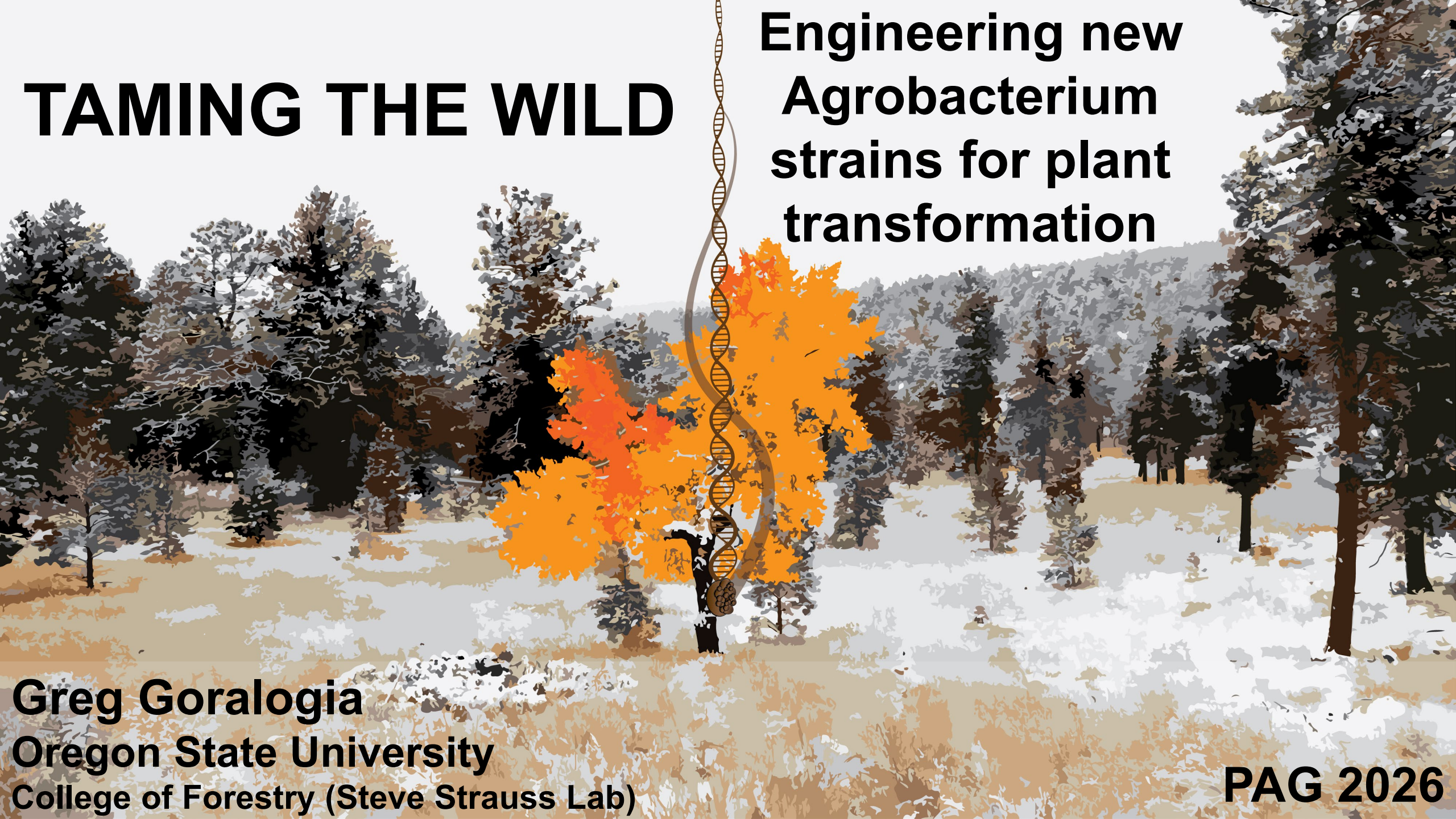
TAMING THE WILD

Engineering new Agrobacterium strains for plant transformation



Greg Goralogia

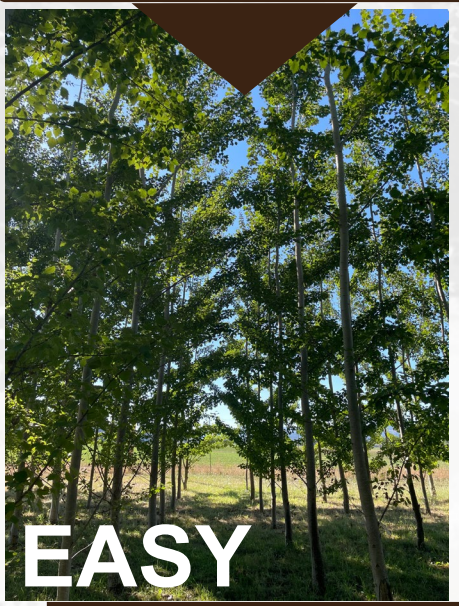
Oregon State University

College of Forestry (Steve Strauss Lab)

PAG 2026

Inefficient transformation remains a bottleneck for biotechnology in many species, including forest trees and specialty crops

Hybrid Poplar
Populus tremula x alba 717-1B4
Populus tremula x tremuloides 353-53

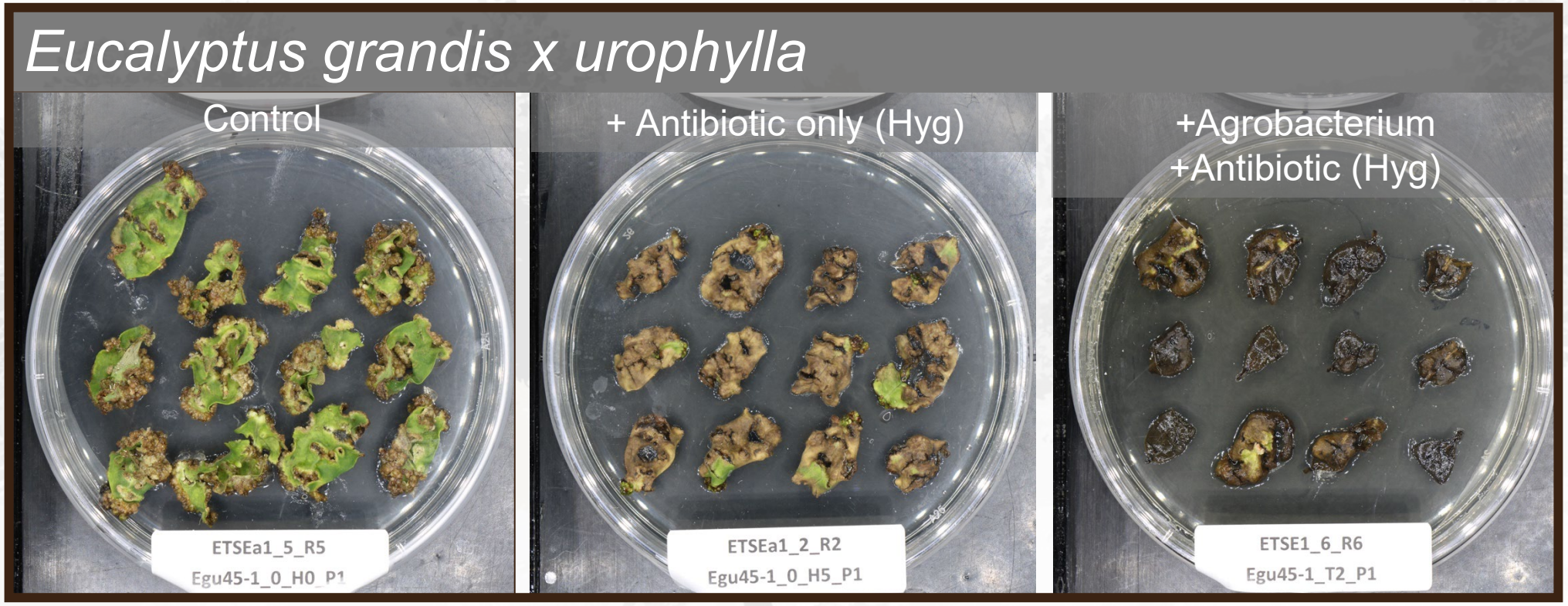


Black Cottonwood
Populus trichocarpa
(~1500 genotype GWAS population)
SKWB24-2, SLMB28-1, Nisqually-1

Hop
Humulus lupulus
Fuggle, Cascade, Saaz

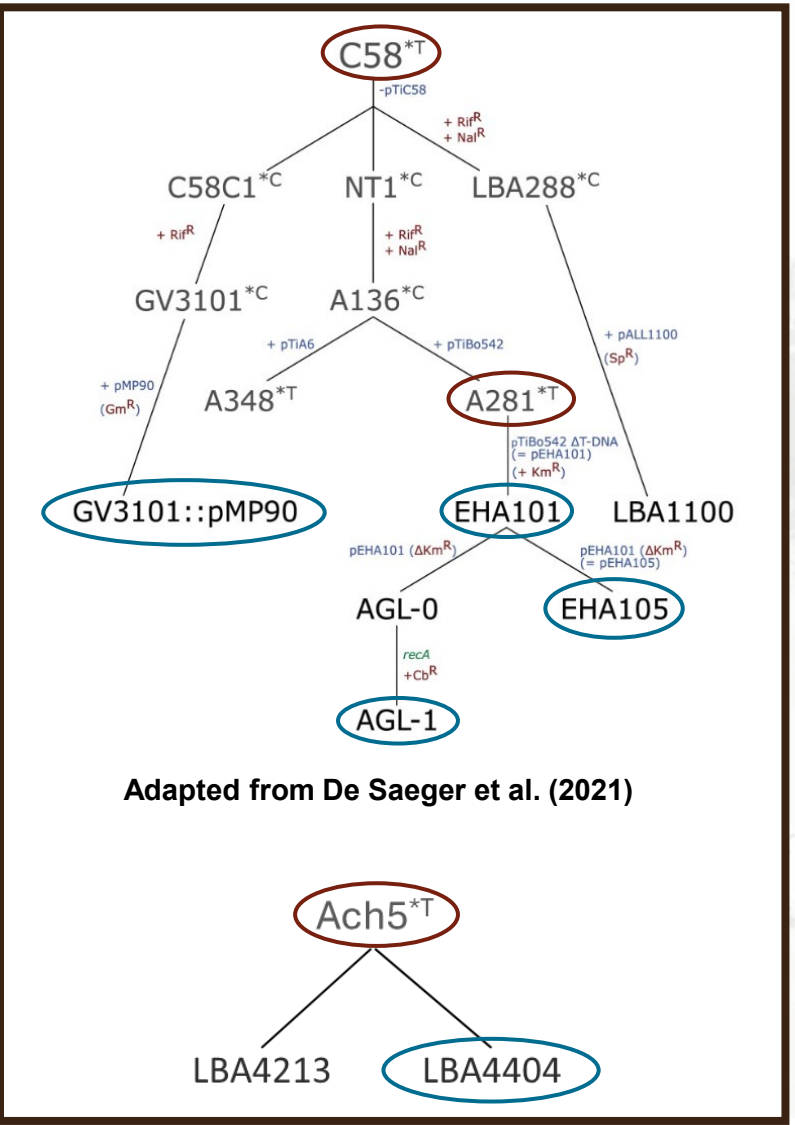
Eucalypts
Eucalyptus grandis x urophylla
E. globulus, E. nitens, E. dunnii

Poor DNA delivery, strong defense responses, and difficult regeneration are common themes in our species

