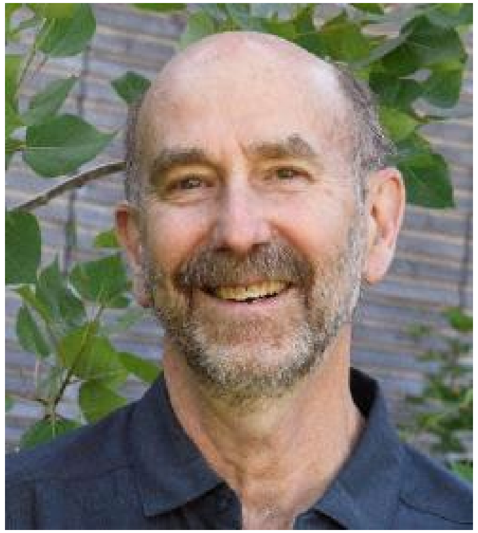
Leveraging developmental genes from a shooty *Agrobacterium* strain for altruistic transformation

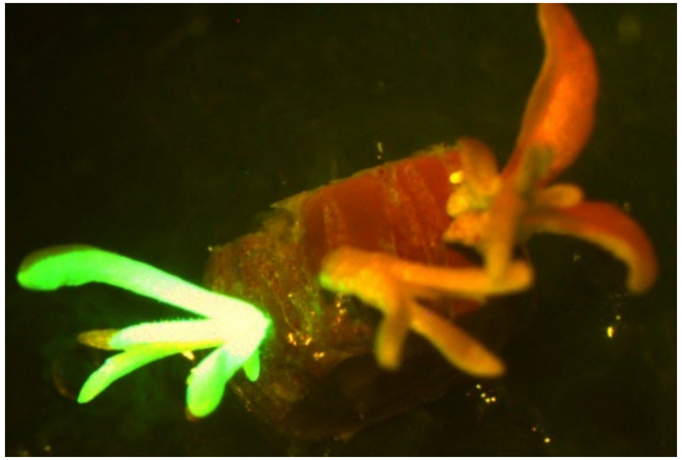
Greg Goralogia & Steve Strauss

Oregon State University



Agenda

- Perspectives & experimental system
- Some stuff we are excited about
  - “Shooty” developmental genes from Agrobacterium



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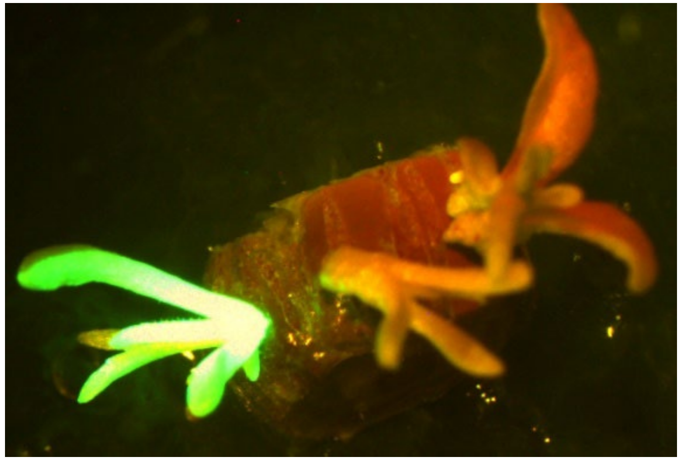
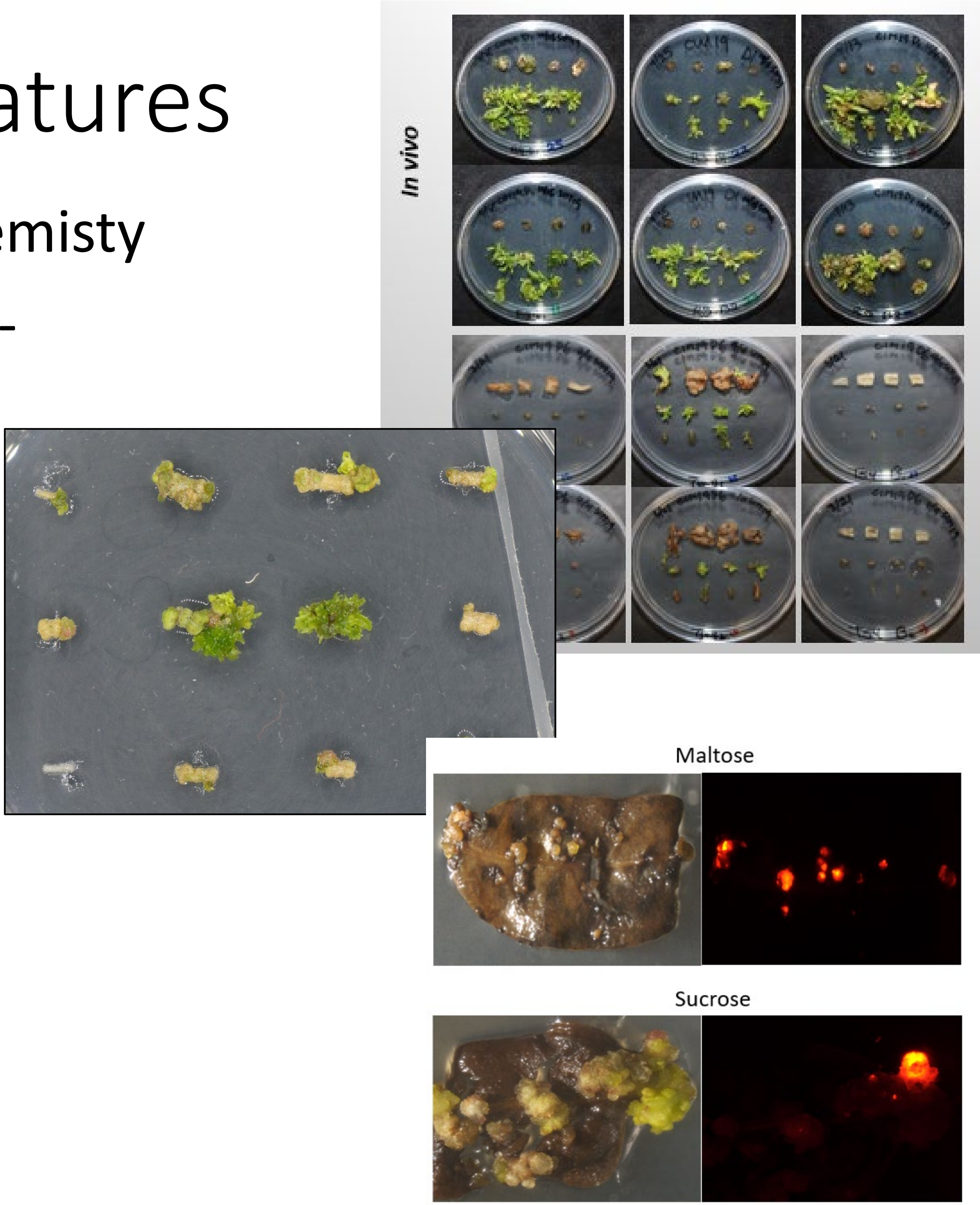


Regeneration & transformation continue to be major limiting factors for gene editing & engineering in plants, and especially trees

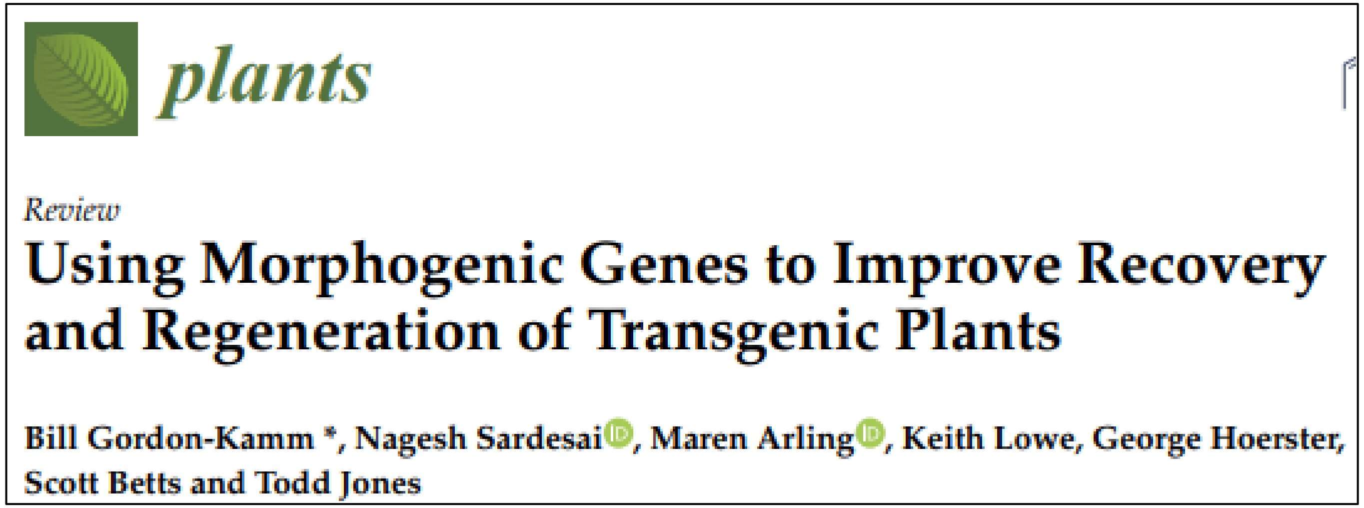
- Species and genotypic differences often dramatic
- Slow, costly, complex customization efforts usually needed
- On top of often large social/regulatory constraints, often a “deal breaker”

Our experimental system features

- Woody (forest) trees – slow, tough biochemisty
- Elite clones, mature propagules, not seed-derived
- High physiological diversity
  - Growth environment, age, explant type and source
- Great tissue sample heterogeneity in response
- Common necrotic responses
- Very high genetic diversity of forest trees
- Large interactions among all of the above



“DEV” genes can work, are they the miracles we hope for?



plants

Review

Using Morphogenic Genes to Improve Recovery and Regeneration of Transgenic Plants

Bill Gordon-Kamm *, Nagesh Sardesai, Maren Arling, Keith Lowe, George Hoerster, Scott Betts and Todd Jones

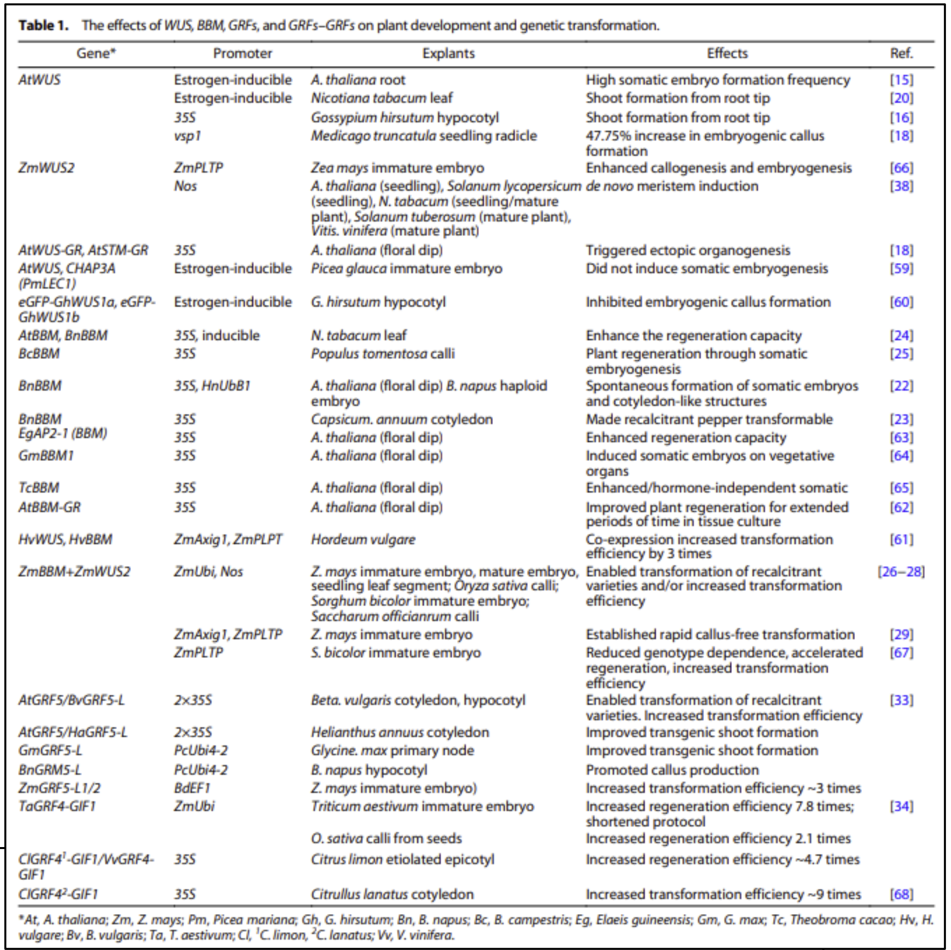
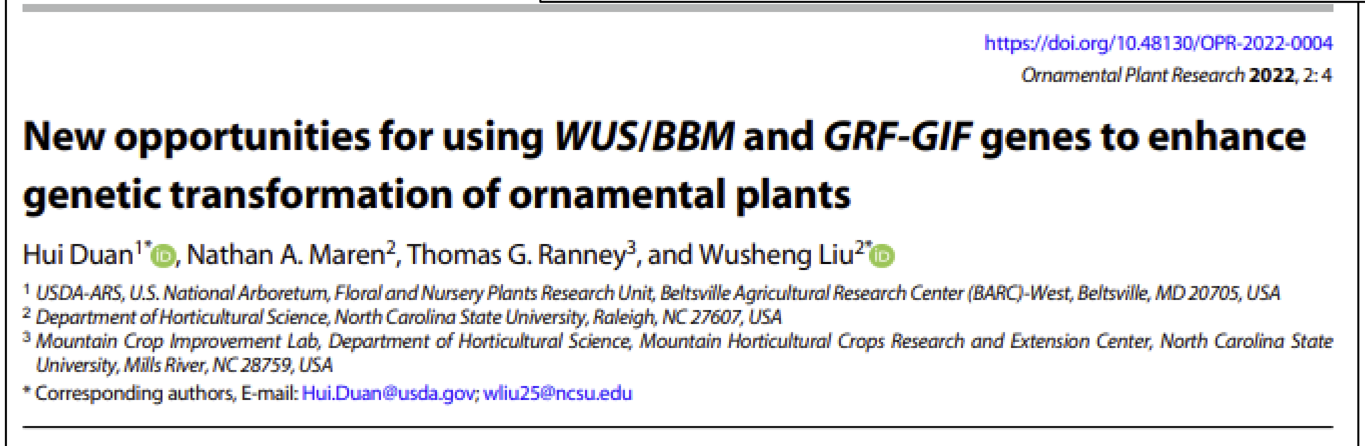


