

NEW AGROBACTERIUM STRAINS AND GENES FOR WOODY PLANT TRANSFORMATION

GREG GORALOGIA



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OREGON STATE UNIVERSITY
COLLEGE OF FORESTRY

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



MEDIUM



Hop

Humulus lupulus

Fuggle, Cascade, Saaz



Eucalypts

Eucalyptus grandis x urophylla

E. globulus, *E. nitens*, *E. dunnii*



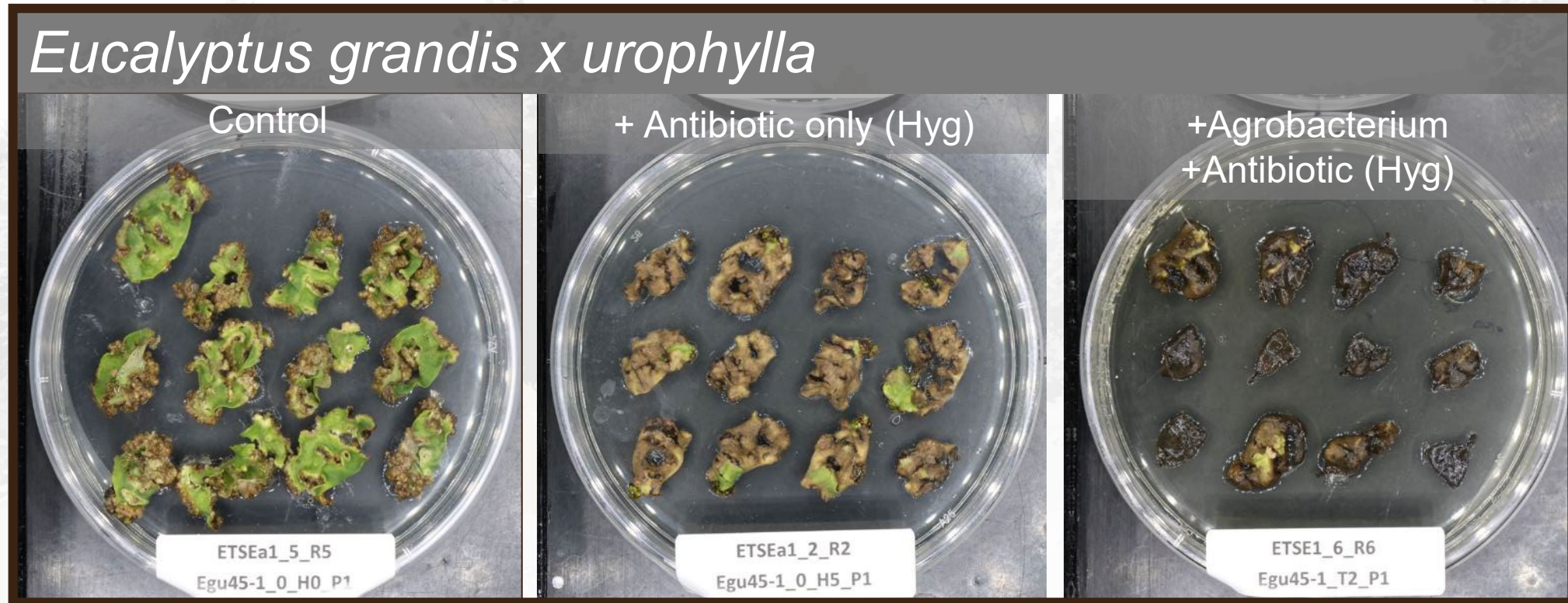
Black Cottonwood

Populus trichocarpa

(~1500 genotype GWAS population)

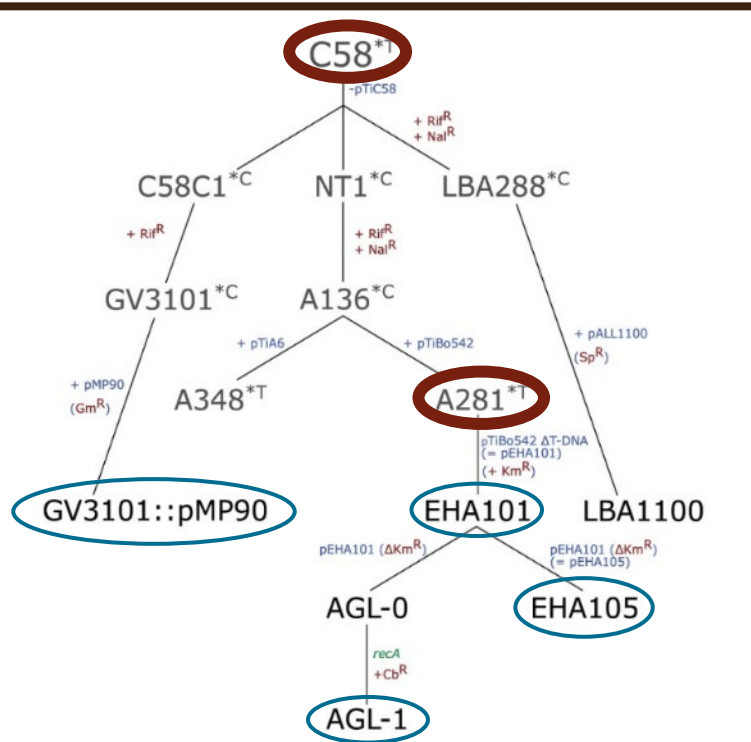
SKWB24-2, SLMB28-1, Nisqually-1

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

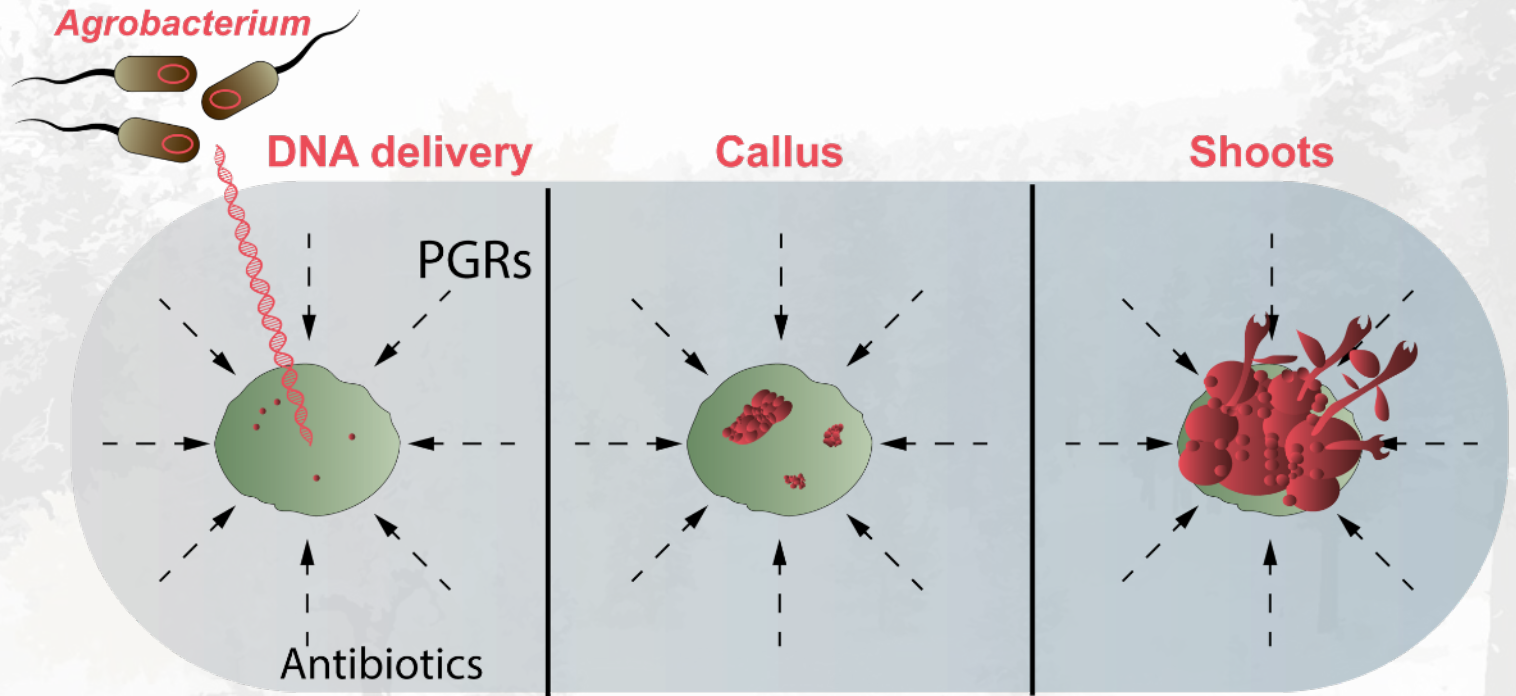
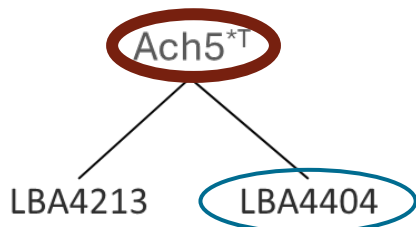


- Elite clones, not seed-derived
- High heterozygosity: each genotype a new adventure in culture
- High physiological diversity and common defense responses

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)

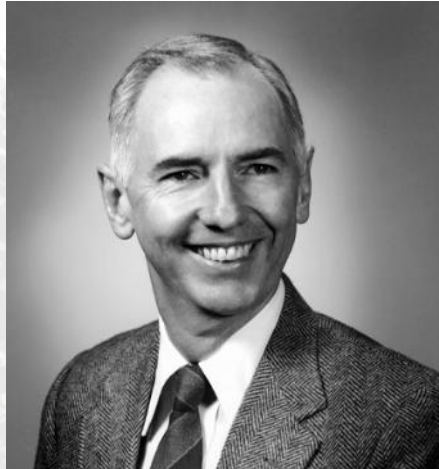


Are there better strains out there?

How do we turn new strains into laboratory tools?

Local history of *Agrobacterium* research enabled this project

Agro strains and molecular biology



Larry Moore



Walt Ream



Melodie Putnam

Strain genomics

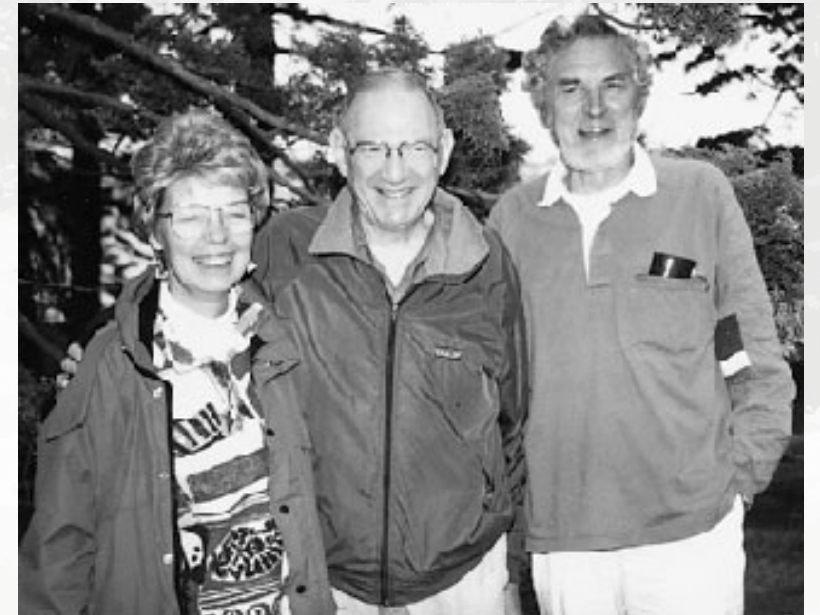


Alex Weisberg



Jeff Chang

Early disarmed and intermediate strains (UW)



Mary-Dell
Chilton

Milton
Gordon

Eugene
Nester

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

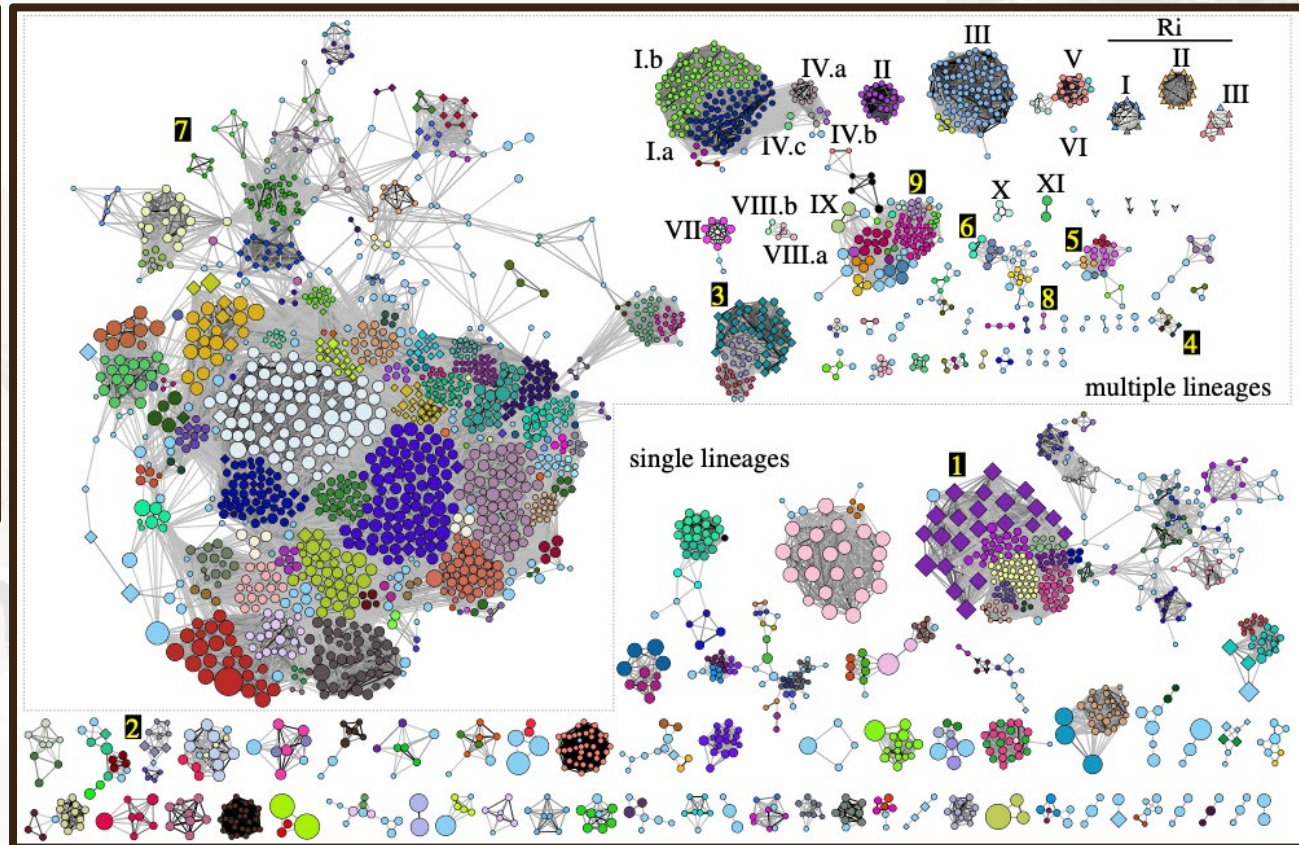
Alexandra J. Weisberg, Edward W. Davis II, Javier Tabima, Michael S. Belcher, Marilyn Miller, Chih-Horng Kuo, Joyce E. Loper, Niklaus J. Grünwald, Melodie L. Putnam, Jeff H. Chang*

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

Alexandra J. Weisberg¹, Marilyn Miller¹, Walt Ream², Niklaus J. Grünwald³ and Jeff H. Chang¹