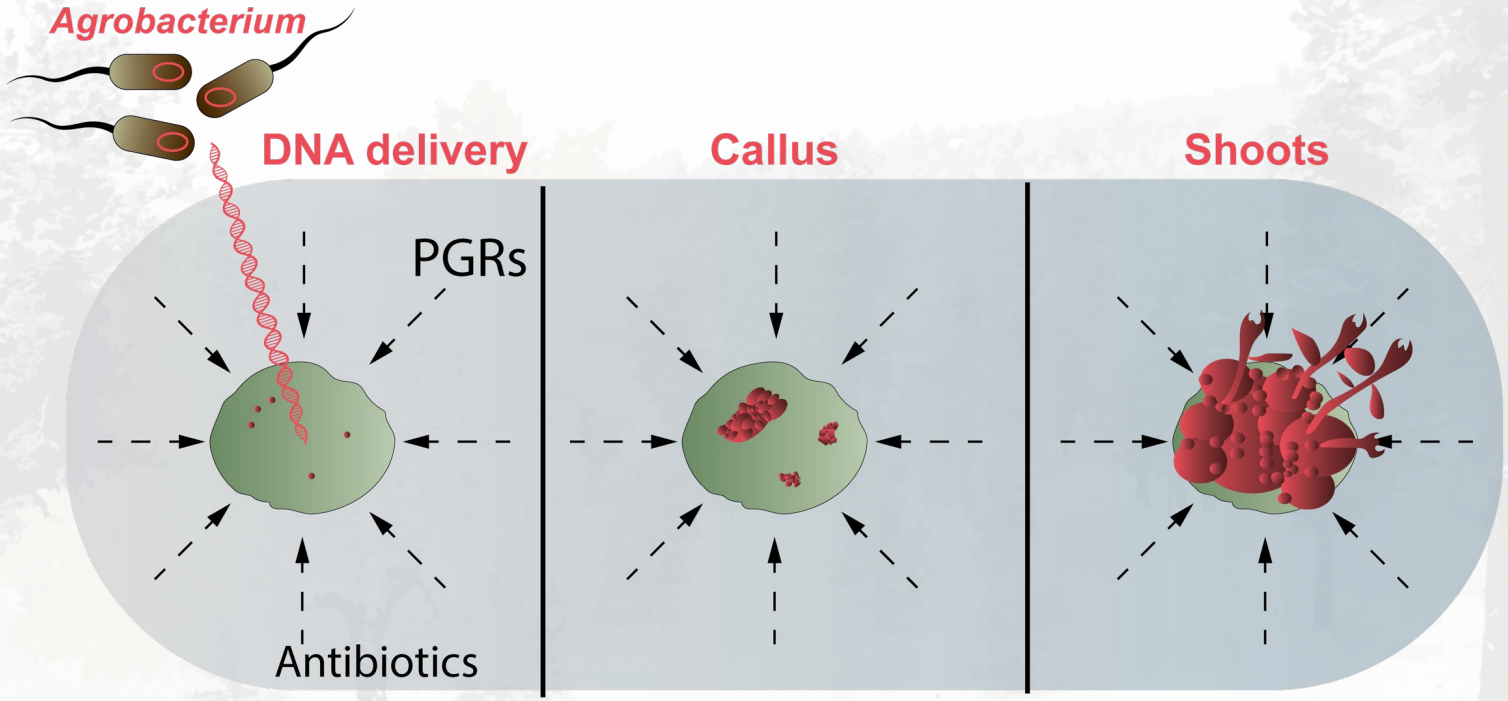


- Elite clones, not seed-derived
- High heterozygosity: each genotype a new adventure in culture
- High physiological diversity and common defense responses

AMT remains the most common method – but little has changed in the 30-40 years since these first lab strains were made



Adapted from De Saeger et al. (2021)



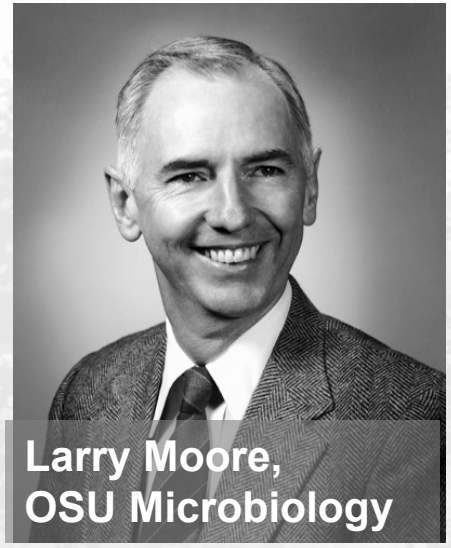
Are there better strains out there?

How do we survey most efficiently?

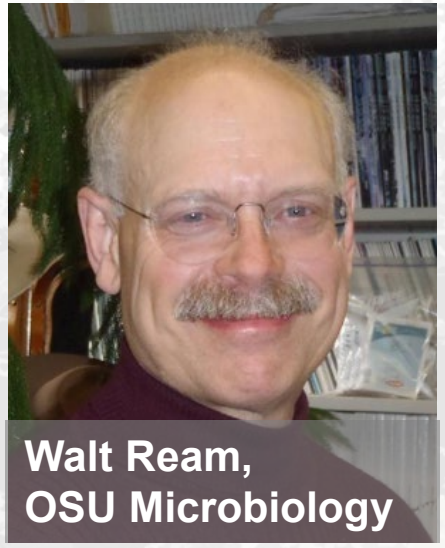
How do we turn new strains into laboratory tools?

Local history of *Agrobacterium* research enabled this project

Agro strains and molecular biology



Larry Moore,
OSU Microbiology

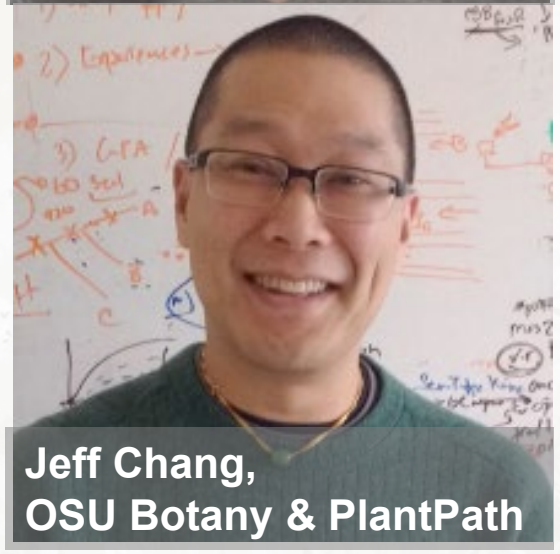


Walt Ream,
OSU Microbiology

Melodie Putnam, OSU Plant Clinic

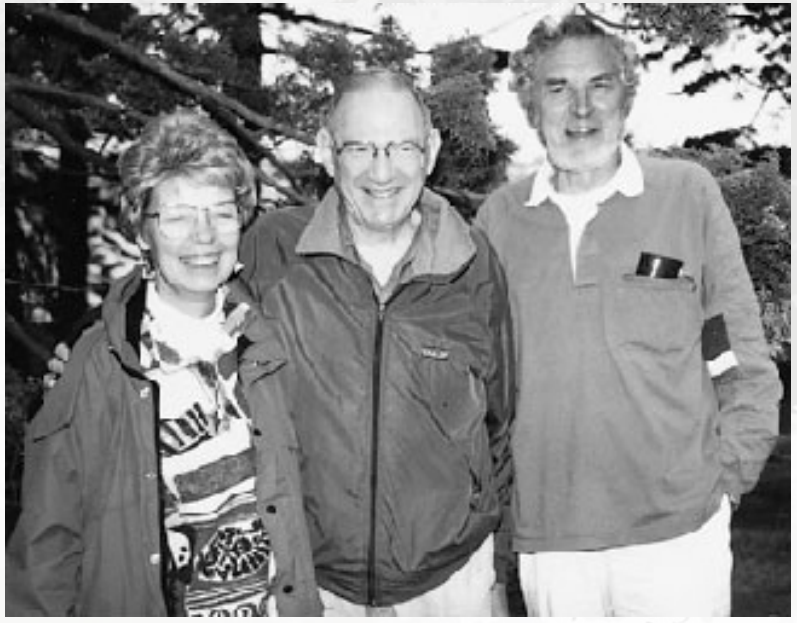
Strain genomics

Alex Weisberg,
OSU Botany & PlantPath



Jeff Chang,
OSU Botany & PlantPath

Early disarmed and intermediate strains (UW)



Mary-Dell Chilton

Milton Gordon

Eugene Nester

Local history of *Agrobacterium* research enabled this project

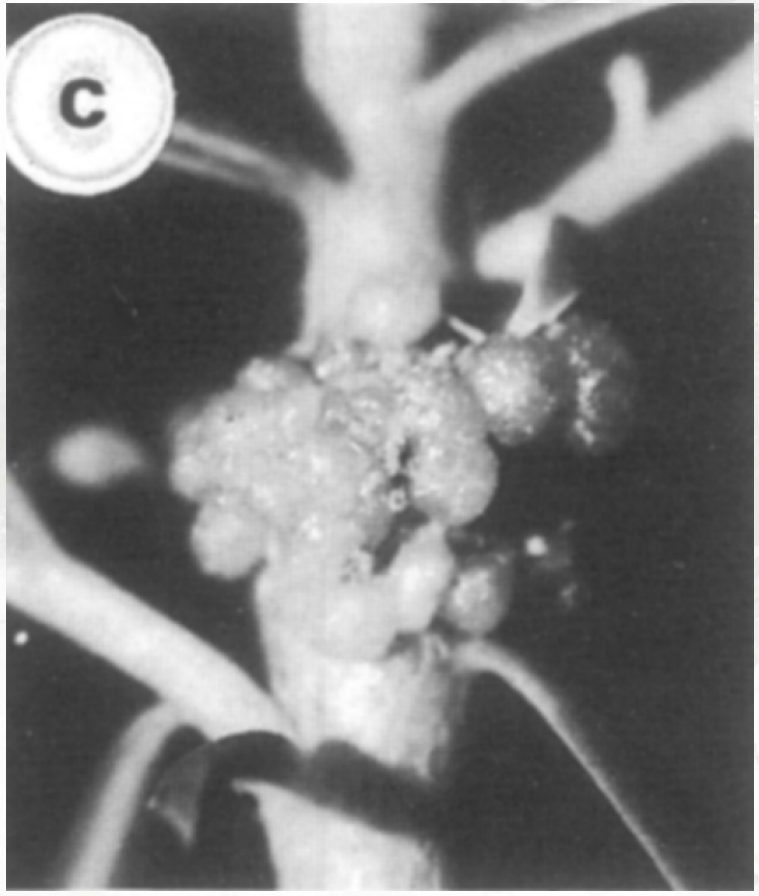
Efficacy of different *Agrobacterium tumefaciens* strains in transformation of pinaceous gymnosperms

JOHN W. MORRIS§, LINDA A. CASTLE and ROY O. MORRIS†*

Department of Biochemistry, 322 Chemistry Building, University of Missouri-Columbia, Columbia MO 65211, U.S.A.;
† Departments of Forest Science and Agricultural Chemistry, Oregon State University, Corvallis, Oregon 97331, U.S.A.

(*Accepted for publication October 1988*)

	Host						
	Pseudotsuga	*Pinus*	*Tsuga*	*Abies*	Opine	Plant origin	Reference
Agrobacterium tumefaciens							
15955	20	0	20	21	oct	*Lycopersicon*	25
3667	100	25	65	70	nop	*Rosa*	33
A6	10	0	25	32	oct	*Rubus*	25
Ach5	40	0	25	53	oct	*Prunus*	17
Ach5C3	0	0	0	0	none		14
B1/74	85	30	75	85	nop	*Prunus*	1
B234	75	40	20	70	nop	unknown	1
B3/73	100	30	70	90	nop	*Acer*	1
B4/73	40	35	55	75	nop	*Acer*	1
C2/74	80	60	50	70	nop	*Prunus*	1
C58	25	9	7	29	nop	*Prunus*	25



Induced gall (strain B3/73) on Douglas fir seedling

A fully sequenced public collection of diverse *Agrobacterium* is the main resource for our study

RESEARCH

RESEARCH ARTICLE SUMMARY

PLASMID EVOLUTION

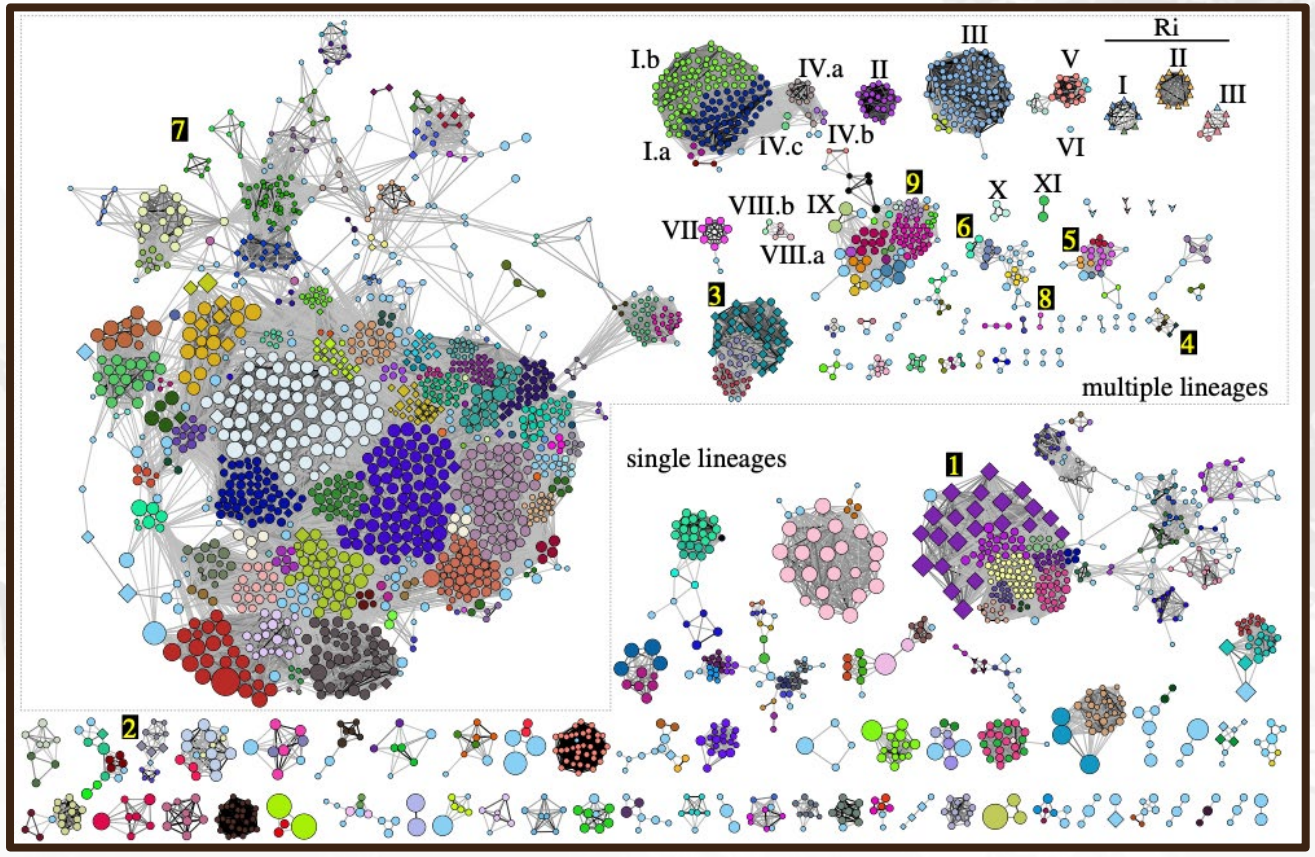
Unexpected conservation and global transmission of agrobacterial virulence plasmids