Table 1. The effects of *WUS, BBM, GRFs*, and *GRFs–GRFs* on plant development and genetic transformation.

Gene*	Promoter	Explants	Effects	Ref.
AtWUS	Estrogen-inducible	*A. thaliana* root	High somatic embryo formation frequency	[15]
	Estrogen-inducible	*Nicotiana tabacum* leaf	Shoot formation from root tip	[20]
	35S	*Gossypium hirsutum* hypocotyl	Shoot formation from root tip	[16]
	vsp1	*Medicago truncatula* seedling radicle	47.75% increase in embryogenic callus formation	[18]
ZmWUS2	*ZmPLTP*	*Zea mays* immature embryo	Enhanced callogenesis and embryogenesis	[66]
	Nos	*A. thaliana* (seedling), *Solanum lycopersicum* (seedling), *N. tabacum* (seedling/mature plant), *Solanum tuberosum* (mature plant), *Vitis. vinifera* (mature plant)	*de novo* meristem induction	[38]
AtWUS-GR, AtSTM-GR	*35S*	*A. thaliana* (floral dip)	Triggered ectopic organogenesis	[18]
AtWUS, CHAP3A (PmLEC1)	Estrogen-inducible	*Picea glauca* immature embryo	Did not induce somatic embryogenesis	[59]
eGFP-GhWUS1a, eGFP-GhWUS1b	Estrogen-inducible	*G. hirsutum* hypocotyl	Inhibited embryogenic callus formation	[60]
AtBBM, BnBBM	*35S*, inducible	*N. tabacum* leaf	Enhance the regeneration capacity	[24]
BcBBM	*35S*	*Populus tomentosa* calli	Plant regeneration through somatic embryogenesis	[25]
BnBBM	*35S, HnUbB1*	*A. thaliana* (floral dip) *B. napus* haploid embryo	Spontaneous formation of somatic embryos and cotyledon-like structures	[22]
BnBBM	*35S*	*Capsicum. annuum* cotyledon	Made recalcitrant pepper transformable	[23]
EgAP2-1 (BBM)	*35S*	*A. thaliana* (floral dip)	Enhanced regeneration capacity	[63]
GmBBM1	*35S*	*A. thaliana* (floral dip)	Induced somatic embryos on vegetative organs	[64]
TcBBM	*35S*	*A. thaliana* (floral dip)	Enhanced/hormone-independent somatic	[65]
AtBBM-GR	*35S*	*A. thaliana* (floral dip)	Improved plant regeneration for extended periods of time in tissue culture	[62]
HvWUS, HvBBM	*ZmAxig1, ZmPLPT*	*Hordeum vulgare*	Co-expression increased transformation efficiency by 3 times	[61]
ZmBBM+ZmWUS2	*ZmUbi, Nos*	*Z. mays* immature embryo, mature embryo, seedling leaf segment; *Oryza sativa* calli; *Sorghum bicolor* immature embryo; *Saccharum officianrum* calli	Enabled transformation of recalcitrant varieties and/or increased transformation efficiency	[26–28]
	ZmAxig1, ZmPLTP	*Z. mays* immature embryo	Established rapid callus-free transformation	[29]
	ZmPLTP	*S. bicolor* immature embryo	Reduced genotype dependence, accelerated regeneration, increased transformation efficiency	[67]
AtGRF5/BvGRF5-L	*2×35S*	*Beta. vulgaris* cotyledon, hypocotyl	Enabled transformation of recalcitrant varieties. Increased transformation efficiency	[33]
AtGRF5/HaGRF5-L	*2×35S*	*Helianthus annuus* cotyledon	Improved transgenic shoot formation	
GmGRF5-L	*PcUbi4-2*	*Glycine. max* primary node	Improved transgenic shoot formation	
BnGRM5-L	*PcUbi4-2*	*B. napus* hypocotyl	Promoted callus production	
ZmGRF5-L1/2	*BdEF1*	*Z. mays* immature embryo)	Increased transformation efficiency ~3 times	
TaGRF4-GIF1	*ZmUbi*	*Triticum aestivum* immature embryo	Increased regeneration efficiency 7.8 times; shortened protocol	[34]
		O. sativa calli from seeds	Increased regeneration efficiency 2.1 times	
ClGRF4^1-GIF1/VvGRF4-GIF1	*35S*	*Citrus limon* etiolated epicotyl	Increased regeneration efficiency ~4.7 times	
ClGRF4^2-GIF1	*35S*	*Citrullus lanatus* cotyledon	Increased transformation efficiency ~9 times	[68]

**At, A. thaliana; Zm, Z. mays; Pm, Picea mariana; Gh, G. hirsutum; Bn, B. napus; Bc, B. campestris; Eg, Elaeis guineensis; Gm, G. max; Tc, Theobroma cacao; Hv, H. vulgare; Bv, B. vulgaris; Ta, T. aestivum; Cl, ^{1}C. limon, ^{2}C. lanatus; Vv, V. vinifera.*

Ornamental
Plant Research



https://doi.org/10.48130/OPR-2022-0004
Ornamental Plant Research **2022**, 2: 4

New opportunities for using *WUS/BBM* and *GRF-GIF* genes to enhance genetic transformation of ornamental plants

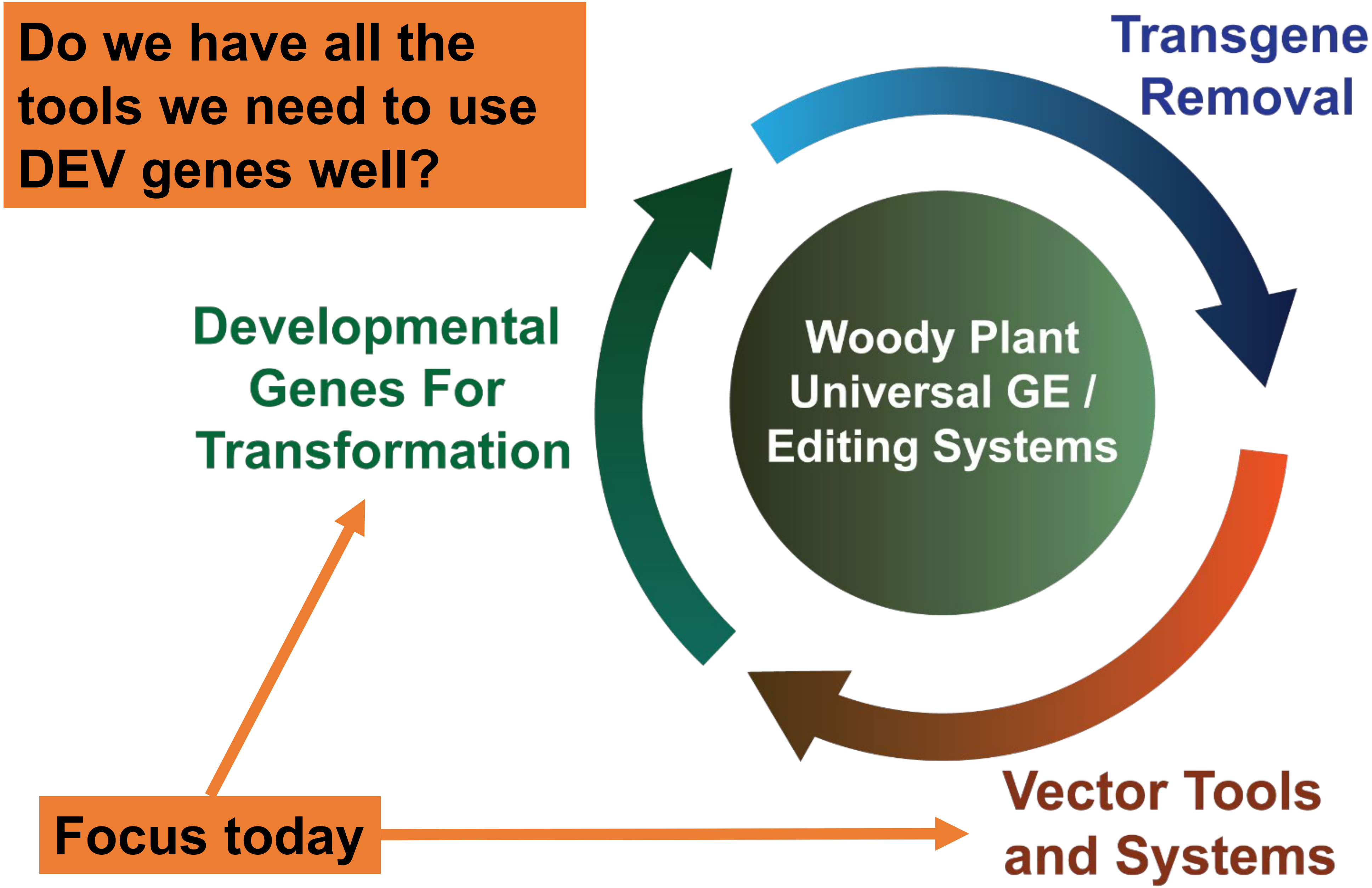
Hui Duan[1]*, Nathan A. Maren[2], Thomas G. Ranney[3], and Wusheng Liu[2]*

[1] *USDA-ARS, U.S. National Arboretum, Floral and Nursery Plants Research Unit, Beltsville Agricultural Research Center (BARC)-West, Beltsville, MD 20705, USA*
[2] *Department of Horticultural Science, North Carolina State University, Raleigh, NC 27607, USA*
[3] *Mountain Crop Improvement Lab, Department of Horticultural Science, Mountain Horticultural Crops Research and Extension Center, North Carolina State University, Mills River, NC 28759, USA*
* Corresponding authors, E-mail: Hui.Duan@usda.gov; wliu25@ncsu.edu

***Focus of GREAT TREES Coop*:**

> ***“Developmental genes as methods to enhance gene editing and transformation in eucalypts”***

Do we have all the tools we need to use DEV genes well?



