

Back to the future:  
**Altruistic use of Agrobacterium  
morphogenic genes to aid transformation of  
difficult plant species**

*Steve Strauss*

*College of Forestry, Oregon State University*



**Oregon State**  
University

# Prof. Bill Powell / 1956 - 2023

“Where there be mountains, there be chestnuts.”

De Soto's expedition, 1540



TEDx Do Extinction

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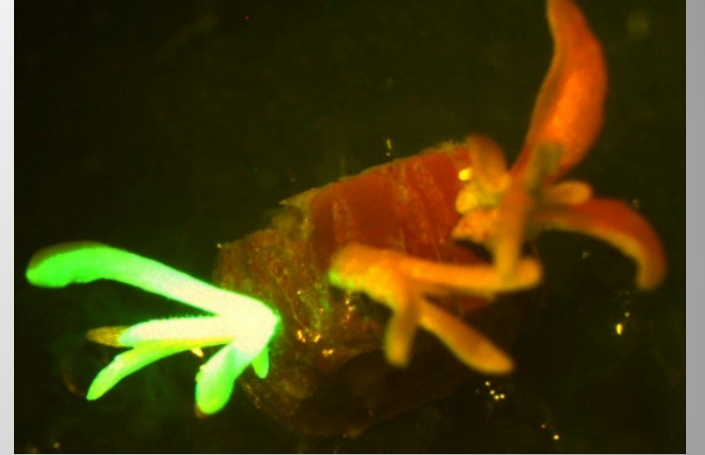
**Oregon State**  
University



**Greg Goralogia**  
Postdoc

# Agenda

- Perspectives & experimental system
- Experiences from some of the “DEV” genes we have tried, mostly unhappily
- “Shooty” morphogenic genes from *Agrobacterium*



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

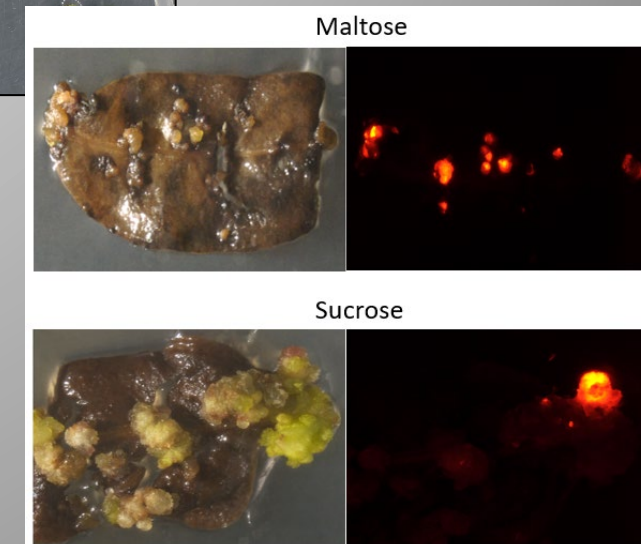
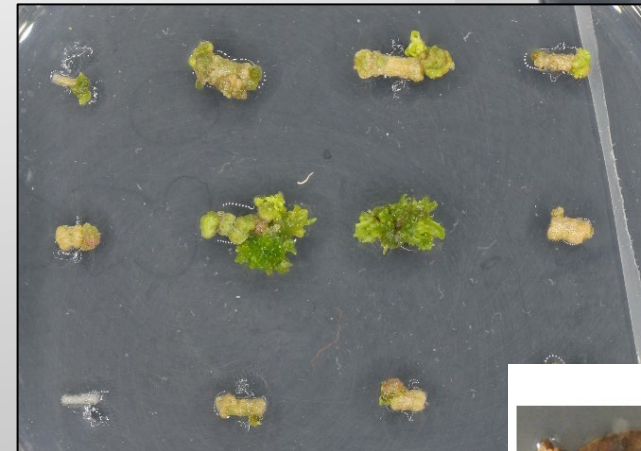
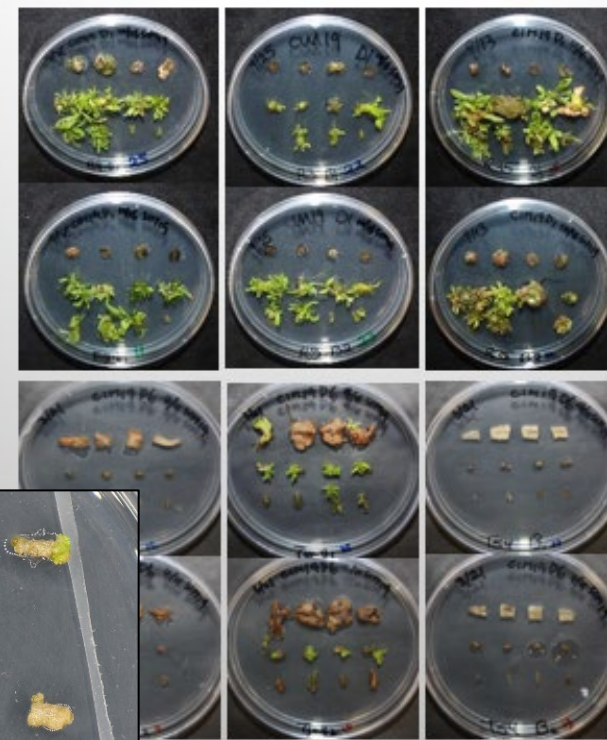