Alexandra J. Weisberg, Edward W. Davis II, Javier Tabima, Michael S. Belcher, Marilyn Miller, Chih-Horng Kuo, Joyce E. Loper, Niklaus J. Grünwald, Melodie L. Putnam, Jeff H. Chang*

Diversification of plasmids in a genus of pathogenic and nitrogen-fixing bacteria

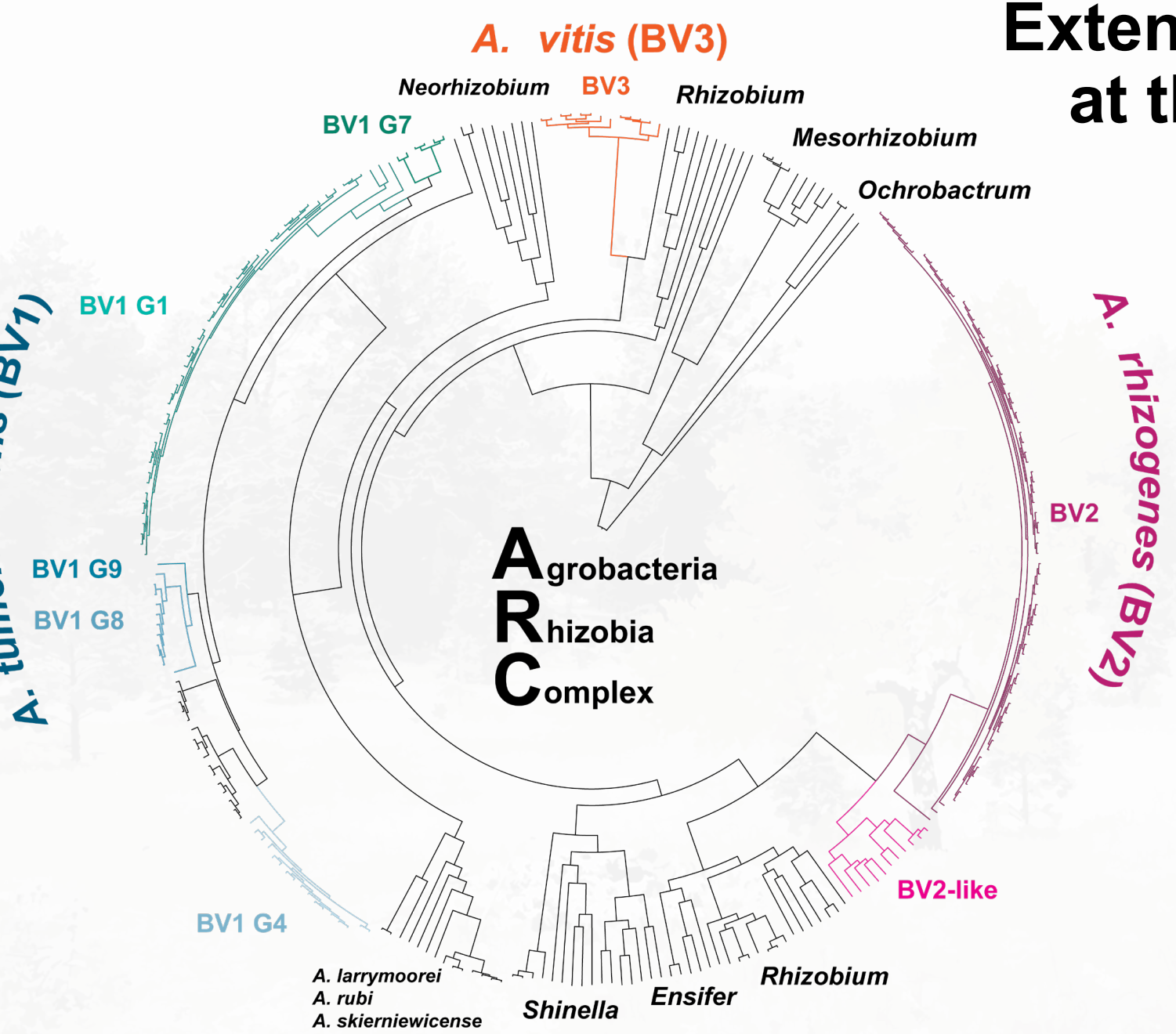
Alexandra J. Weisberg[1], Marilyn Miller[1], Walt Ream[2], Niklaus J. Grünwald[3] and Jeff H. Chang[1]

[1]Department of Botany and Plant Pathology, and [2]Department of Microbiology, Oregon State University, Corvallis, OR 97331, USA
[3]Horticultural Crops Research Laboratory, United States Department of Agriculture and Agricultural Research Service, Corvallis, OR 97330, USA



Complex network of accessory plasmids in Agrobacteria and Rhizobia species (**From Weisberg et al. 2021 *Phil. Trans. R. Soc. B***)

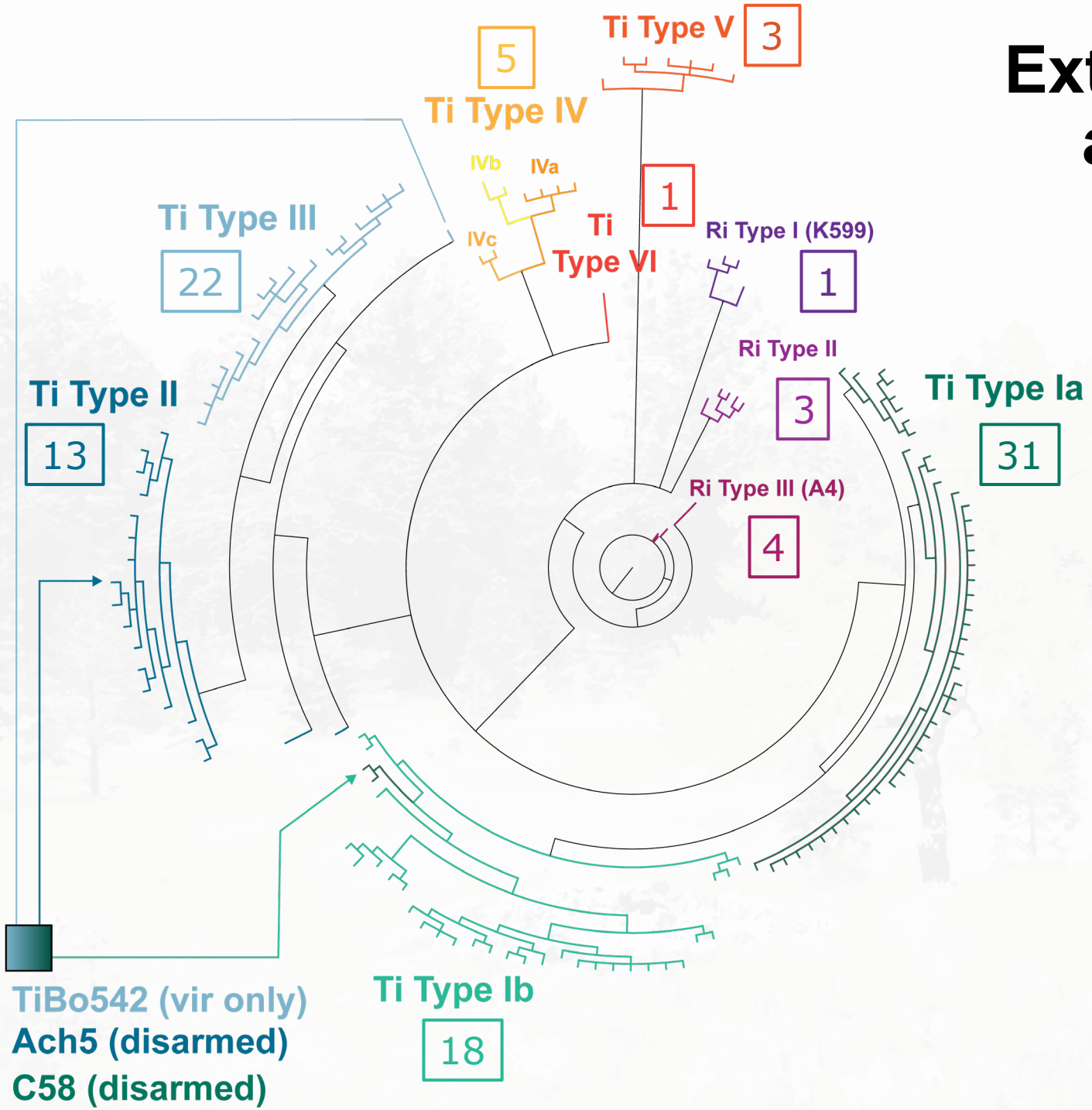
Extensive collection diversity at the chromosomal level



- At least ten species-level taxonomic groups based on sequence
- Parasitic (+Ti/Ri) strains are paraphyletic
- Biovar 2 (*A. rhizogenes*) have had a recent evolutionary bottleneck (~2mya)
- Related ARC species can be used for transformation if Ti/Ri is introduced

Adapted from Weisberg et al. (2020) *Science*

Extensive collection diversity at the Ti/Ri plasmid level

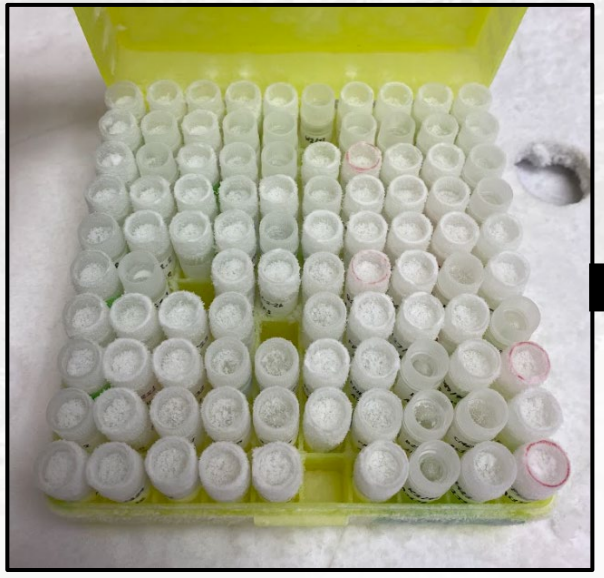


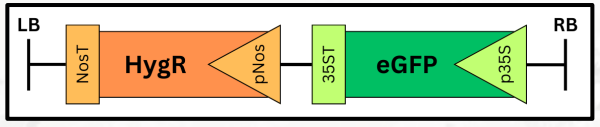
- Plasmids include DNA transfer machinery and T-DNA region (crown gall plant hormone genes)
- Most types can be biovar 1 (*tumefasciens*) or biovar 2 (*rhizogenes*)
- Strains isolated from diverse dicots, with type II and type III Ti more likely to be herbaceous than woody
- Ri plasmid diversity underrepresented in public collections

Adapted from Weisberg et al. (2020) *Science*

We used a high throughput phenomics platform to quantify transformation and regeneration using wild strains

