Exploring the *Agrobacterium* genome to facilitate *in planta* transformation

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Oregon State University

Agenda

1. Perspectives & experimental system

2. Hopes and approaches for tissue culture-free “*in planta*” transformation

3. Surveying wild *Agrobacterium* strains for novel morphogenic capability

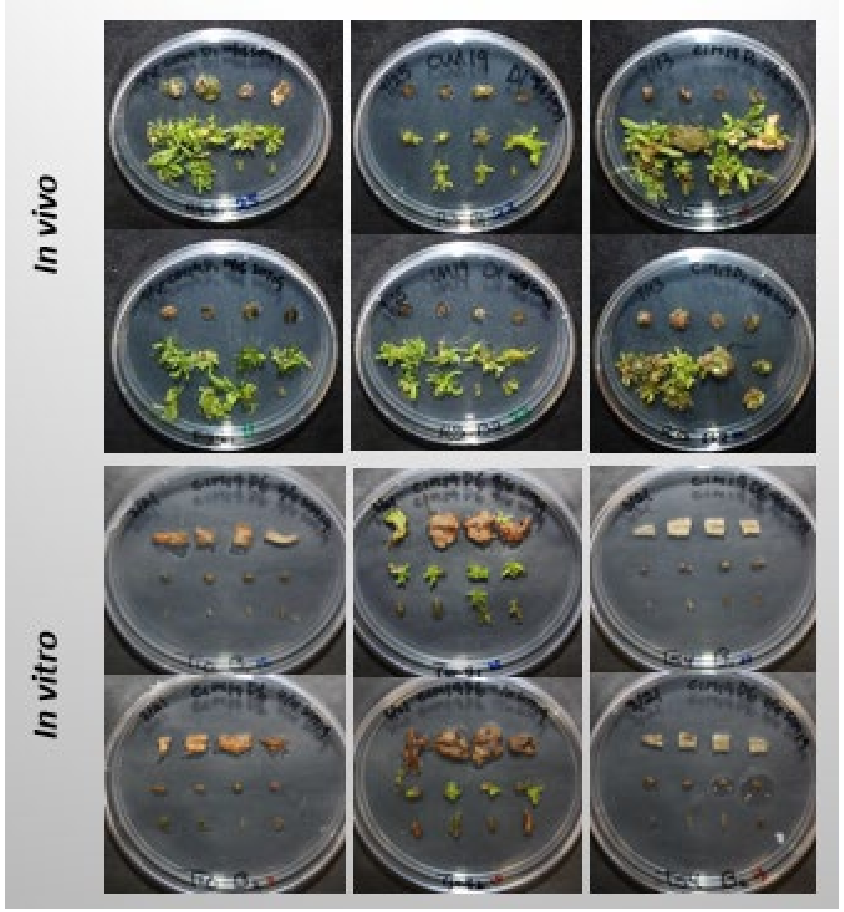
Regeneration & transformation continue to be major limiting factors for gene editing & engineering in plants, and especially trees

- Species and genotypic differences often dramatic
- Slow, costly, complex customization efforts usually needed
- On top of often large social/regulatory constraints, often a “deal breaker”

Our experimental system features

- Woody (forest) trees
- Elite clones, not seed-derived
- High physiological diversity
    - Growth environment, age, explant type and source
- Great tissue sample heterogeneity
- Common necrotic responses
- Very high genetic diversity of forest trees

Populus trichocarpa



Eucalyptus grandis x urophylla

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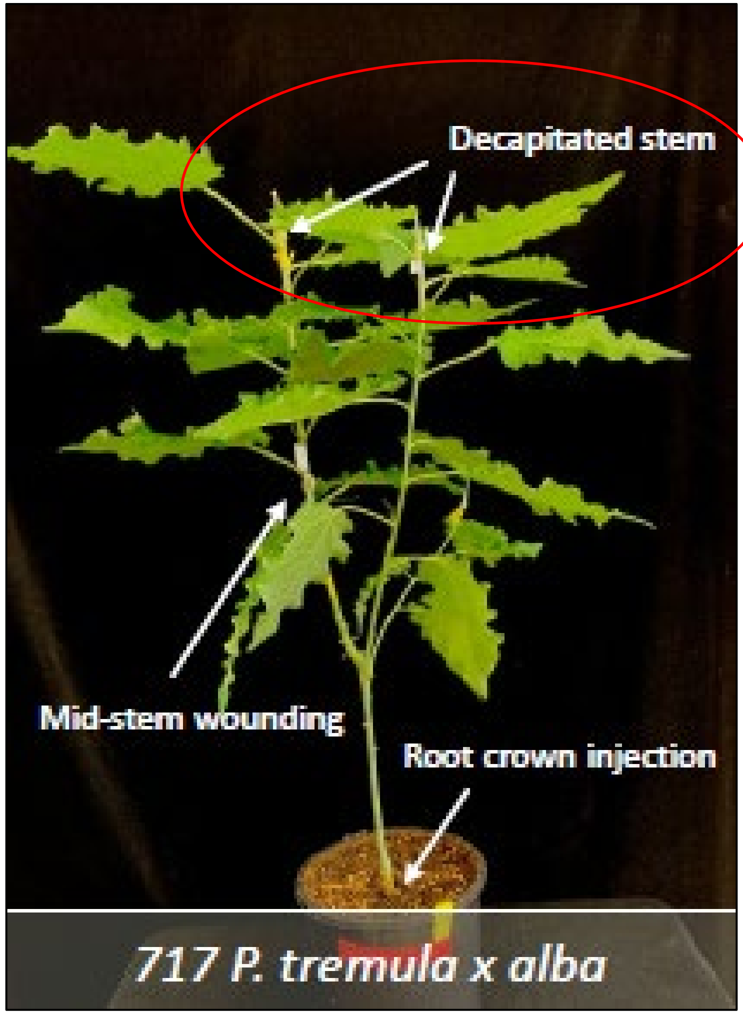
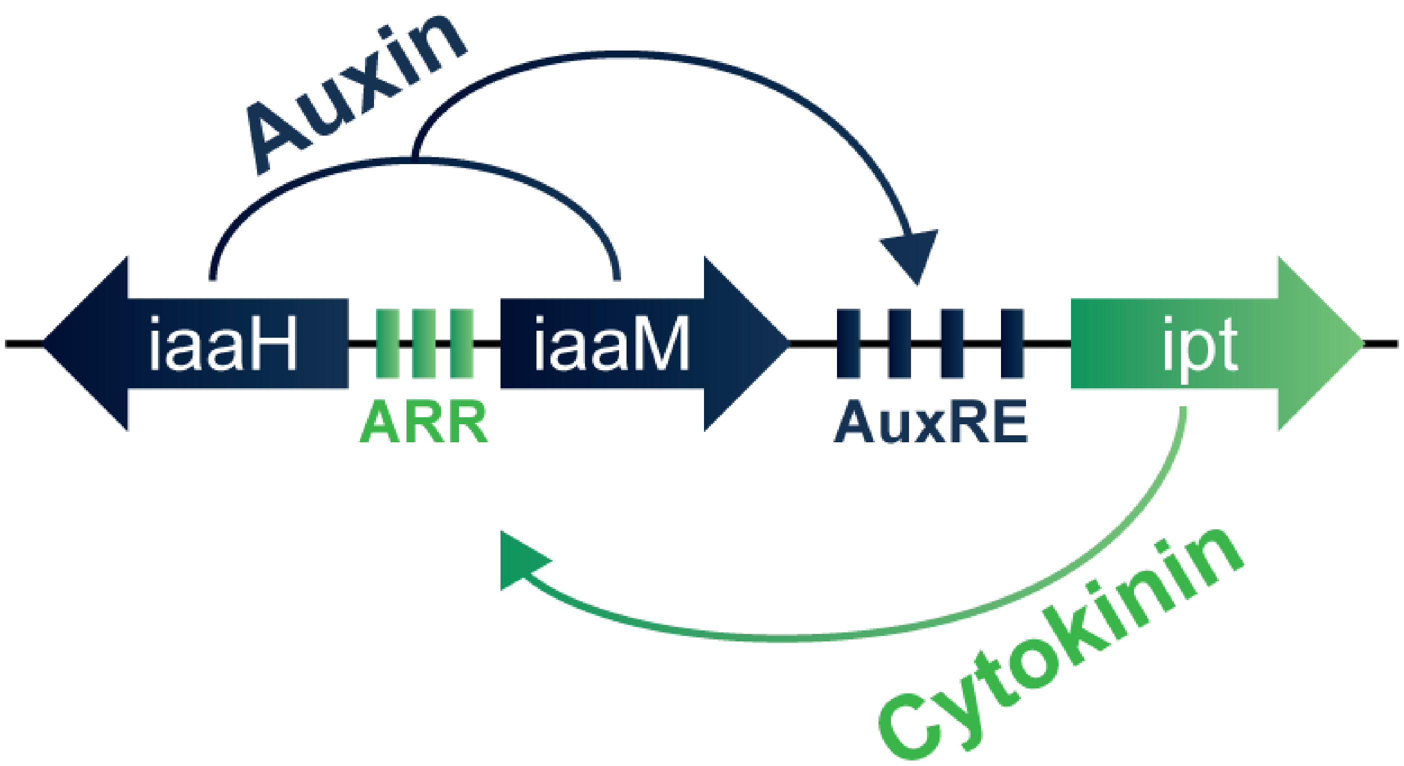
In planta transformation of great interest

- Lower cost in media, facilities?
- Reduced customization efforts?
- Less specialized personnel could do it?
- Less genotype-dependent?
- DEV genes can help?

