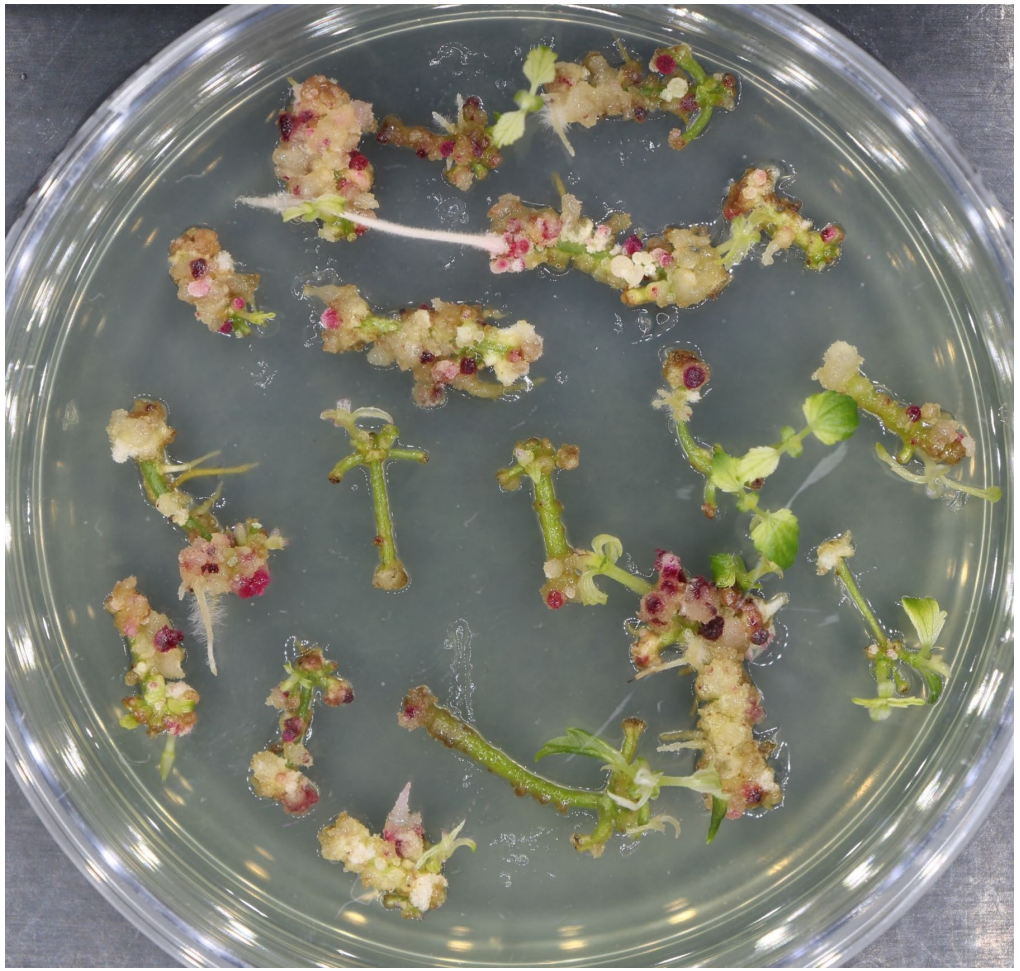
Strain 12 shows high rates of transformation in seven Eucalyptus genotypes