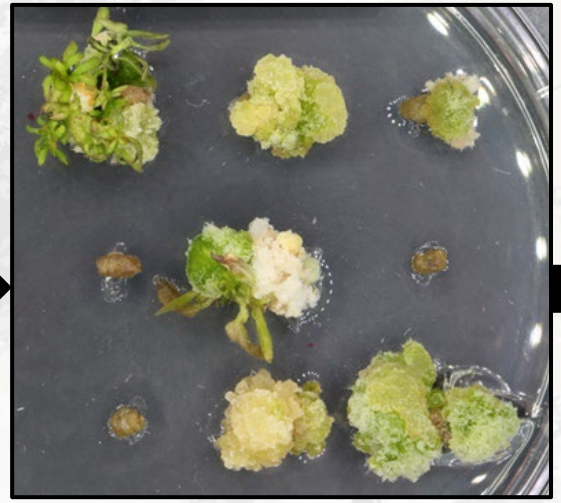
Insert binary vector





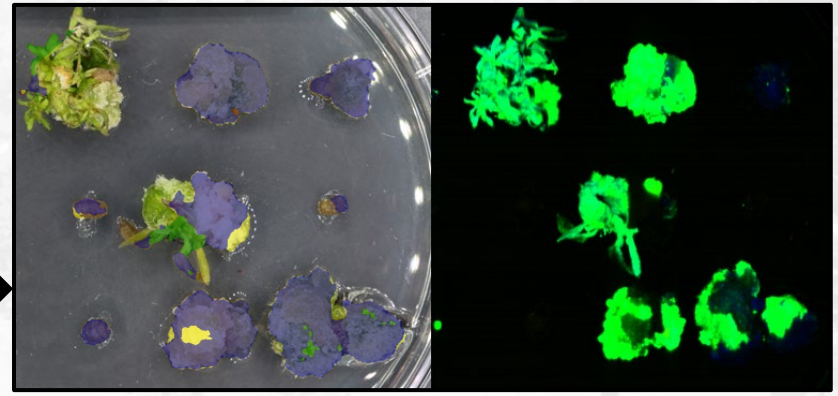
Kanamycin selection for binary plasmid in Agro

Transform plants



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

Phenomics Analysis



- Transgenic tissue
- Regeneration
- Browning

Screening was performed using a two-stage strategy

Hybrid Poplar
Populus tremula x alba 717-1B4

Stage I

100 strains

Low replication (3 plates)

No plant hormones

Minimal selection (Hygromycin)

3w 7w 10w

- **Transgenic tissue**
- **Regeneration**
- **Browning**

Stage II

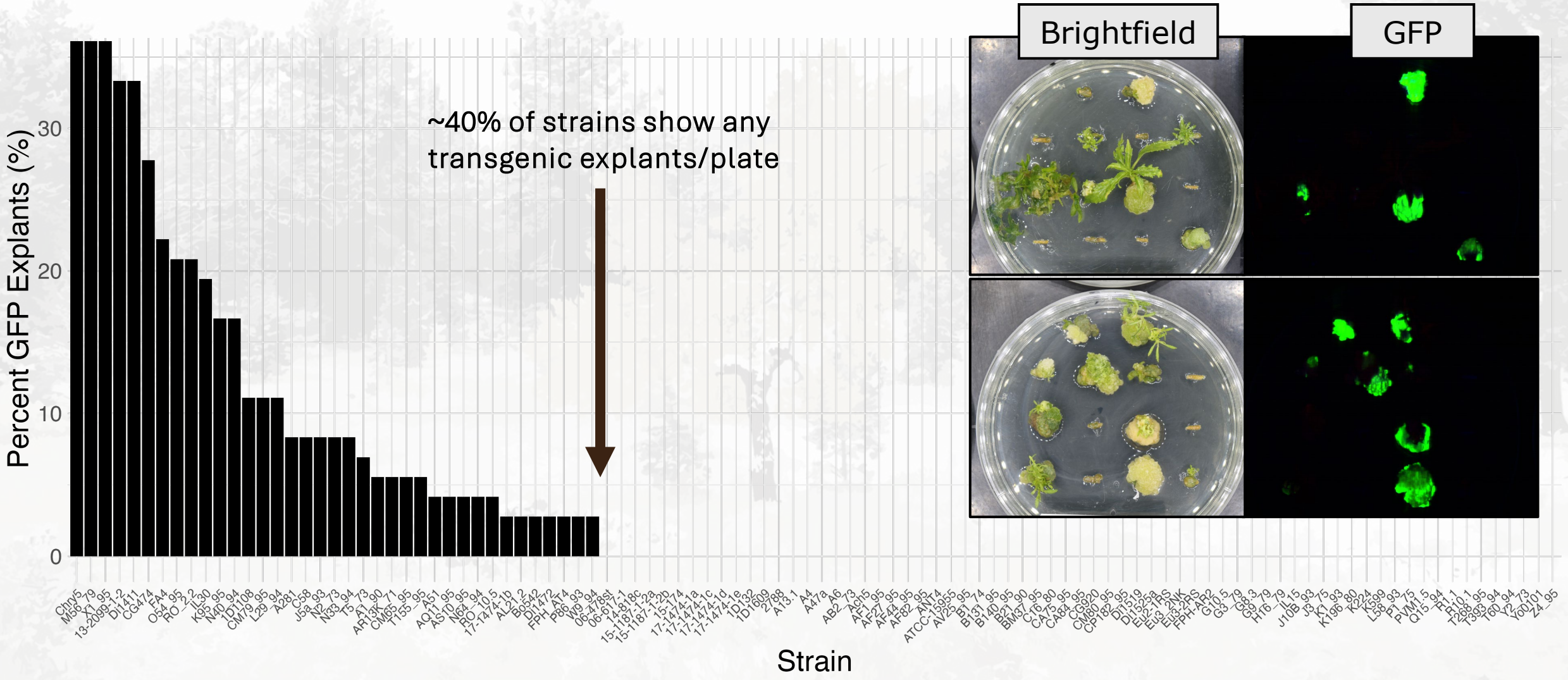
Select:
20 good strains
10 bad* strains

30 strains
high replication
(10 plates)

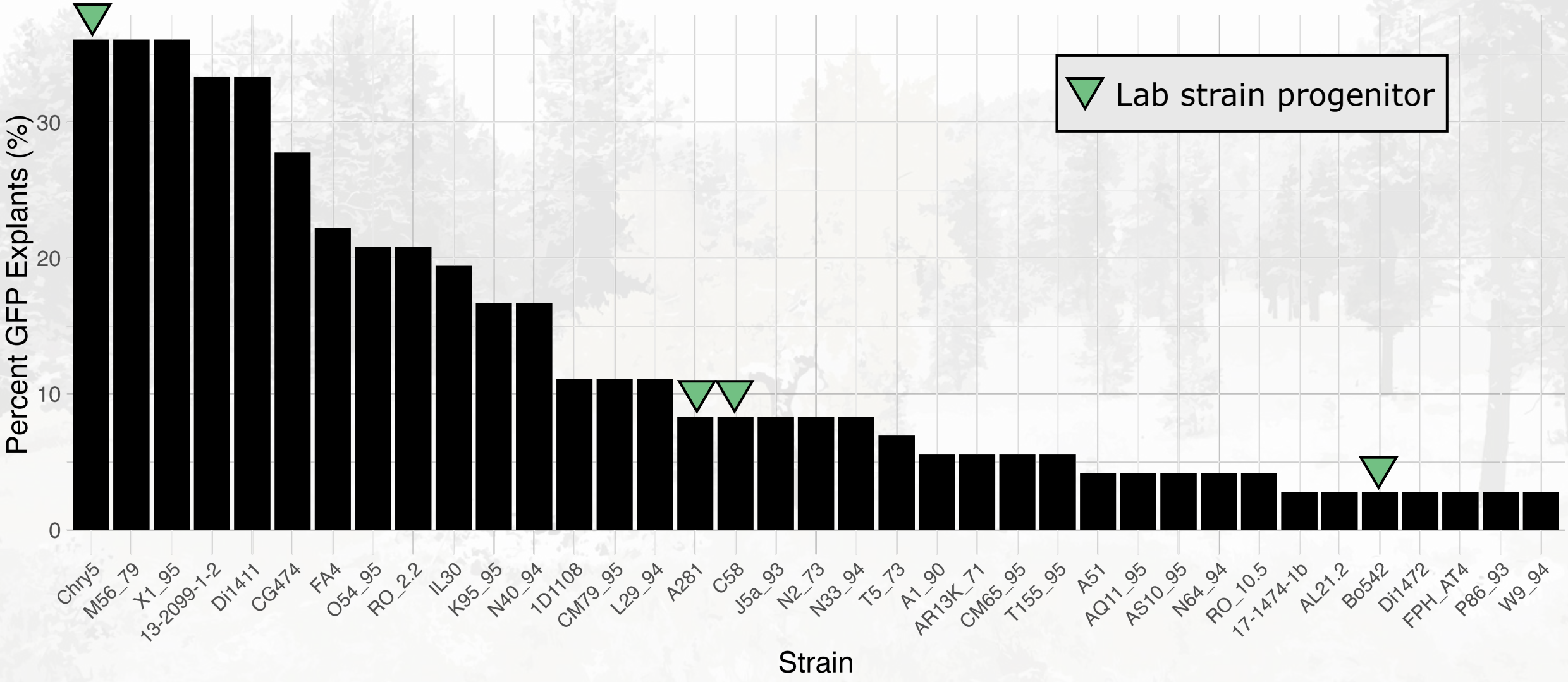
Repeat in other species or treatment conditions

(*bad strains retained for mechanistic insight)

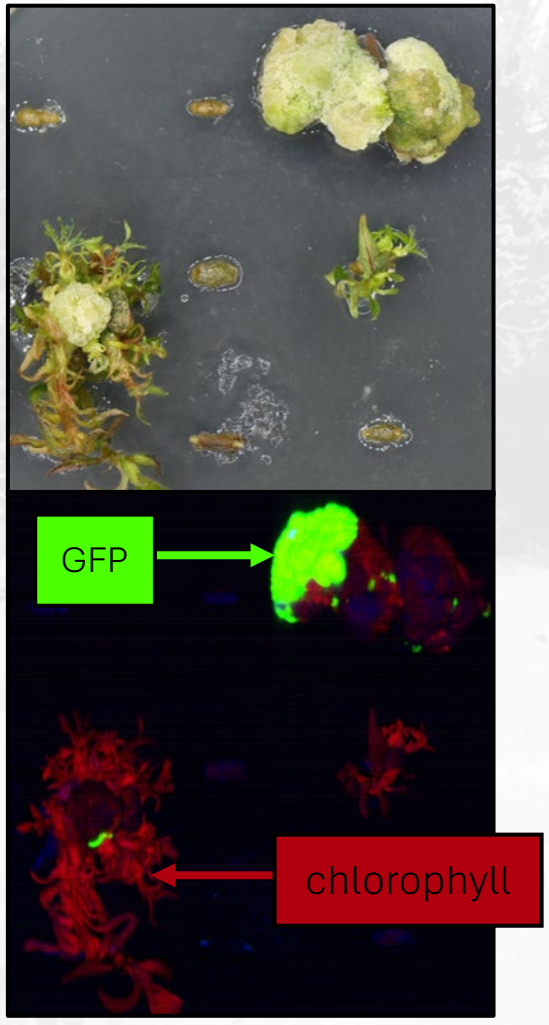
Wild strains show wide variability in transformation and regeneration in hybrid poplar

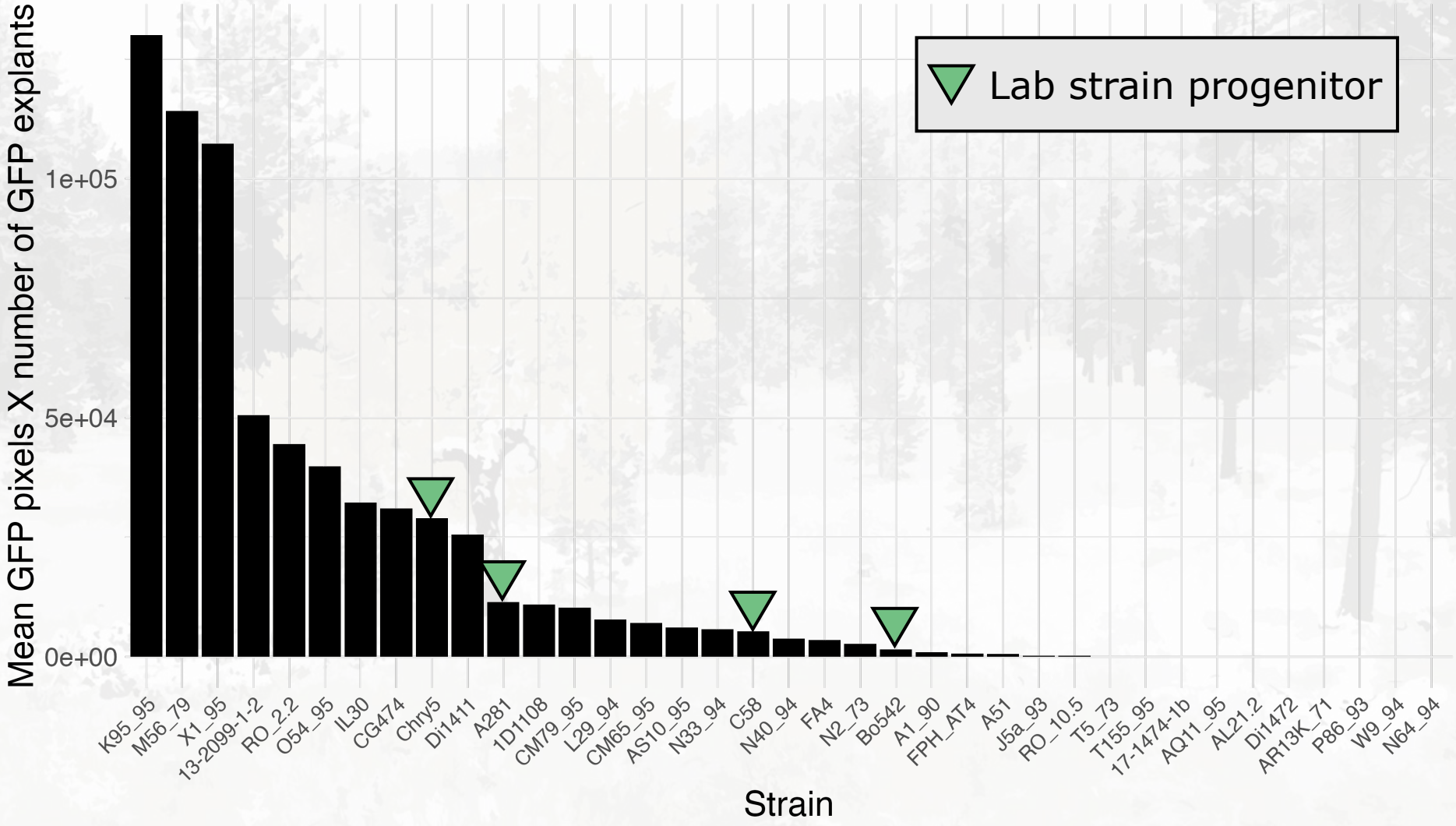


Many wild strains in our initial screens were competent transformers compared to lab strain progenitors