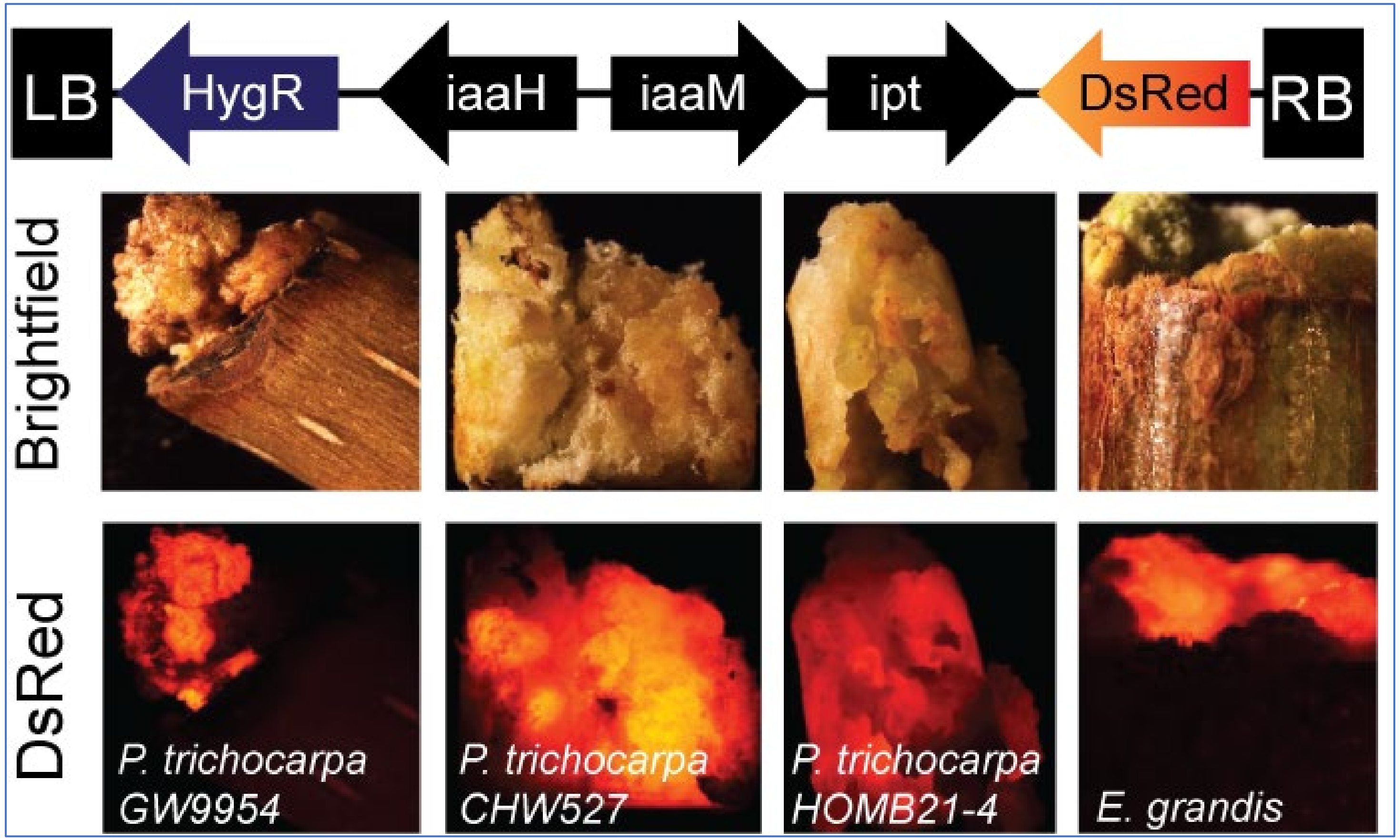


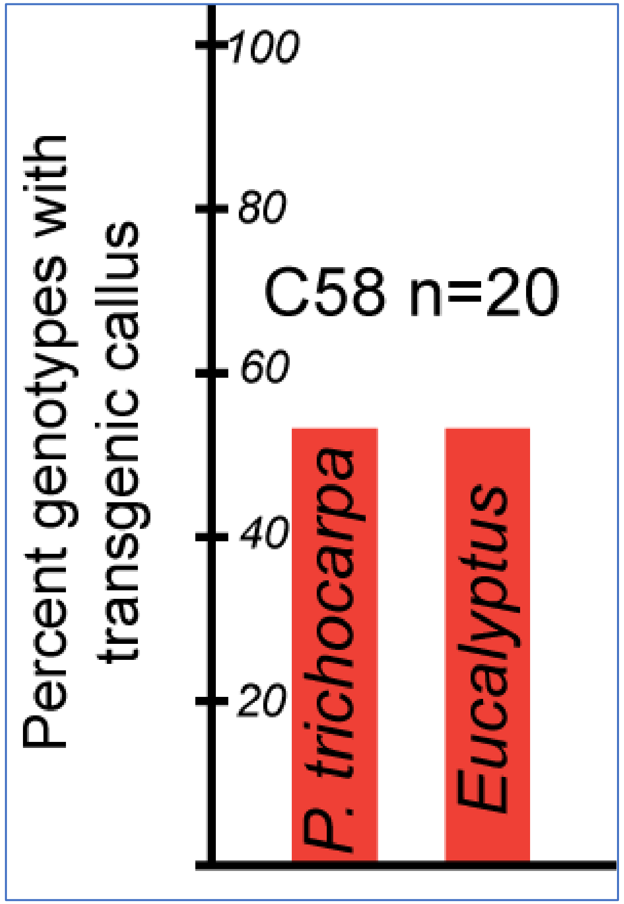
A. tumefaciens hormone genes promote regeneration useful for *in planta* methods?



iaa/ipt genes form a positive feedback loop to reinforce undifferentiated cell growth

iaaH/M and ipt genes from *Agrobacterium* were effective *in planta* inducers of transgenic cells in diverse genotypes





Can we find more useful, developmentally flexible Agro genes?

Plant Molecular Biology **17**: 441–452, 1991.

An alternative approach for gene transfer in trees using wild-type *Agrobacterium* strains†

Ana Cristina Miranda Brasileiro [1], Jean-Charles Leplé [2], Joris Muzzin [2,3], Dalila Ounnoughi [2], Marie-France Michel [2†] and Lise Jouanin [1*]
[1] *Laboratoire de Biologie Cellulaire, INRA, route de Saint-Cyr, F-78026 Versailles Cedex, France (* author for correspondence);* [2] *Station d'Amélioration des Arbres Forestiers, INRA, Ardon, F-45160 Olivet, France;* [3] present address: *Piccoplant Mikrovermehrungen, Brockhauser Weg 75, D-2900 Oldenburg, Germany*

Received 3 January 1991; accepted in revised form 24 May 1991

Key words: Agrobacterium, crown gall, poplar, tree transformation, wild cherry

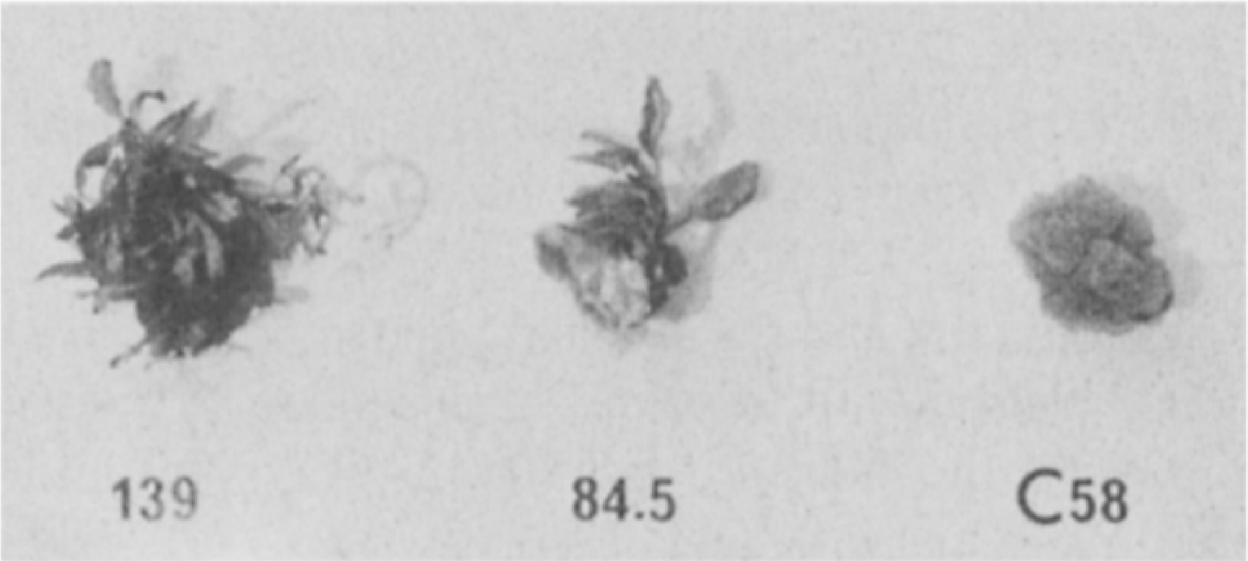
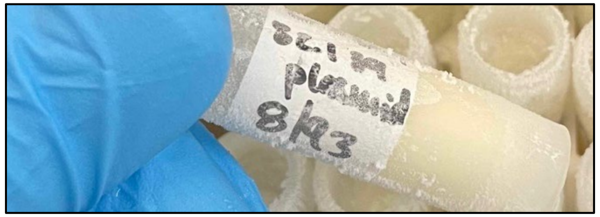
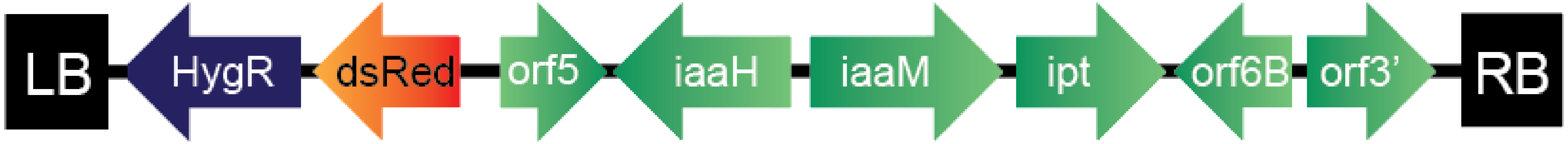


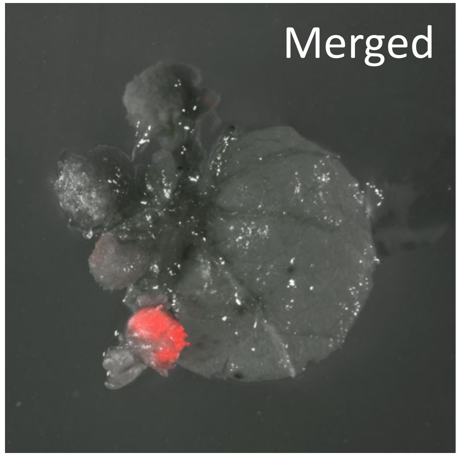
Fig. 1. Tumors and shoot differentiation from poplar tumors induced by *A. tumefaciens* strains 82.139, 84.5 and C58 and cultivated on MS medium, 6 weeks after inoculation.

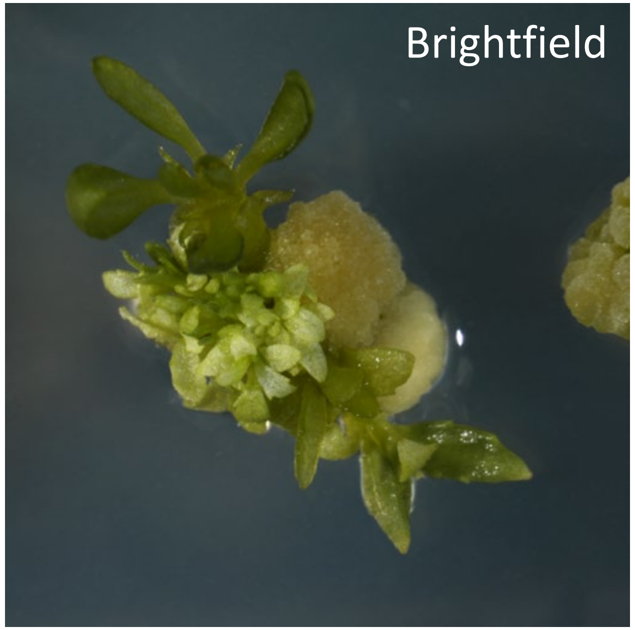
We cloned out the DEV genes from our resurrected clone in deep freeze