- Species and genotypic differences often dramatic
- Slow, costly, complex customization efforts usually needed
- On top of often large social/regulatory constraints, often a “deal breaker”

# Our experimental system features

- Woody (forest) trees – slow, tough biochemistry
- 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

In vivo



# Morphogenic, or “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<sup>1</sup>, Maren Arling<sup>1</sup>, Keith Lowe, George Hoerster, Scott Betts and Todd Jones

### Focus of GREAT TREES Coop:

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



### Ornamental Plant Research

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

Hui Duan<sup>1\*</sup>, Nathan A. Maren<sup>2</sup>, Thomas G. Ranney<sup>3</sup>, and Wusheng Liu<sup>2\*</sup>

<sup>1</sup> USDA-ARS, U.S. National Arboretum, Floral and Nursery Plants Research Unit, Beltsville Agricultural Research Center (BARC)-West, Beltsville, MD 20705, USA

<sup>2</sup> Department of Horticultural Science, North Carolina State University, Raleigh, NC 27607, USA

<sup>3</sup> 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

**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	<i>A. thaliana</i> root	High somatic embryo formation frequency	[15]
	Estrogen-inducible	<i>Nicotiana tabacum</i> leaf	Shoot formation from root tip	[20]
	35S	<i>Gossypium hirsutum</i> hypocotyl	Shoot formation from root tip	[16]
	vsp1	<i>Medicago truncatula</i> seedling radicle	47.75% increase in embryogenic callus formation	[18]
ZmWUS2	ZmPLTP	<i>Zea mays</i> immature embryo	Enhanced callogenesis and embryogenesis	[66]
	Nos	<i>A. thaliana</i> (seedling), <i>Solanum lycopersicum</i> (seedling), <i>N. tabacum</i> (seedling/mature plant), <i>Solanum tuberosum</i> (mature plant), <i>Vitis vinifera</i> (mature plant)	de novo meristem induction	[38]
AtWUS-GR, AtSTM-GR	35S	<i>A. thaliana</i> (floral dip)	Triggered ectopic organogenesis	[18]
AtWUS, CHAP3A (PmLEC1)	Estrogen-inducible	<i>Picea glauca</i> immature embryo	Did not induce somatic embryogenesis	[59]
eGFP-GhWUS1a, eGFP-GhWUS1b	Estrogen-inducible	<i>G. hirsutum</i> hypocotyl	Inhibited embryogenic callus formation	[60]
AtBBM, BnBBM	35S, inducible	<i>N. tabacum</i> leaf	Enhance the regeneration capacity	[24]
BcBBM	35S	<i>Populus tomentosa</i> calli	Plant regeneration through somatic embryogenesis	[25]
BnBBM	35S, HnUbB1	<i>A. thaliana</i> (floral dip) <i>B. napus</i> haploid embryo	Spontaneous formation of somatic embryos and cotyledon-like structures	[22]
BnBBM	35S	<i>Capsicum annuum</i> cotyledon	Made recalcitrant pepper transformable	[23]
EgAP2-1 (BBM)	35S	<i>A. thaliana</i> (floral dip)	Enhanced regeneration capacity	[63]
GmBBM1	35S	<i>A. thaliana</i> (floral dip)	Induced somatic embryos on vegetative organs	[64]