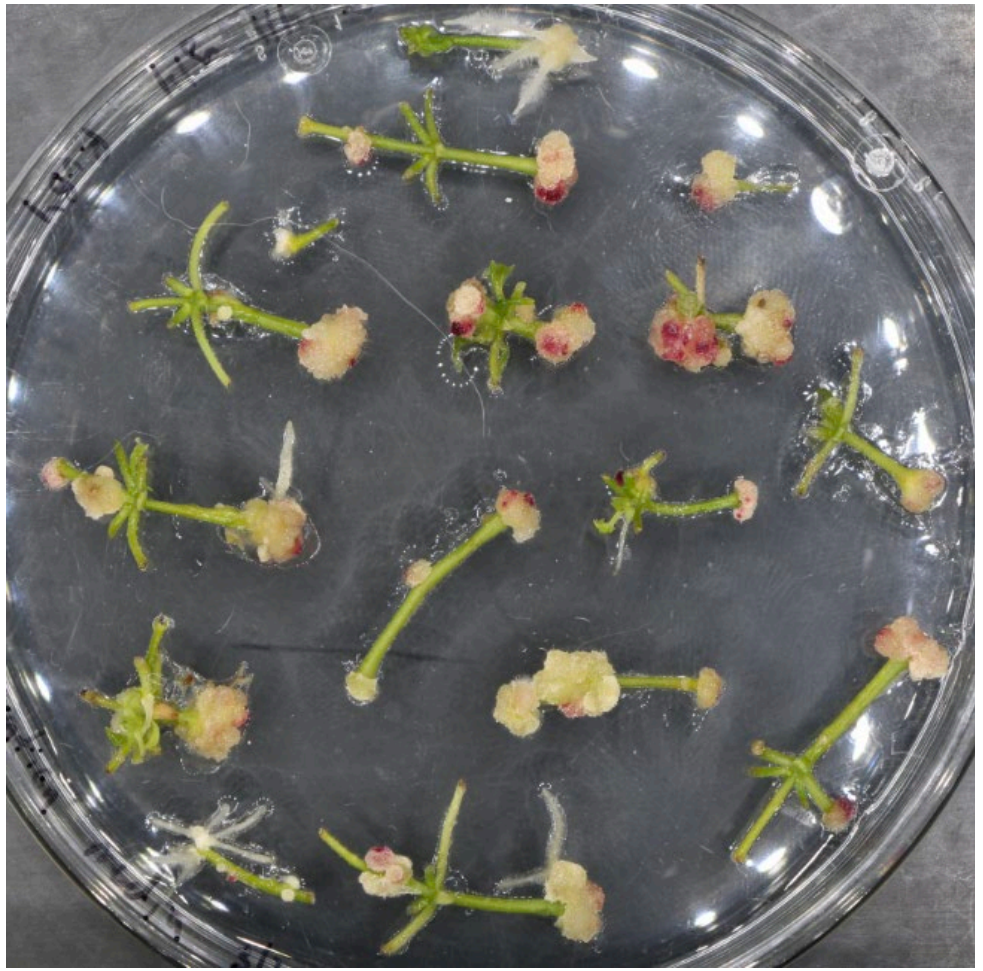




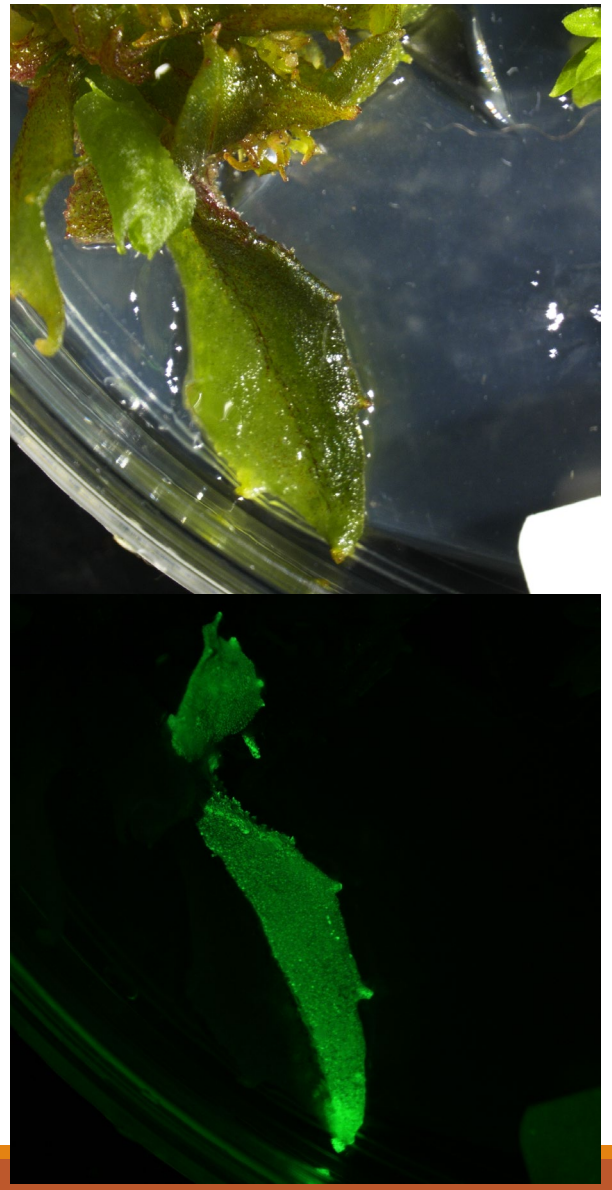
- 8-37% RUBY callus formation among all seven Eucalyptus genotypes tested