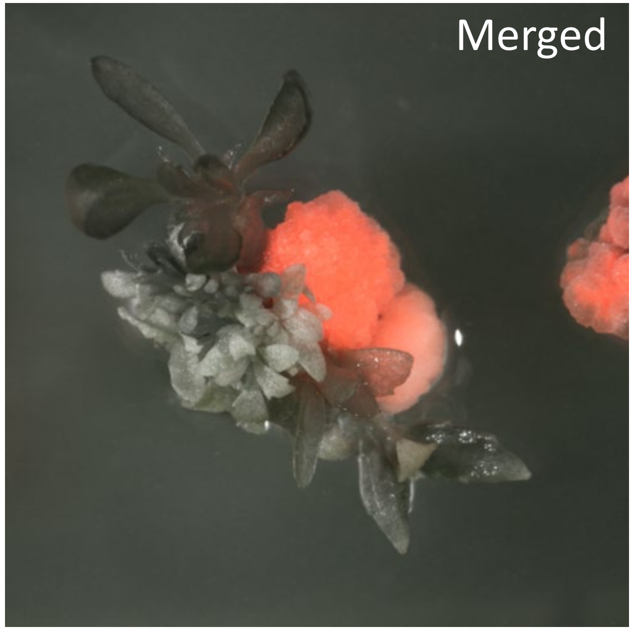




Agro transformed tissues promoted regeneration of shoots

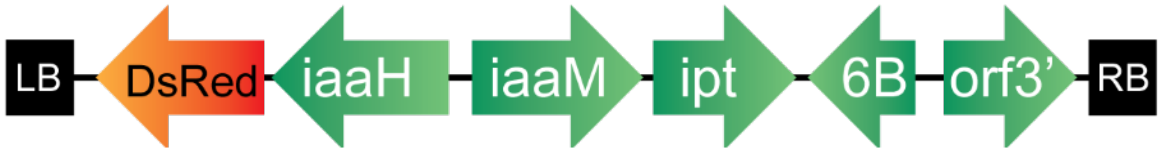




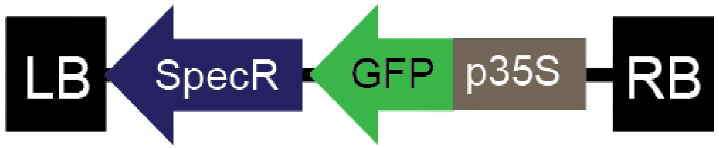


Mixed co-transformation with *Agrobacterium* genes

S82 (DsRed)



Trait vector (GFP)

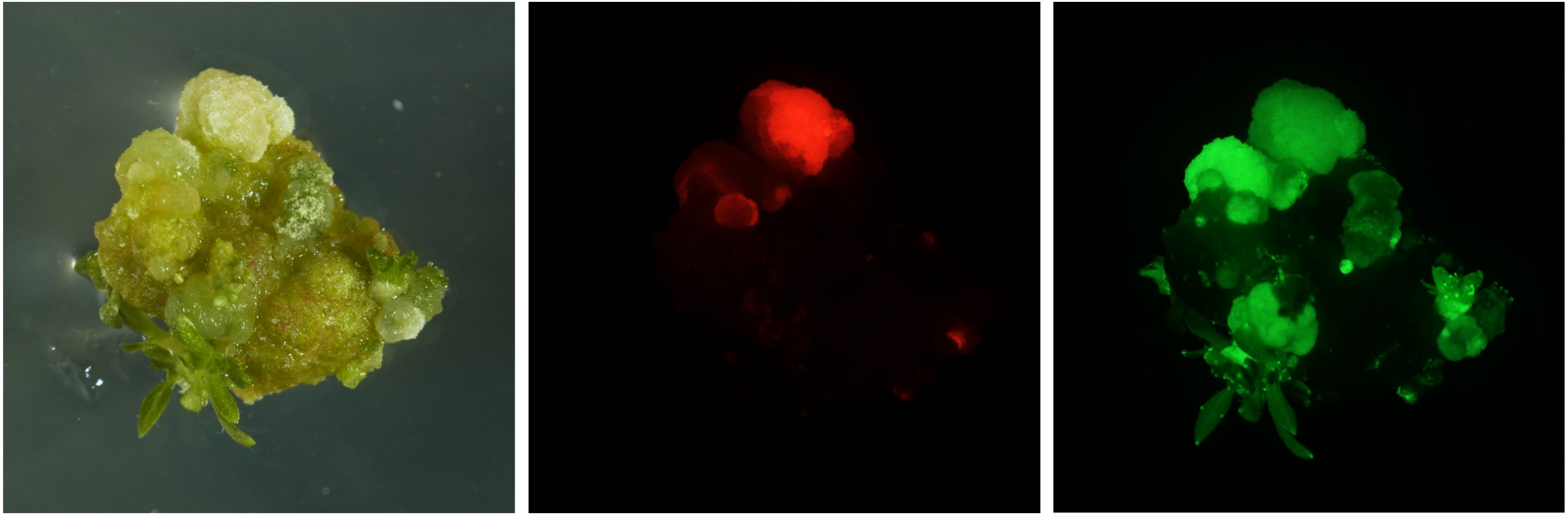


No hormones to induce regeneration

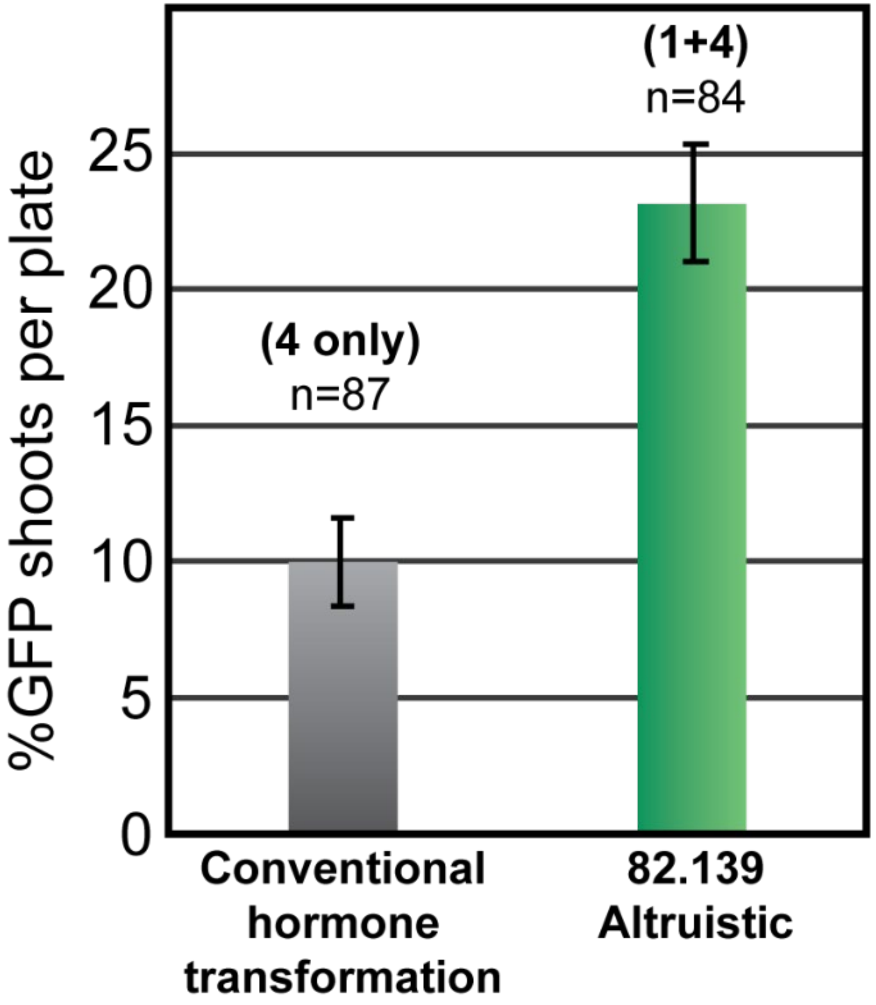
Only spec selection

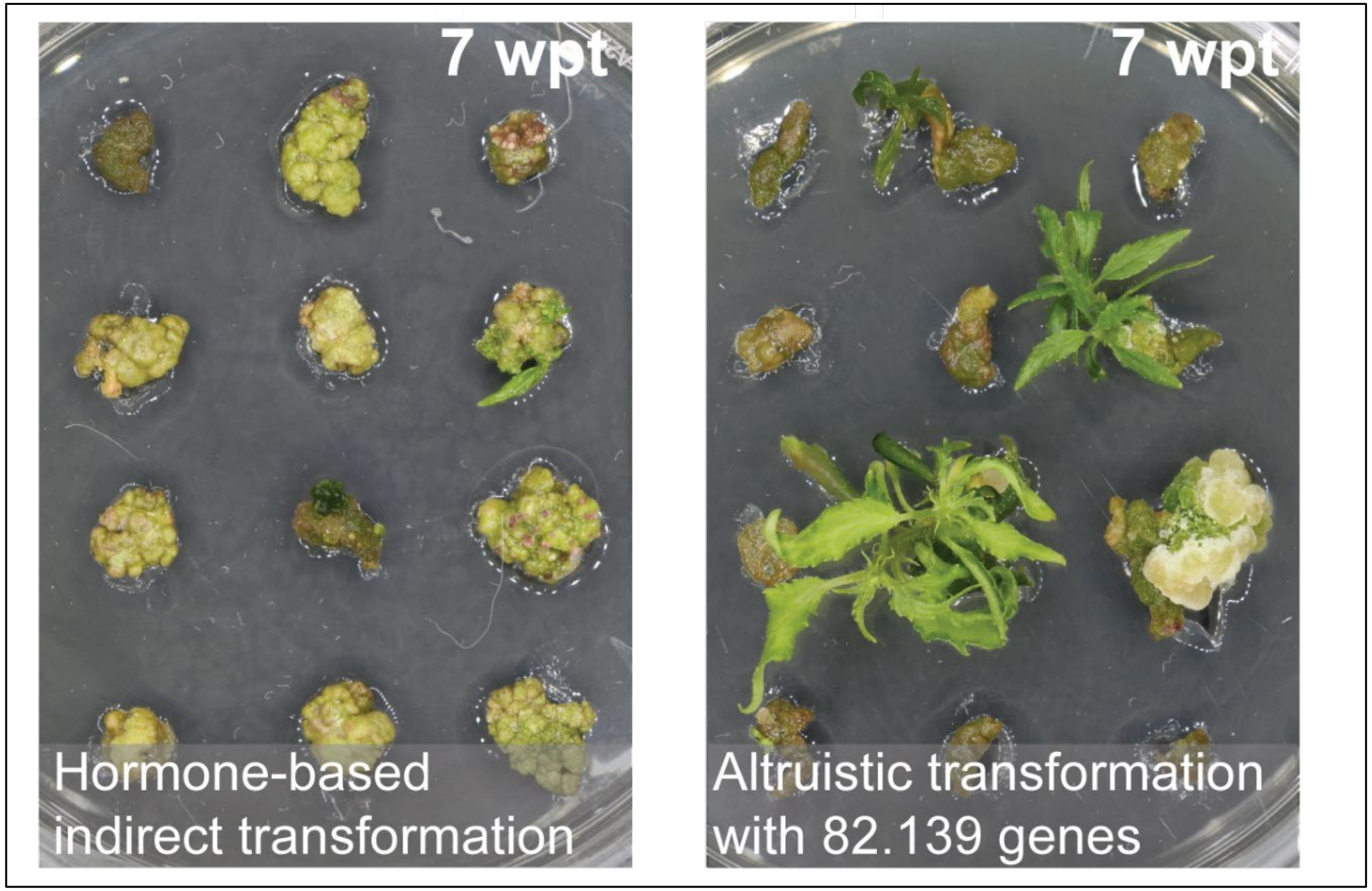
82.139 DEV genes spur the regeneration of trait vector cells