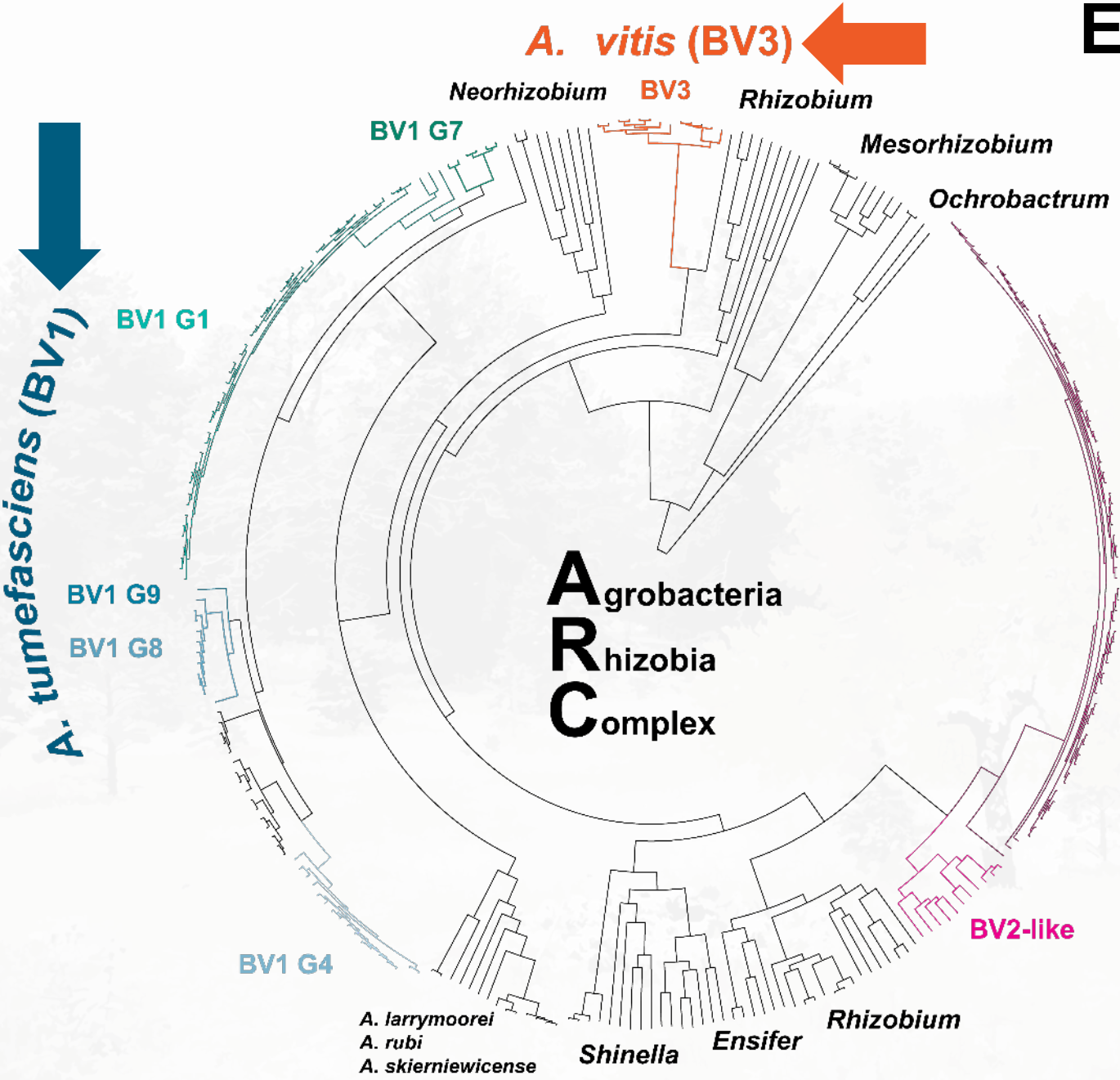
¹Department of Botany and Plant Pathology, and ²Department of Microbiology, Oregon State University, Corvallis, OR 97331, USA

³Horticultural Crops Research Laboratory, United States Department of Agriculture and Agricultural Research Service, Corvallis, OR 97330, USA



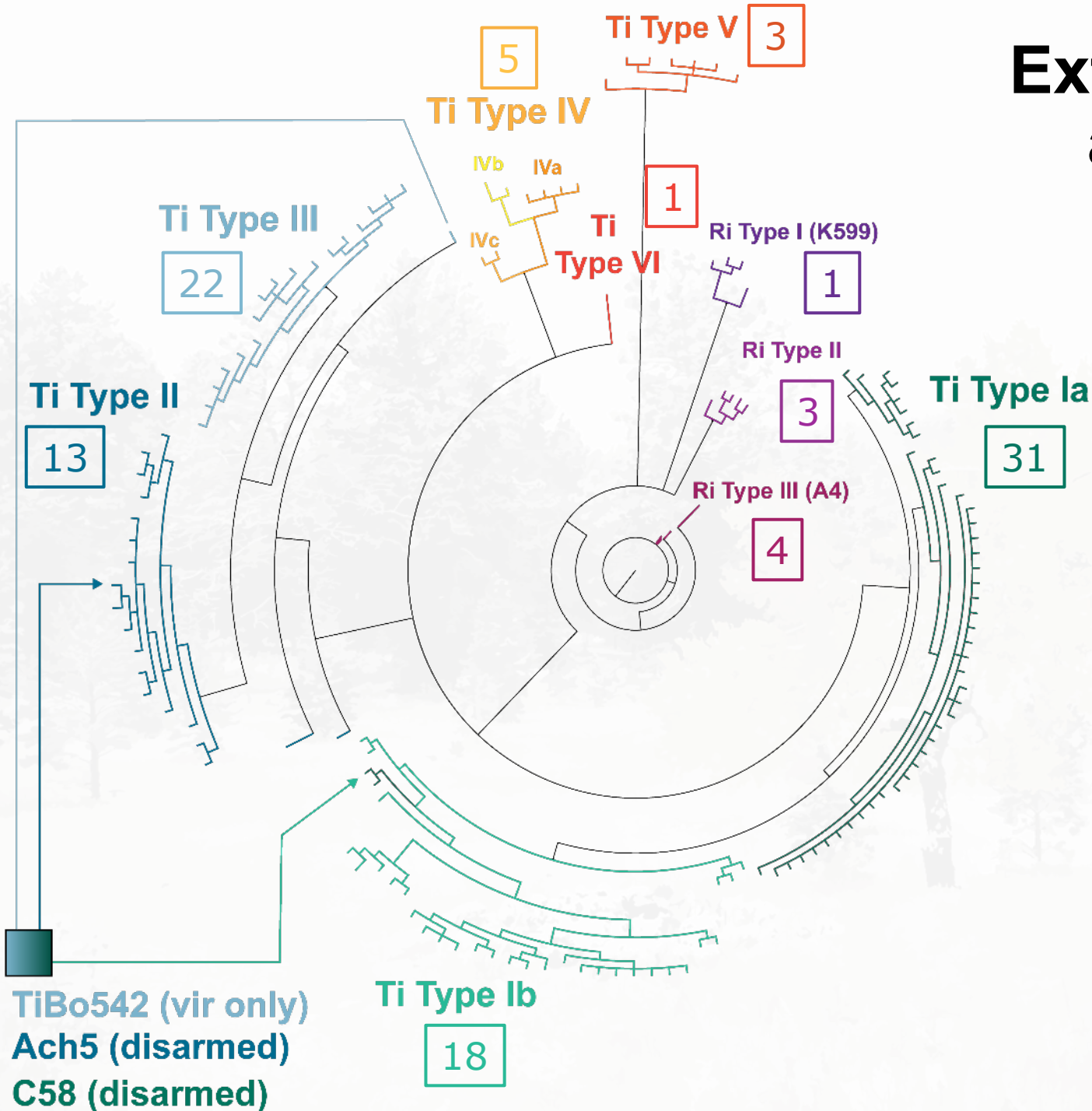
Complex network of accessory plasmids in *Agrobacteria* and *Rhizobia* species (From Weisberg et al. 2021 *Phil. Trans. R. Soc. B*)

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

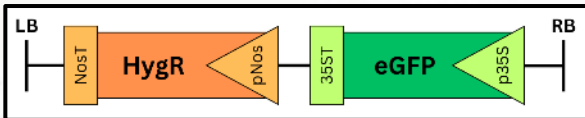
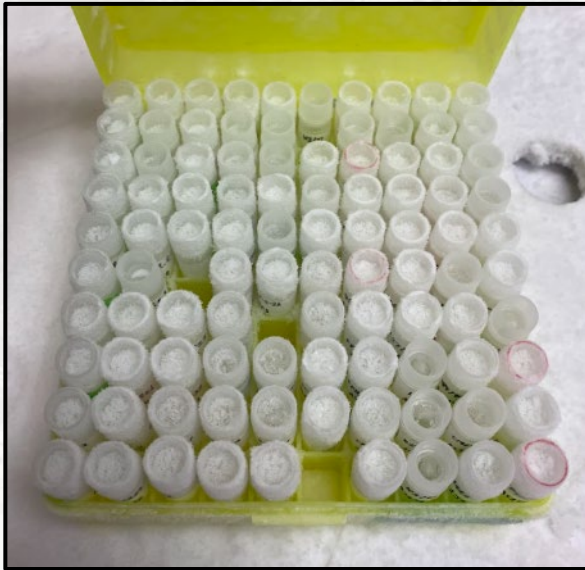
Extensive collection diversity at the Ti/Ri plasmid level



- 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

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

Insert binary vector



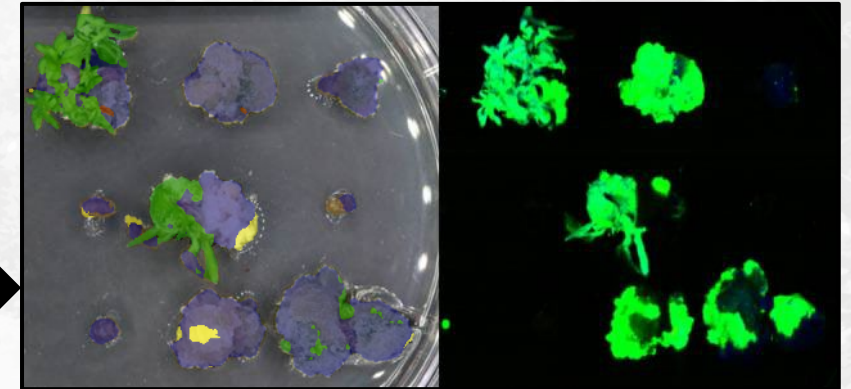
Kanamycin selection for binary plasmid in Agro

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

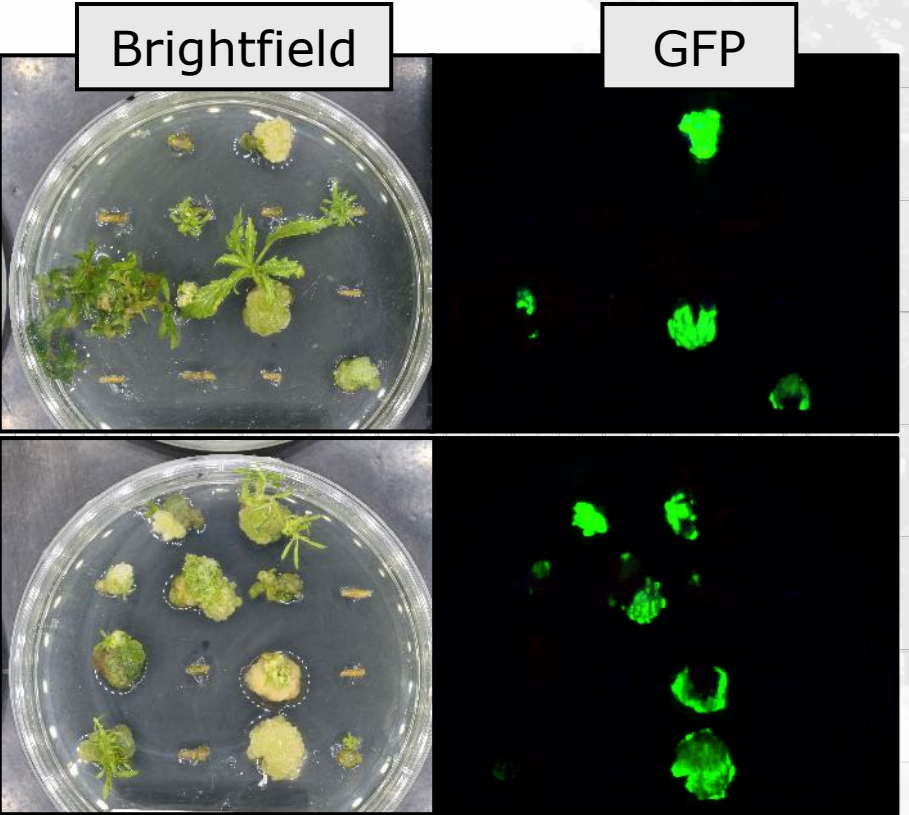
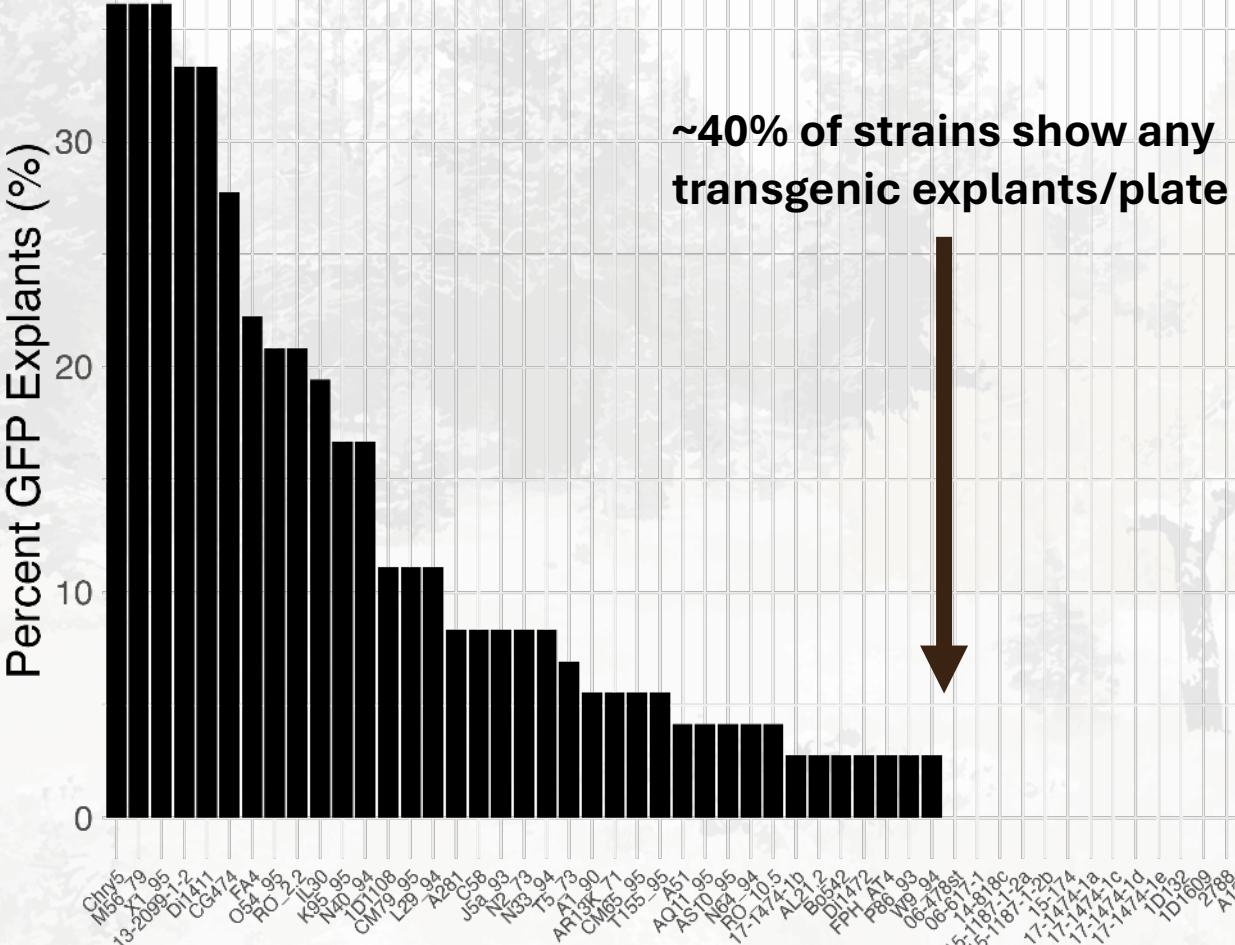
Repeat in other species
or treatment conditions