TcBBM	35S	<i>A. thaliana</i> (floral dip)	Enhanced/hormone-independent somatic	[65]
AtBBM-GR	35S	<i>A. thaliana</i> (floral dip)	Improved plant regeneration for extended periods of time in tissue culture	[62]
HvWUS, HvBBM	ZmAxig1, ZmPLTP	<i>Hordeum vulgare</i>	Co-expression increased transformation efficiency by 3 times	[61]
ZmBBM+ZmWUS2	ZmUbi, Nos	<i>Z. mays</i> immature embryo, mature embryo, seedling leaf segment; <i>Oryza sativa</i> calli; <i>Sorghum bicolor</i> immature embryo; <i>Saccharum officinarum</i> calli	Enabled transformation of recalcitrant varieties and/or increased transformation efficiency	[26–28]
	ZmAxig1, ZmPLTP	<i>Z. mays</i> immature embryo	Established rapid callus-free transformation	[29]
AtGRFs/BvGRFs-L	2x35S	<i>Beta. vulgaris</i> cotyledon, hypocotyl	Reduced genotype dependence, accelerated regeneration, increased transformation efficiency	[67]
AtGRFs/HaGRFs-L	2x35S	<i>Helianthus annuus</i> cotyledon	Enabled transformation of recalcitrant varieties. Increased transformation efficiency	[33]
GmGRFs-L	PcUbi4-2	<i>Glycine max</i> primary node	Improved transgenic shoot formation	
BnGRMs-L	PcUbi4-2	<i>B. napus</i> hypocotyl	Improved transgenic shoot formation	
ZmGRFs-L1/2	BdEF1	<i>Z. mays</i> immature embryo	Promoted callus production	
TaGRF4-GIF1	ZmUbi	<i>Triticum aestivum</i> immature embryo	Increased transformation efficiency ~3 times	
CIGRF4 <sup>1</sup> -GIF1/VVGRF4-GIF1	35S	<i>O. sativa</i> calli from seeds	Increased regeneration efficiency 7.8 times; shortened protocol	[34]
CIGRF4 <sup>2</sup> -GIF1	35S	<i>Citrus limon</i> etiolated epicotyl	Increased regeneration efficiency 2.1 times	
CIGRF4 <sup>3</sup> -GIF1	35S	<i>Citrus limon</i> , <sup>1</sup> <i>C. limon</i> , <sup>2</sup> <i>C. lanatus</i> ; Vv, <i>V. vinifera</i>	Increased regeneration efficiency ~4.7 times	
CIGRF4 <sup>4</sup> -GIF1	35S	<i>Citrus limon</i> etiolated epicotyl	Increased regeneration efficiency ~4.7 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, <sup>1</sup>*C. limon*, <sup>2</sup>*C. lanatus*; Vv, *V. vinifera*.

<https://doi.org/10.48130/OPR-2022-0004>

Ornamental Plant Research 2022, 2: 4

# What are DEV genes?

- Many names in literature – including “morphogenic genes”
- **DEV gene** = any gene whose expression is useful in promoting the transformation or regeneration (TR) of transgenic or gene-edited tissues
- Genes derived from basic studies of development and pathology
- But use often deviate substantially from natural roles due to the radical interventions that are part of TR
- These include....
  - Redifferentiation from terminally differentiated somatic tissues
  - Wounding and pathogen attack (Agrobacterium)
  - Complexity of natural meristem / embryo / organ regeneration pathways
  - Interactions among all of the above