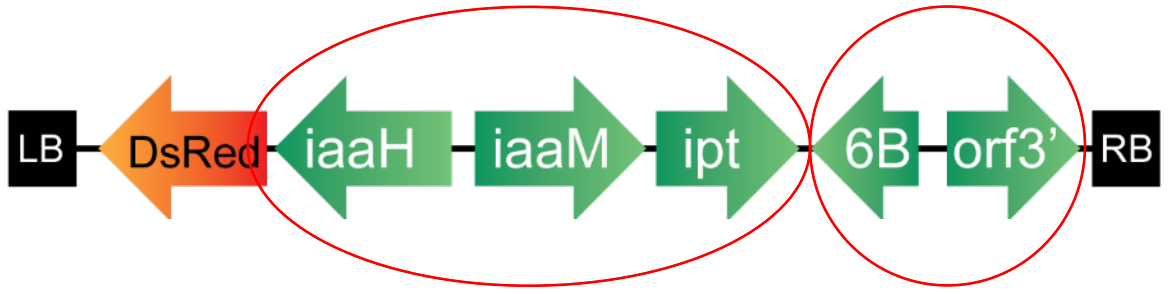
Bright-field | DsRed (82.139) | GFP (SpecR trait)



82.139 altruistic transformation was superior to routine hormone-based indirect transformation

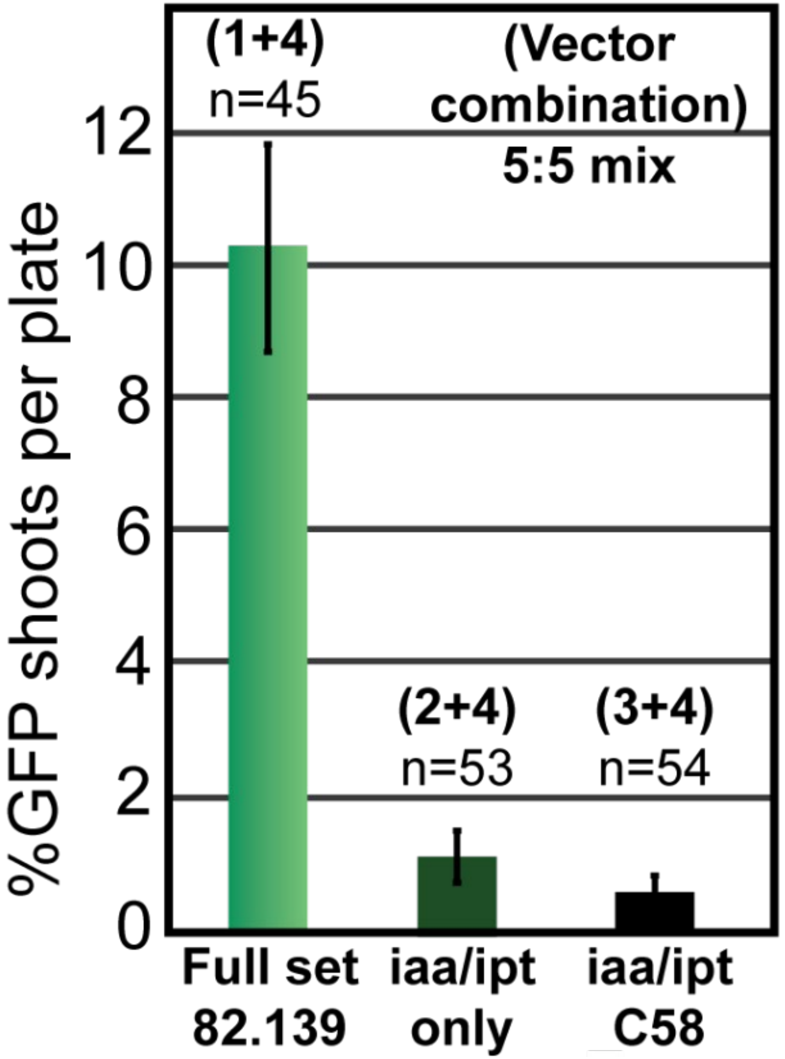




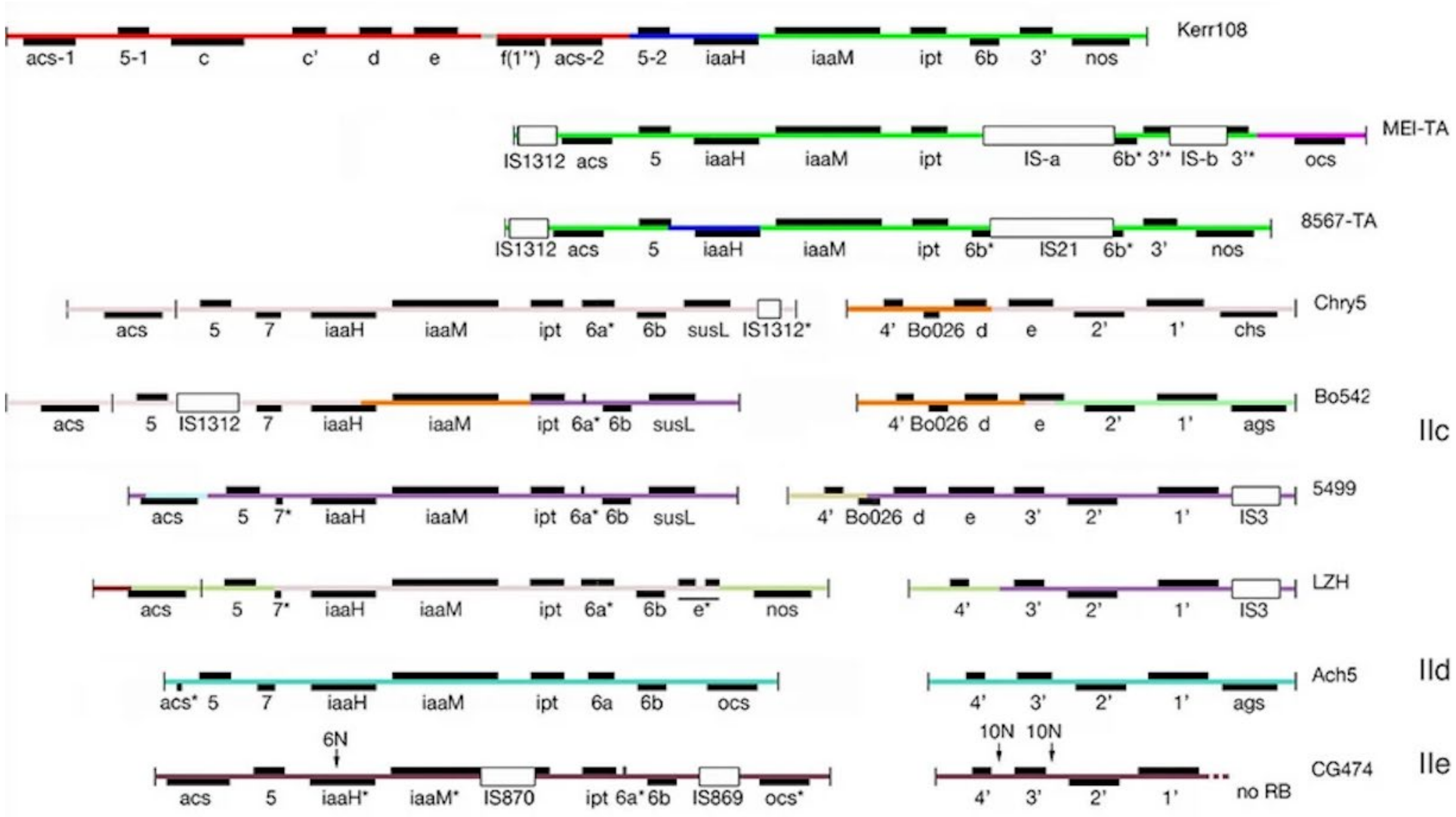


Which genes are most important for non-cell autonomous shoot promotion?

iaa/ipt genes alone did not support high rates of altruistic shoot induction



Many T-DNA genes and structures, most completely unexplored



Otten 2021, Plant Mol Biol

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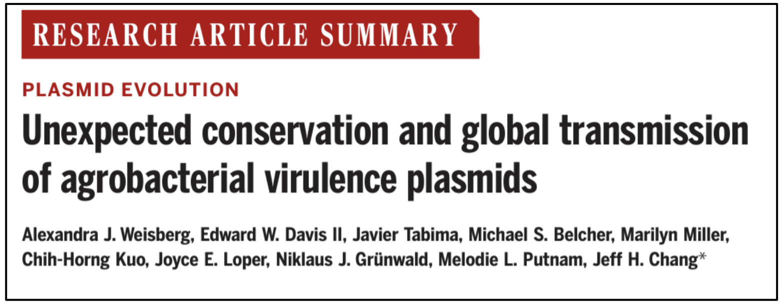
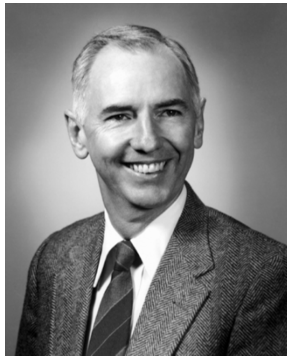
Are there other useful DEV genes? Agro diversity hardly studied


RESEARCH ARTICLE SUMMARY

PLASMID EVOLUTION

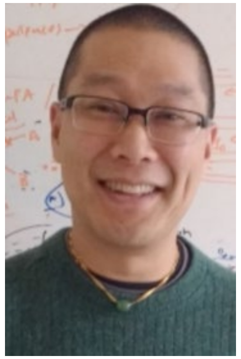
Unexpected conservation and global transmission of agrobacterial virulence plasmids

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*



Larry Moore

Alexandra Weisberg (BPP)



Jeff Chang (BPP)