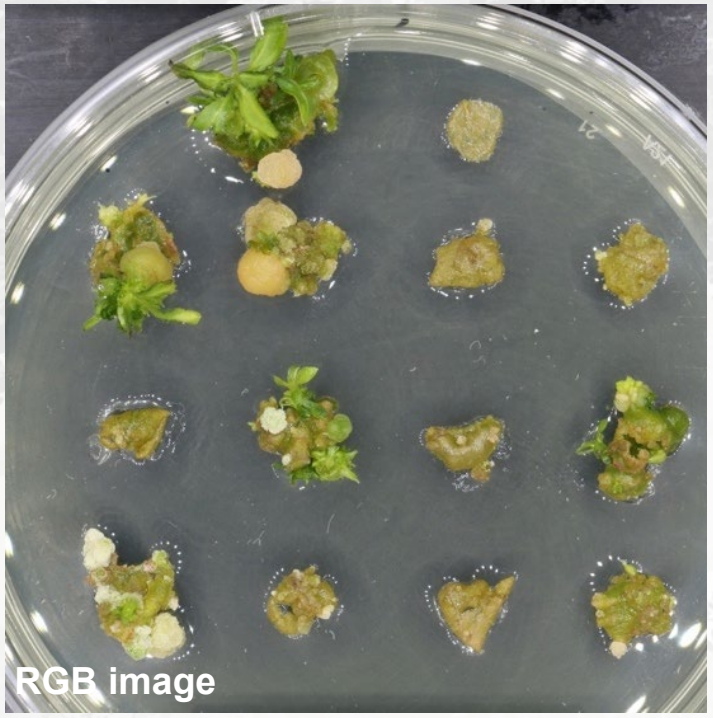


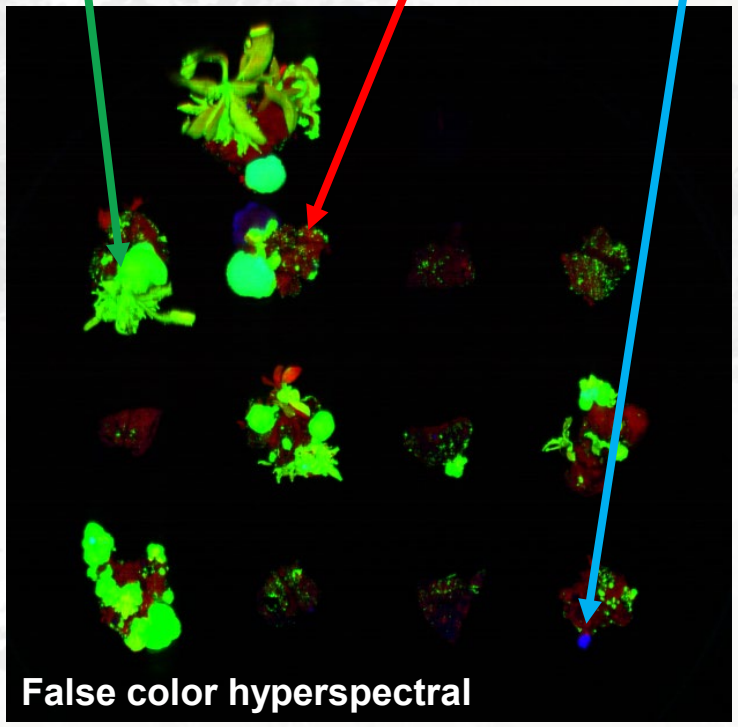
We are cautious about using transgenic callus area phenotypes, as gall size is highly variable among strains

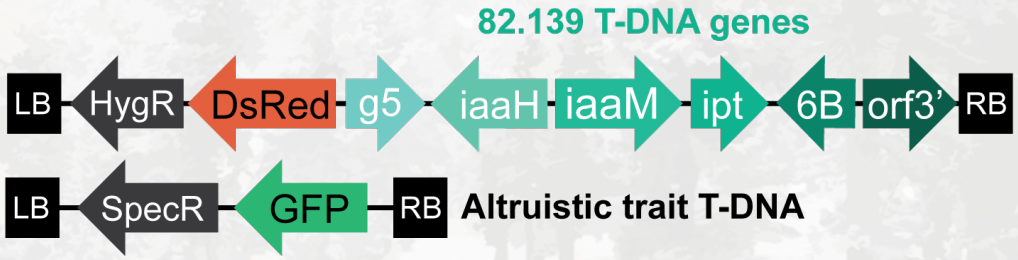




We are also interested in passive “shooty” regeneration behavior as a tool using a co-transformation approach







Plant Biotechnology Journal

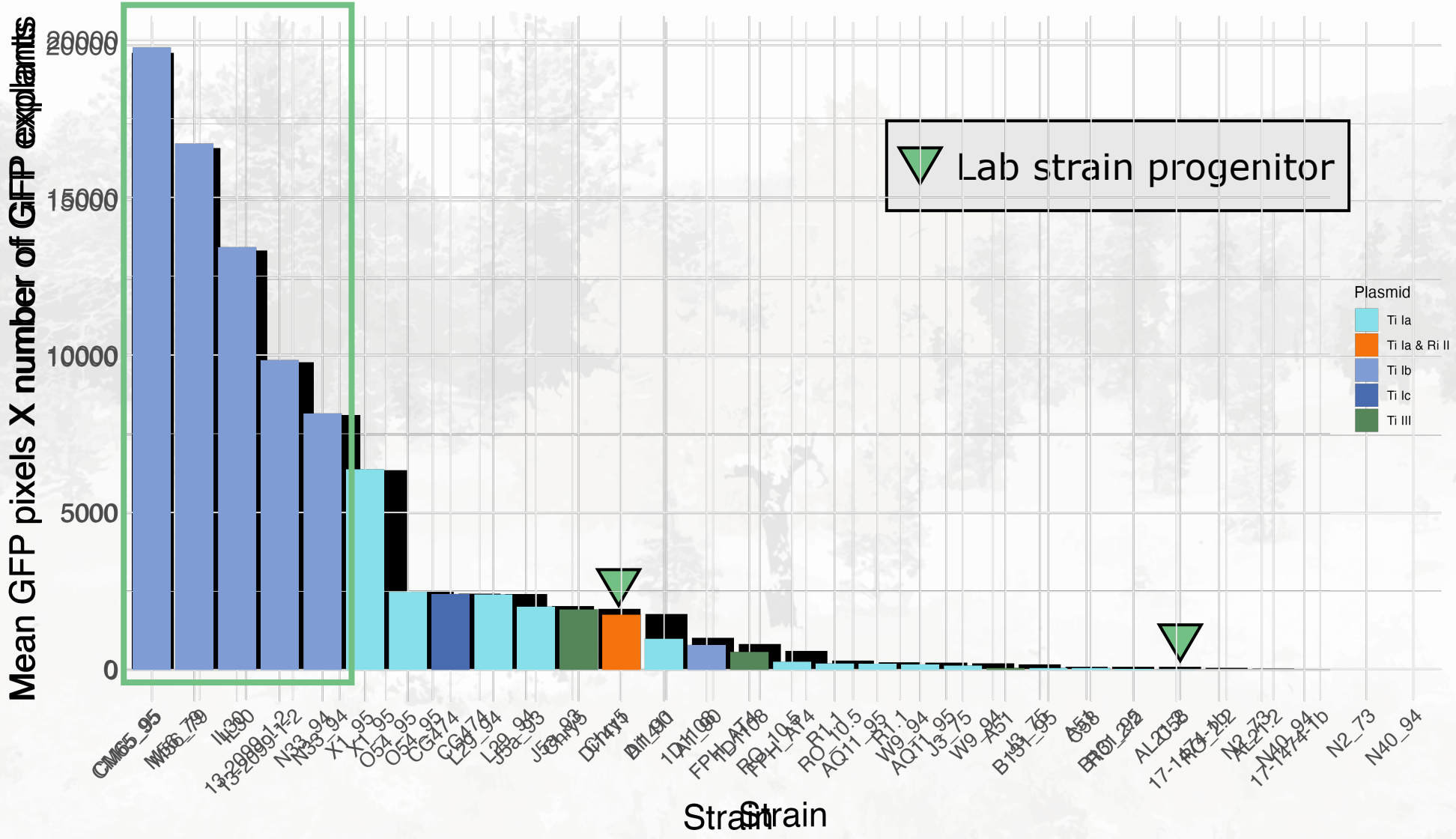
Plant Biotechnology Journal (2025) **23**, pp. 3841–3850 doi: 10.1111/pbi.70159

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

Greg S. Goralogia, Cathleen Ma, David S. Taylor, Abigail Lawrence, Victoria Conrad, Ekaterina Peremyslova and Steven H. Strauss*

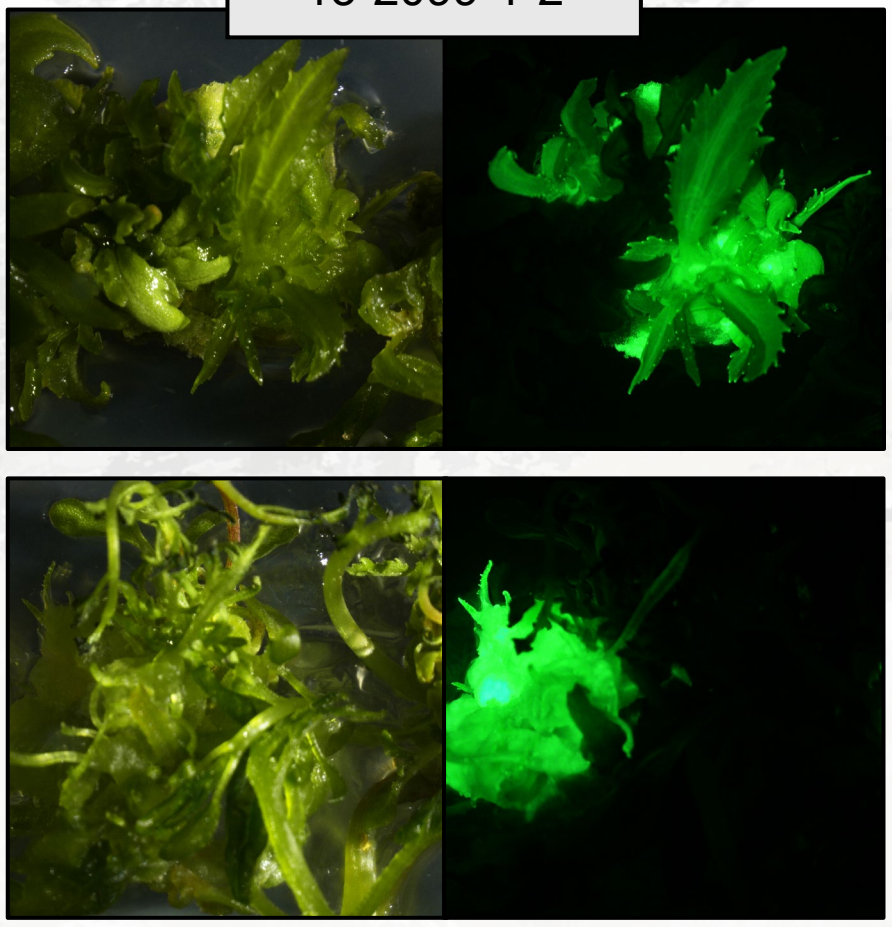
Department of Forest Ecosystems and Society, Oregon State University, Corvallis, Oregon, USA