30 strains
high replication
(10 plates)

Select:
20 good strains
10 bad* strains

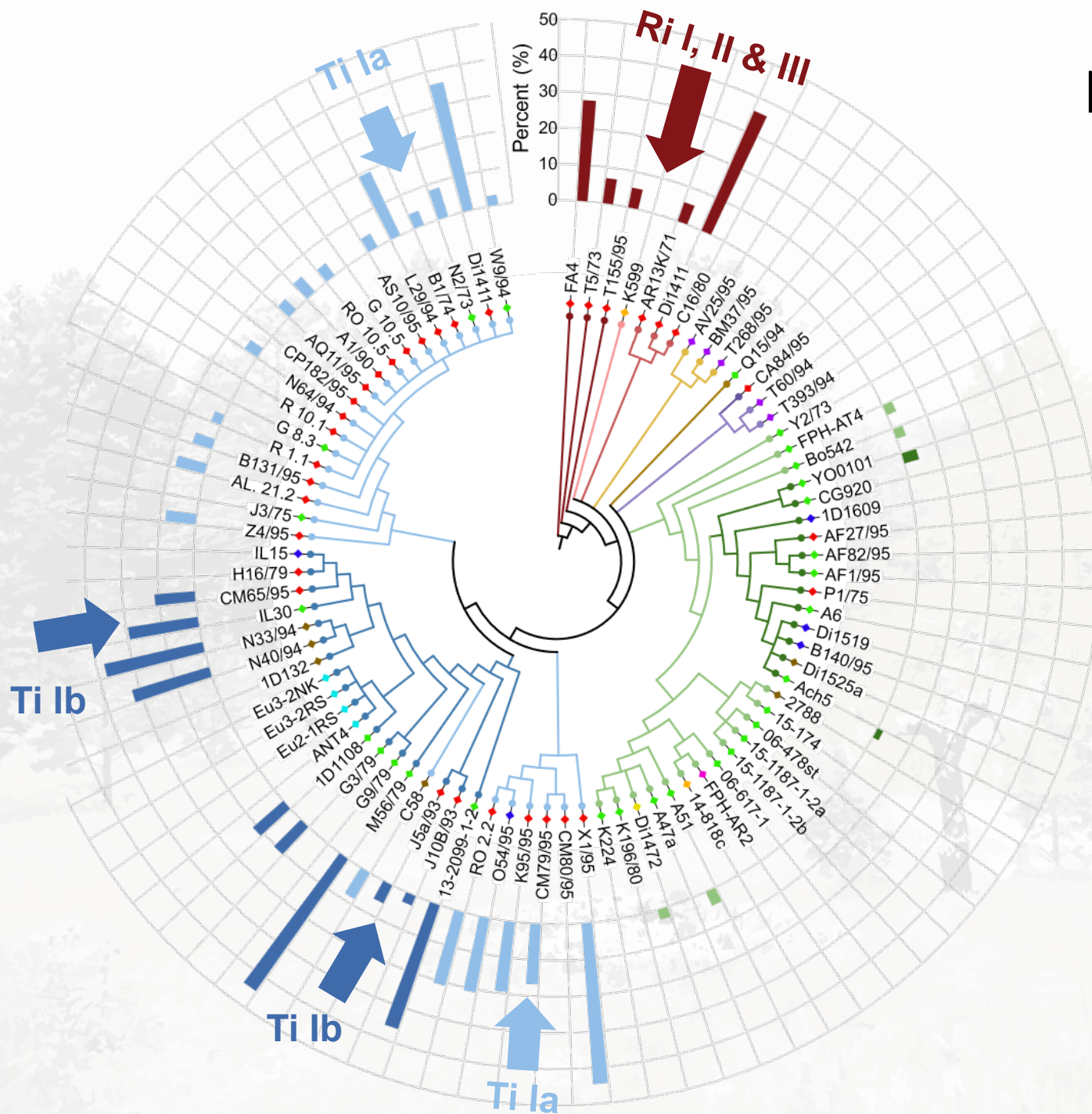
(*bad strains retained for mechanistic insight)

Wild strains show wide variability in transformation and regeneration in poplar



Strain

Many good performing strains were found, particularly from ones with type I Ti plasmids



- | | |
|----------------|-------------------------------|
| Plasmid | Phenotype |
| ● Ri I | ■ GFP explants |
| ● Ri II | |
| ● Ri III | |
| ● Ti Ia | Species |
| ● Ti Ib | ◆ Agrobacterium deltaense |
| ● Ti II | ◆ Agrobacterium fabrum |
| ● Ti III | ◆ Agrobacterium larrymoorei |
| ● Ti IVa | ◆ Agrobacterium radiobacter |
| ● Ti IVc | ◆ Agrobacterium rubi |
| ● Ti V | ◆ Agrobacterium skirniwicense |
| ● Ti VI | ◆ Agrobacterium tumefaciens |
| | ◆ Allorhizobium vitis |
| | ◆ Rhizobium rhizogenes |

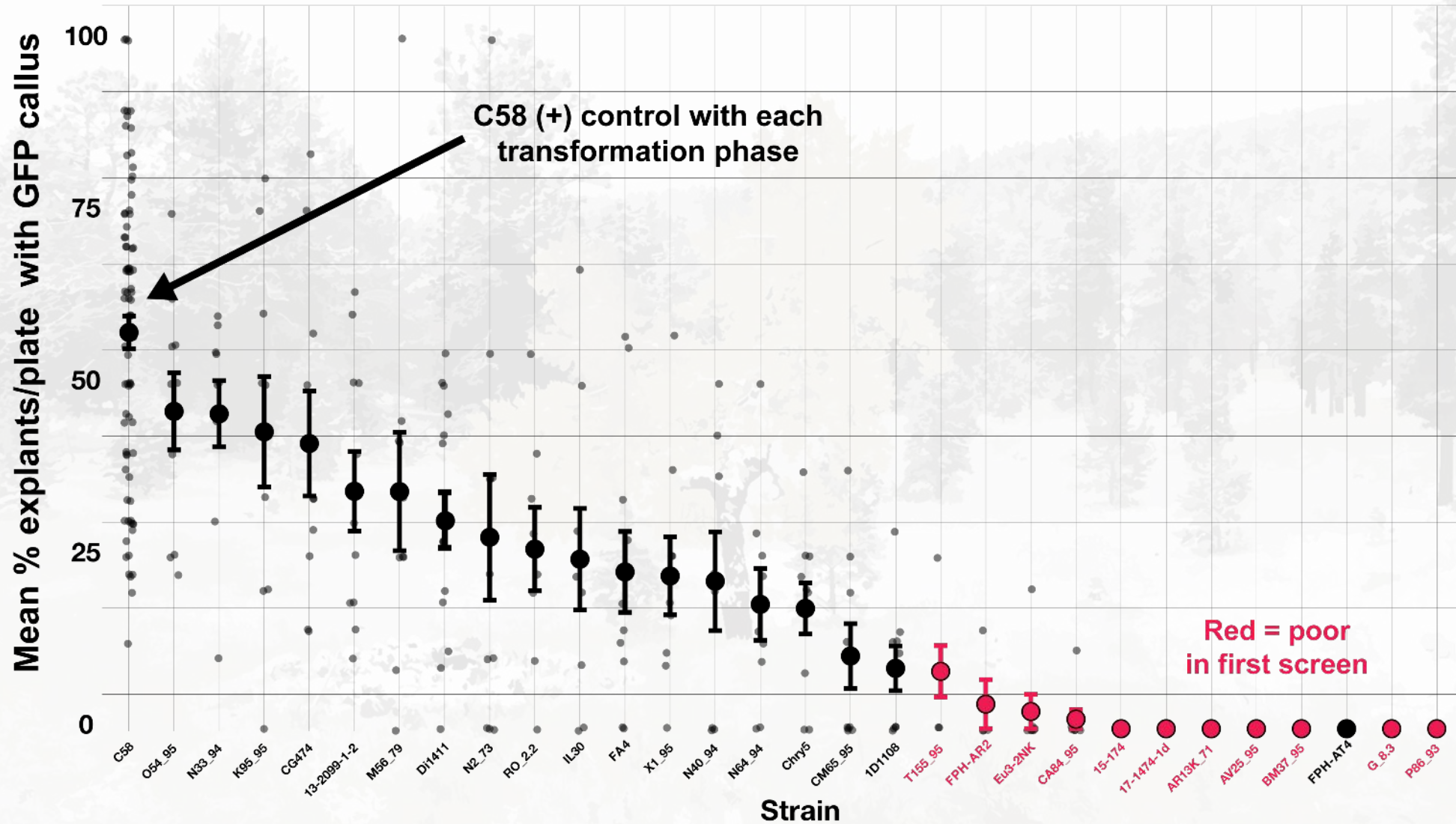
Chromosomal background impact less clear

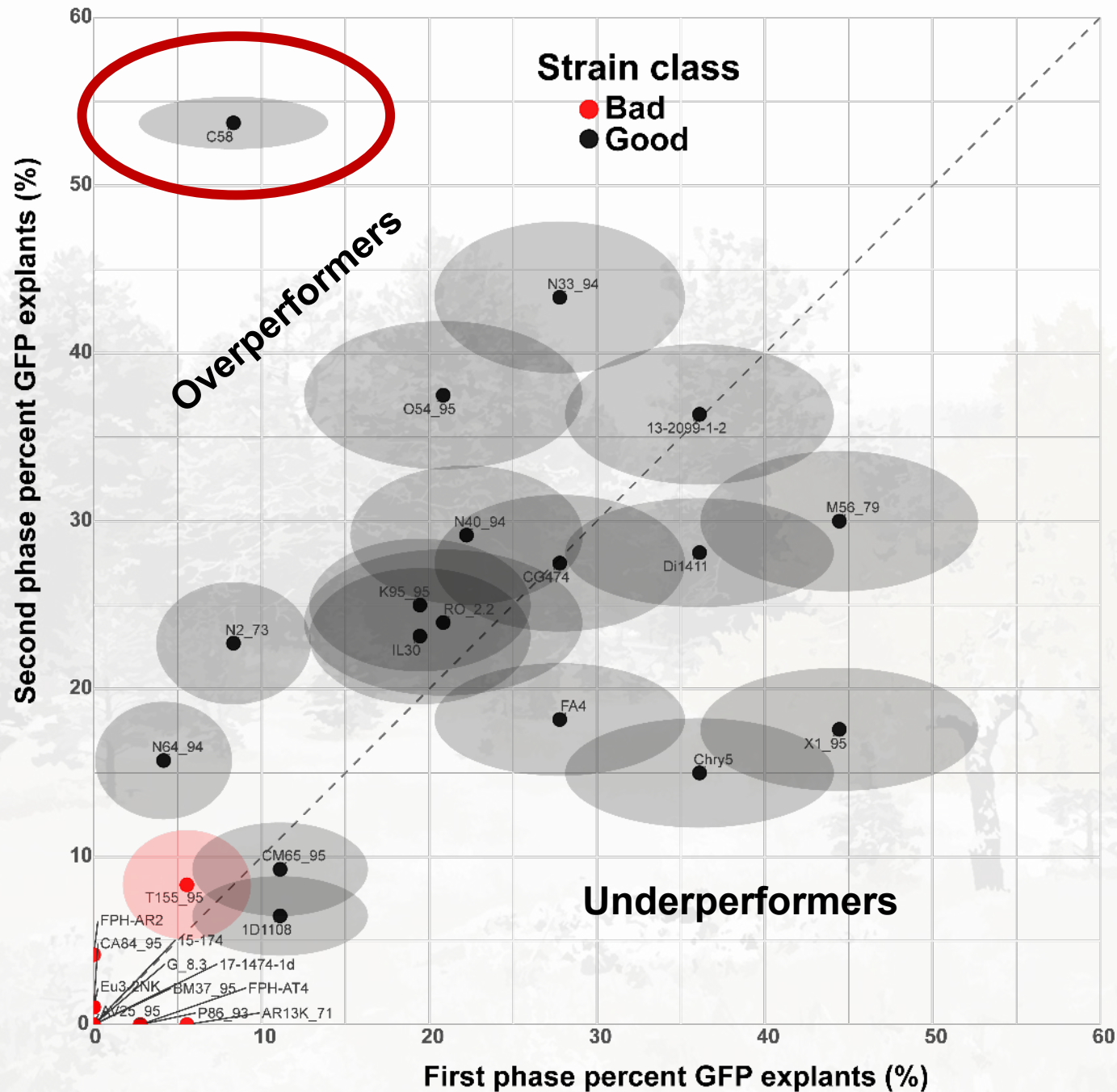
Differences in growth media and antibiotic susceptibility were noted

**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

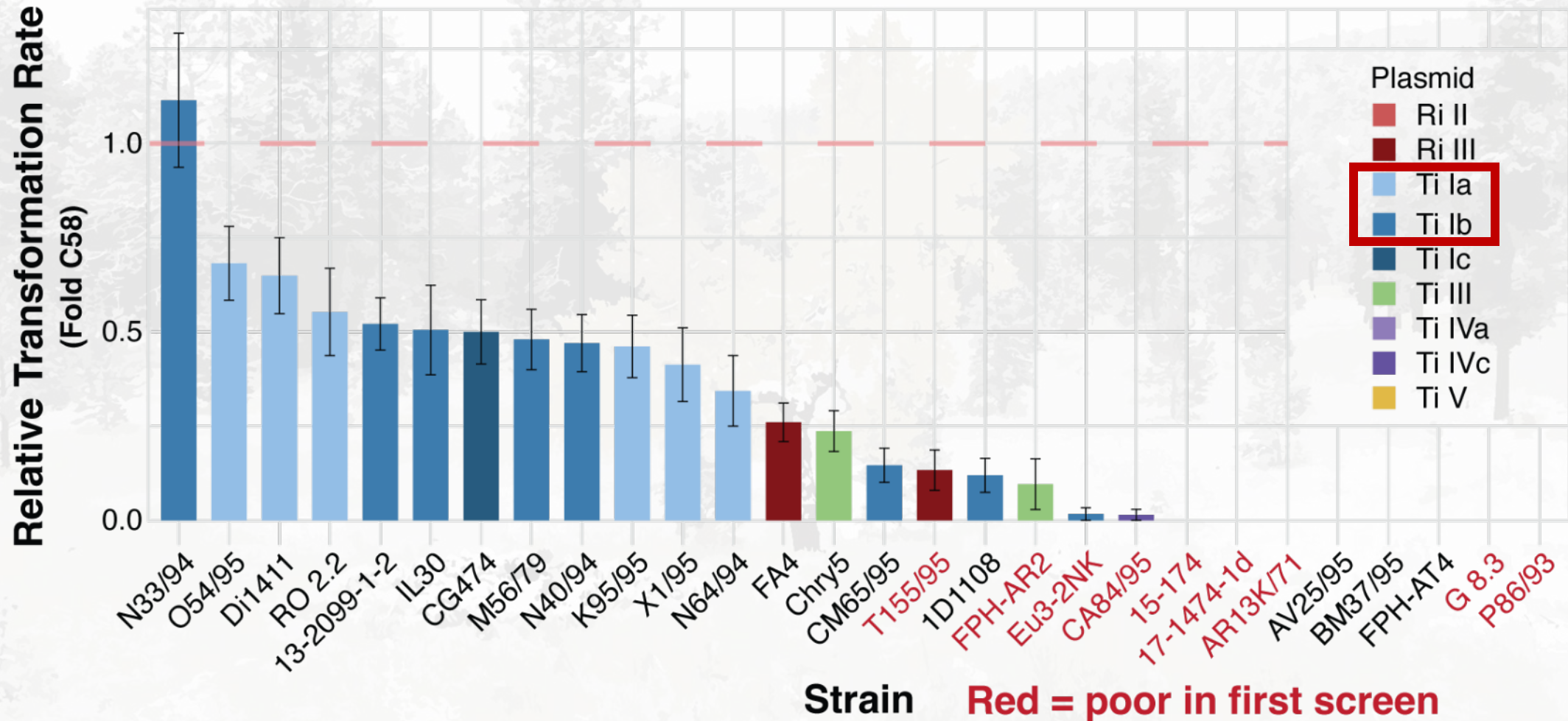




First and second screening trials were consistent in strain transformation behavior

- C58 an outlier between initial and higher replication trials
- C58 included in second screen as a positive control with each transformation group

Strains from several Ti/Ri backgrounds and chromosomal groups show high rates of transgenic tissues



Collecting is never finished!