Woody Plant Universal GE / Editing Systems

Transgene Removal

Vector Tools and Systems

Developmental Genes For Transformation

Focus today

Types of DEV genes we have studied in poplars or eucalypts – many both *in vitro* and *in planta*

- *LEC* 1, 2 – *LEAFY COTYLEDON*
- *EBB1 - EARLY BUD BREAK 1* (ESR family)
- *BBM – BABY BOOM*
- *WOX* 5, 11 -- *WUSCHEL RELATED HOMEOBOX*
- *IPT – ISOPENTYL TRANSFERASE* (cytokinin) – Agrobacterium
- Agrobacterium oncogenes
- *ROL* – Hairy root-inducing genes – Agrobacterium
- *WUS – WUSCHEL*
- *GRF-GIF – GROWTH REGULATOR FACTOR 4* and *GRF INTERACTING FACTOR* 1

Most have failed with simple overexpression, or given highly genotype-specific enhancement or inhibition

- *LEC* 1, 2 – *LEAFY COTYLEDON*
- *EBB1 - EARLY BUD BREAK 1* (ESR family)
- *BBM – BABY BOOM*
- *WOX* 5, 11 -- *WUSCHEL RELATED HOMEOBOX*
- *IPT – ISOPENTYL TRANSFERASE* (cytokinin) – Agrobacterium
- **Agrobacterium oncogenes**
- ***ROL* – Hairy root-inducing genes – Agrobacterium**
- *WUS – WUSCHEL*
- *GRF-GIF – GROWTH REGULATOR FACTOR 4* and *GRF INTERACTING FACTOR* 1

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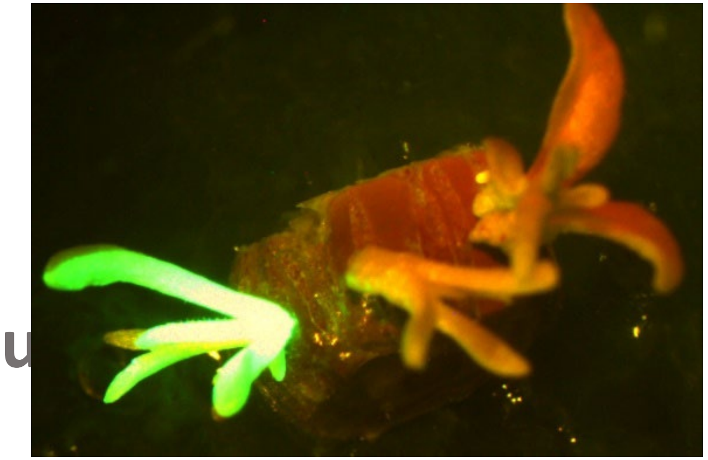
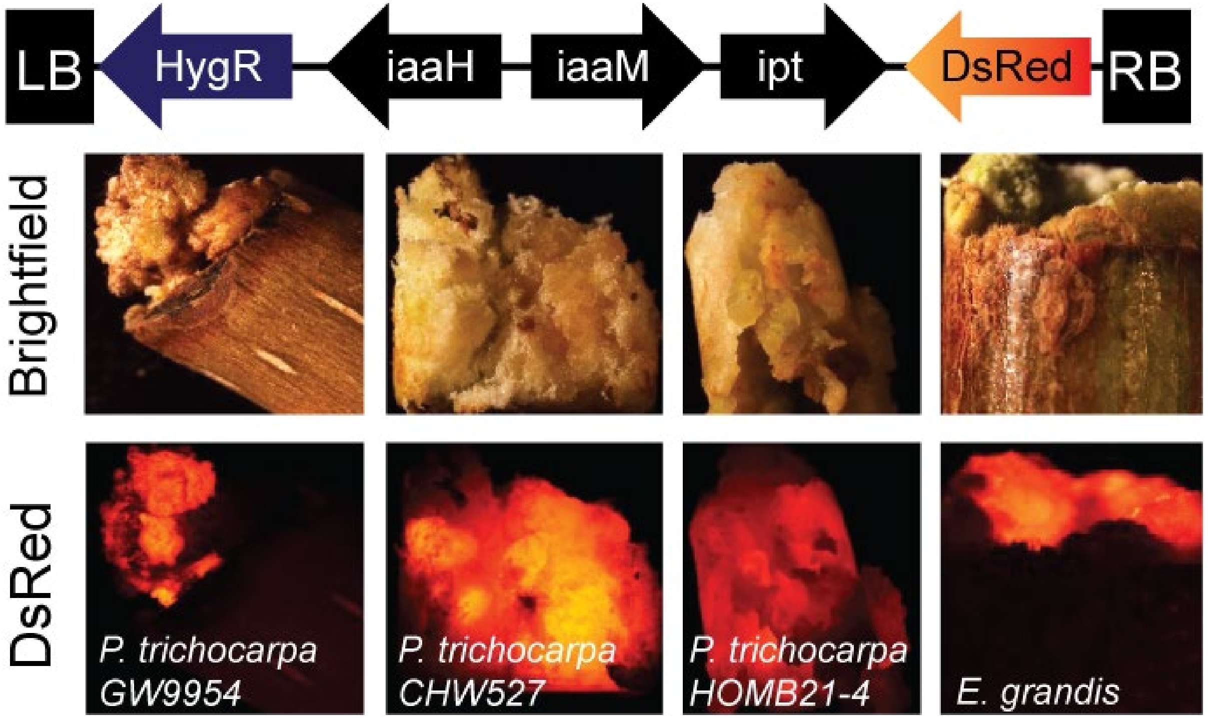
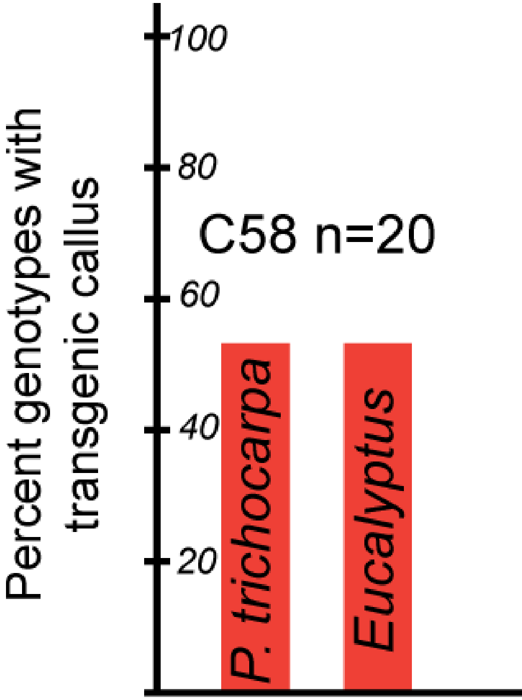
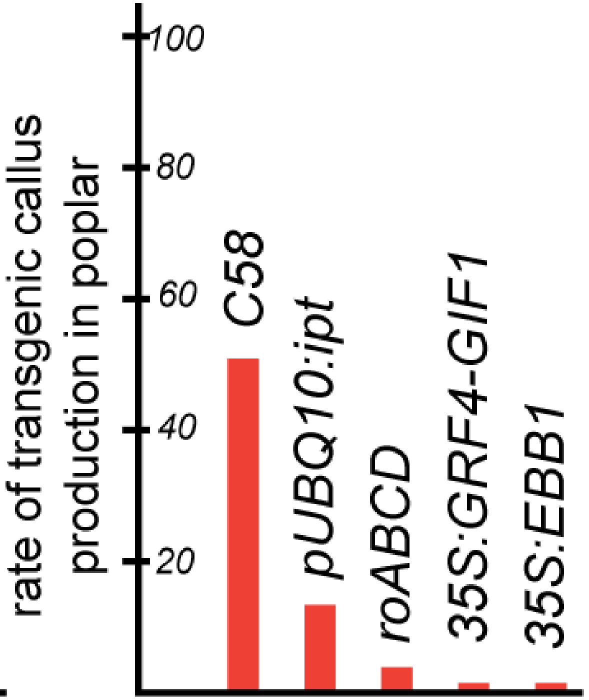


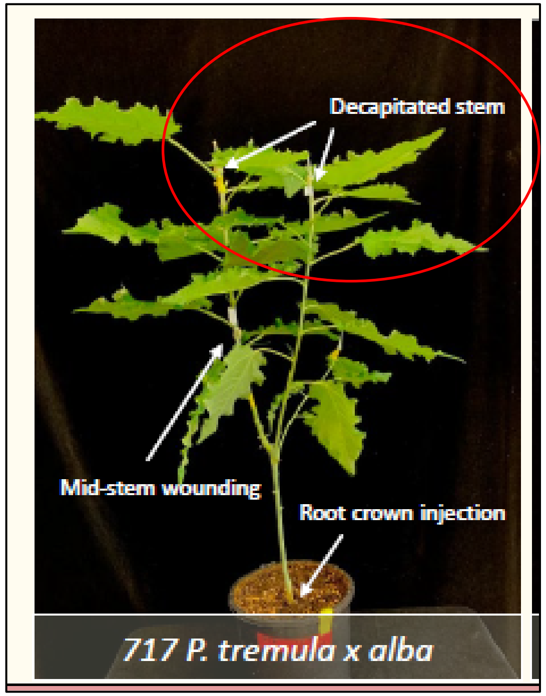
iaaH/M and ipt genes from *Agrobacterium* (C58) were effective *in planta* inducers of transgenic galls in diverse poplar and eucalypt genotypes