Some wild strains spur transgenic shoot regeneration in the absence of exogenous hormones

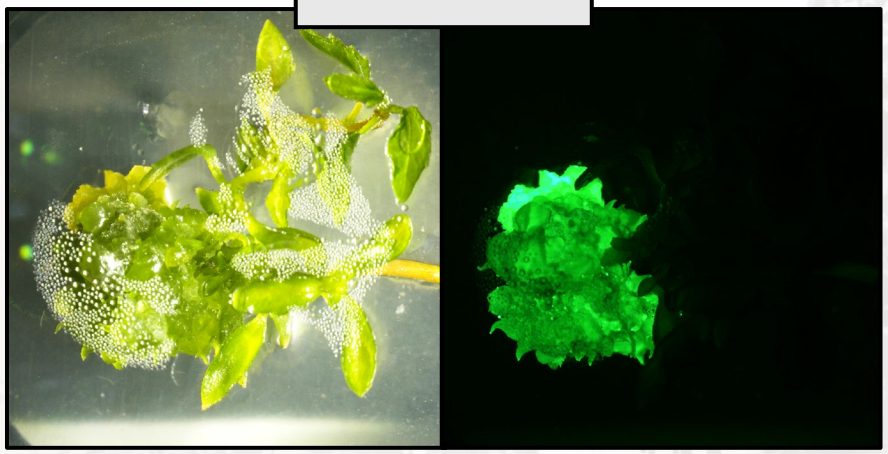


Ti plasmid type 1b was the most efficient at transgenic shoot formation

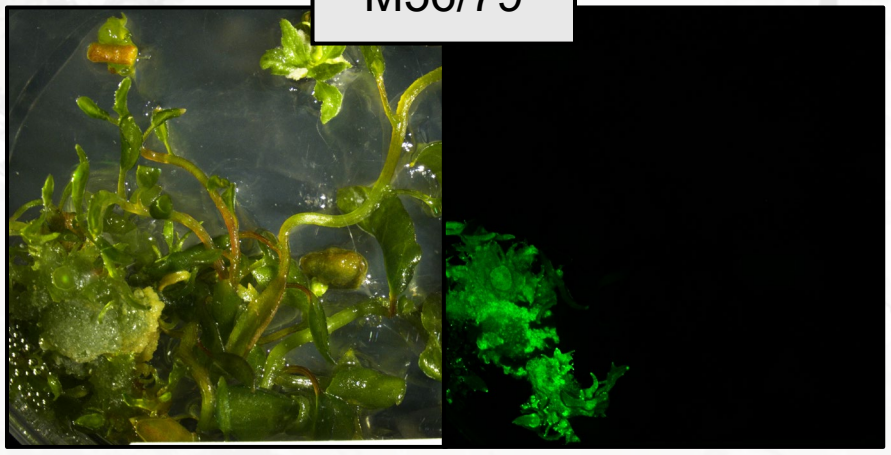
13-2099-1-2



CM65/95

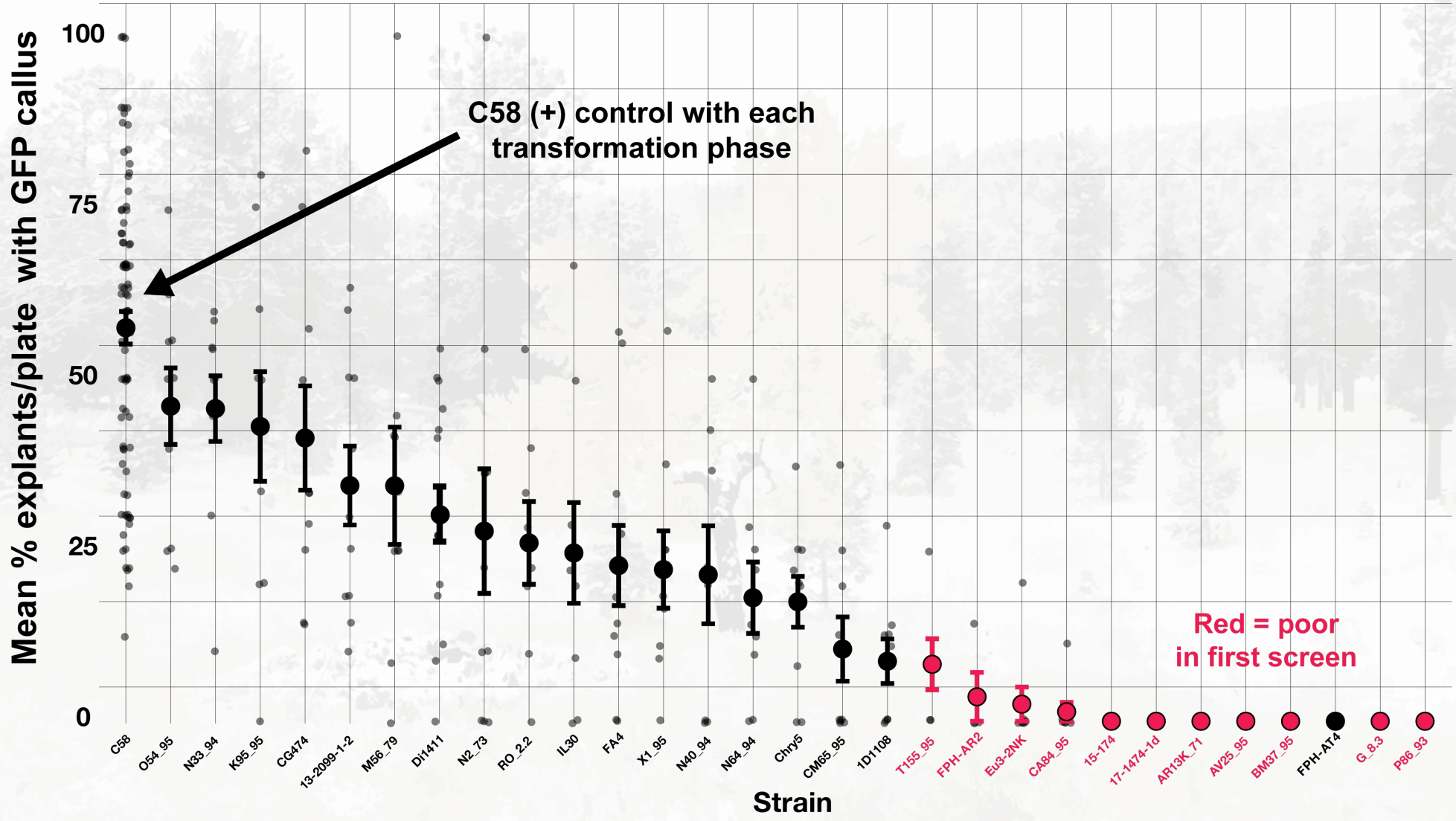


M56/79

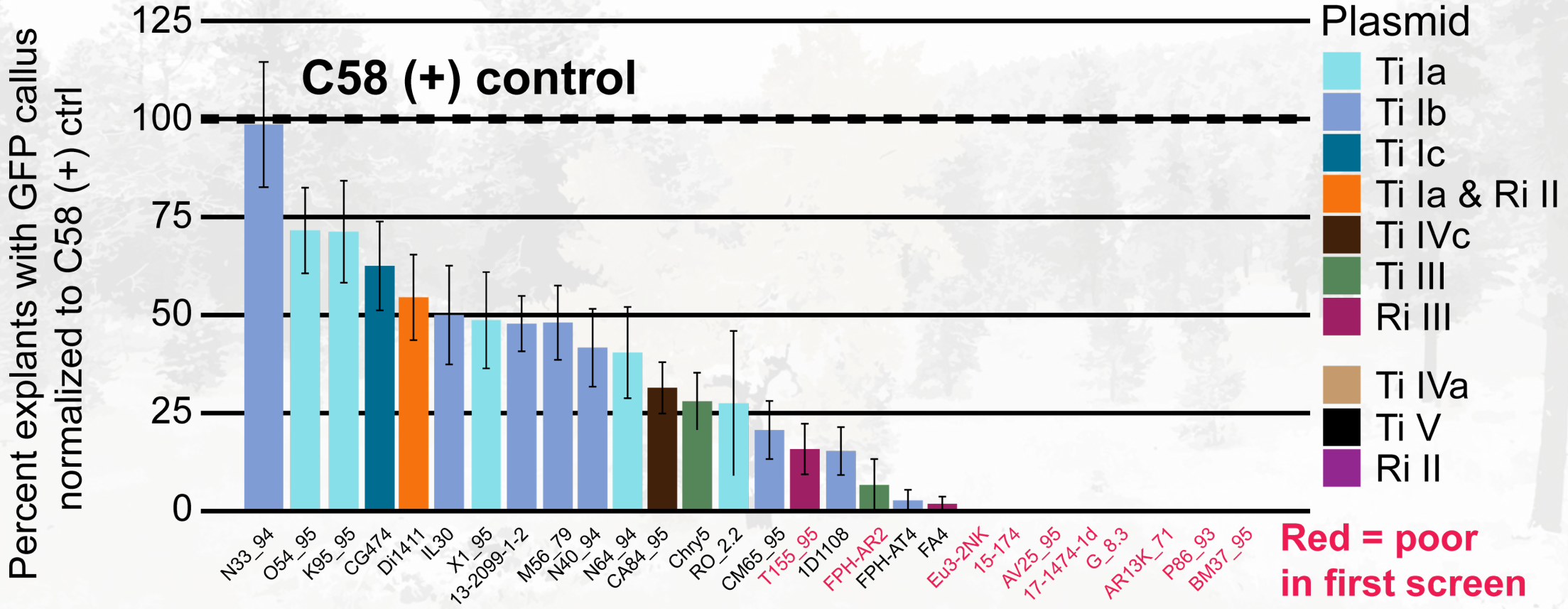


After our initial screens, do the strains behave similarly in higher replication trials?

Higher replication trials show consistent behavior in hybrid poplar, though C58 outperformed other strains