When opportunity strikes we are trying to isolate new strains



Michele Wiseman
PhD, OSU Bot&PP
now postdoc at Cornell

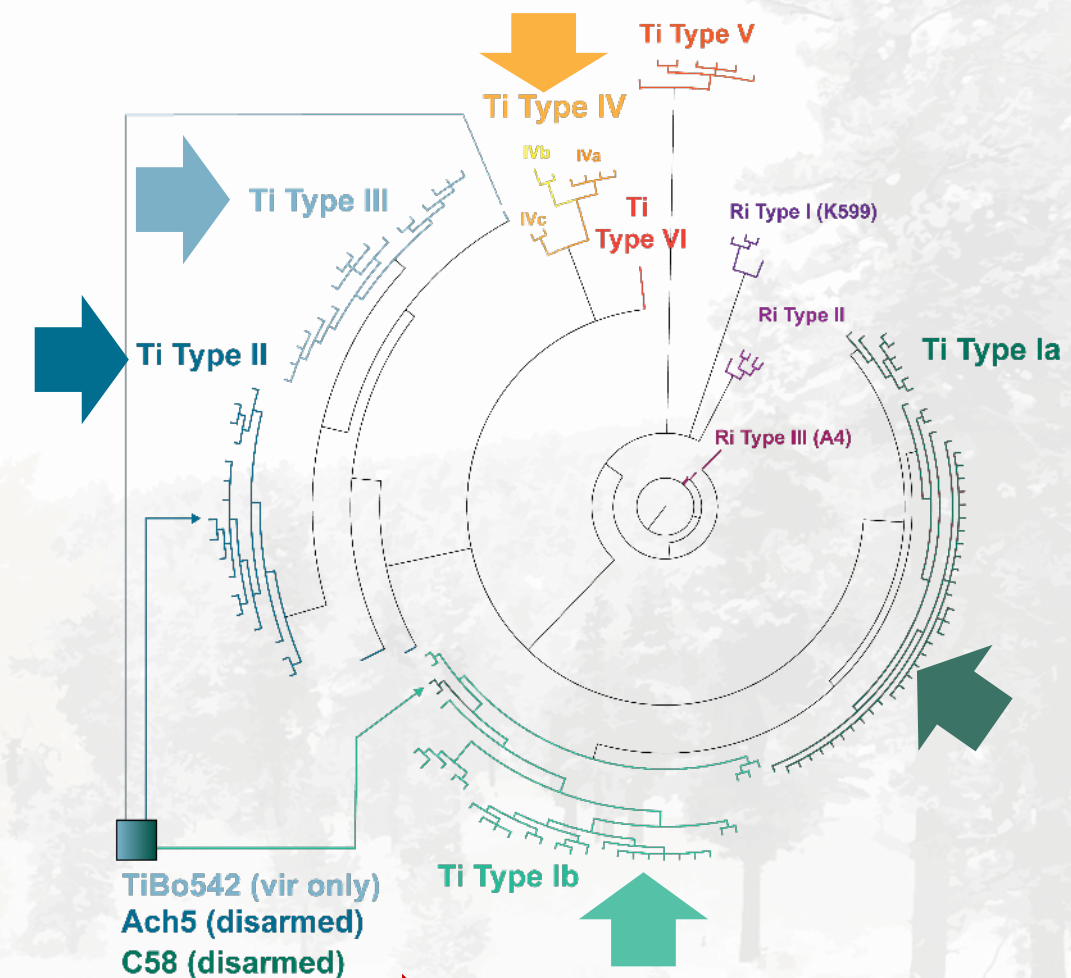
We were able to isolate a strain (BV1, Ti type Ia) from a hop gall

How best to disarm and engineer promising strains?



We have so far disarmed 16 strains using double crossover recombination

Strain designation	Host plant source	Virulence plasmid type	Chromosomal background / taxonomy
STR_H01_DE	Hop (<i>Humulus lupulus</i>)	Ti Ia	<i>Agrobacterium fabrum</i> (biovar 1)
STR_L01_DE	Pear (<i>Pyrus betulifolia</i>)	Ti Ia	<i>Rhizobium rhizogenes</i> (biovar 2)
STR_R01_DE	Rose (<i>Rosa</i> sp.)	Ti Ia	<i>Rhizobium rhizogenes</i> (biovar 2)
STR_P01_DE	Peach (<i>Prunus persica</i>)	Ti Ia	<i>Rhizobium rhizogenes</i> (biovar 2)
STR_P02_DE	Apricot (<i>Prunus siberica</i>)	Ti Ia	<i>Rhizobium rhizogenes</i> (biovar 2)
STR_P03_DE	Cherry (<i>Prunus avium</i>)	Ti Ia	<i>Agrobacterium radiobacter</i> (biovar 1)
STR_A01_DE	Quaking aspen (<i>Populus tremuloides</i>)	Ti Ib	<i>Agrobacterium tumefaciens</i> (biovar 1)
STR_A02_DE	Cottonwood (<i>Populus</i> sp.)	Ti Ib	<i>Agrobacterium tumefaciens</i> (biovar 1)
STR_G02_DE	Grape (<i>Vitis vinifera</i>)	Ti Ib	<i>Rhizobium rhizogenes</i> (biovar 2)
STR_R02_DE	Rose (<i>Rosa</i> sp.)	Ti Ib	<i>Agrobacterium tumefaciens</i> (biovar 1)
STR_S02_DE	Red raspberry (<i>Rubus idaeus</i> x <i>strigosus</i>)	Ti Ib	<i>Agrobacterium fabrum</i> (biovar 1)
STR_Y01_DE	Yarrow (<i>Achillea</i> sp.)	Ti II	<i>Agrobacterium tumefaciens</i> (biovar 1)
STR_B01_DE	Lilac (<i>Syringa vulgaris</i>)	Ti II	<i>Agrobacterium tumefaciens</i> (biovar 1)
STR_C01_DE	Chrysanthemum (<i>Chrysanthemum</i> sp.)	Ti III	<i>Agrobacterium tumefaciens</i> (biovar 1)
STR_G01_DE	Grape (<i>Vitis vinifera</i>)	Ti IVa	<i>Allorhizobium vitis</i> (biovar 3)
STR_R03_DE	Rose (<i>Rosa</i> sp.)	Ti Ia	<i>Rhizobium rhizogenes</i> (biovar 2)



Talk on Monday about strain disarmament

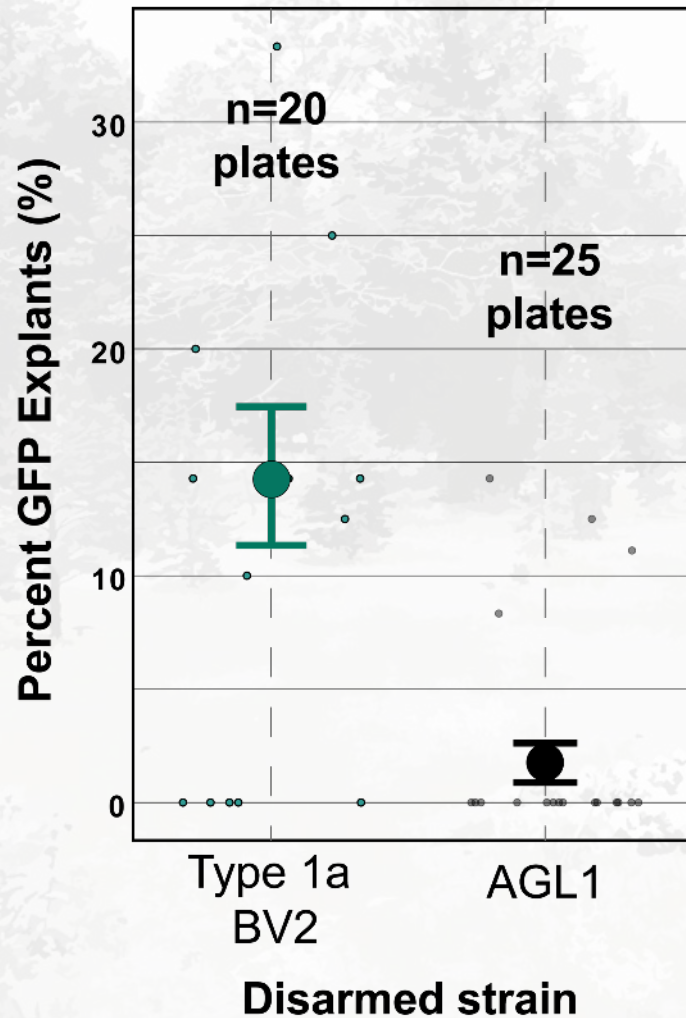


Zachary Heinhold
PhD student

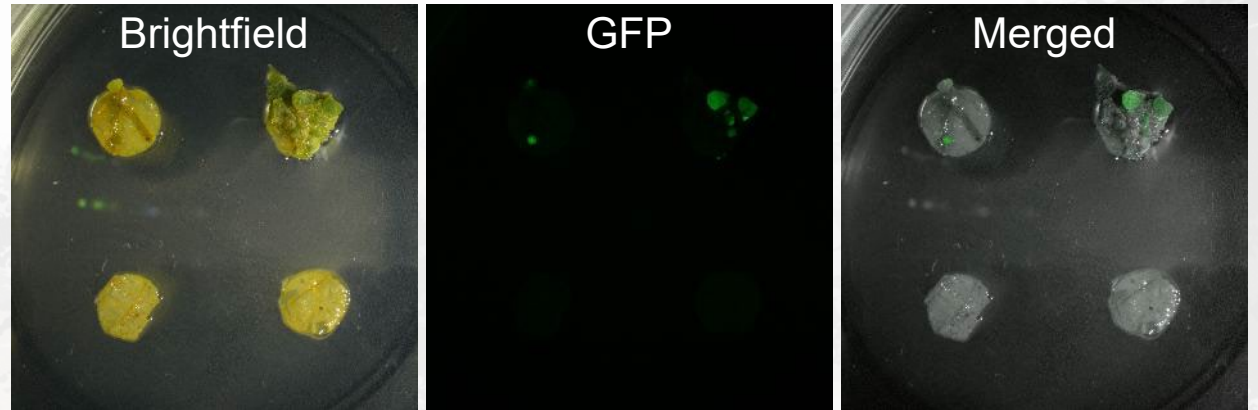


Chris Willig
Postdoc

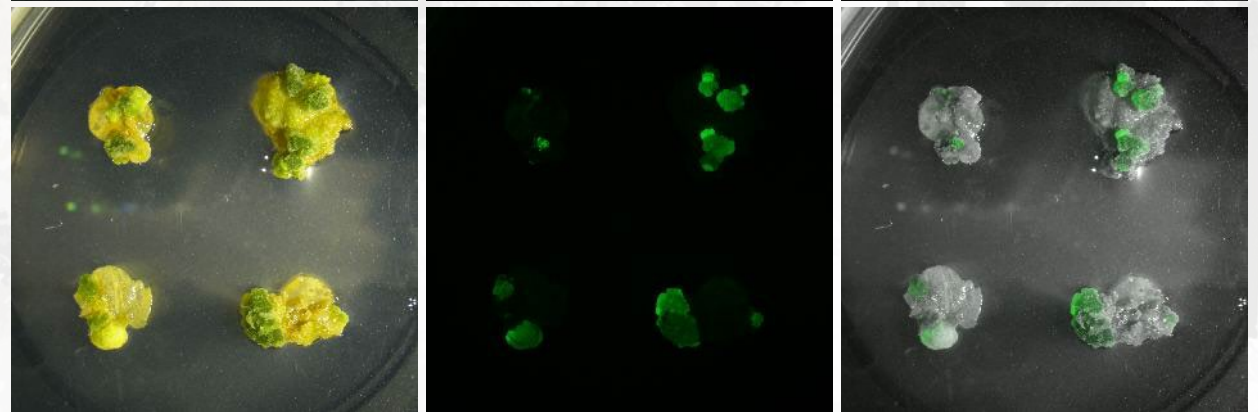
Initial testing of a disarmed Ti type 1a (BV2) against the AGL1 lab strain showed improved transformation in poplar



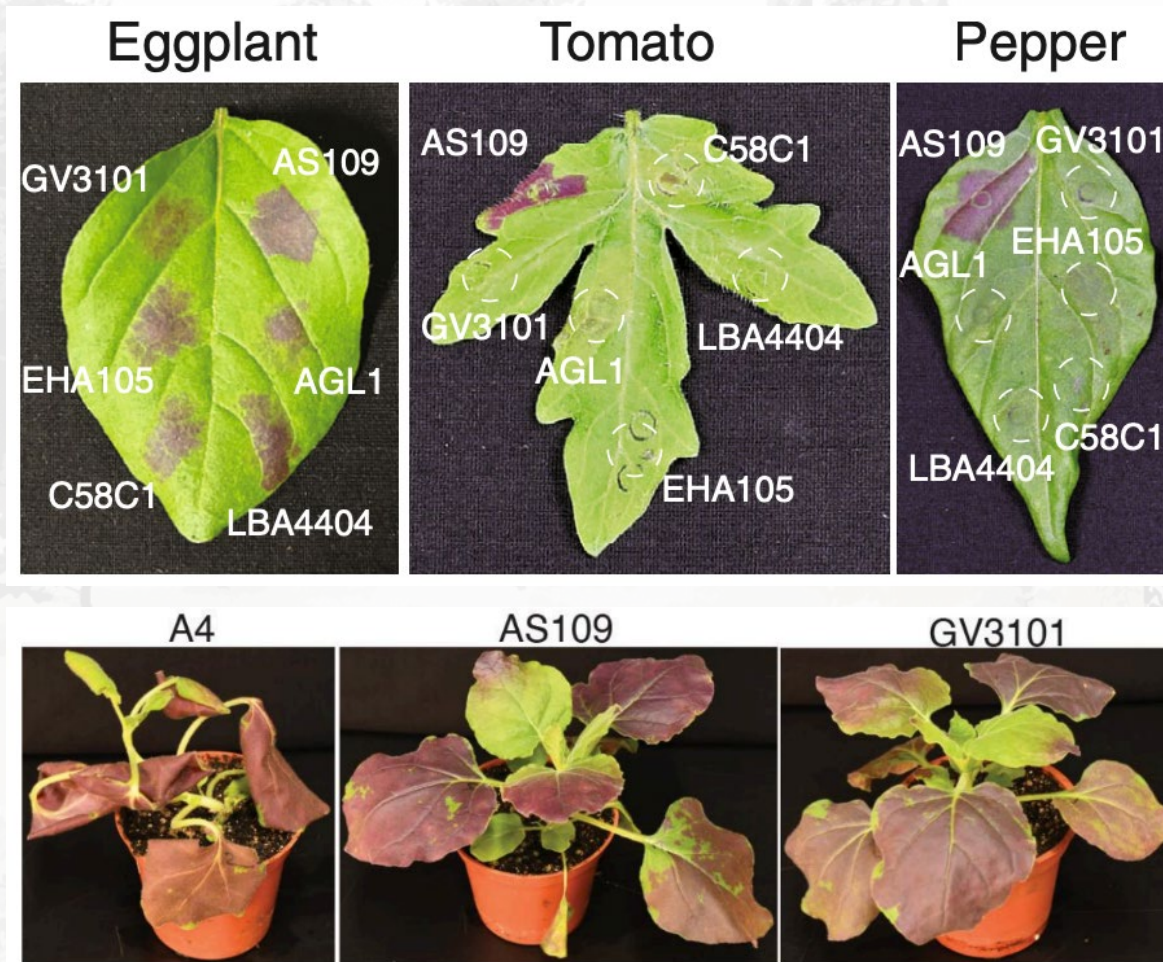
AGL1



T1aBV2



Other recent strain engineering like A4 shows promise as transformation tools



Plant Biotechnology Journal



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***Rhizobium rhizogenes* A4-derived strains mediate hyper-efficient transient gene expression in *Nicotiana benthamiana* and other solanaceous plants**