But shoots could not be regenerated from transgenic galls



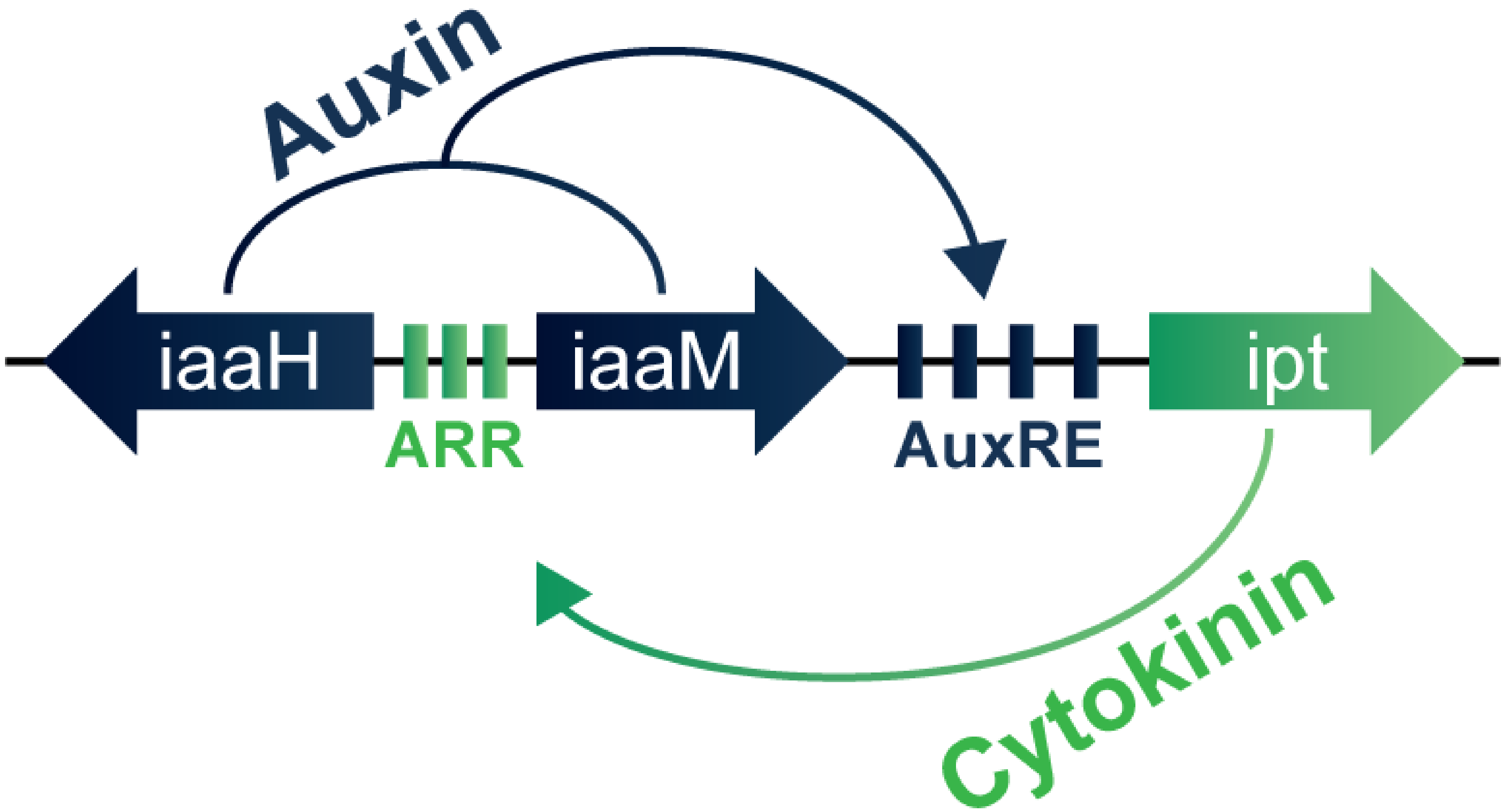






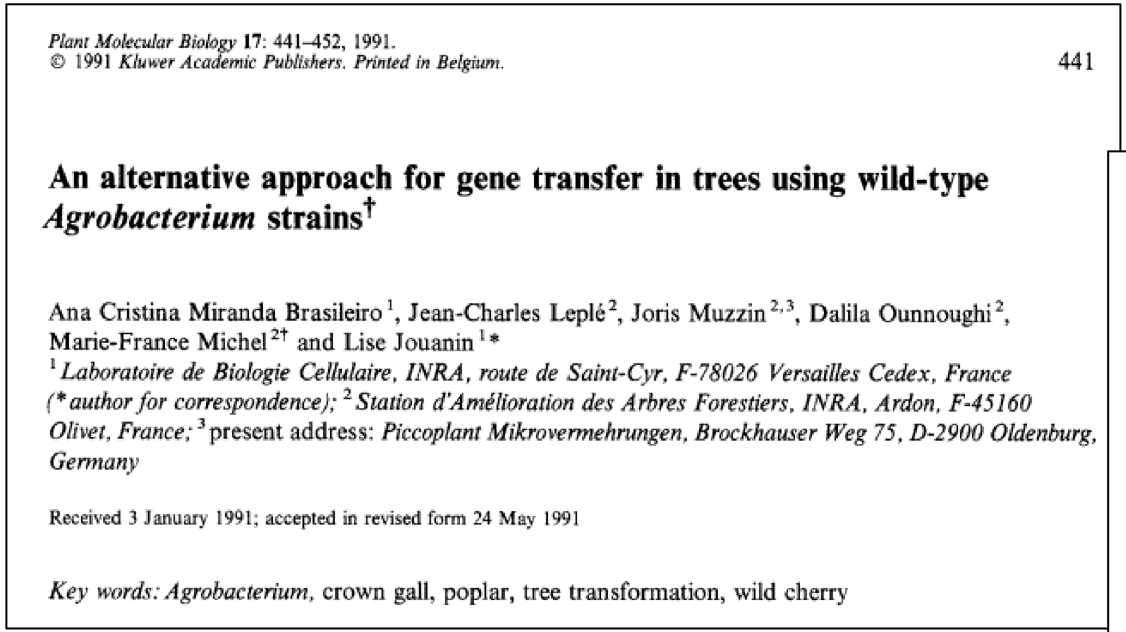
Back to the future: *A. tumefaciens* development genes revisited with new techniques? Useful for *in planta* transformation?

iaa/ipt genes form a positive feedback loop to induce and gall development



Can we find more useful, developmentally flexible galls?

Jouanin group (INRA-France) characterized a shooty agro strain, and leveraged it for *in planta* regeneration in the 1990s



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

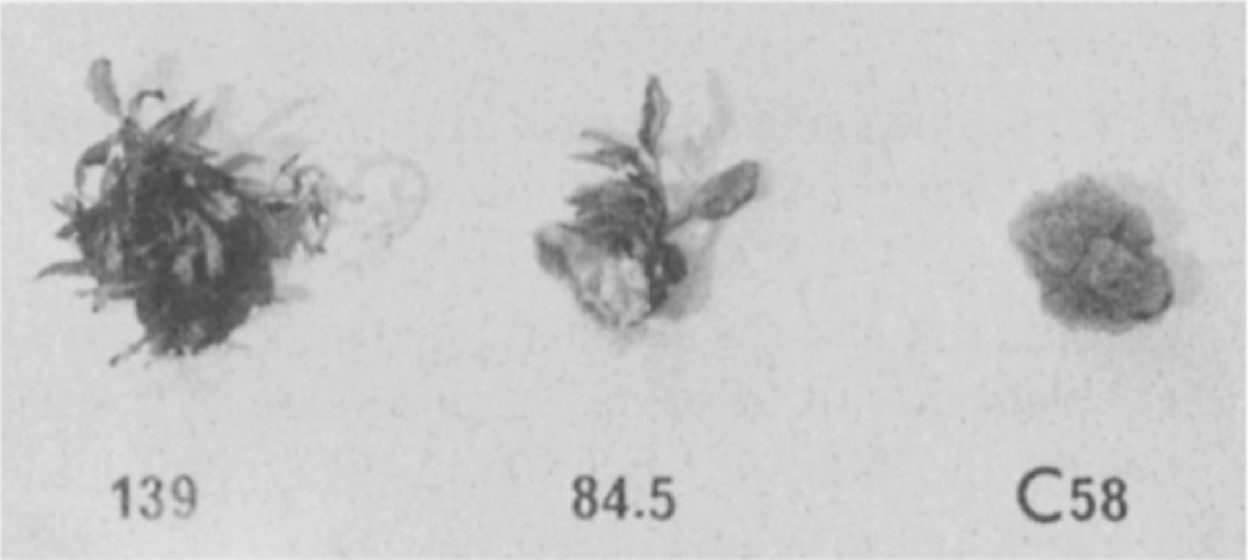
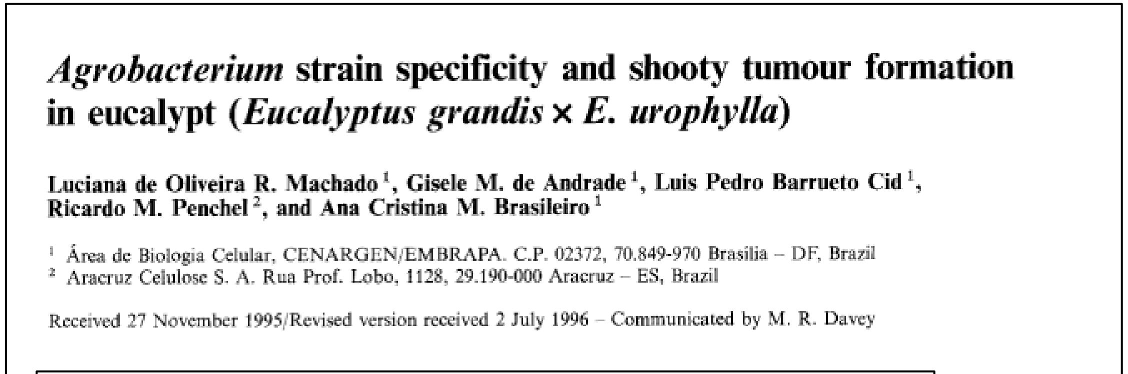


Fig. 1. Tumors and shoot differentiation from poplar tumors induced by *A. tumefaciens* strains 82.139, 84.5 and C58 and cultivated on MS medium, 6 weeks after inoculation.

The method also reportedly worked in *Eucalyptus*, less well in birch, using the wild strain



Agrobacterium strain specificity and shooty tumour formation in eucalypt (_Eucalyptus grandis_ × _E. urophylla_)

Luciana de Oliveira R. Machado[1], Gisele M. de Andrade[1], Luis Pedro Barrueto Cid[1], Ricardo M. Penchel[2], and Ana Cristina M. Brasileiro[1]

[1] Área de Biologia Celular, CENARGEN/EMBRAPA. C.P. 02372, 70.849-970 Brasília – DF, Brazil
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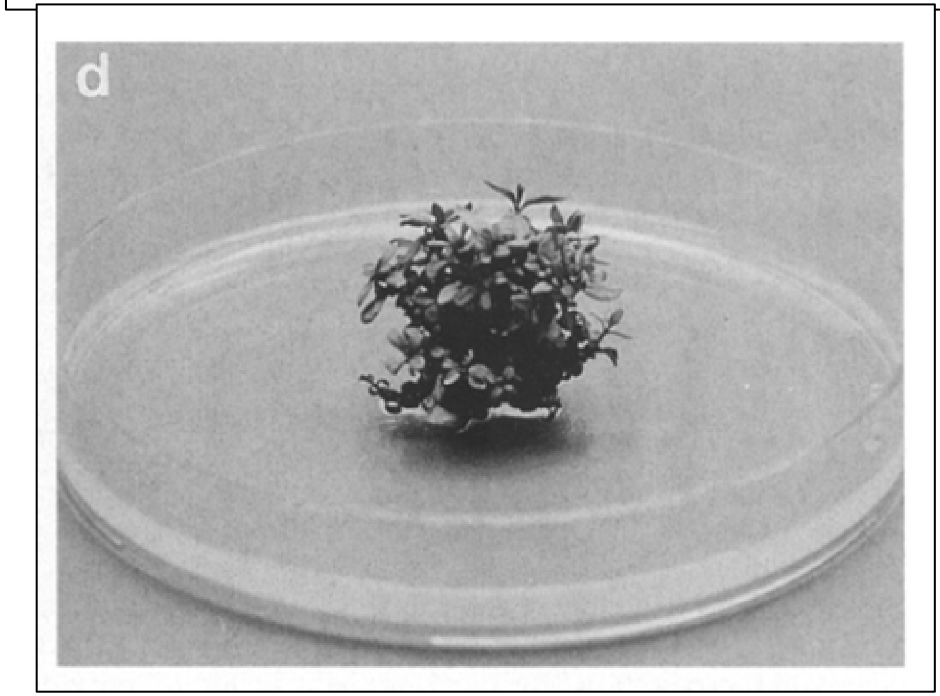
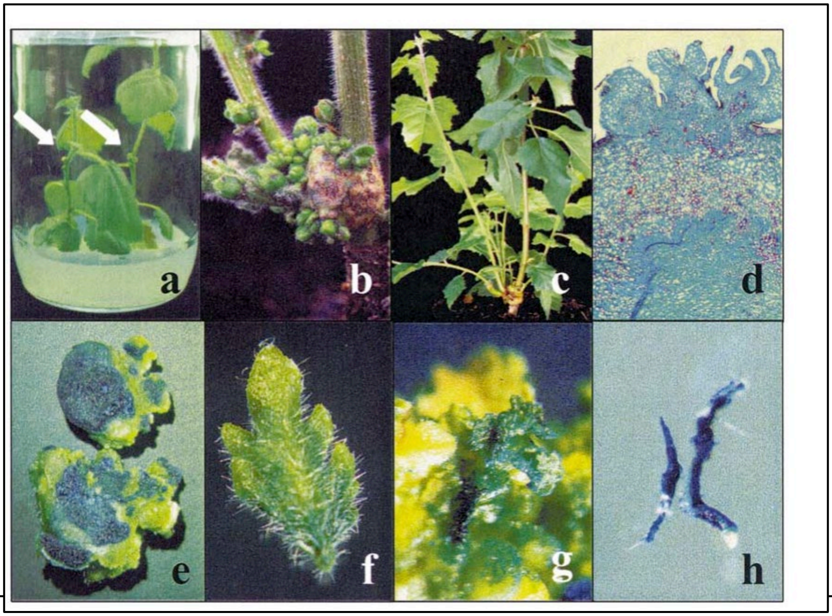
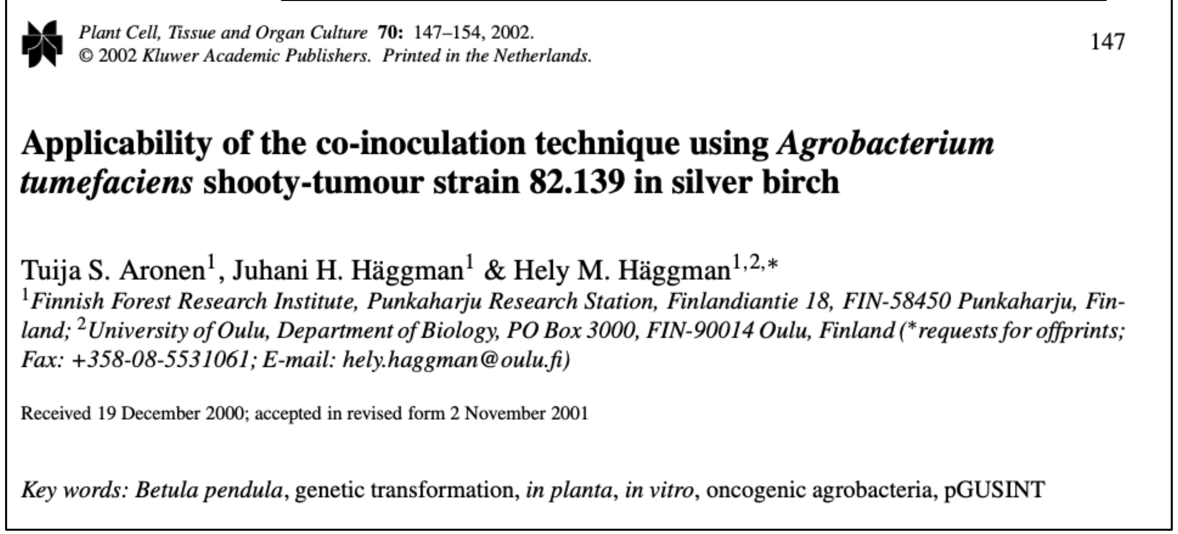
Received 27 November 1995/Revised version received 2 July 1996 – Communicated by M. R. Davey