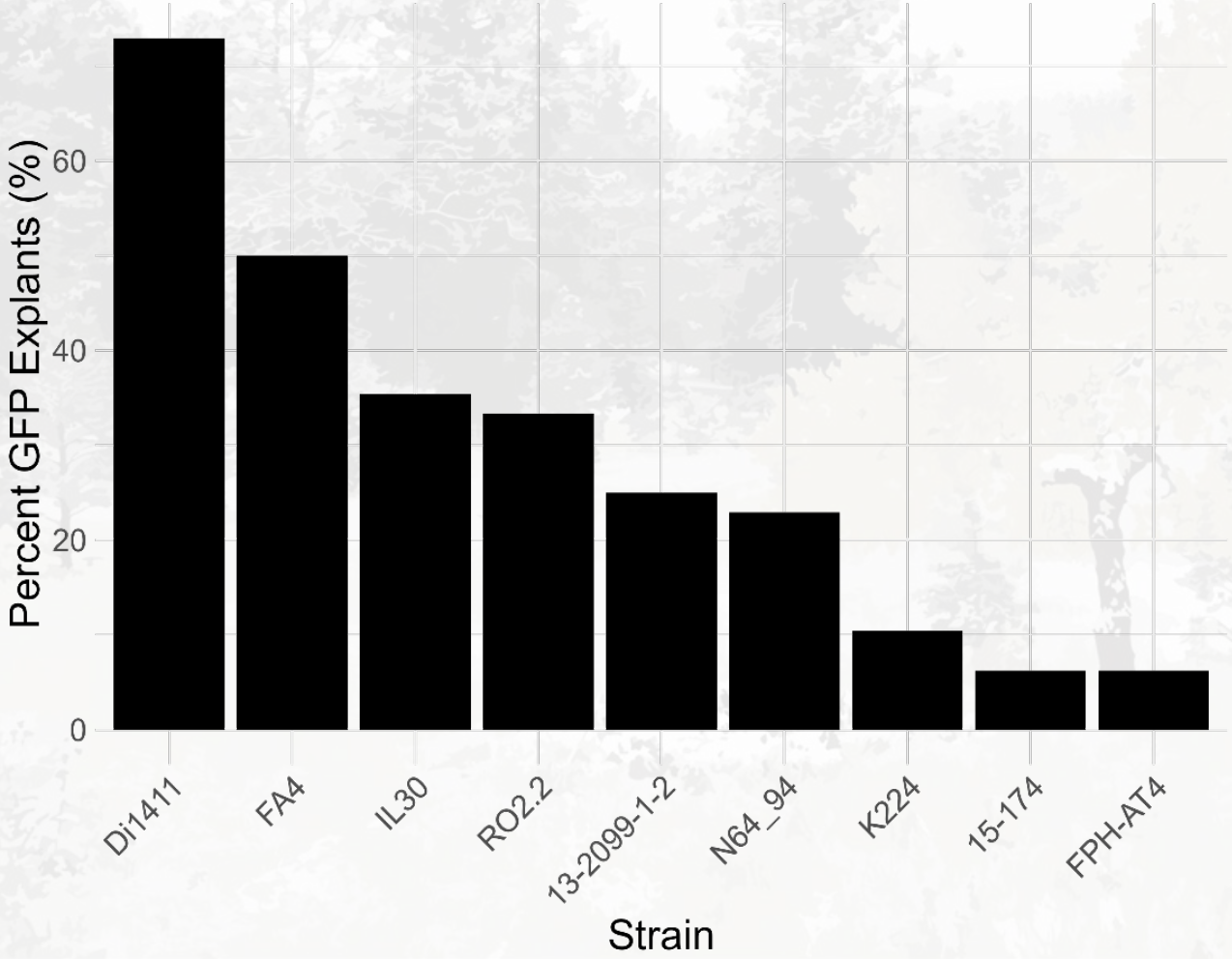


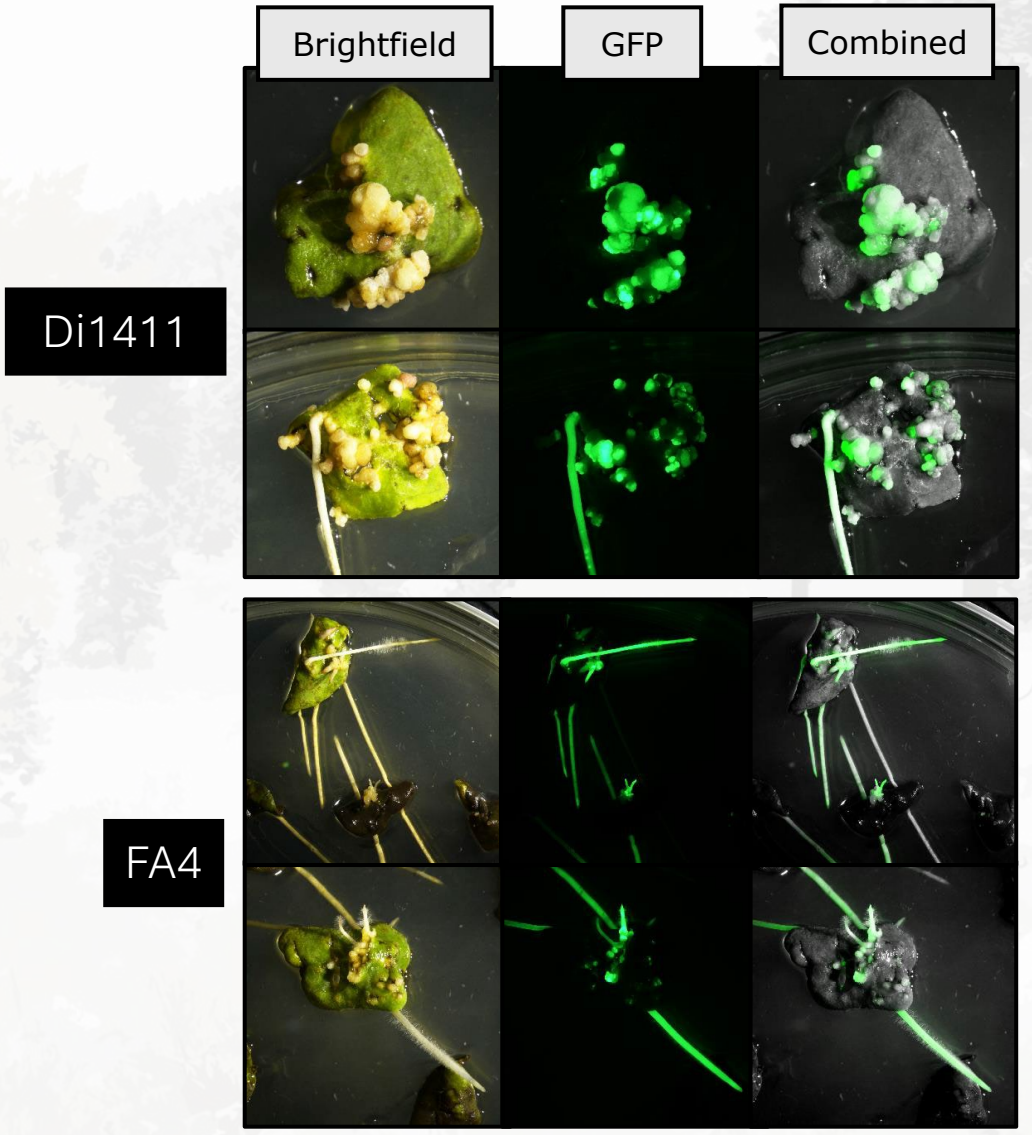
Strains from several Ti/Ri backgrounds and chromosomal groups show high rates of TG callus production



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

GREAT Trees Cooperative





Collecting is never finished!

When opportunity strikes are also isolating new strains

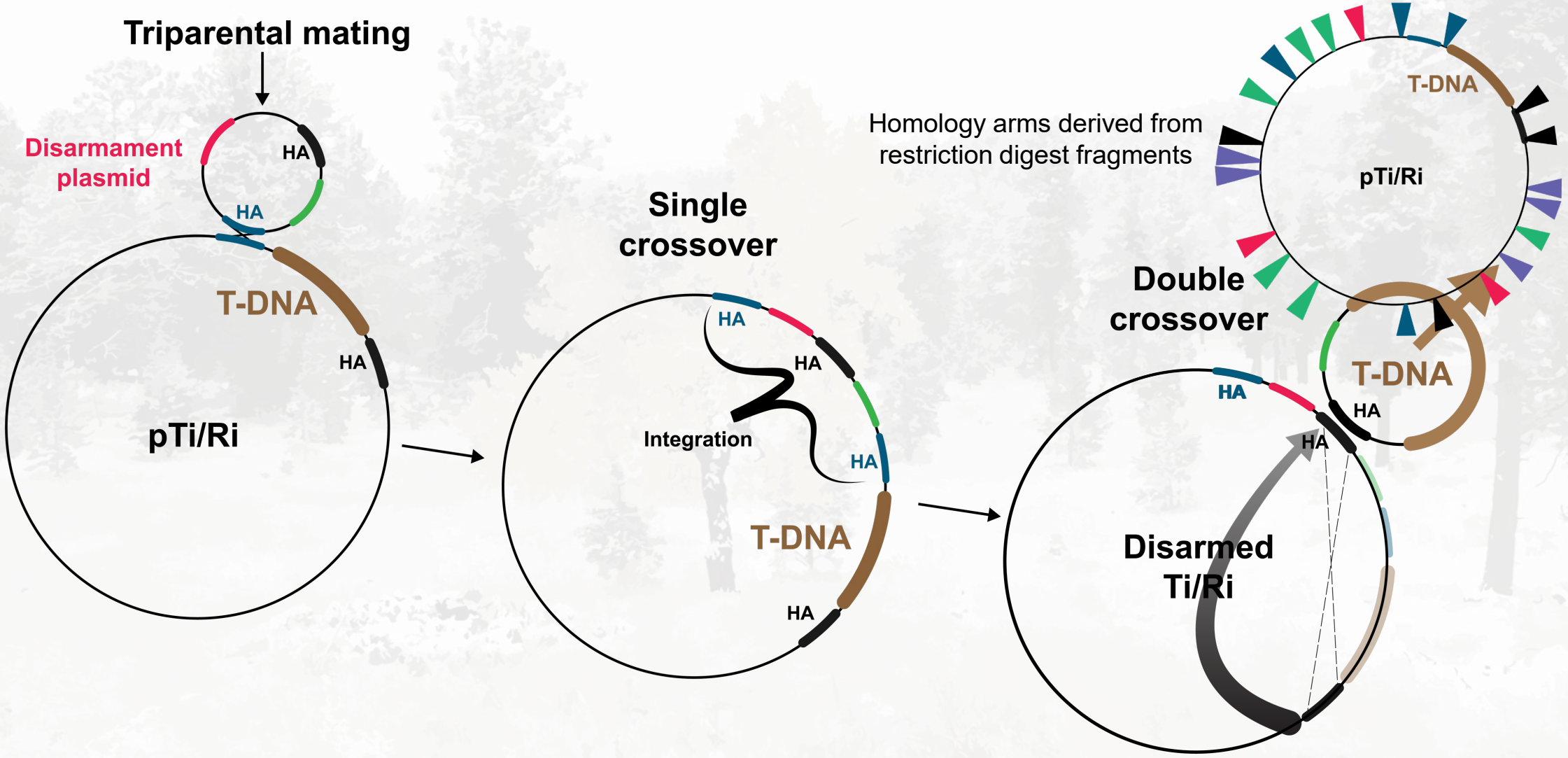
Galls among stand of interior Douglas fir in Uinta-Wasatch Cache National Forest *(No strain isolated)*

Michele Wiseman
PhD Candidate
Gent Lab
OSU Bot&PP

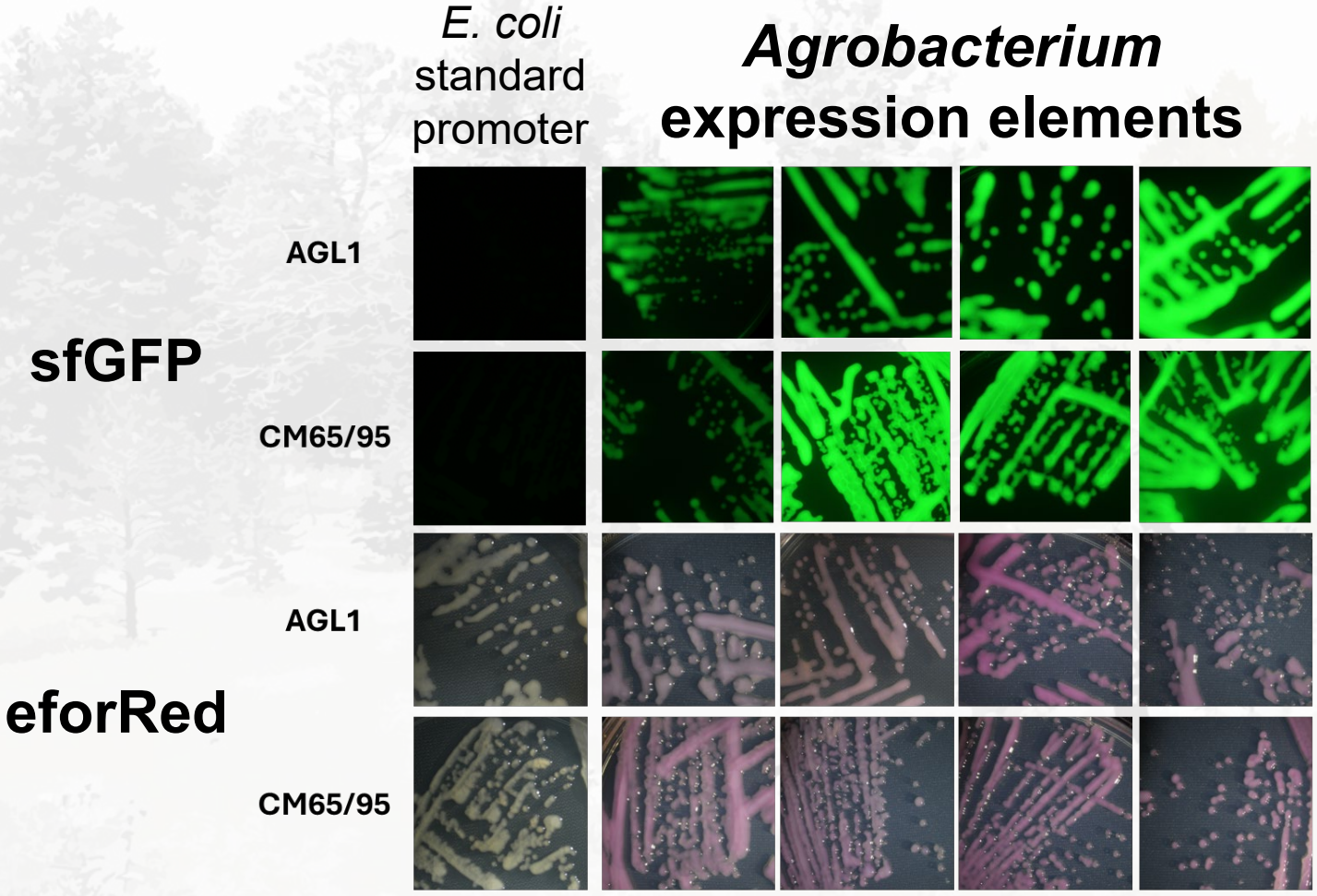
We were able to isolate a strain (BV1, Ti type Ia) from a hop gall

How best to disarm and engineer promising strains?