[Juan Carlos Lopez-Agudelo](#), [Foong-Jing Goh](#), [Sopio Tchabashvili](#), [Yu-Seng Huang](#), [Ching-Yi Huang](#), [Kim-Teng Lee](#), [Yi-Chieh Wang](#), [Yu Wu](#), [Hao-Xun Chang](#), [Chih-Horng Kuo](#), [Erh-Min Lai](#), [Chih-Hang Wu](#) ✉

“Shooty” strategies for transformation using Agrobacterium T-DNA genes



Strains of *Agrobacterium* can form “shooty” crown galls

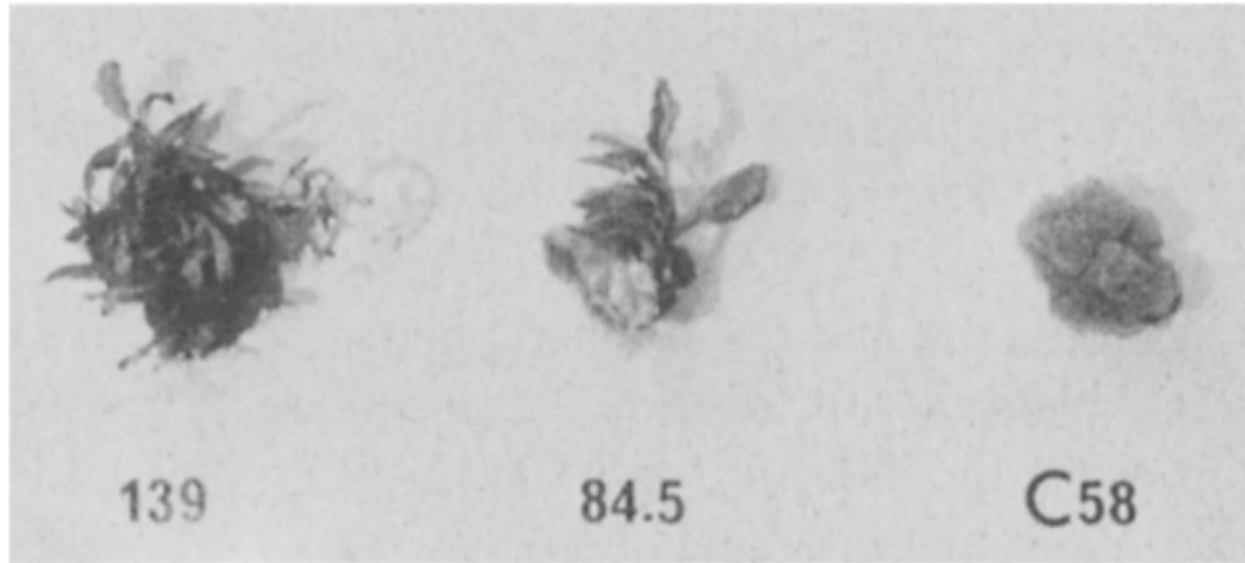
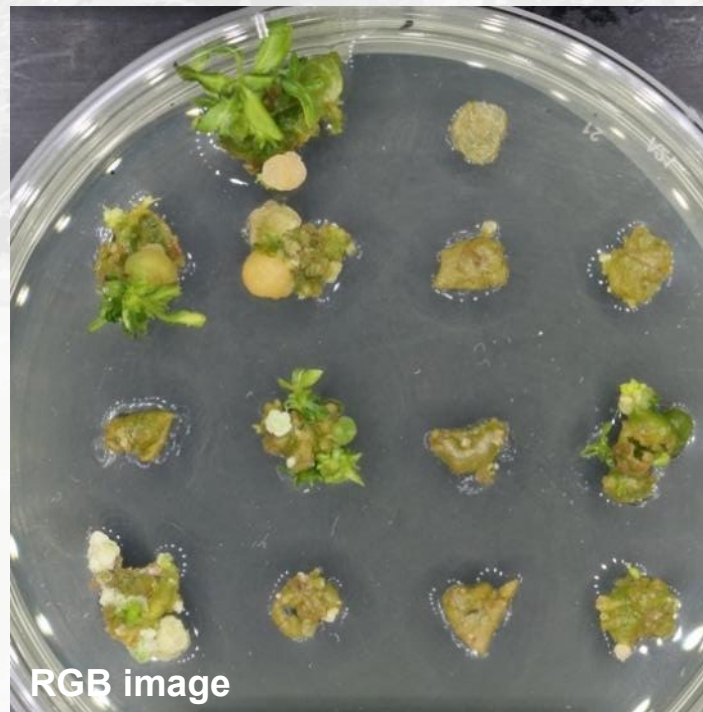


Fig. 1. Tumors and shoot differentiation from poplar tumors induced by *A. tumefaciens* strains 82.139, 84.5 and C58 and cultivated on MS medium, 6 weeks after inoculation.

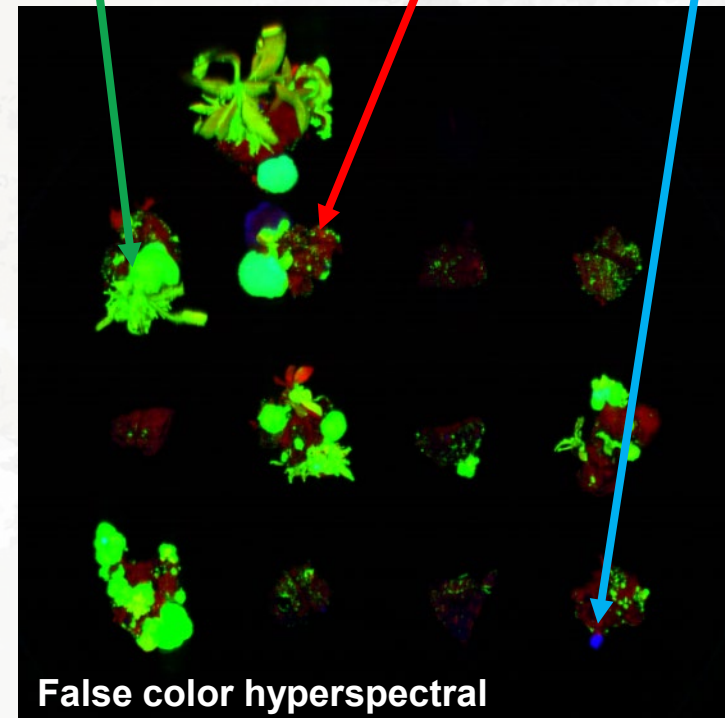


Populus tremula x tremuloides (82.139 infection)

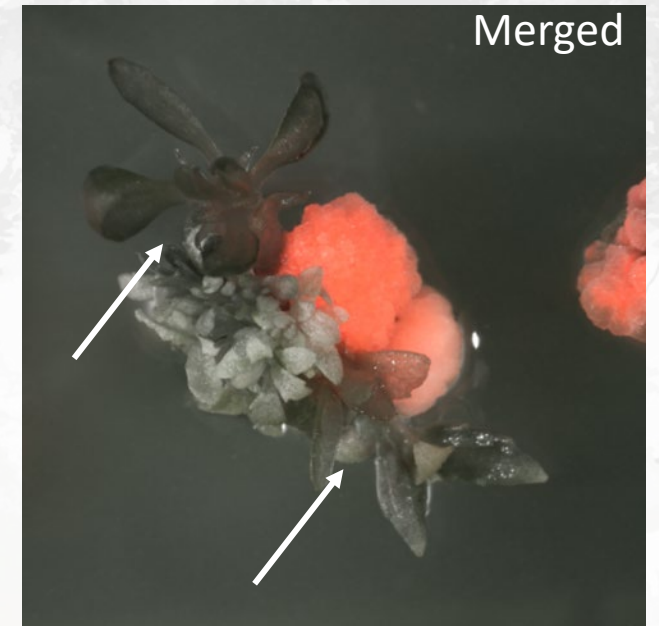
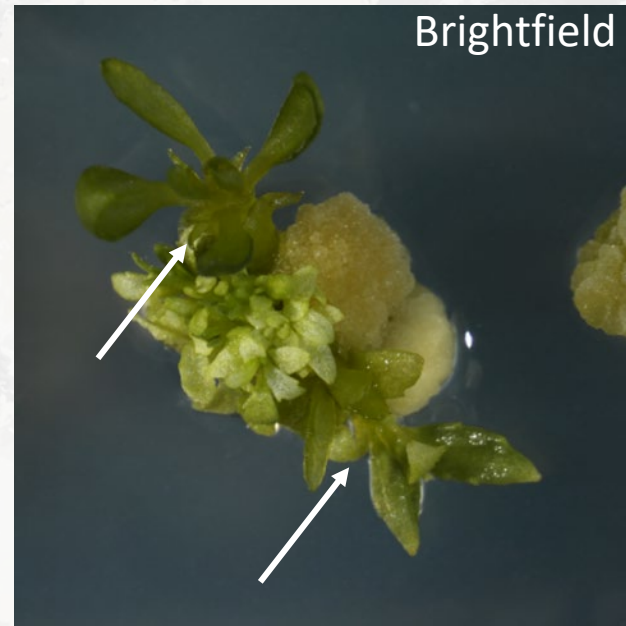
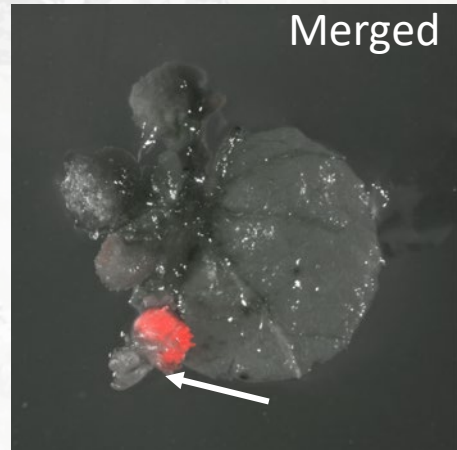
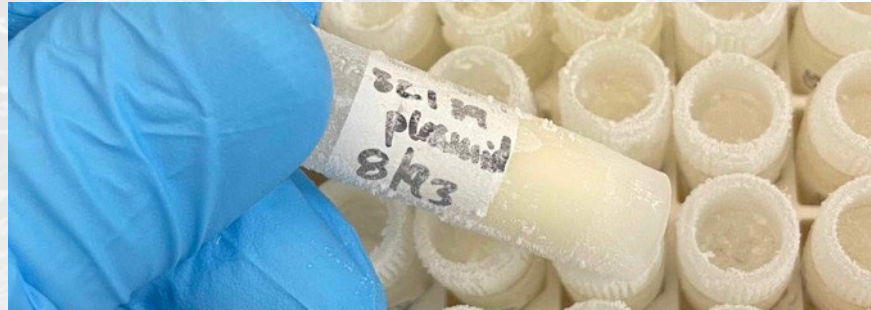
Passive “shooty” regeneration behavior as a tool using a co-transformation approach



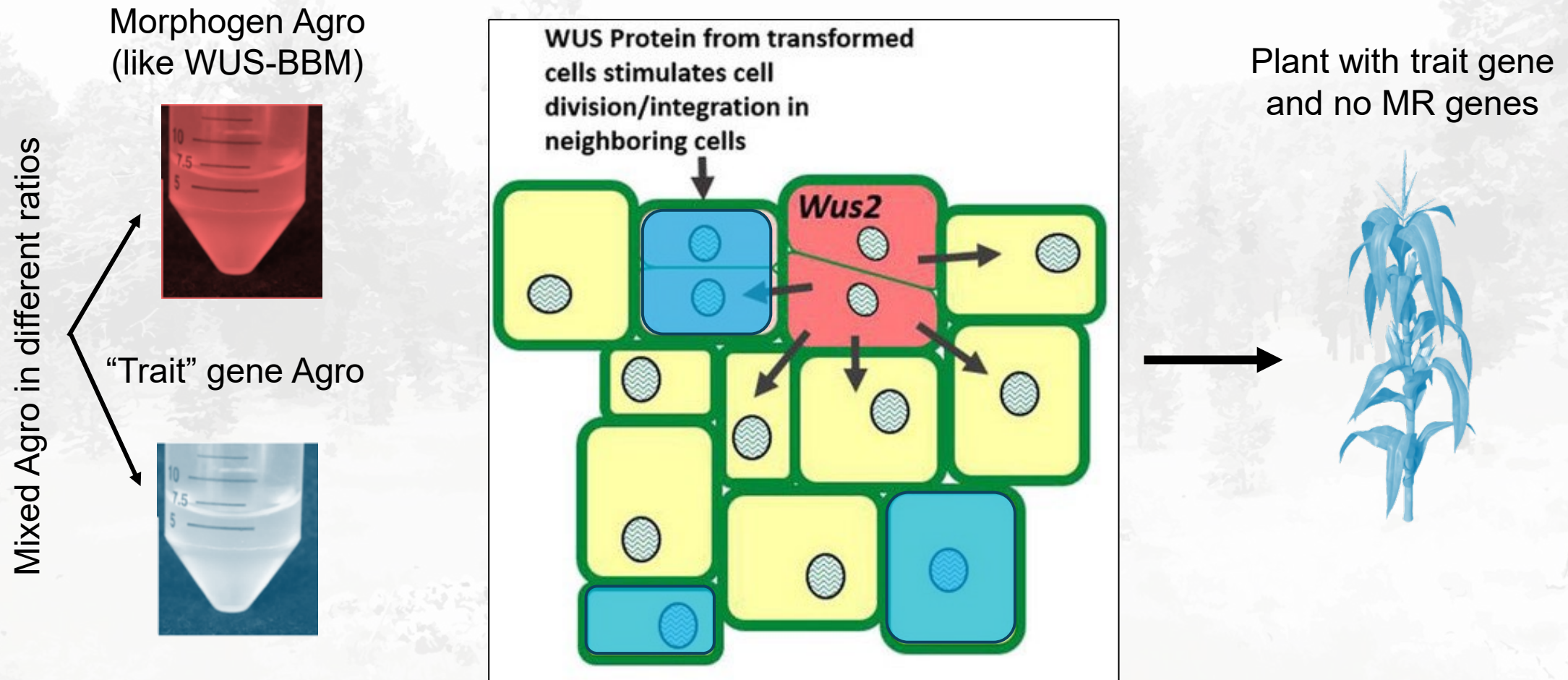
GFP (Trait T-DNA) Chlorophyll DsRed (Agro T-DNA)