Plant Cell, Tissue and Organ Culture **70:** 147–154, 2002.
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Applicability of the co-inoculation technique using _Agrobacterium tumefaciens_ shooty-tumour strain 82.139 in silver birch

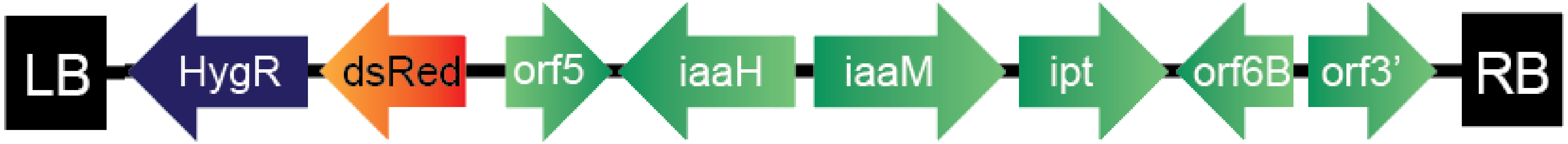
Tuija S. Aronen[1], Juhani H. Häggman[1] & Hely M. Häggman[1,2,*]
*[1]Finnish Forest Research Institute, Punkaharju Research Station, Finlandiantie 18, FIN-58450 Punkaharju, Finland; [2]University of Oulu, Department of Biology, PO Box 3000, FIN-90014 Oulu, Finland (*requests for offprints; Fax: +358-08-5531061; E-mail: hely.haggman@oulu.fi)*

Received 19 December 2000; accepted in revised form 2 November 2001

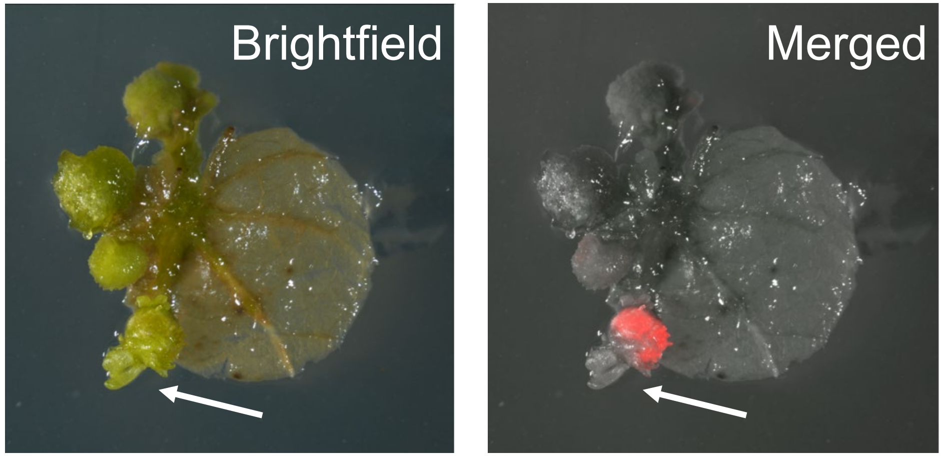
Key words: Betula pendula, genetic transformation, *in planta*, *in vitro*, oncogenic agrobacteria, pGUSINT

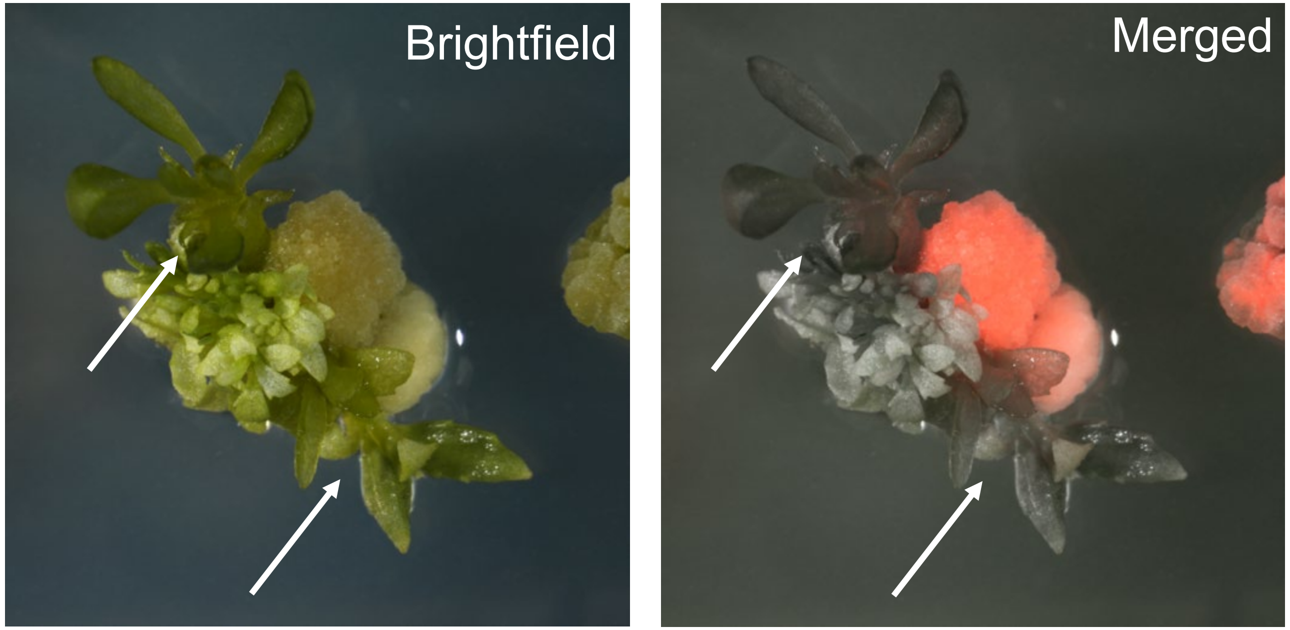
Though very promising, this work essentially came to a halt – due to GMO pushback in Europe – and due to the challenges of dealing with the large Ti plasmids and its many *vir* and development genes prior to high throughput sequencing and advanced gene cloning systems

We cloned out the genes from our resurrected clone in deep freeze, and added modern amenities like DsRed (called "S82")



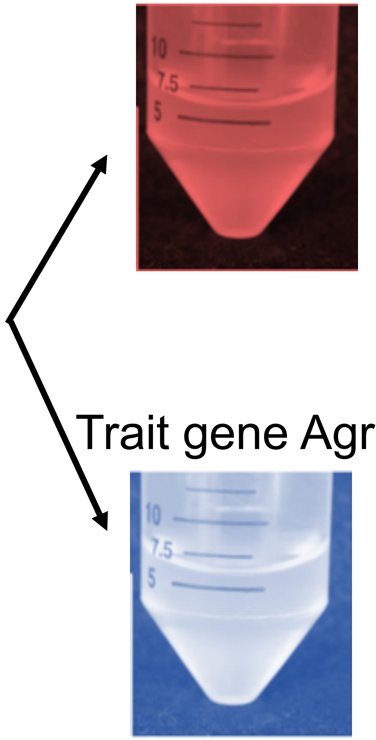
Transgenic galls promoted regeneration of galls and shoots





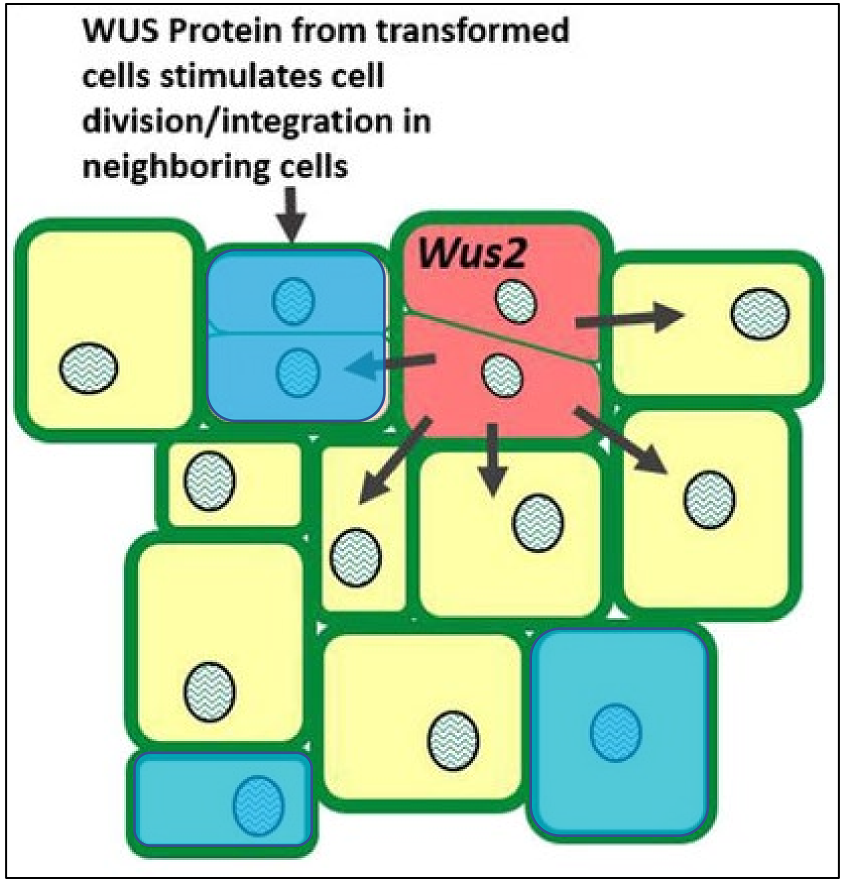
“Altruistic” transformation approach – strain mixtures

Mixed Agro in different ratios



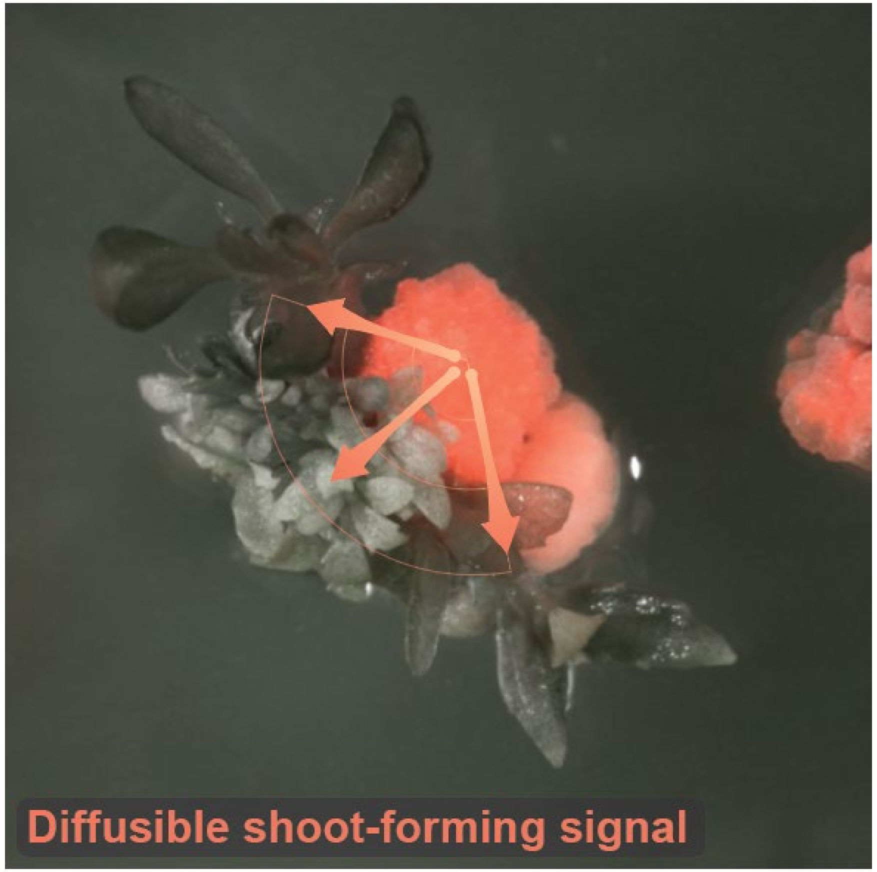
DEV gene Agro
(like *WUS*)