Conventional *Agrobacterium* disarmament



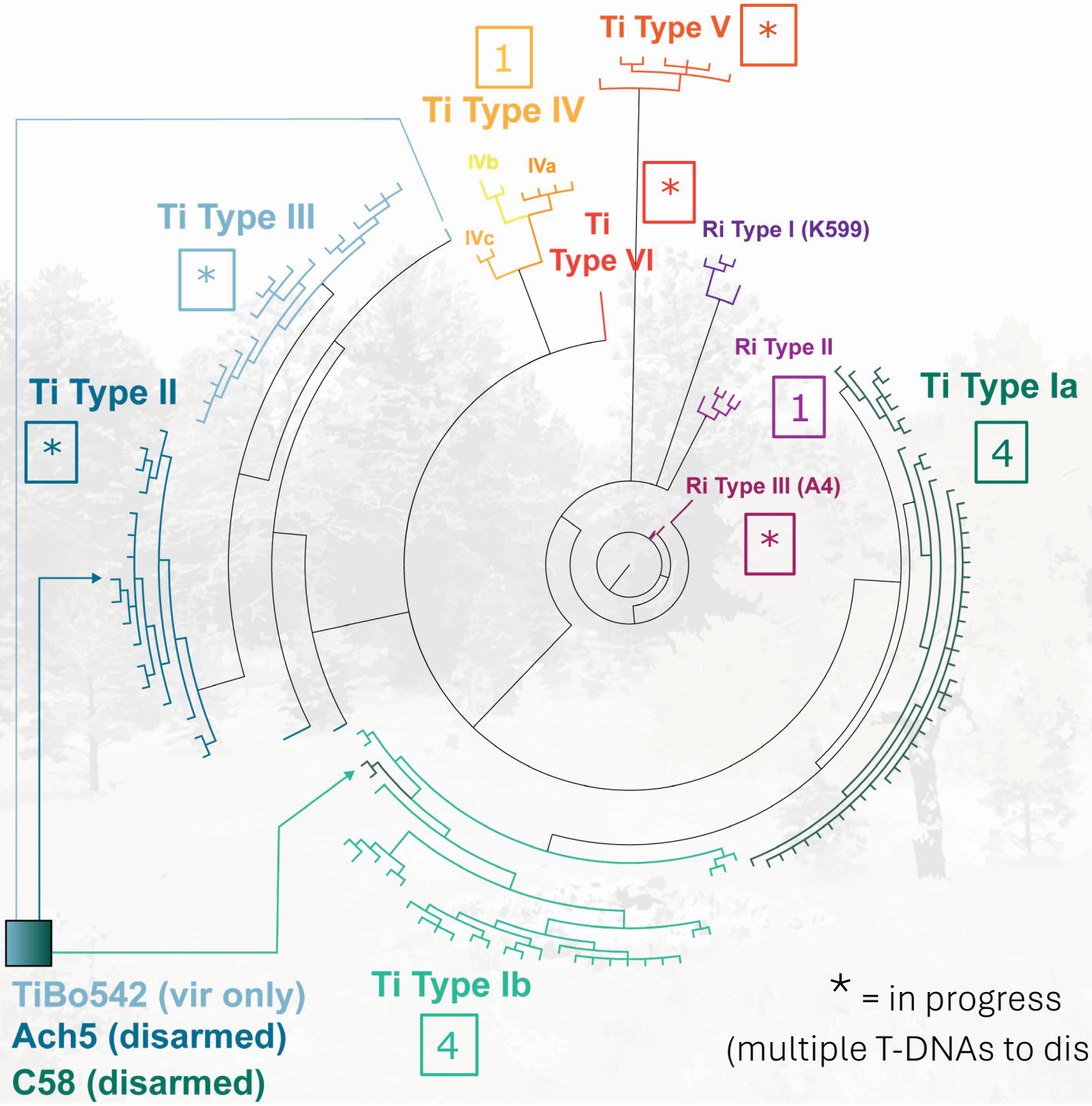
We used conventional strategies with modern cloning tools



- Electroporation rather than mating
- Visualize crossover recombination outcomes
- Counterselection to remove plasmids
- Recombinases to remove residual components or markers after disarmament

Bethani Sutliff
Undergraduate researcher

Chris Willig
Postdoc



* = in progress
(multiple T-DNAs to disarm)

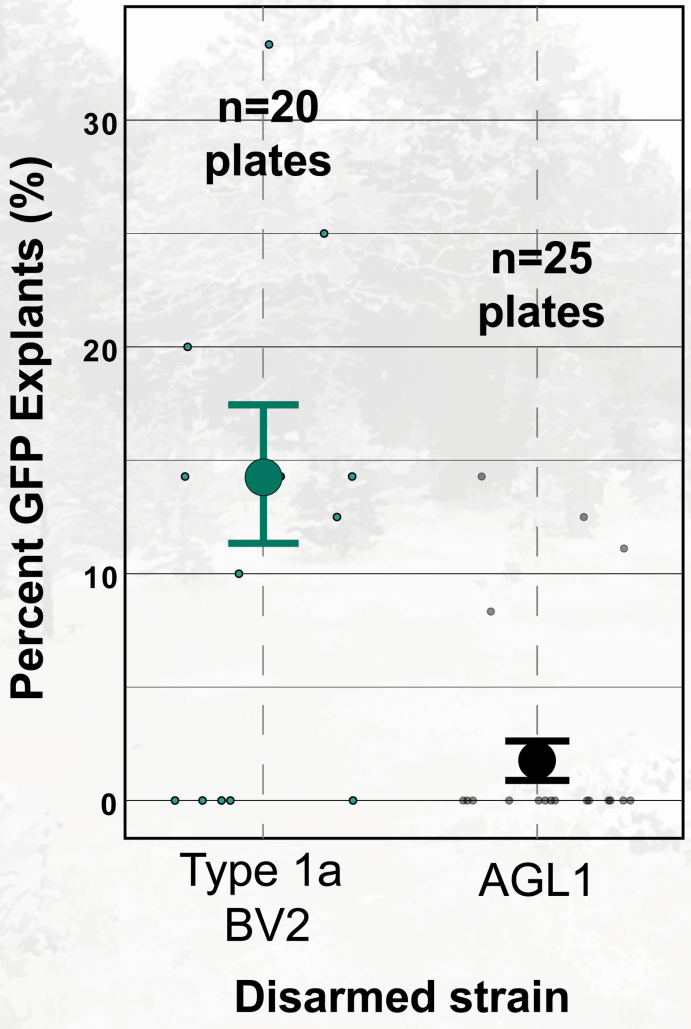
We have so far disarmed 10 strains using this approach

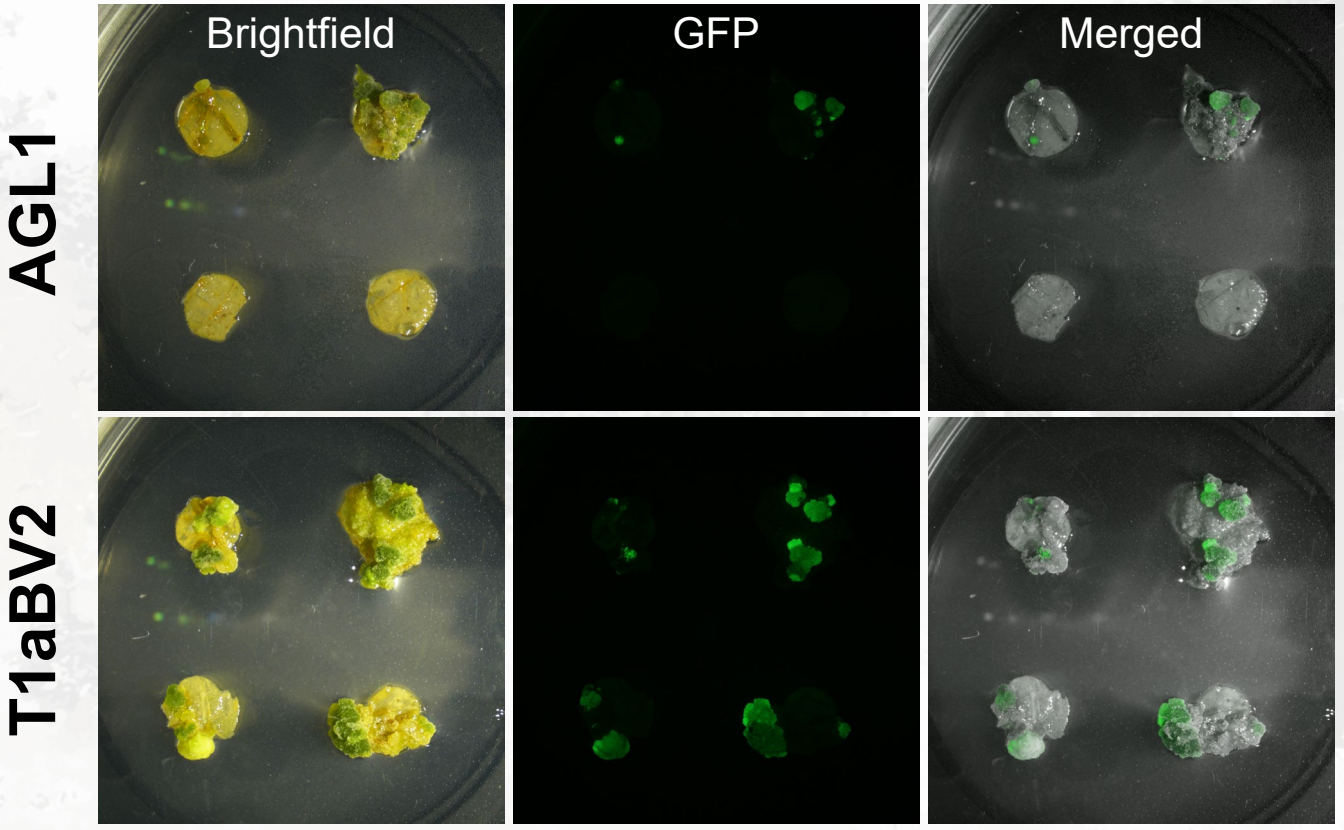
…with more complex edits coming soon

Zachary Heinhold
PhD student

Chris Willig
Postdoc

Initial testing of a disarmed Ti type 1a (BV2) against the AGL1 lab strain showed improved transformation in poplar





Summary:

Genomics and modern cloning tools make strain discovery and engineering simple compared to a few years ago

- 40% of strains had any transformation, leaving 10-20% as candidates for strain engineering
- Higher replication studies in 717 show consistent strain behavior
- Some wild strains show improved traits (browning, TG callus) in other species
- Disarmament using classical approaches but with modern amenities is efficient and simple

Next steps:

Is having more diverse lab strains useful?

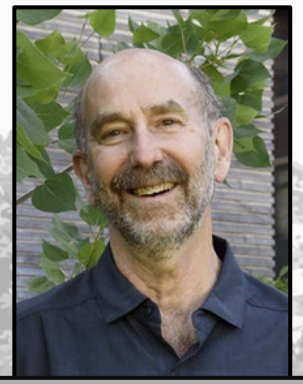
- Testing in a wider variety of plant species
  - *Populus tremula x tremuloides* (353-53) with 100 strain panel
  - Systematic tests in eucalypts and hops
  - Coordinating with other groups to test in other species

- Measuring virulence gene expression when exposed to different plant species and their exudates (Jeff Anderson, OSU Botany & PP)

- Using disarmed and Ti/Ri swapped strains to understand chromosome vs. plasmid contribution to plant transformation

- Using ”shooty” T-DNA genes as morphogens to improve regeneration

Acknowledgements



Steve Strauss
Professor FES

Cathleen Ma
Transformation & Greenhouse Experiments

Kate Peremyslova
Transformation Experiments

Zachary Heinhold
PhD student

Chris Willig
Postdoc

Jeff Anderson
Associate Professor BPP

Forest Biotechnology Lab
Colette Richter (FRA)
Parker Wheeler (FRA)
Dylan Gregory (BPP Anderson Lab)
Bethani Sutliff (Undergraduate)
Jay Adams (Undergraduate)
Michael Nagle (LIBD)
Anthony Marroquin (Greenhouse Manager)
Dana Howe (Lab Manager)

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

GREAT Trees Cooperative

Oregon State University

SIVB CELEBRATES 80

SOCIETY FOR IN VITRO BIOLOGY 1946-2026

2026 IN VITRO BIOLOGY MEETING

RENO, NEVADA • JUNE 6-10, 2026

PLANT AND ANIMAL BIOTECHNOLOGY AND GENOMICS

Dr. Indra K. Vasil, PhD

A Witness to the History of Plant Biotechnology in the SIVB

Dr. Delia R. Bethell, PhD

The Society for In Vitro Biology, 80 Years of Commitment to the Teaching and Standardization of the Science of In Vitro Cell and Tissue Culture

PROGRAM

Join us for this once-in-a-lifetime scientific event!

REGISTER


Thank you for listening!