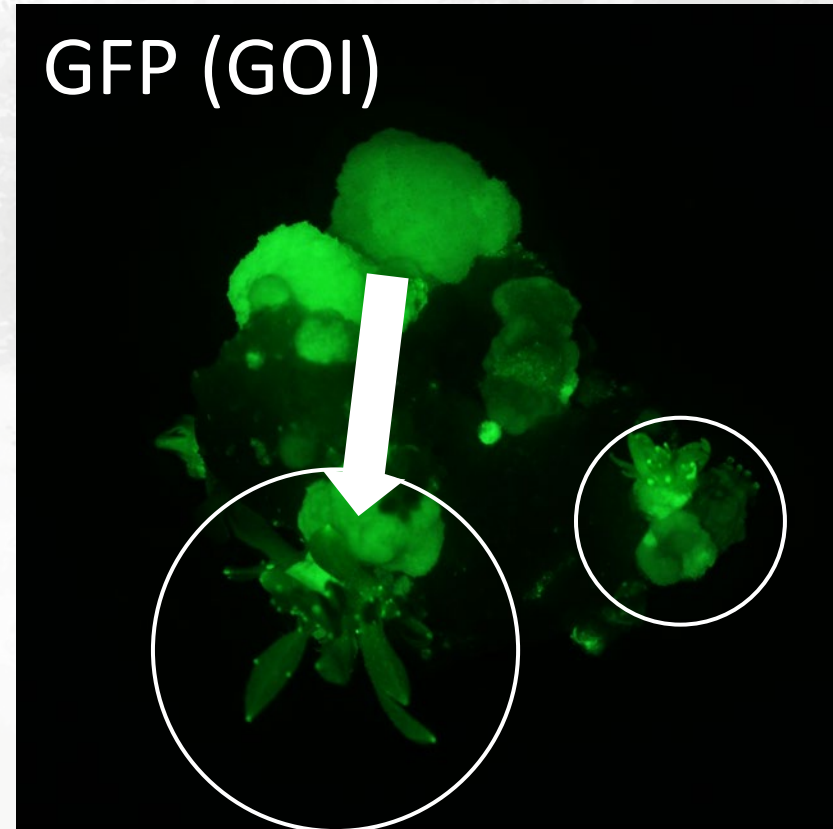
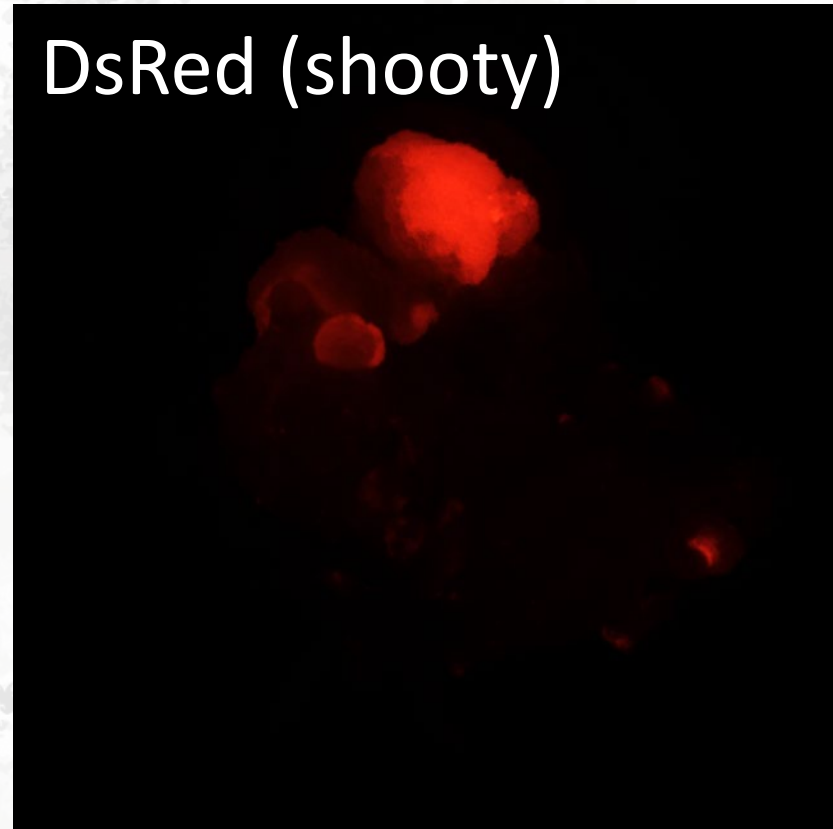
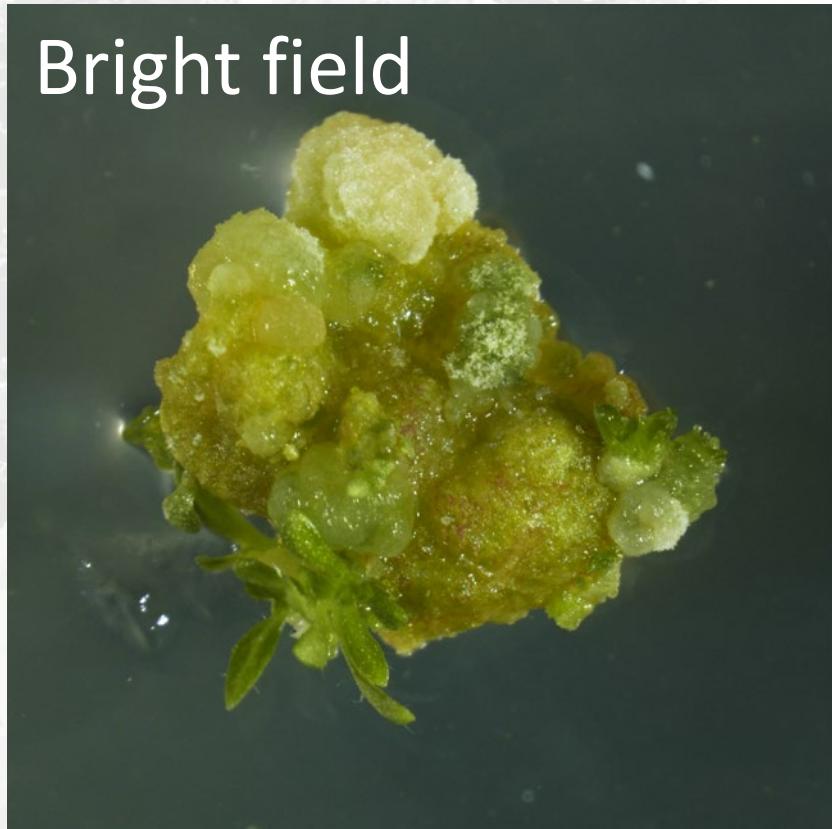
Genes from T-DNA of shooty strain 82.139 spur shoot regeneration outside transgenic callus



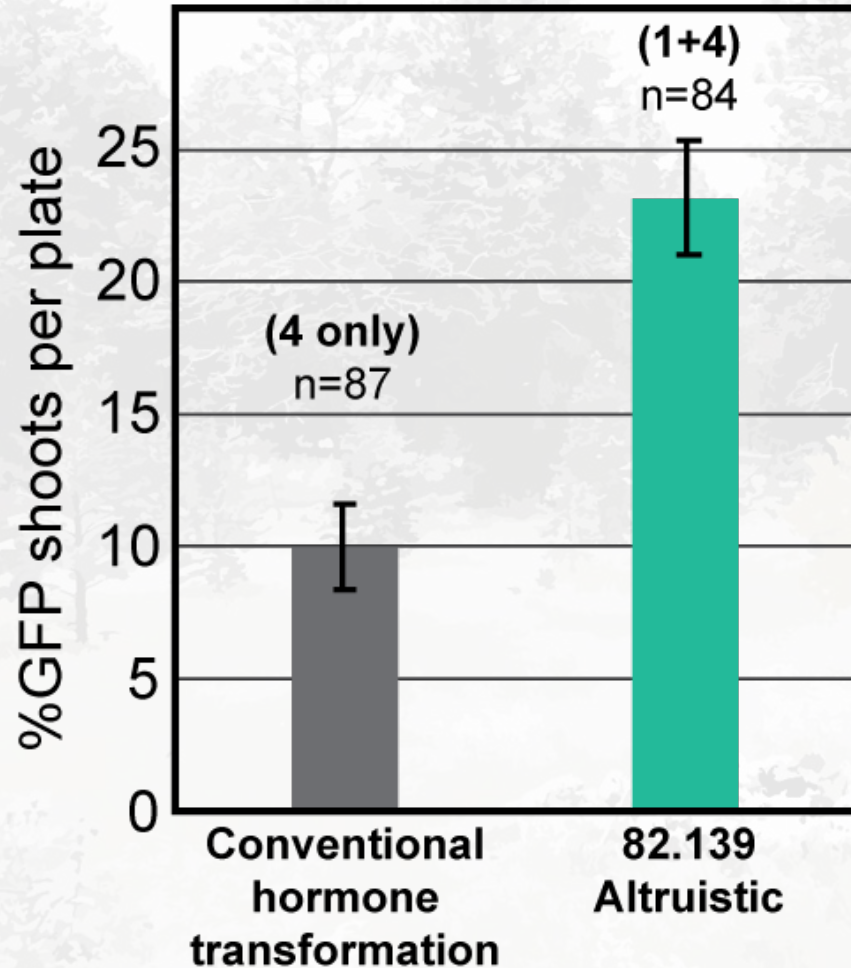
“Altruistic” transformation approach – strain mixtures



Cells distal to those transformed with 82.139 regenerate into transgenic trait T-DNA only shoots



82.139 altruistic transformation was superior to routine hormone-based transformation in poplar

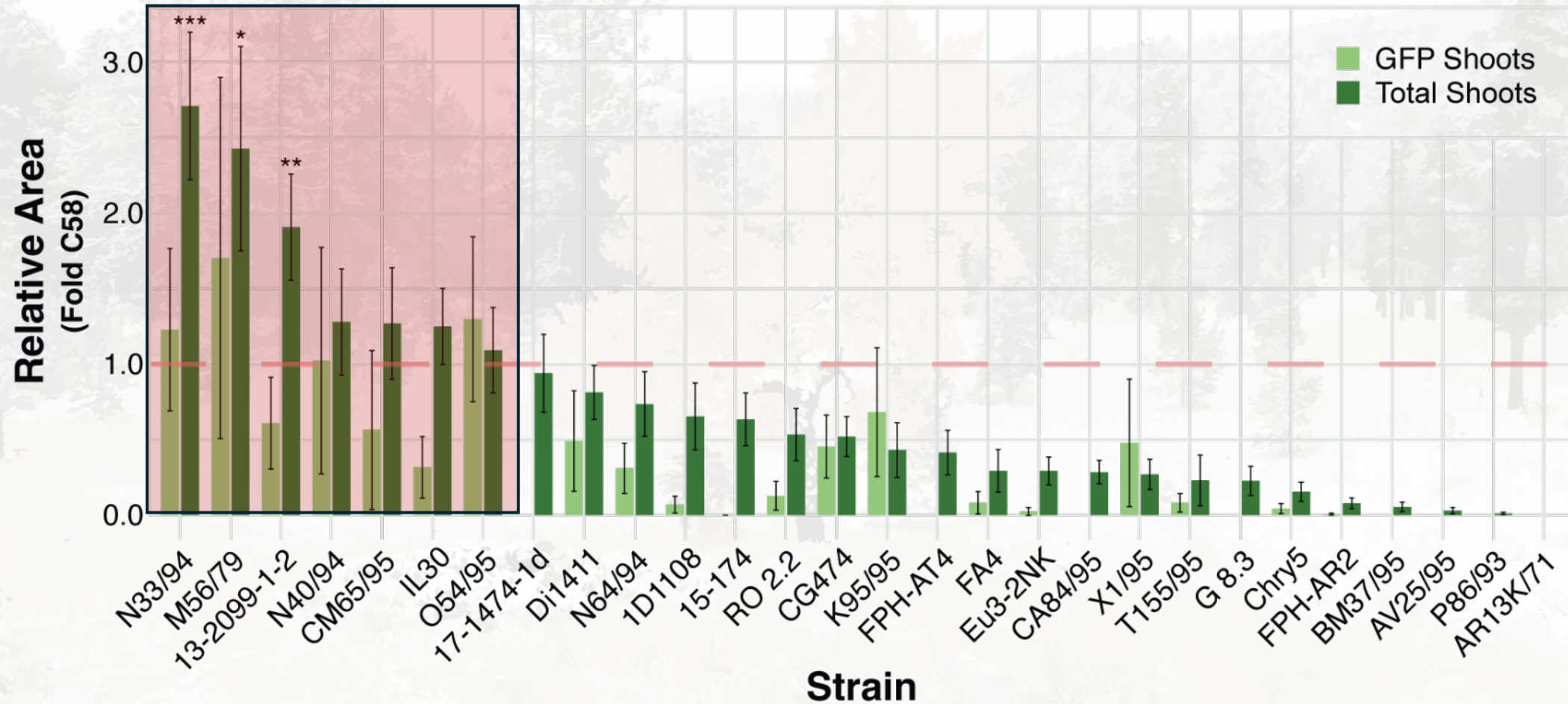


Vector types used



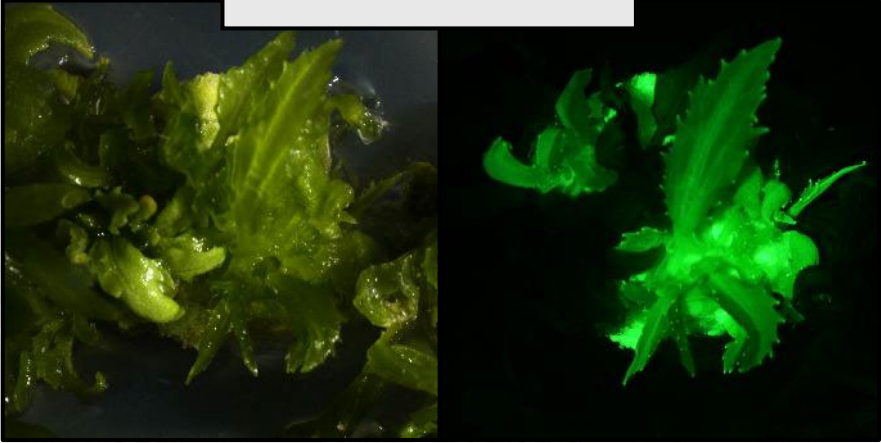
n = number of plates of 12 explants each

Several strains with Ti plasmid type 1b were efficient at transgenic shoot formation