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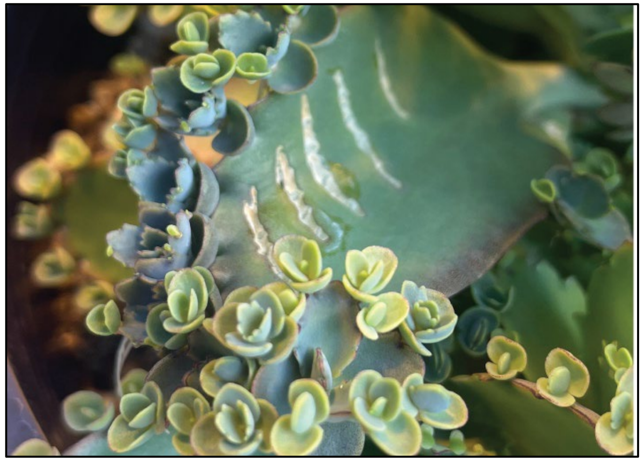
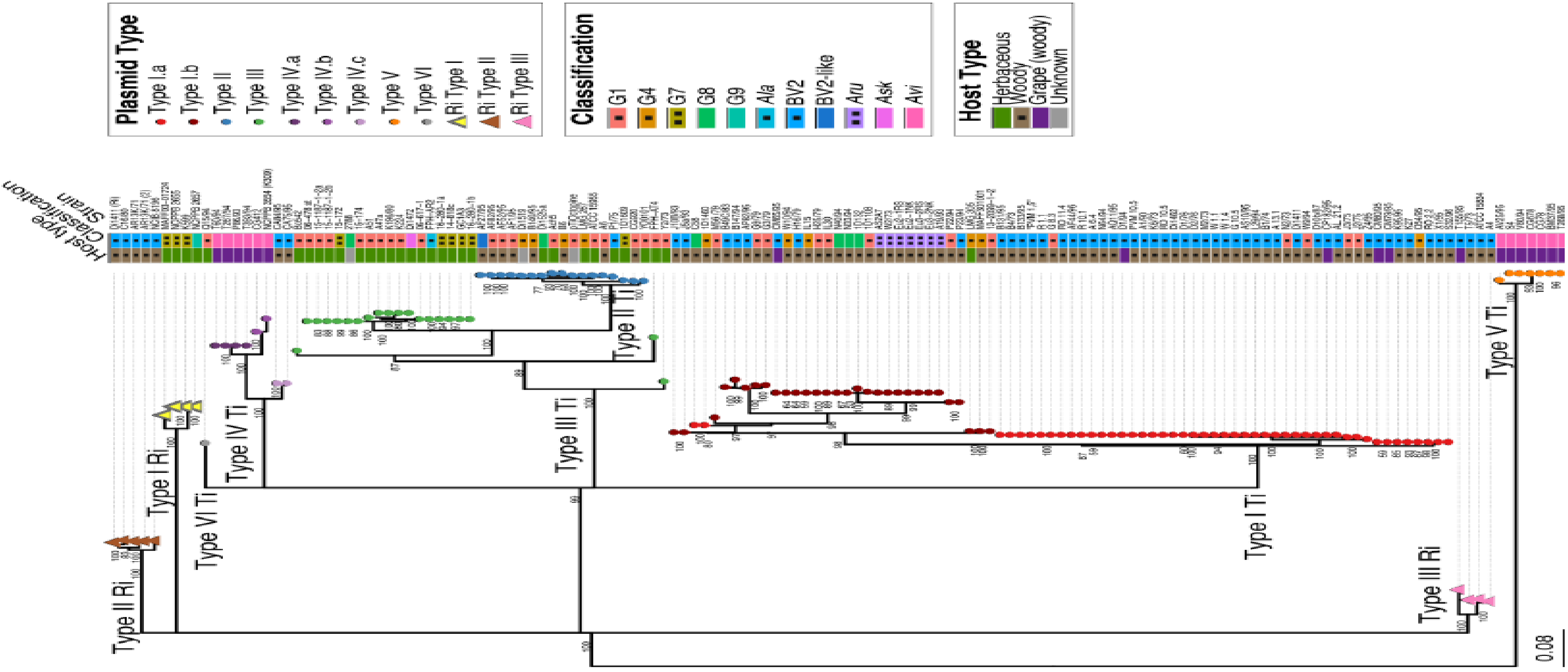


Disarmed lab strain

“Shooty” relative of 82.139

What does the Ti/Ri plasmid diversity look like?



Weisberg et al. 2020 *Science*

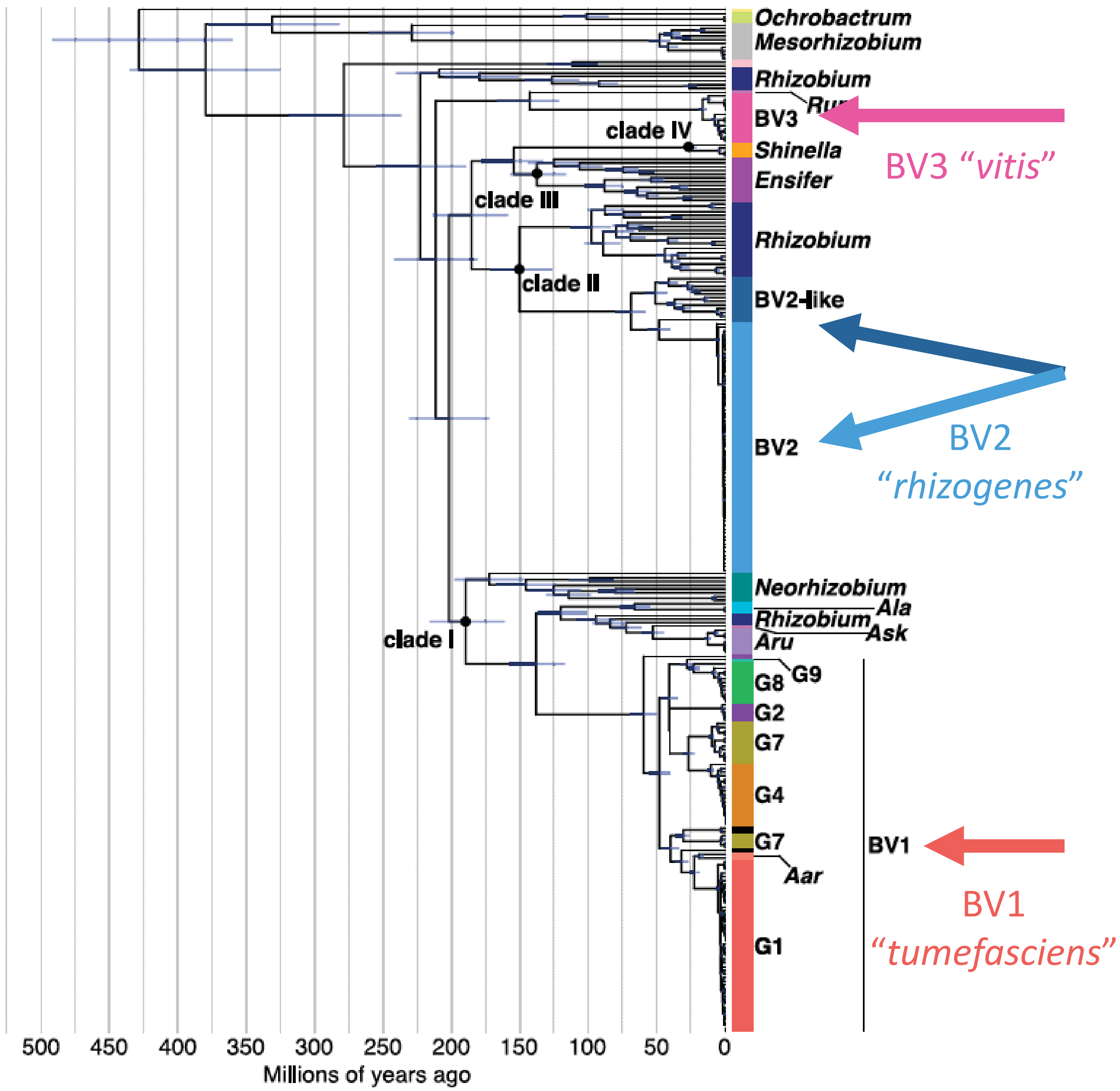


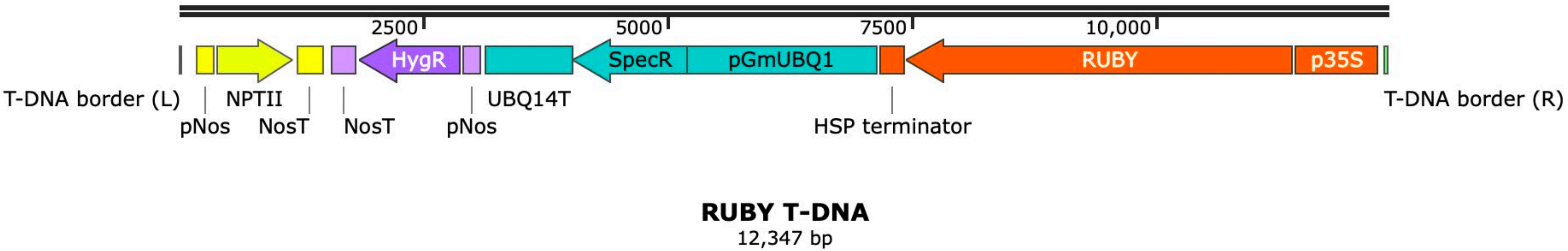
Fig. 1. Time-calibrated phylogenetic tree of the agrobacteria-rhizobia complex. Blue horizontal bars

What about chromosomal diversity?

…….Three independent chromosomal lineages

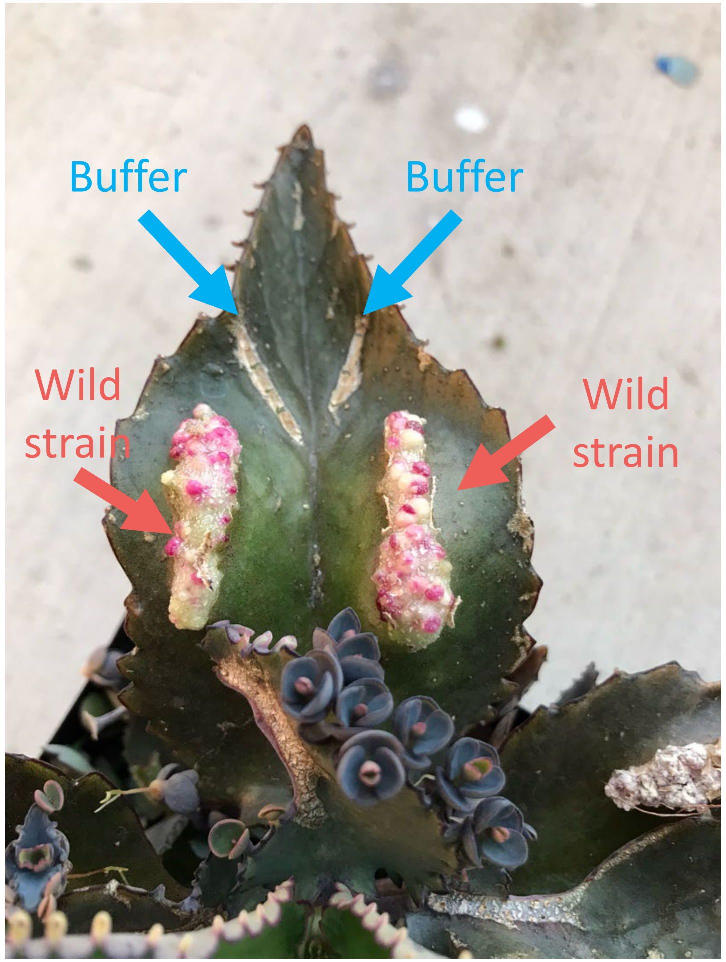
Weisberg et al. 2020 *Science*

To see transgenic tissues and try a variety of selection systems, we adopted a universal binary vector to place into wild strains