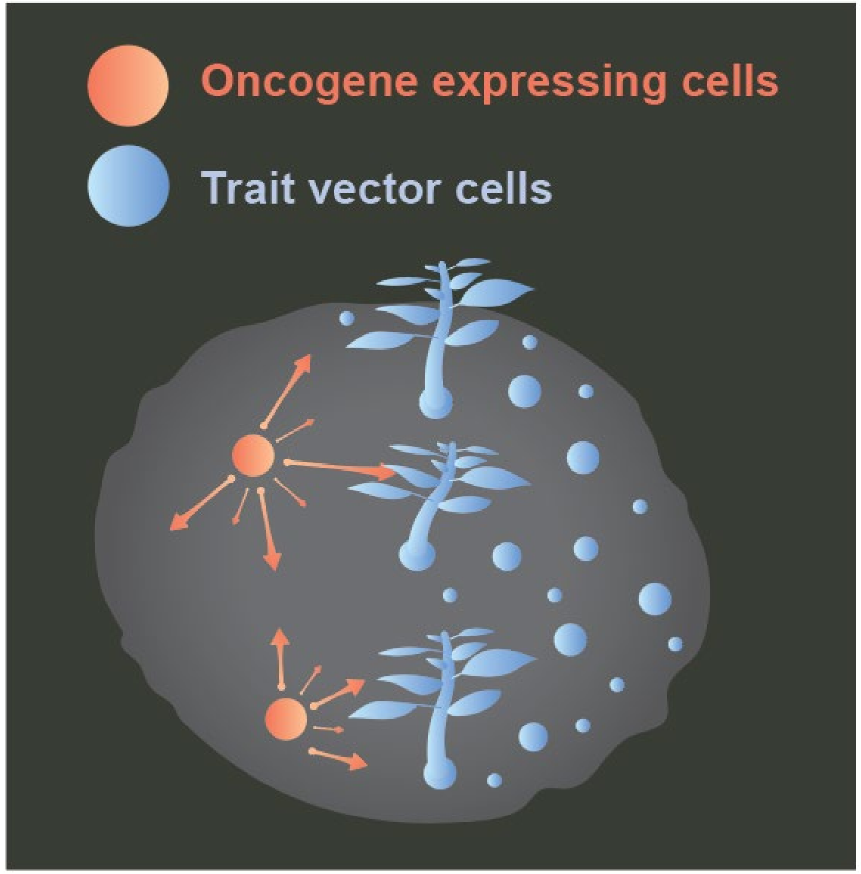
Trait gene Agro



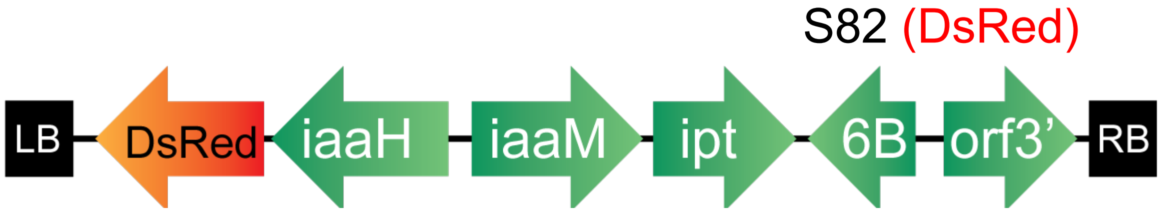
Plant with trait gene
and no DEV genes

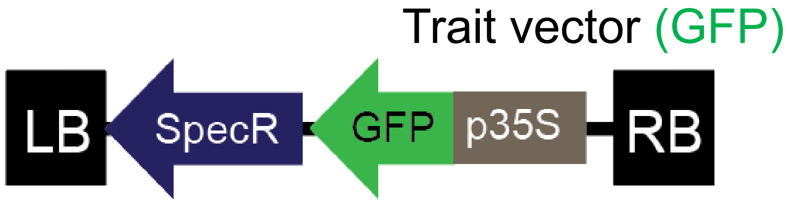
After pilot studies we thought these genes were well suited for “altruistic” transformation





Altruitsic "S82" transformation in hybrid poplar

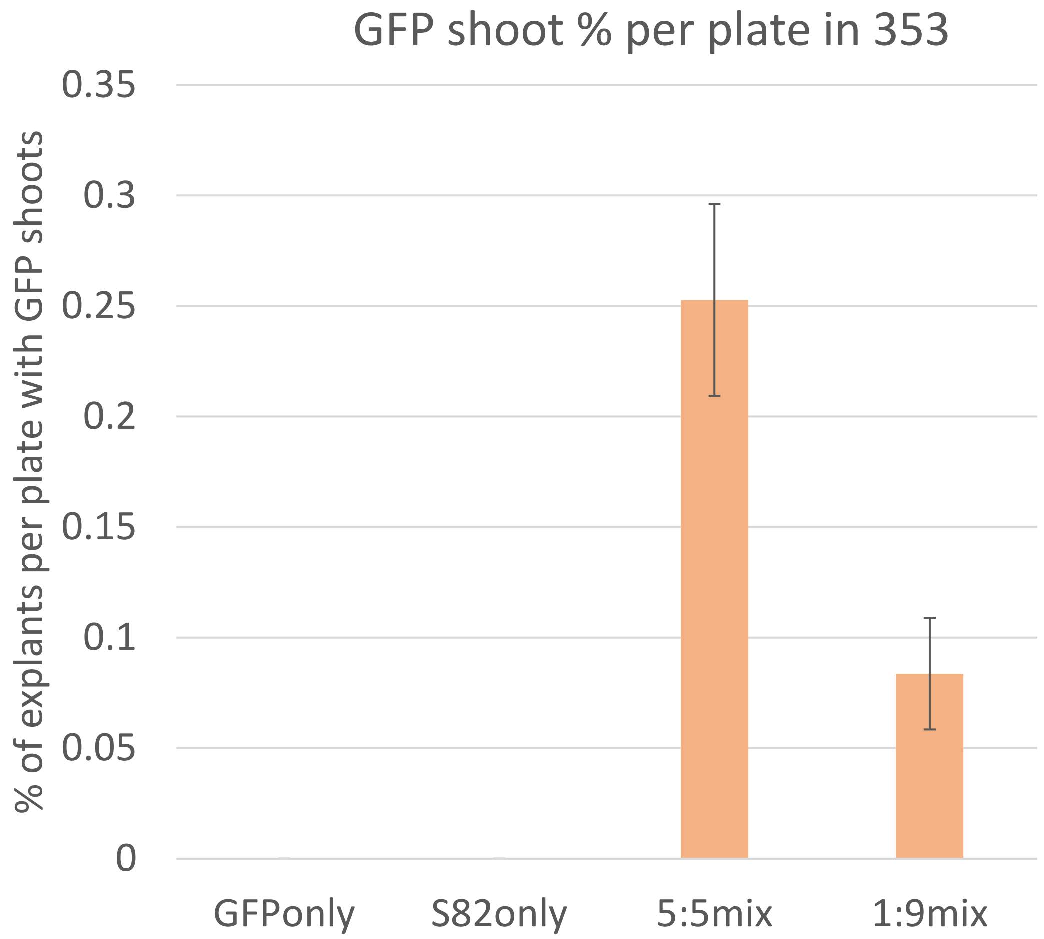


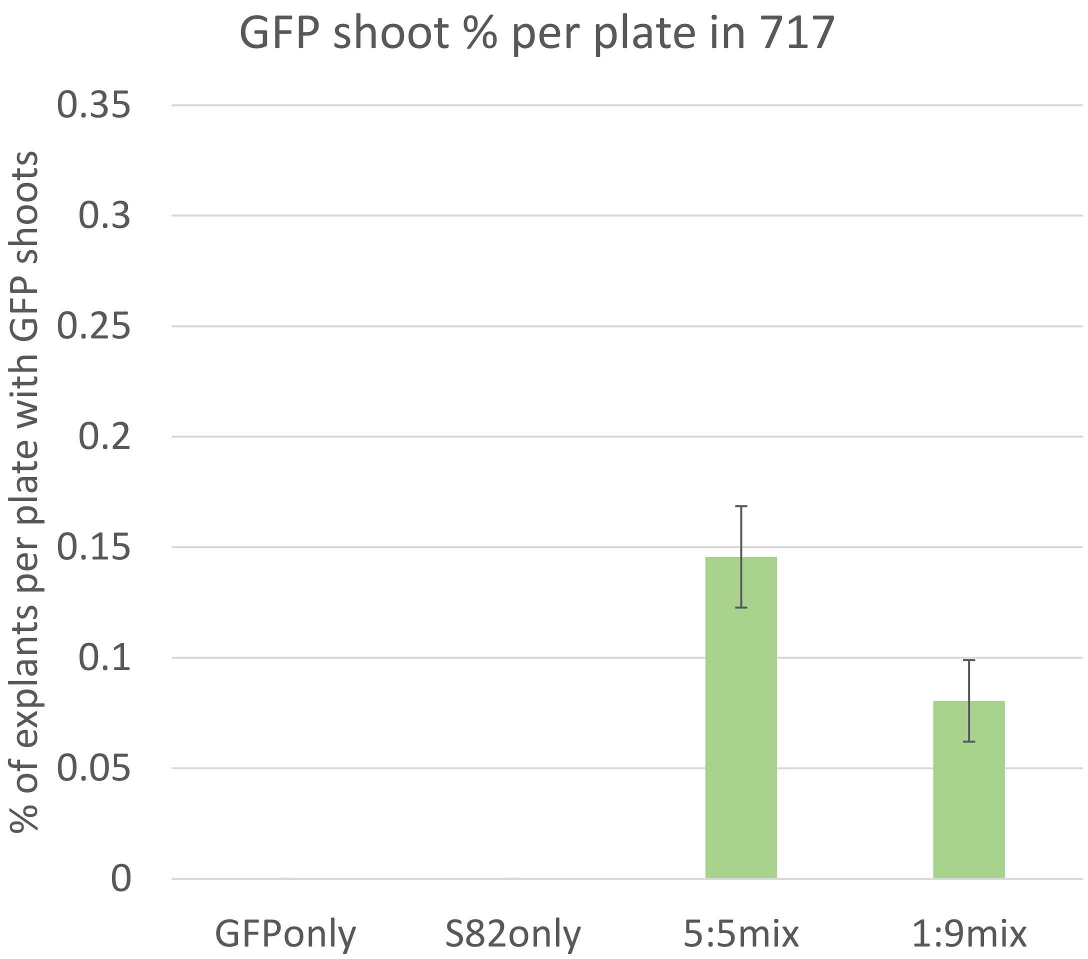


- 4 transformations
  - 100% S82
  - 50% S82 / 50% Trait-GFP
  - 10% S82 / 90% Trait-GFP
  - 100% Trait-GFP