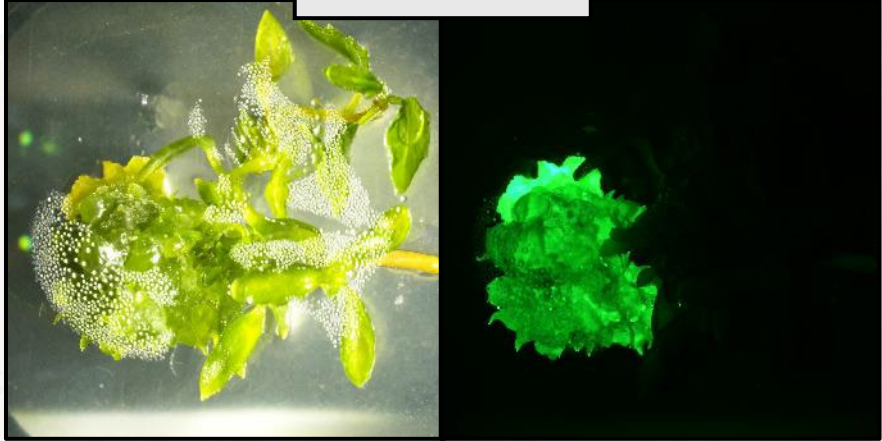


Ti plasmid type 1b was the most efficient at transgenic shoot formation

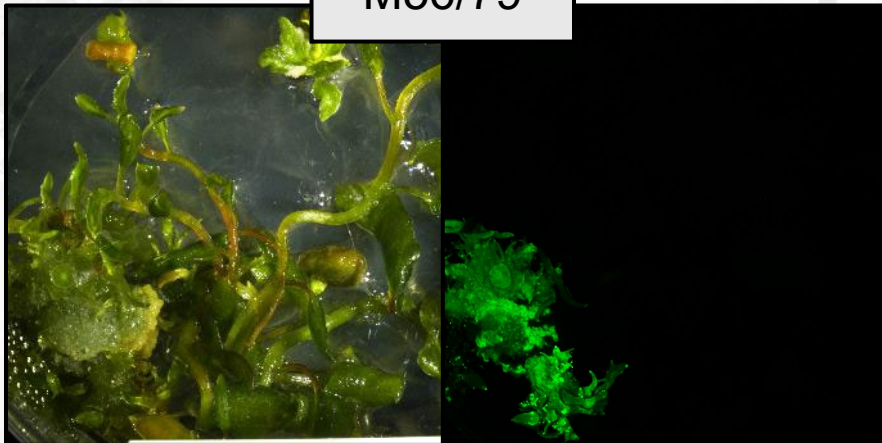
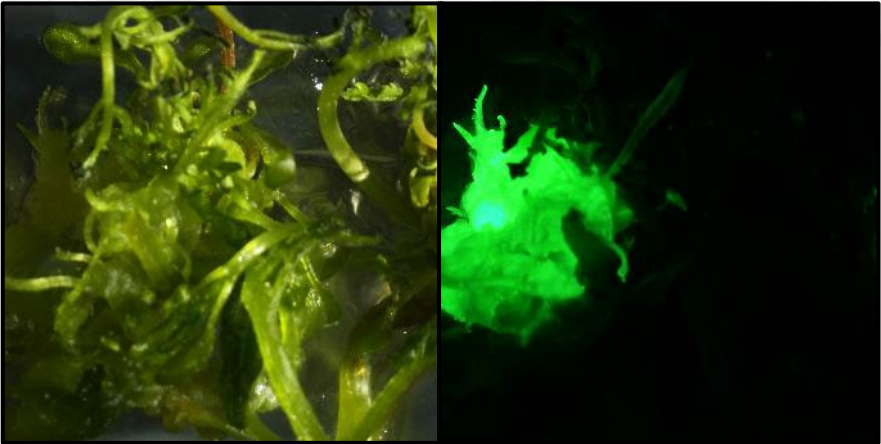
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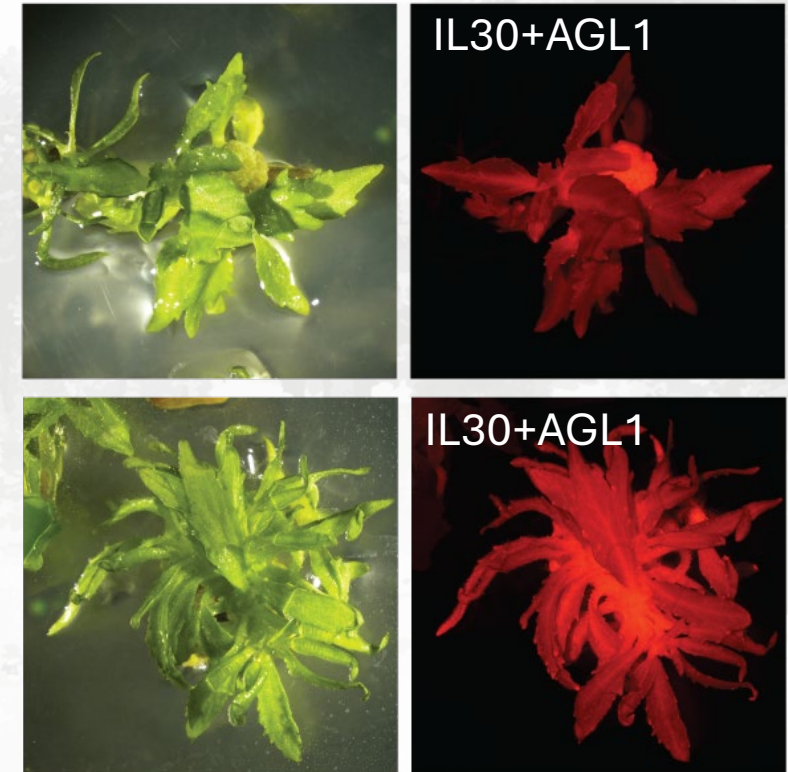
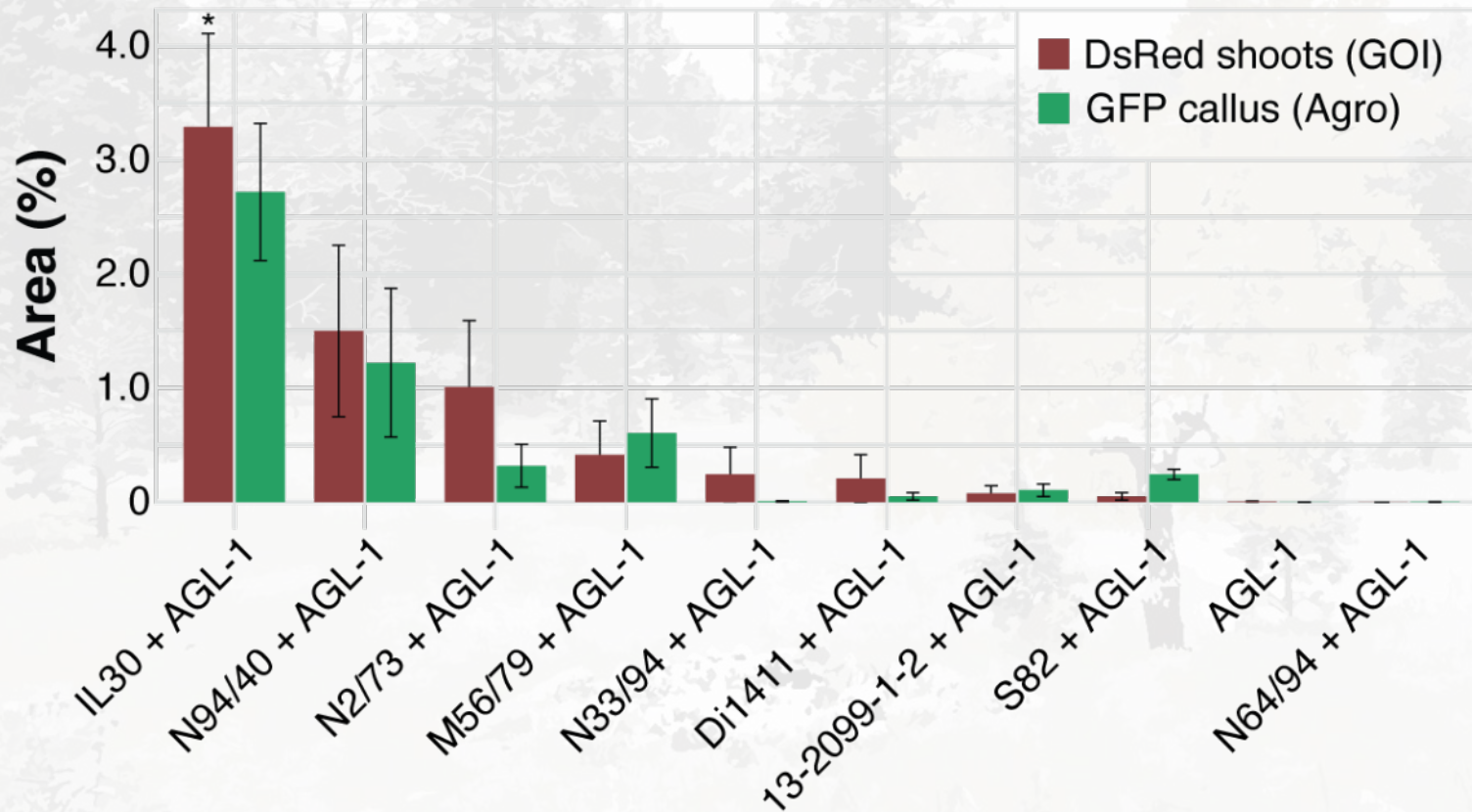
CM65/95



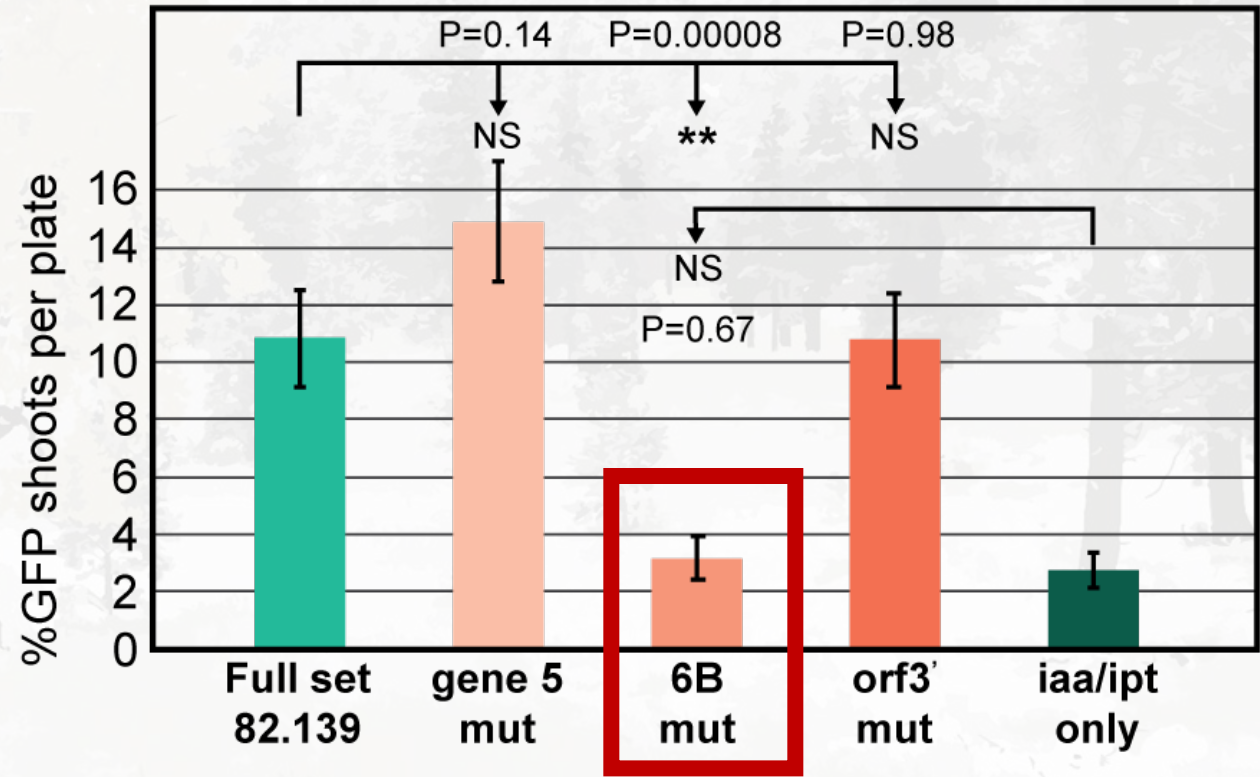
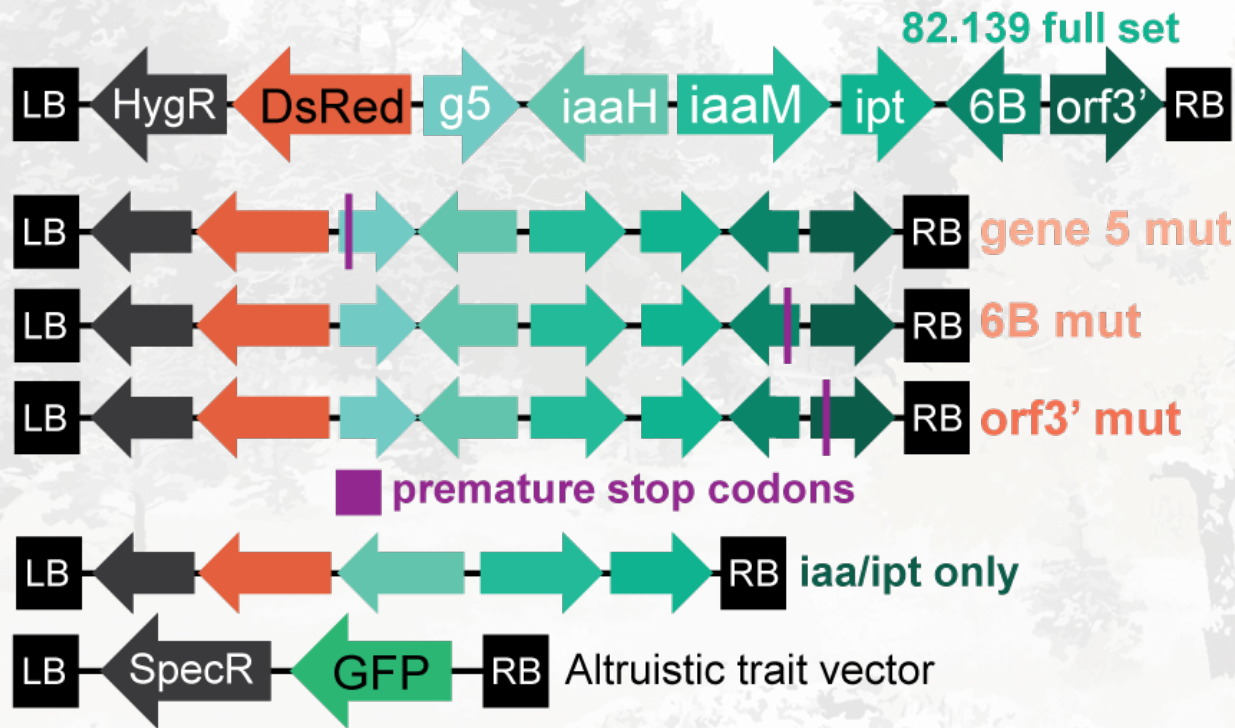
M56/79



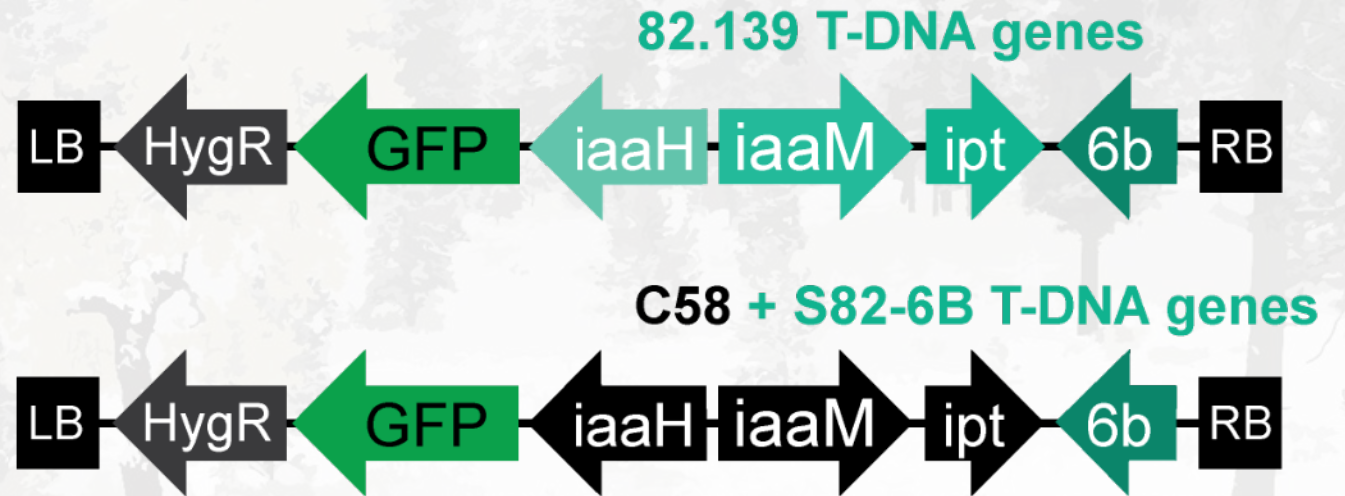
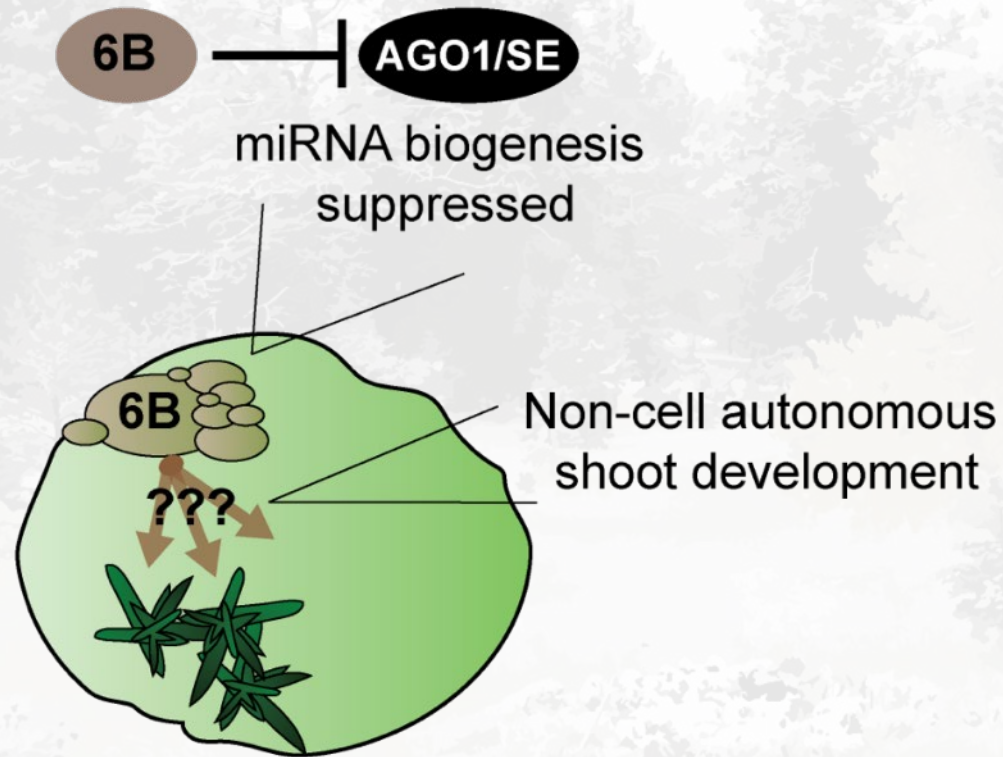
Altruistic co-transformation using these new “shooty” strains was effective in poplar



We know gene 6b is a critical part of shooty strain 82.139, but are others the same?