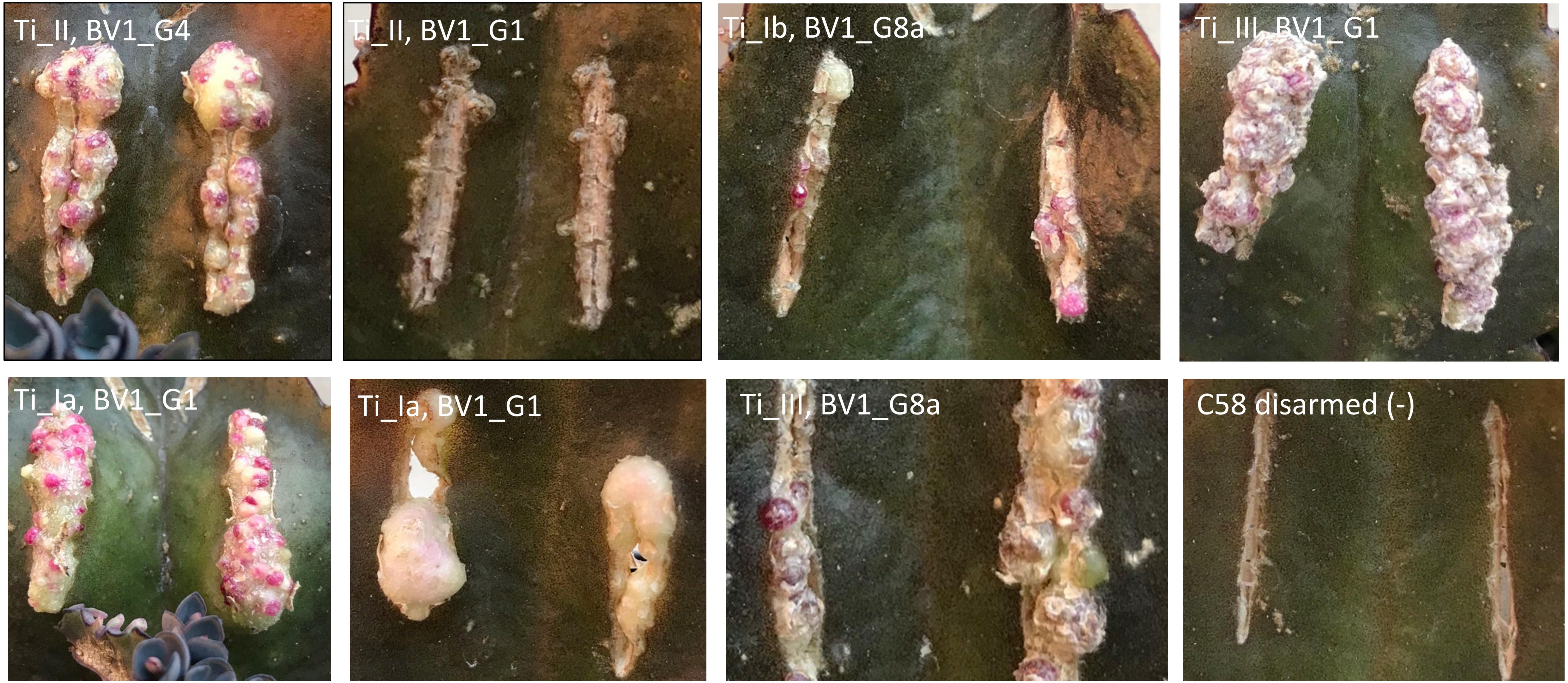
- Selected 10 strains with widely varied Ti/Ri lineages, gene content, chromosomal background
- Testing *in vitro* and *in planta* in several species

Kalanchoe as a simple system to assess strain characteristics



- Rapid callus/gall development
- Easy to propagate
- Simple to wound and apply Agro
- Flat surface for imaging (hyperspectral / machine vision)

In kalanchoe TG tissue tended to be discrete amongst regenerating non-TG tissue



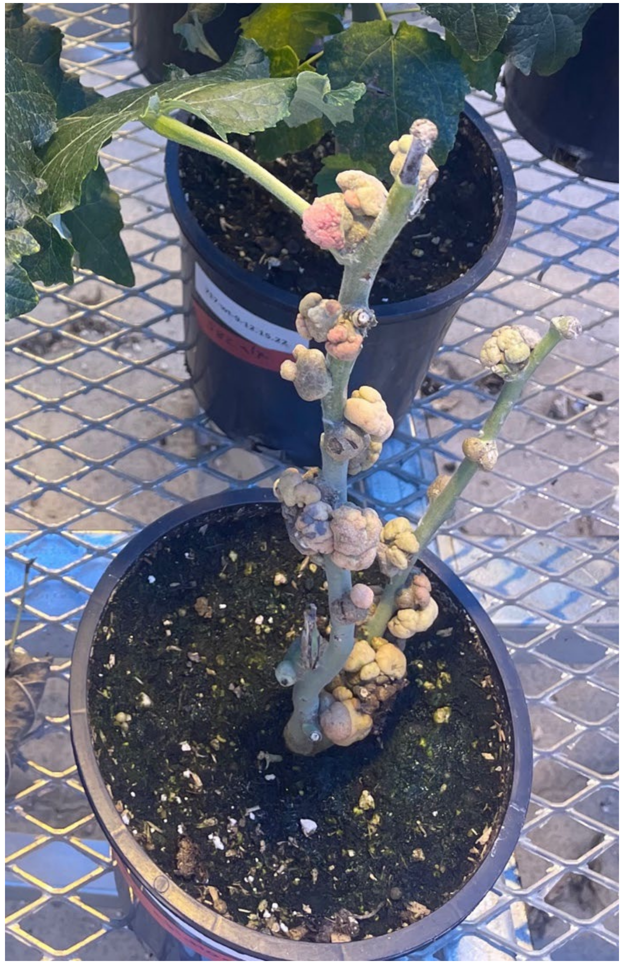
How about woody plants like poplar?

How should we approach *in planta* transformation?

In planta methods are still changing rapidly, require a different way of thinking about transformation

***Populus tremula x alba* 717-1B4**

Apical decapitation



Axillary bud injection

We transformed two poplar genotypes with three treatments using apical decapitation

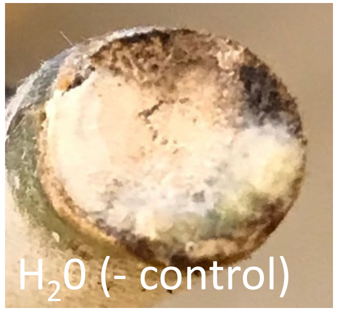
Genotypes:

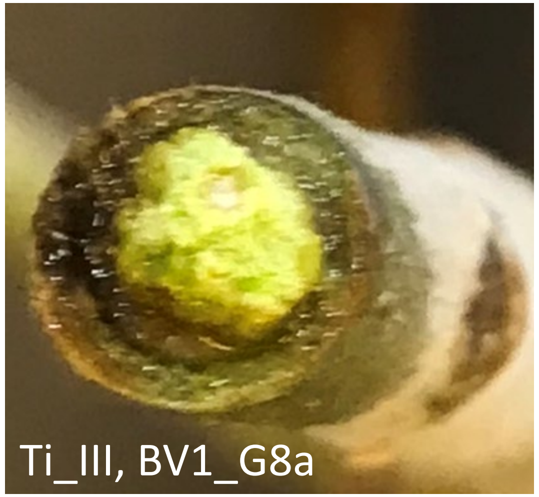
- *Populus tremula x alba* 717-1B4 – transformable hybrid aspen
- *Populus trichocarpa* SKWB-22 (“T10”) –more challenging

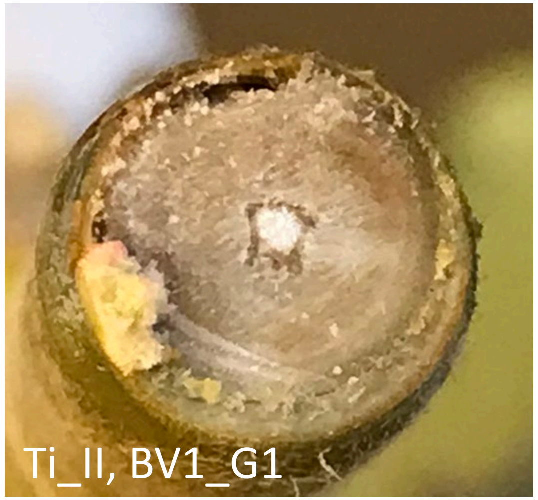
Treatments:

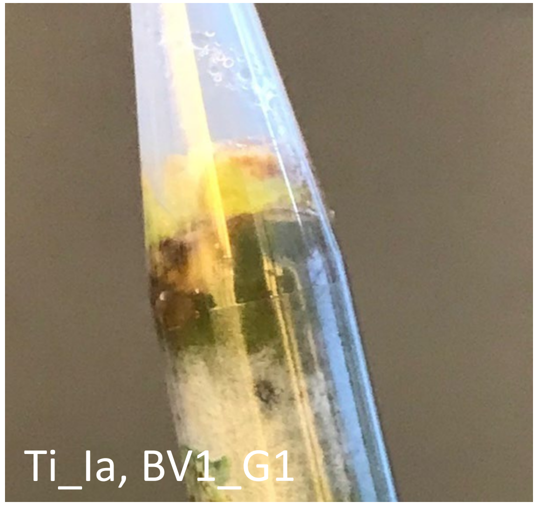
1. No selection or hormones, moisture control with inverted epp. Tube
2. Selection only 1/week (100mg/L kanamycin)
3. Selection + weak cytokinin (tests whether galls are developmentally labile)

No selection or hormones, in 717 hybrid aspen









Kanamycin treatment in 717 hybrid aspen

C58 (+ control) | Ti_Ia, BV1_G1 | Ti_III, BV1_G8a | Ti_III, BV1_G1 | Ti_Ib, BV1_G4

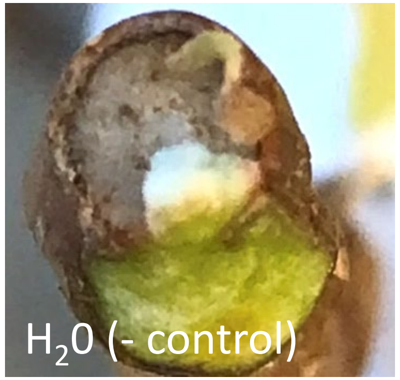
Different trees treated with wild strains

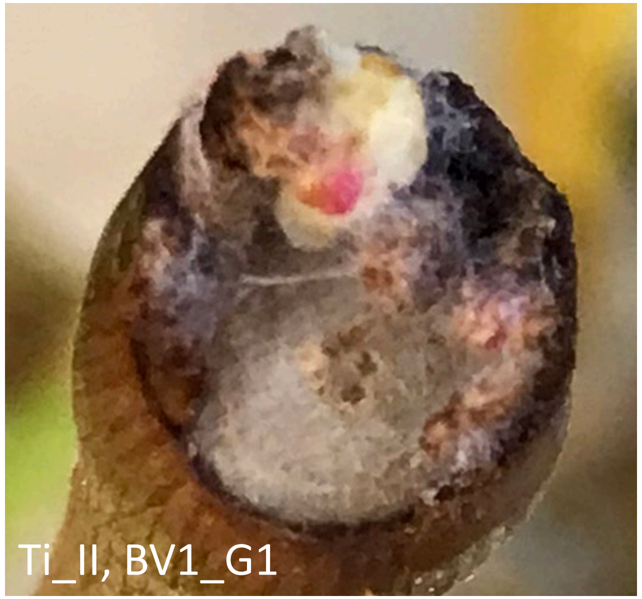
kanamycin + TDZ treatment in 717 hybrid aspen