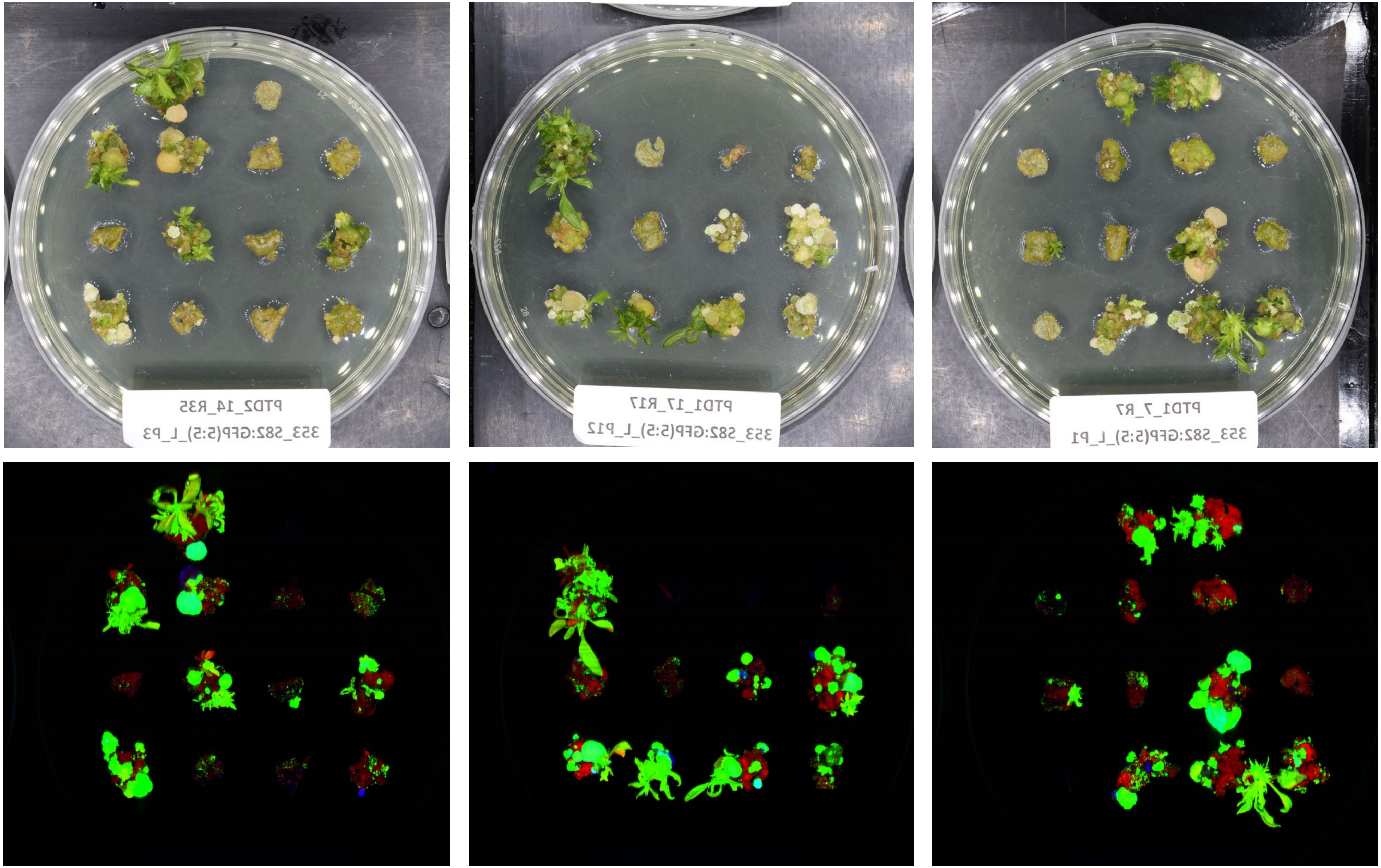
1. Co-culture 2 days (dark)
2. Transfer to 1xMS for 1 week (resting phase, dark)
3. Transfer to 1xMS + Spec to select for Trait-GFP (Dark 2 weeks)
4. Transfer to light, and subculture at 1 month onto same media

5:5 mixes of the two strains worked best in two poplar genotypes





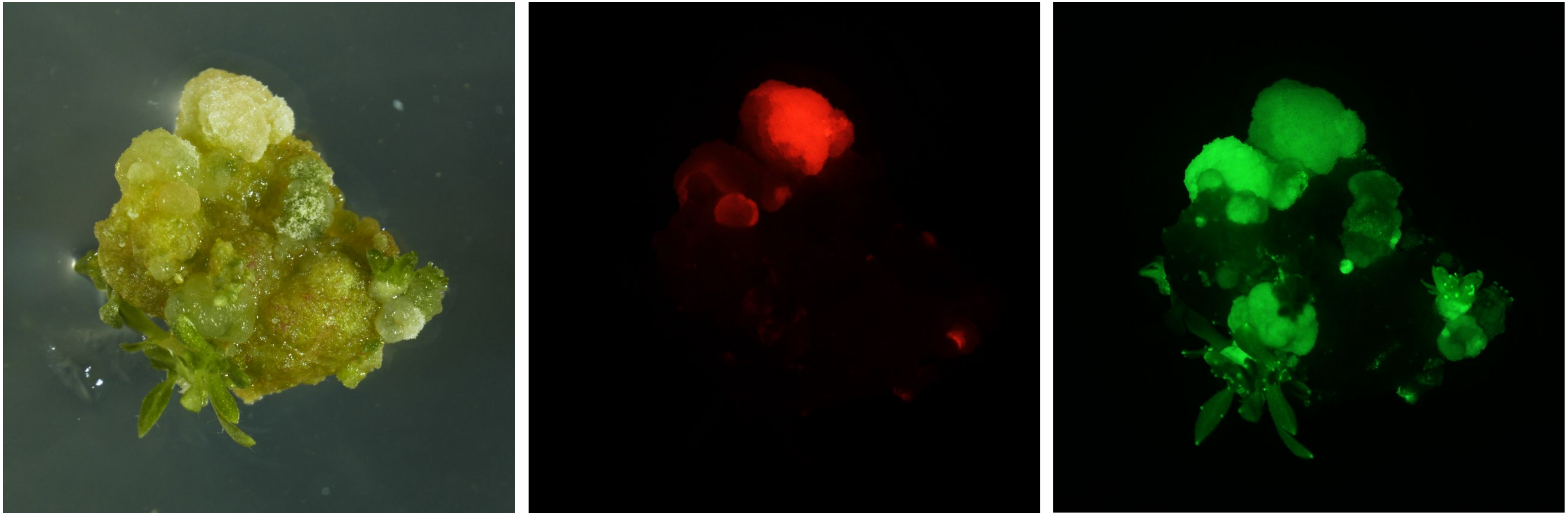
Hyperspectral imaging showed altruistic shoot regeneration



Green = GFP

Red = Chlorophyll

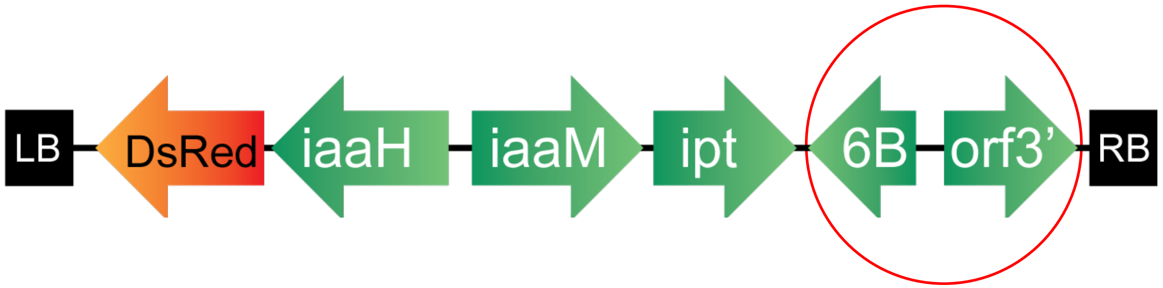
A closer look: 5:5 mix at week 6



Bright-field

DsRed

GFP



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

Is there novel *iaa/ipt* expression in this strain, or are the novel genes there most important?

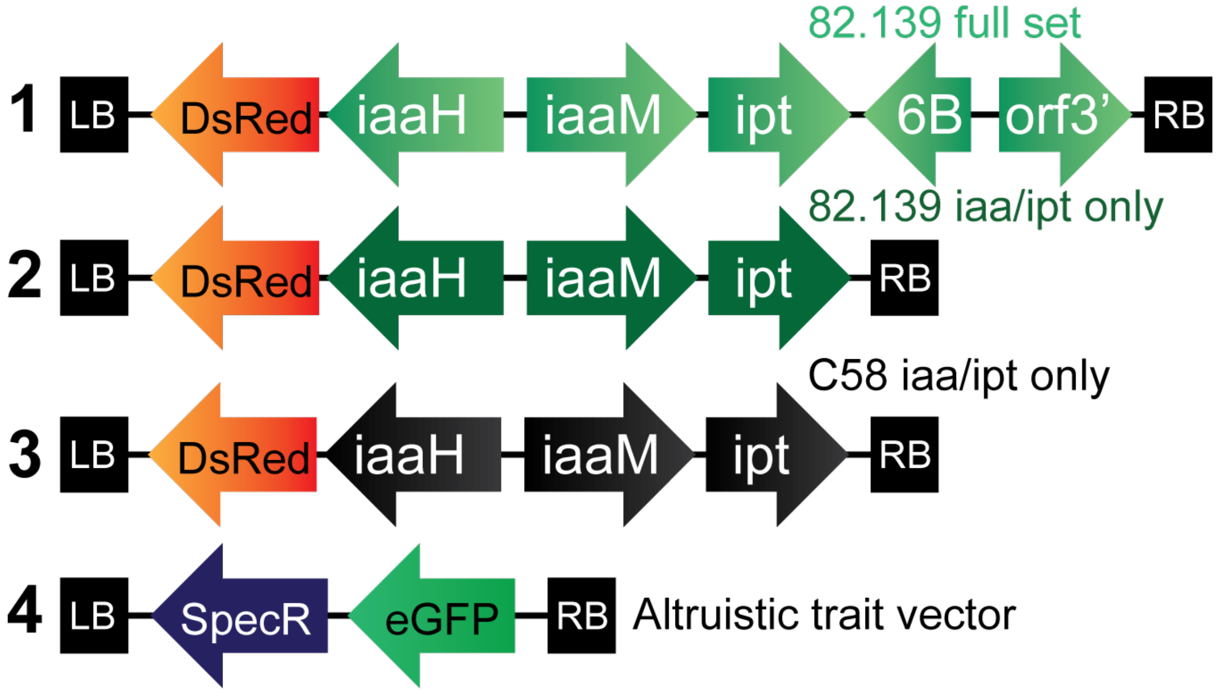
Experimental setup

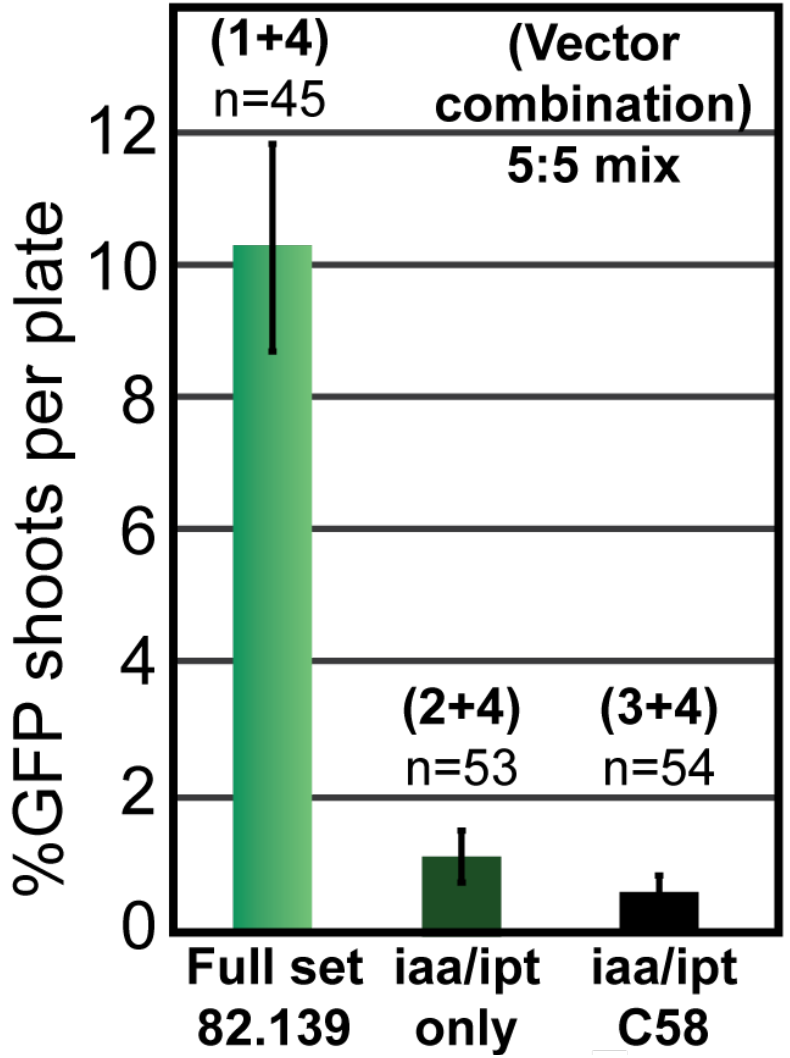
- 3 constructs
  - C58 (just *iaa* and *ipt* genes)
  - S82 (all six cloned genes)
  - S82 (just iaa and ipt genes)

- All constructs mixed 5:5 with SpecR GFP binary vector
- 1 week rest after co-culture without spectinomycin, 6 weeks on MS media without hormones but with spectinomycin