We still don't understand the mechanism of the altruistic effect -and it might be distinct between type Ia and Ib



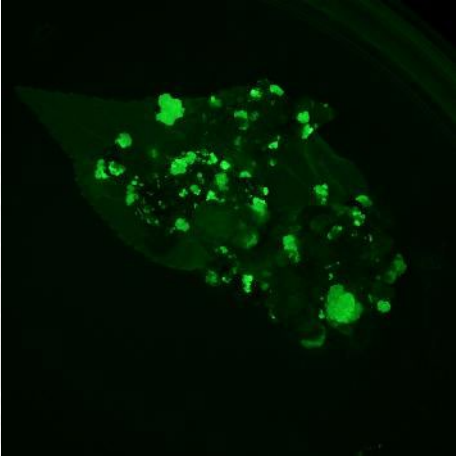
C58 hormone genes paired with 6b do not produce altruistic shoots in poplar

C58 iaa ipt
+ S826b

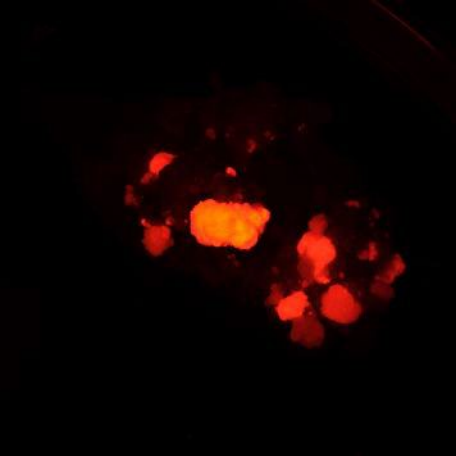
Brightfield



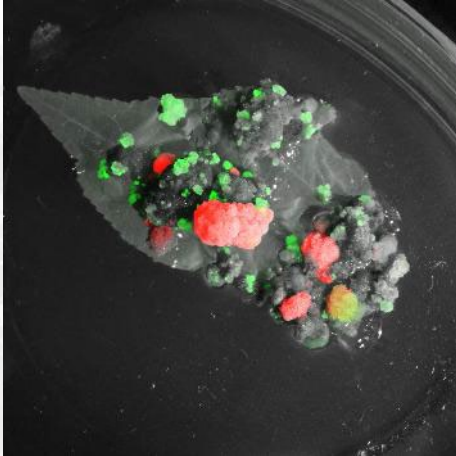
Agro morphogen



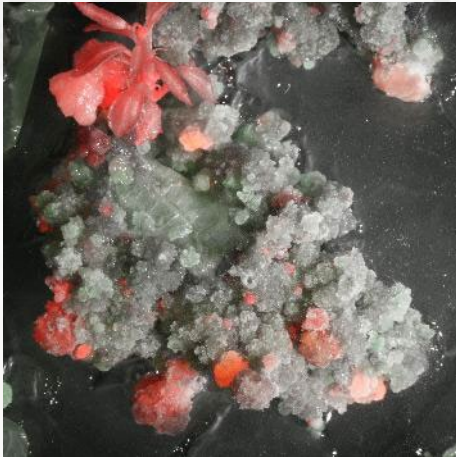
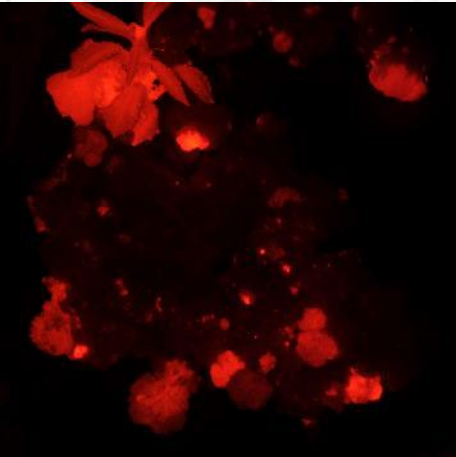
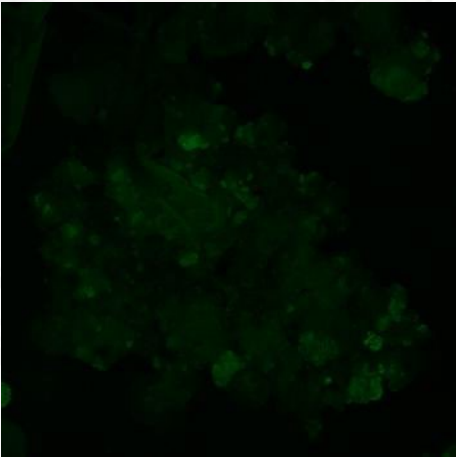
Trait T-DNA



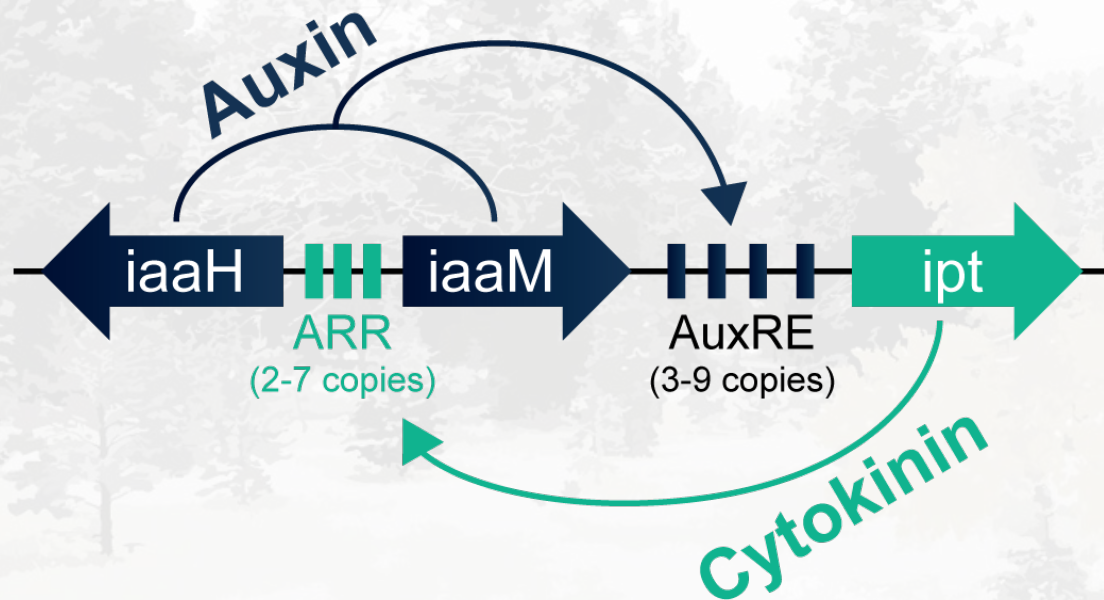
Merged



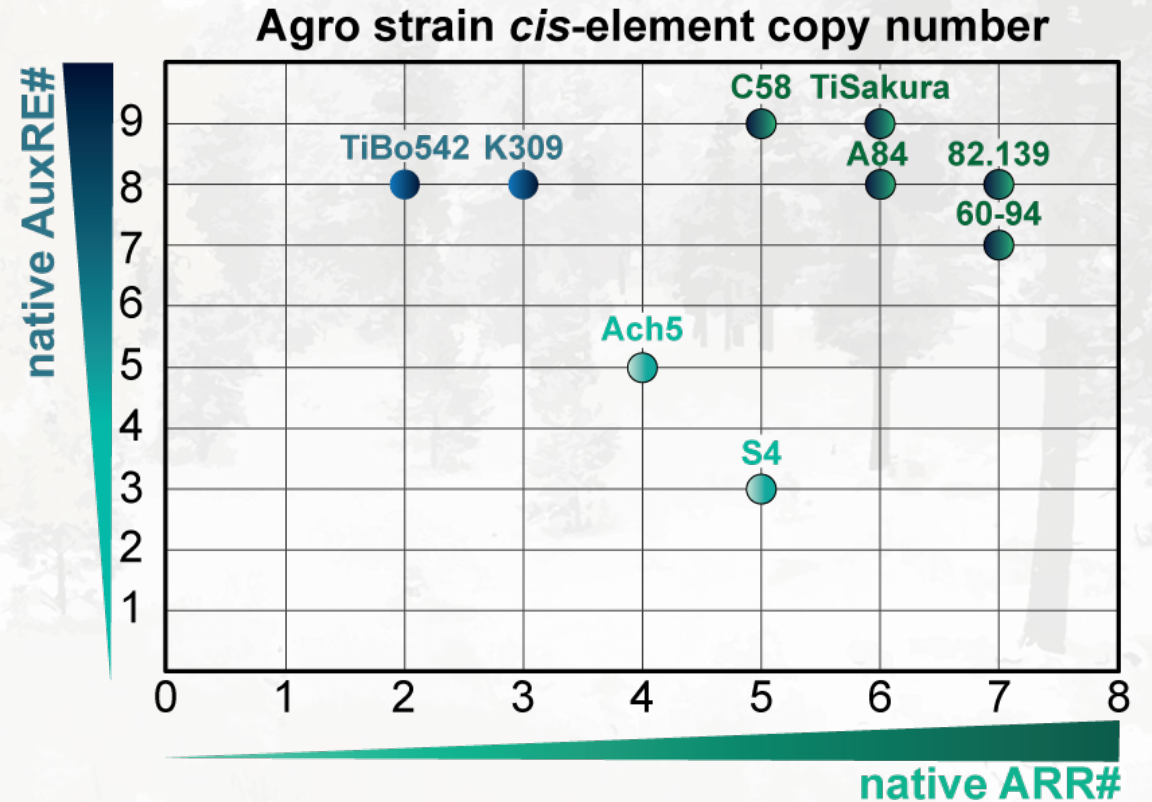
S82 iaa ipt
+ S826b



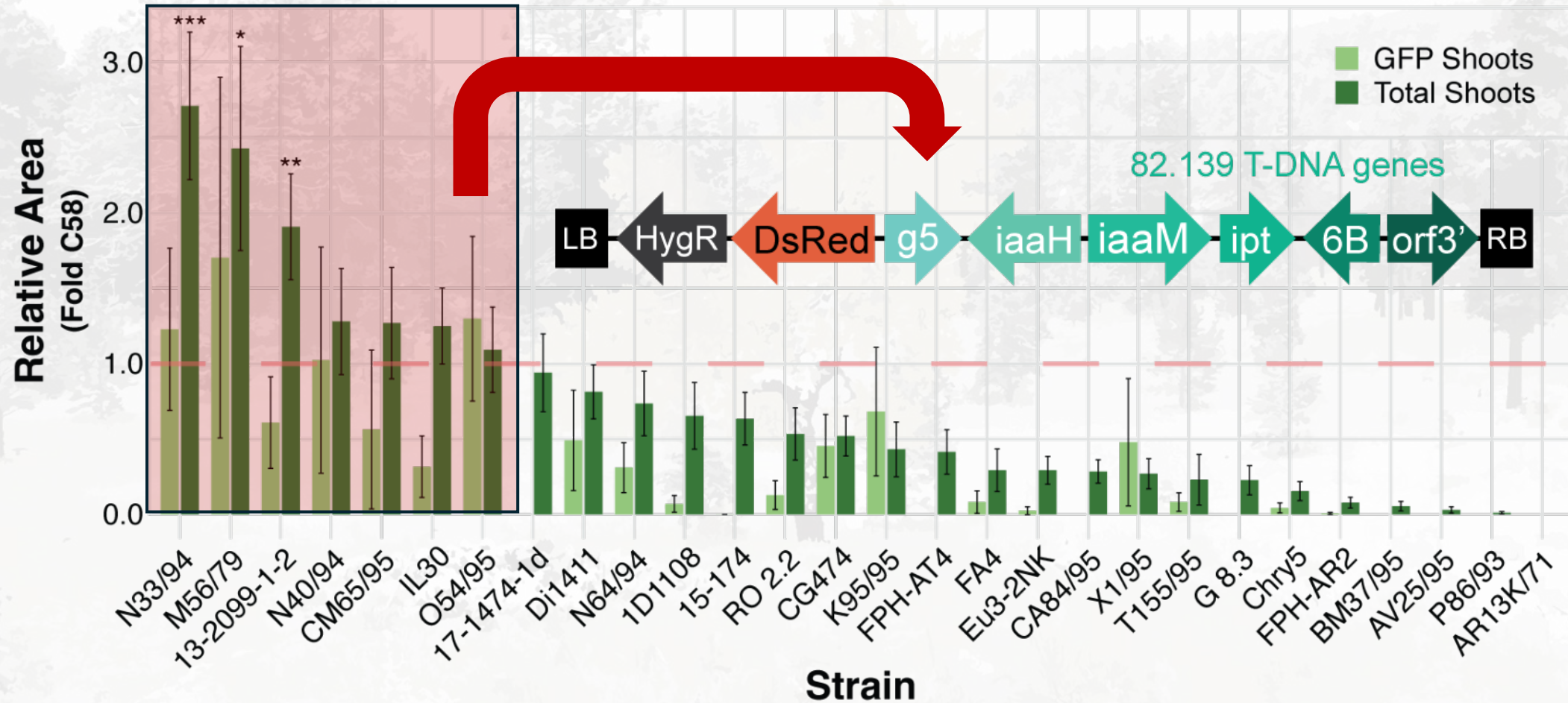
This suggests that *iaa/ipt* gene expression or protein activity, combined with *6b* influences “shootyness”



iaa/ipt promoters could be tailored for different plant species or genotypes



Next step is to clone out T-DNA genes from top-performing shooty strains

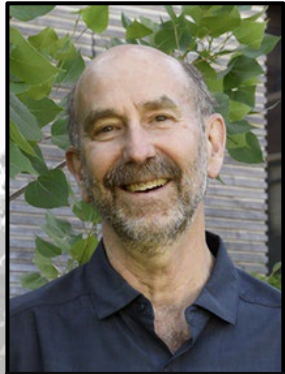


Summary:

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

- 40% of strains had any transformation, leaving 20% as candidates for strain engineering
- Higher replication studies in hybrid poplar show consistent strain behavior
- Shooty strain T-DNAs are useful for altruistic co-transformation approaches
- In future work, we hope to delve into mechanisms of why some strains behave better or worse in particular species or genotypes

Acknowledgements



Steve Strauss
Professor FES



Cathleen Ma
Transformation &
Greenhouse
Experiments



Kate Peremyslova
Transformation
Experiments



Parker Wheeler
Transformation
Experiments



Colette Richter
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)

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University

A stylized illustration of a forest scene. In the center, a tree with vibrant red leaves stands out against a backdrop of green trees. In the foreground, a red DNA double helix structure is depicted, looping across the bottom. To the right, a deer is visible in the background. The overall style is soft and artistic, with a muted color palette.

Thank you for listening!