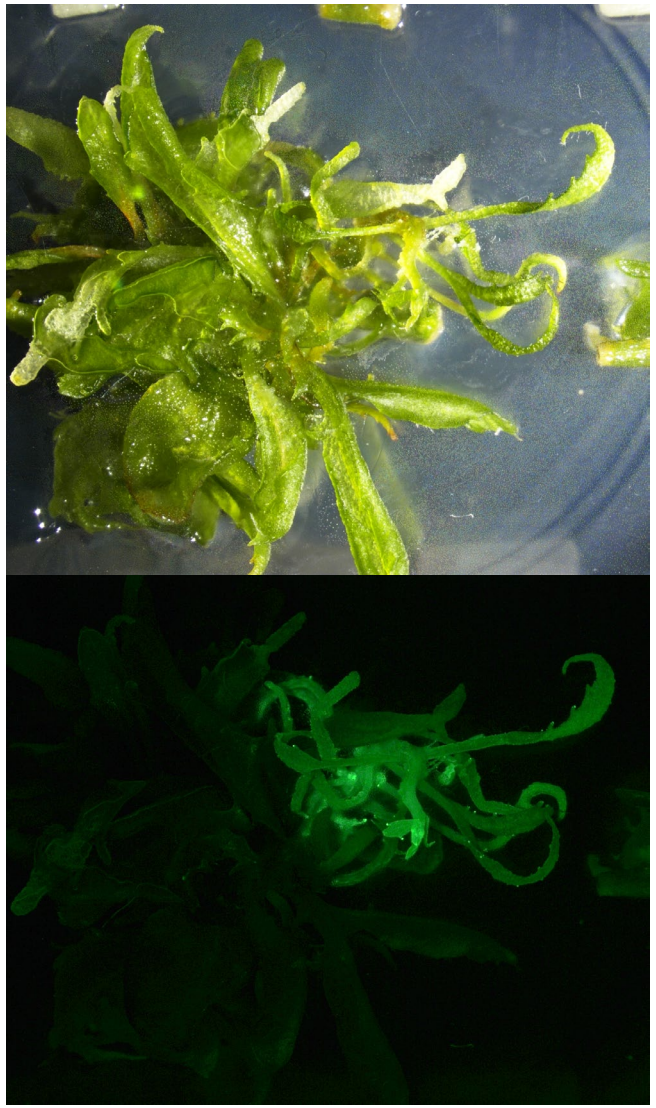
Strain 12 shows high rates of transgenic RUBY callus in Hops

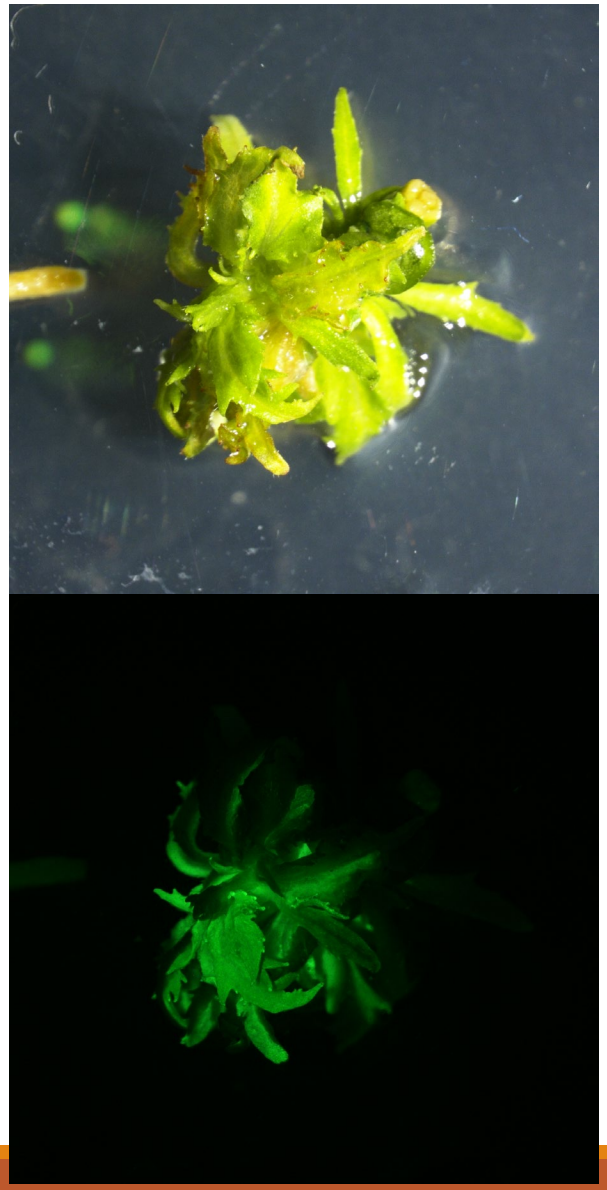




Some strains showed improved regeneration – a key obstacle



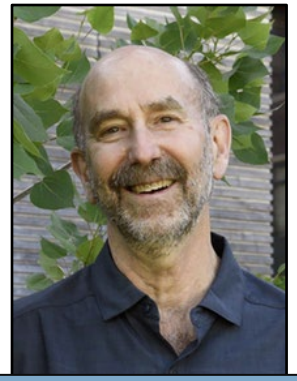




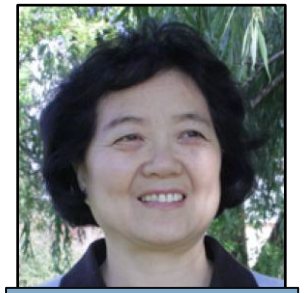
Summary

- Many plant species and genotypes are very difficult to transform – new technology needed
- Very little natural diversity in Agrobacterium has been utilized to date
- Plant defense responses including necrosis in response to Agrobacterium is often great
- Early screening has already identified new wild strains with improved transformation and regeneration rates

Thank you



Steve Strauss
Professor FES



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GREAT Trees Cooperative

Oregon State University

Thank you! Questions?

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