82.139 *iaa/ipt* genes alone are insufficient for altruistic shoot induction

Vector types used

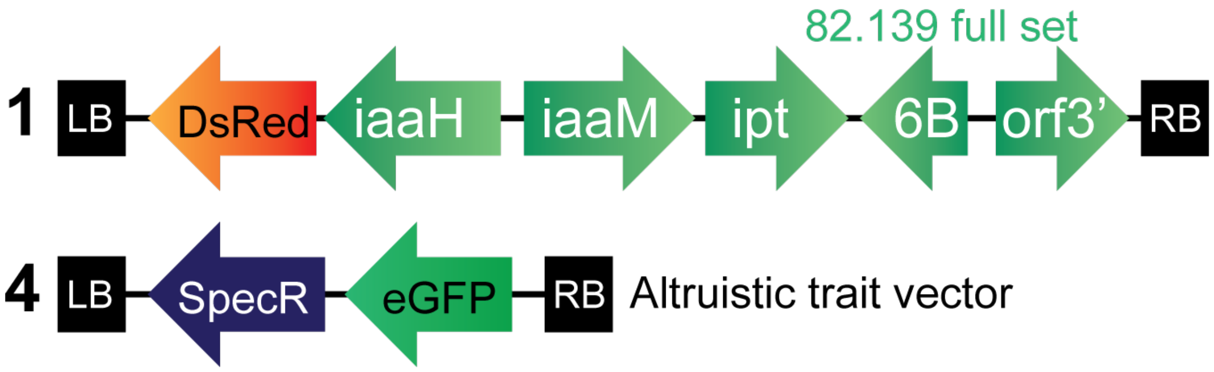




n= number of plates of 12 explants each

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

Vector types used



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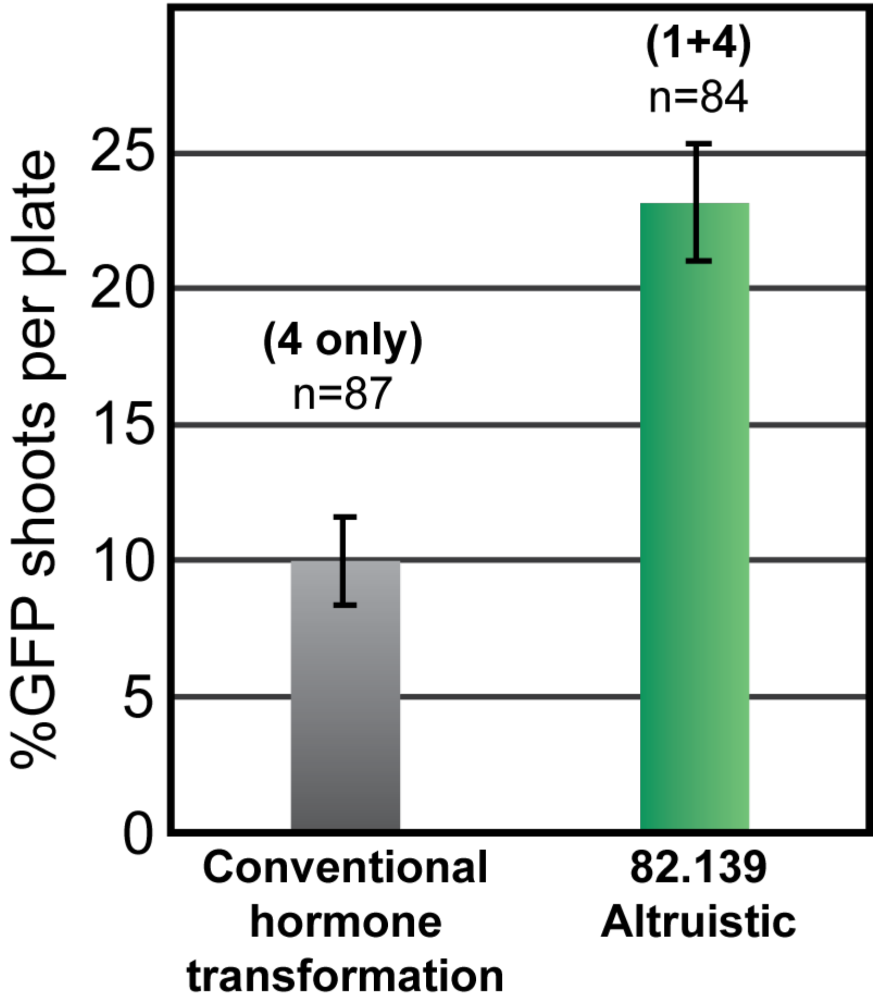
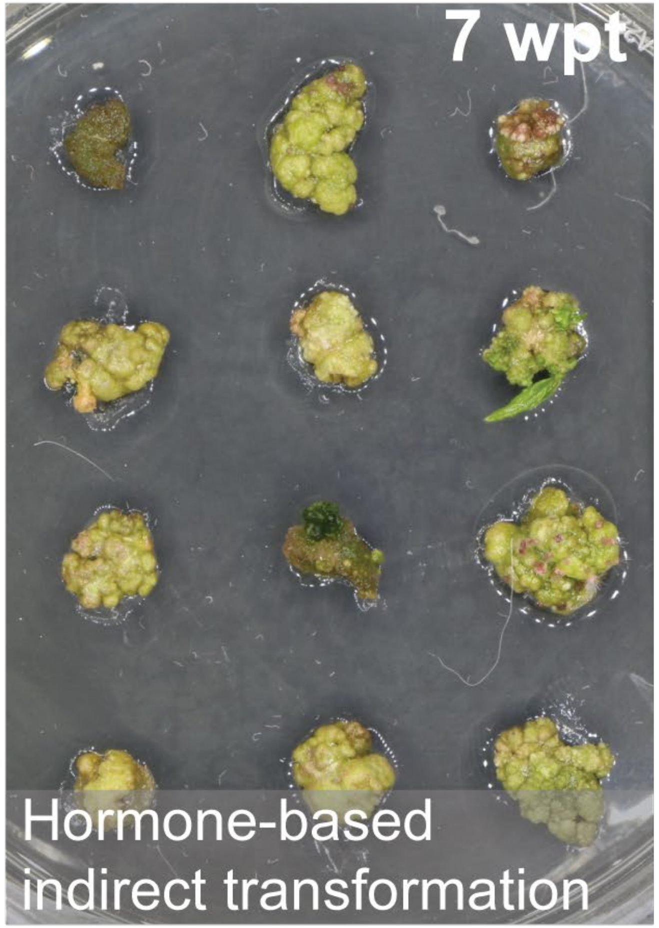


The 82.139 altruistic method was also significantly faster, shortening time to propagation by half



Hormone-based indirect transformation

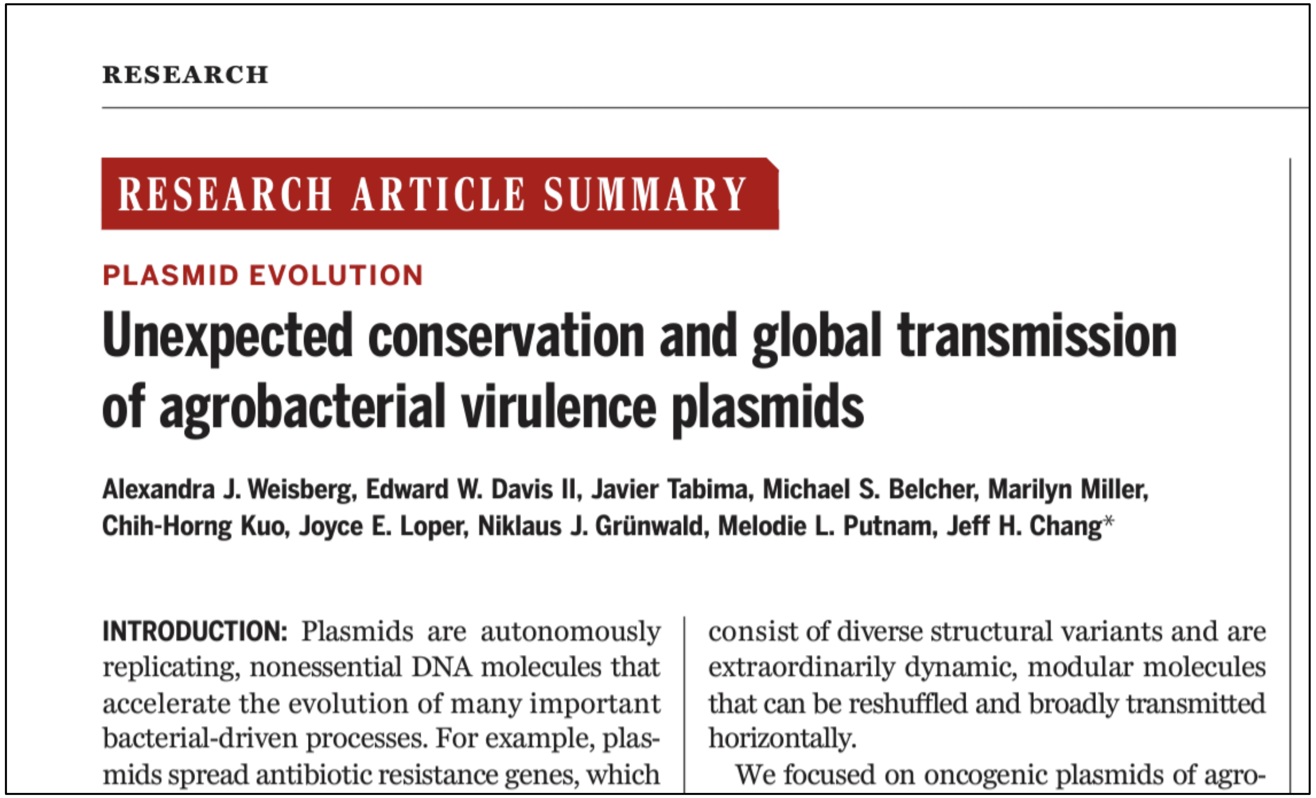
Altruistic transformation with 82.139 genes

Making this a useful tool

- **Delivery of the 82.139 genes is presently in our vir-delivered GAANTRY strain (ARS Albany, J. Thomson), this strain is aggressive, not an auxotroph**
- **We have mobilized the genes into binary-compatible vectors**
- **We aim to test in auxotrophic strains for general ease of use**
- **We have begun further testing to identify which genes are most critical for non-autonomous shoot induction**

Are there other useful development genes? Agro diversity hardly studied

We are starting to test ~300 fully sequenced wild Agrobacterium strains from the Chang lab at Oregon State, to look for increased virulence and shooty phenotypes in altruistic modes



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*

INTRODUCTION: Plasmids are autonomously replicating, nonessential DNA molecules that accelerate the evolution of many important bacterial-driven processes. For example, plasmids spread antibiotic resistance genes, which consist of diverse structural variants and are extraordinarily dynamic, modular molecules that can be reshuffled and broadly transmitted horizontally.

We focused on oncogenic plasmids of agro-

BAYER

Grants
4Ag

Going forward

- Woody species, mature-clonal tissues, are tough and slow – poplars exceptional
- Not shown today: GRF-GIF and WUS results show there is *major league* genetic diversity in response to most everything we try – media amendments and DEV gene type, expression etc.
- Shooty Agro development genes, delivered altruistically, very promising transformation approach now being tested in multiple species

Thanks to our funders and collaborators

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United States
Department of
Agriculture

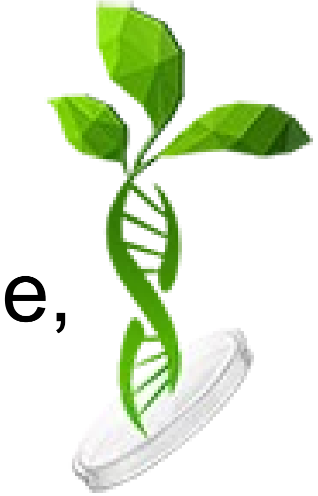
National Institute
of Food and
Agriculture

NSF

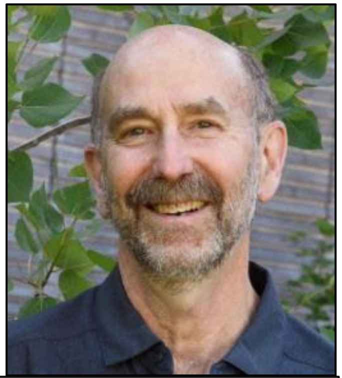
The National
Science Foundation

GREAT TREES Consortium

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



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



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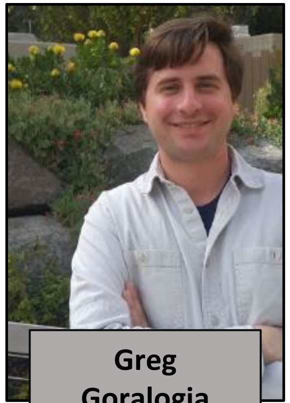
Biotechnology Program Manager



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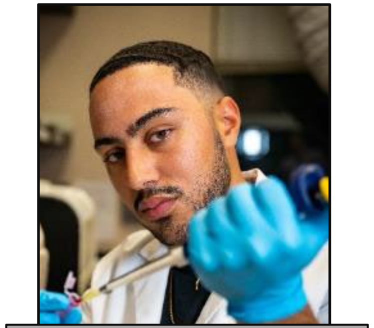

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