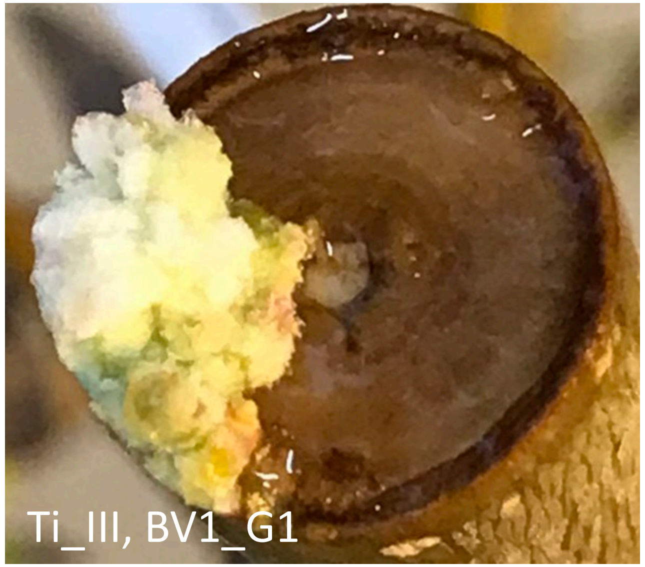
C58 (+ control) | Ti_II, BV1_G4 | Ti_Ib, BV1_G4 | Ti_Ia, BV1_G1 | Ti_III, BV1_G1 | Ti_Ia, BV1_G1

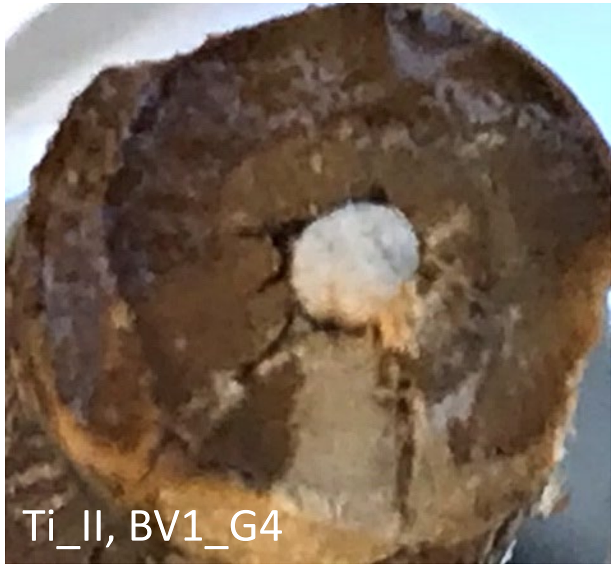
Different trees treated with wild strains

No selection or hormones in T10 black cottonwood









Kanamycin treatment in T10 black cottonwood

Genotypes and existing kill curves for antibiotic often need to be re-worked with *in planta* inoculation

kanamycin + TDZ treatment in T10 black cottonwood

C58 (+ control) | Ti_Ib, BV1_G4 | Ti_II, BV1_G1 | Ti_III, BV1_G1 | Ti_Ia, BV1_G1 | Ti_Ia, BV1_G1 | Ti_II, BV1_G4

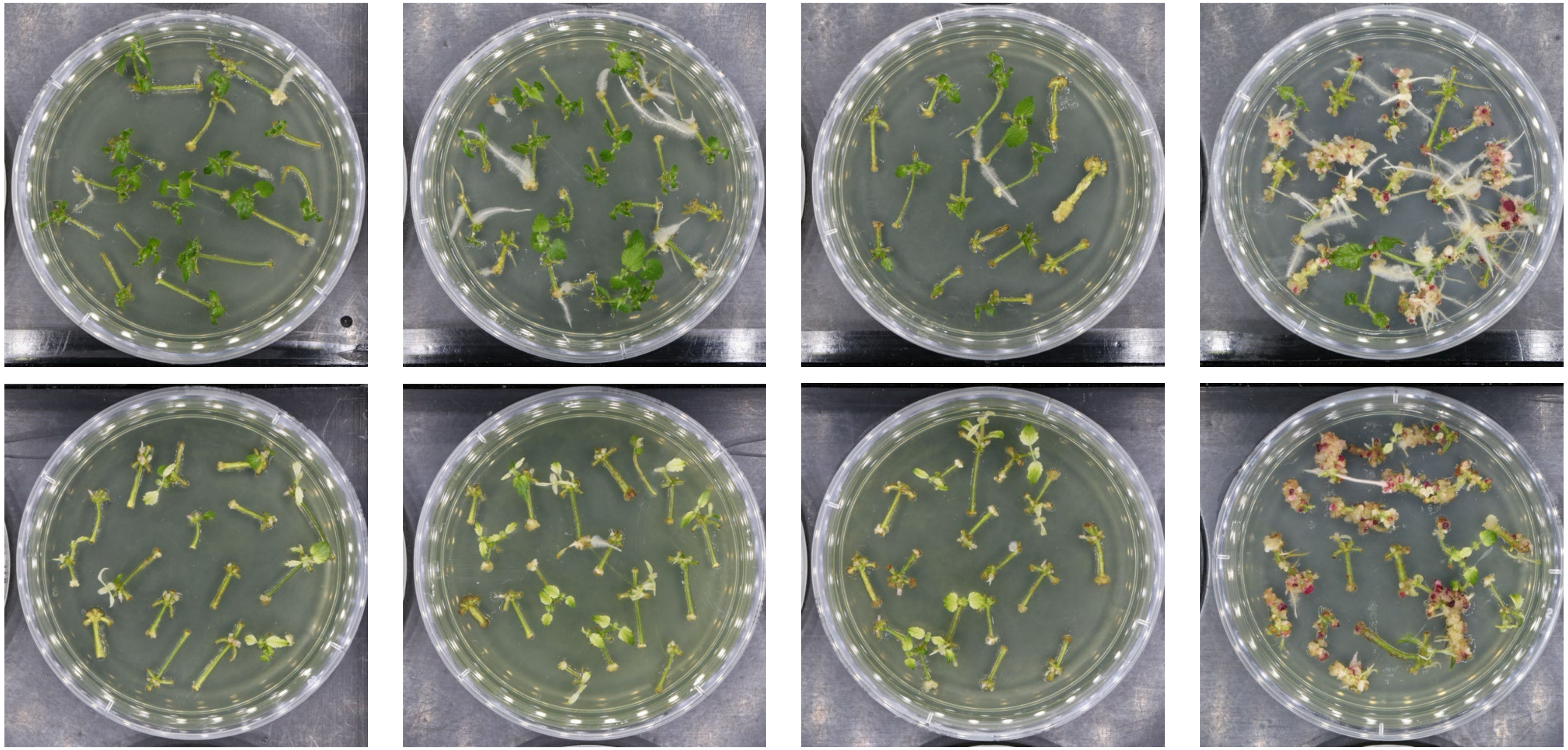
How does *in vitro* differ from *in planta* when transforming with wild strains?

Pilot tests in hop showed strong variance in delivery and regeneration among strains

Ri_I, BV1_G7c | Ri_III, BV2 | Ri_II, BV2 | Ti_Ia/Ri_II, BV2

No selection

10 mg/L spec

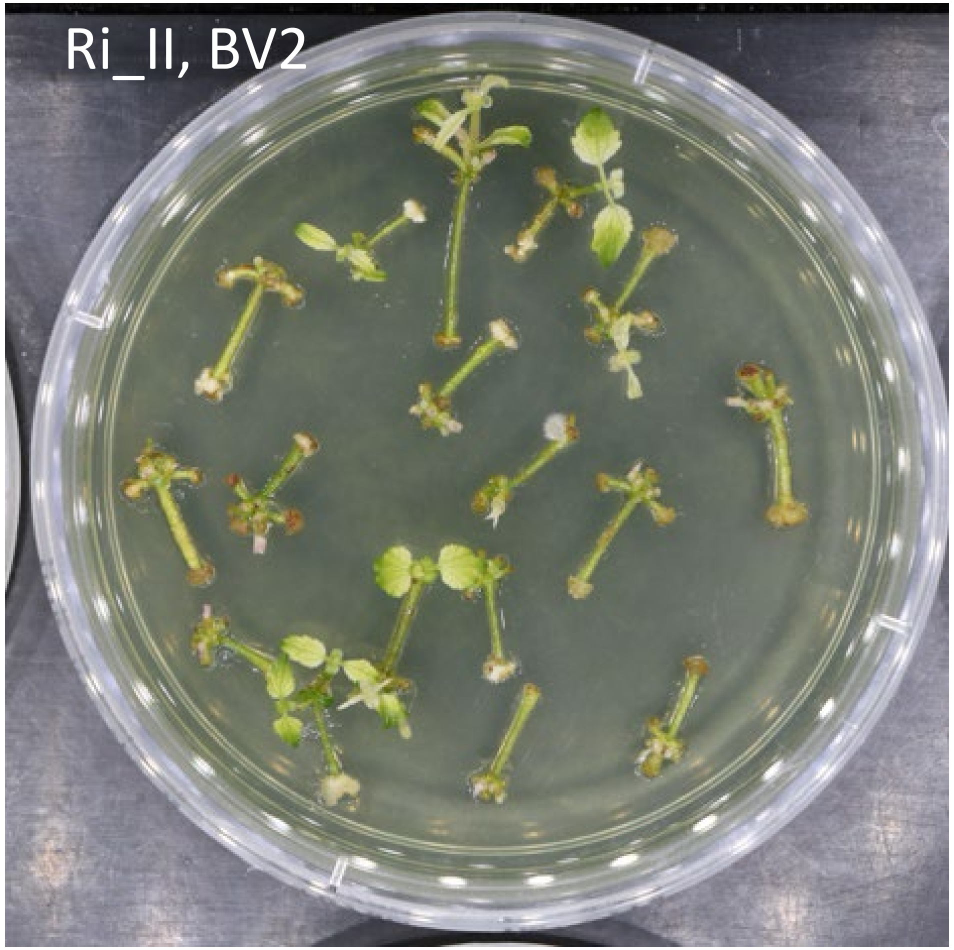


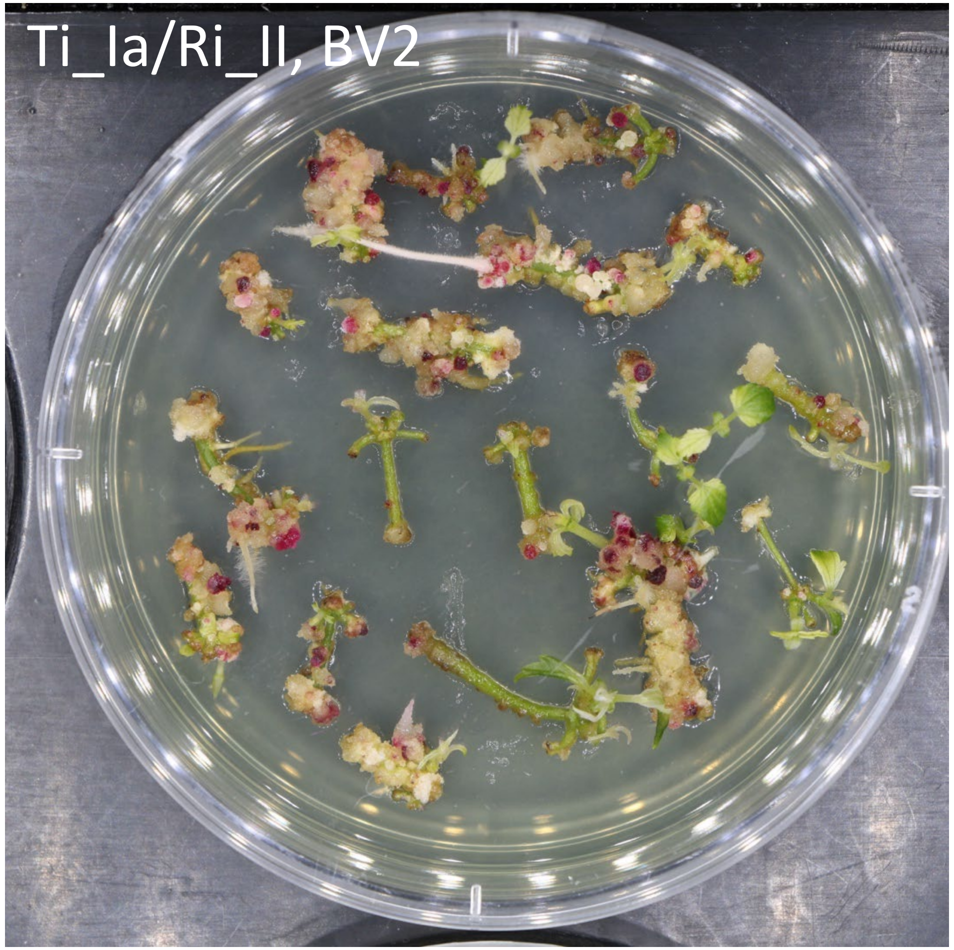
Humulus lupulus cv. "Cascade"

Chris Willig, OSU

Domains of transgenic vs. non-transgenic regenerating tissue often distinct among strains

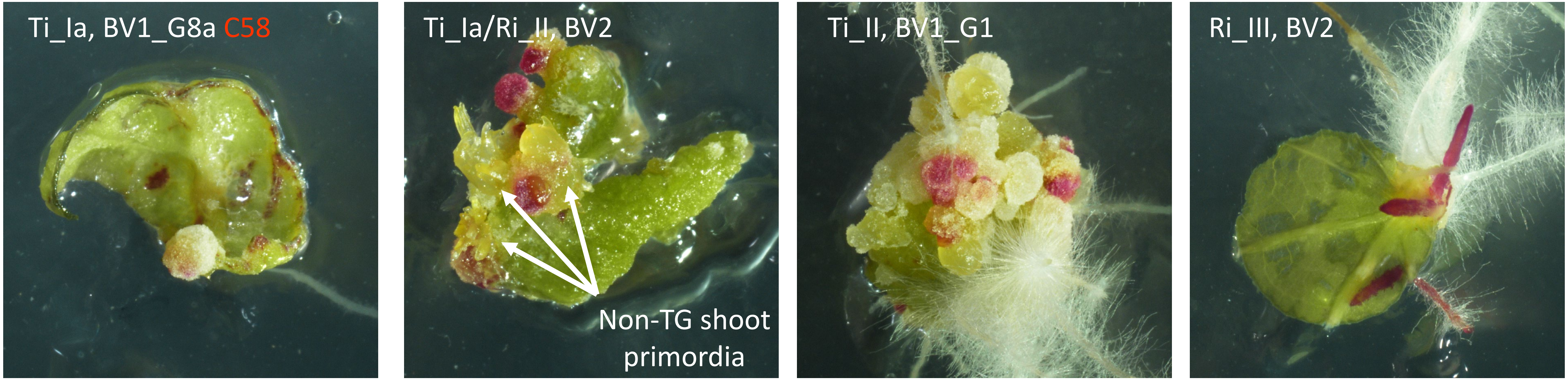
10 mg/L spec





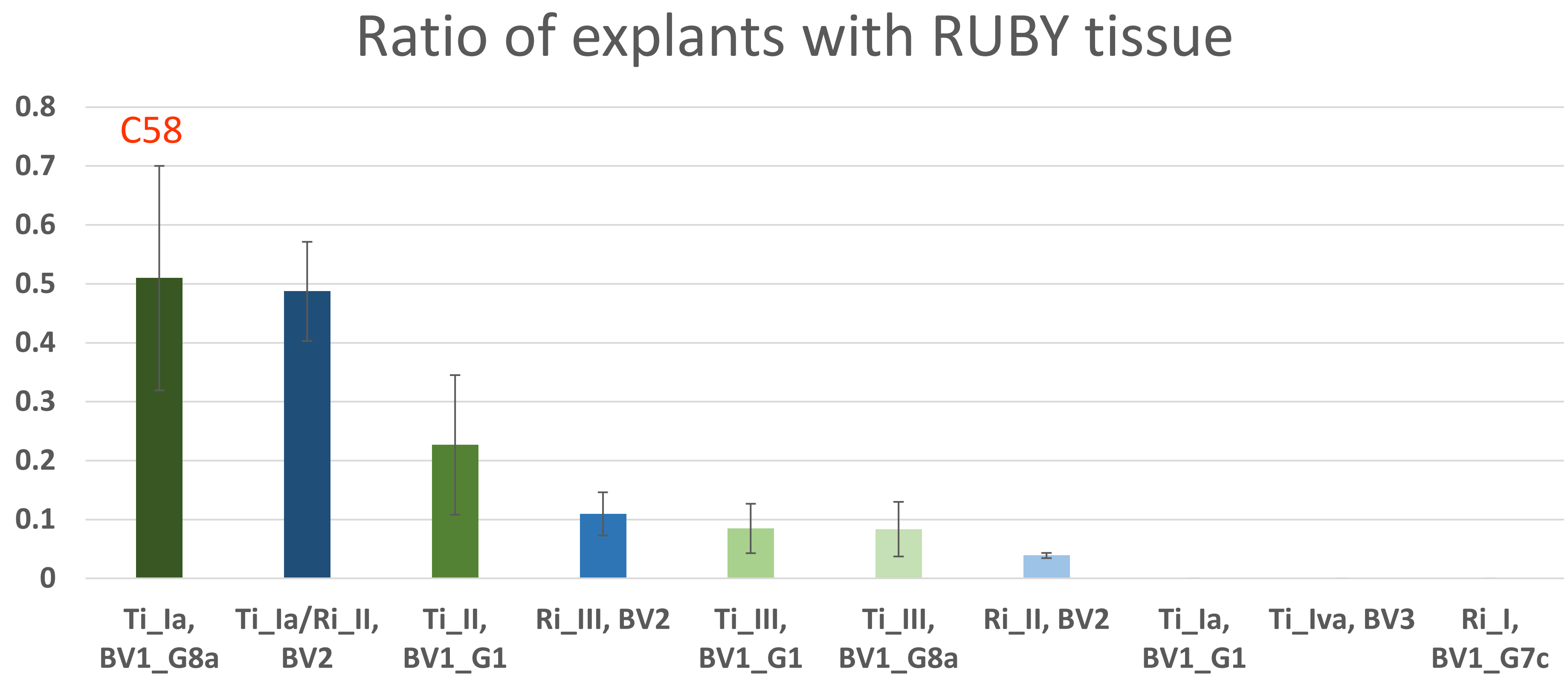
Humulus lupulus cv. “Cascade”

In vitro testing in poplar showed a wide range regeneration outcomes, TG vs. non-TG domains



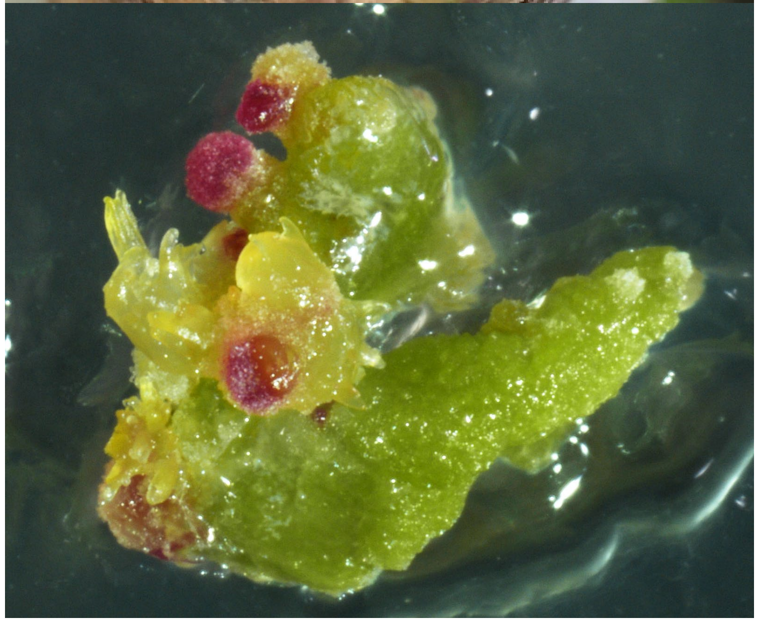
- Transformable genotype 717-1B4 *P. tremula x alba*
- No selection in this experiment

RUBY regenerating callus or hairy roots varied widely amongst strains in poplar



Ti strains = green bars, Ri strains = blue bars

Transforming greenhouse plants is tough, but we are learning!



- Things we take for granted like *Agrobacterium* preparation methods, selection strength, media composition all need to be re-calibrated *in planta*

- So far our wild strains exhibit potent morphogenic outcomes *in vitro*, highlighting many for further study

Summary

- Agro genes have impressive morphogenic potential for transformation
- Many Agro genes are unexplored mechanistically in the ways they change plant development
- Binary vectors using RUBY are effective tools to assess strain characteristics
- Agro strains have wide variability in transgene delivery and regeneration outcomes on different hosts

Going forward

- *Agrobacterium* diversity, both in terms of transgene delivery and regeneration outcomes warrants further study
- Screening population of sequenced strains will highlight unexplored T-DNA genes for further mechanistic study or careful combination
- *In planta*, tissue culture-free systems have many technical challenges in vegetatively propagated crops –co-transformation with Agro genes could be a path forward
- Strain “domestication” a priority in cases where lab strains (C58/Ach5) are inadequate for the desired plant species

Thanks to our funders and collaborators

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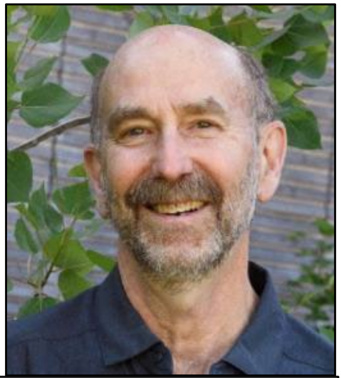
USDA
United States Department of Agriculture
National Institute of Food and Agriculture

NSF
The National Science Foundation

<u>GREAT TREES Consortium</u>

Suzano, SAPPI, Arauco, Klabin, SweTree, Corteva Agriscience

Thank you to all the people in the lab who contributed!



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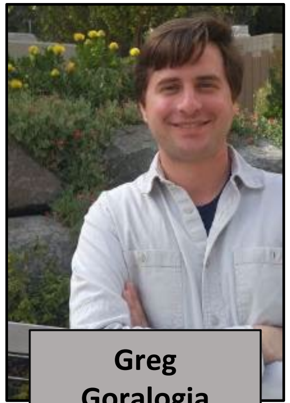

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