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

Developmental  
Genes For  
Transformation


Woody Plant  
Universal GE /  
Editing Systems

Transgene  
Removal

Vector Tools  
and Systems

JOURNAL ARTICLE

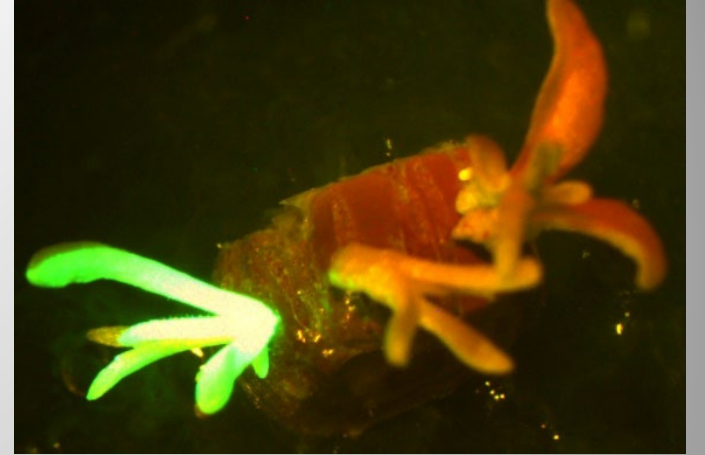
### GWAS identifies candidate genes controlling adventitious rooting in *Populus trichocarpa*

Michael F Nagle , Jialin Yuan, Damanpreet Kaur, Cathleen Ma, Ekaterina Peremyslova, Yuan Jiang, Bahiya Zahl, Alexa Niño de Rivera, Wellington Muchero, Li Fuxin ... [Show more](#)

*Horticulture Research*, Volume 10, Issue 8, August 2023, uhad125,  
<https://doi.org/10.1093/hr/uhad125>

# Agenda

- Perspectives & experimental system
- Experiences from some of the “DEV” genes we have tried, mostly unhappily
- “Shooty” morphogenic genes from Agrobacterium



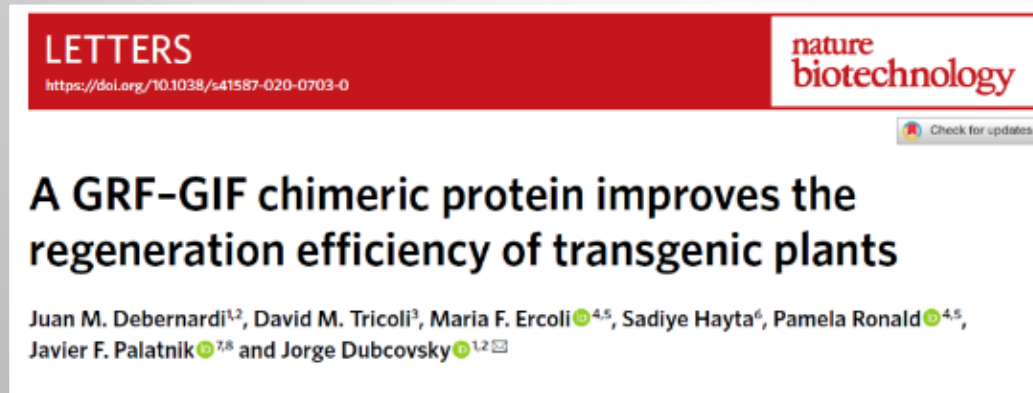
Poplar or eucalypt organogenesis: Most have failed with simple overexpression, or given highly genotype-specific enhancement, *or inhibition*

- *LEC 1, 2 – LEAFY COTYLEDON*
- *EBB1 - EARLY BUD BREAK 1* (ESR family)
- *BBM – BABY BOOM*
- *WOX 5, 11 -- WUSCHEL RELATED HOMEODOMAIN*
- *WUS – WUSCHEL*
- *IPT – ISOPENTYL TRANSFERASE* (cytokinin) – Agrobacterium
- Agrobacterium hormone biosynthesis/signaling genes
  - *Tumefaciens and rhizogenes origins*
- *GRF-GIF – GROWTH REGULATOR FACTOR 4 and GRF INTERACTING FACTOR 1*

## A few have shown some promise

- *LEC 1, 2 – LEAFY COTYLEDON*
- *EBB1 - EARLY BUD BREAK 1* (ESR family)
- *BBM – BABY BOOM*
- *WOX 5, 11 -- WUSCHEL RELATED HOMEODOMAIN*
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- *IPT – ISOPENTYL TRANSFERASE* (cytokinin) – Agrobacterium
- **Agrobacterium hormone biosynthesis/signaling genes**
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- ***GRF-GIF – GROWTH REGULATOR FACTOR 4 and GRF INTERACTING FACTOR 1***

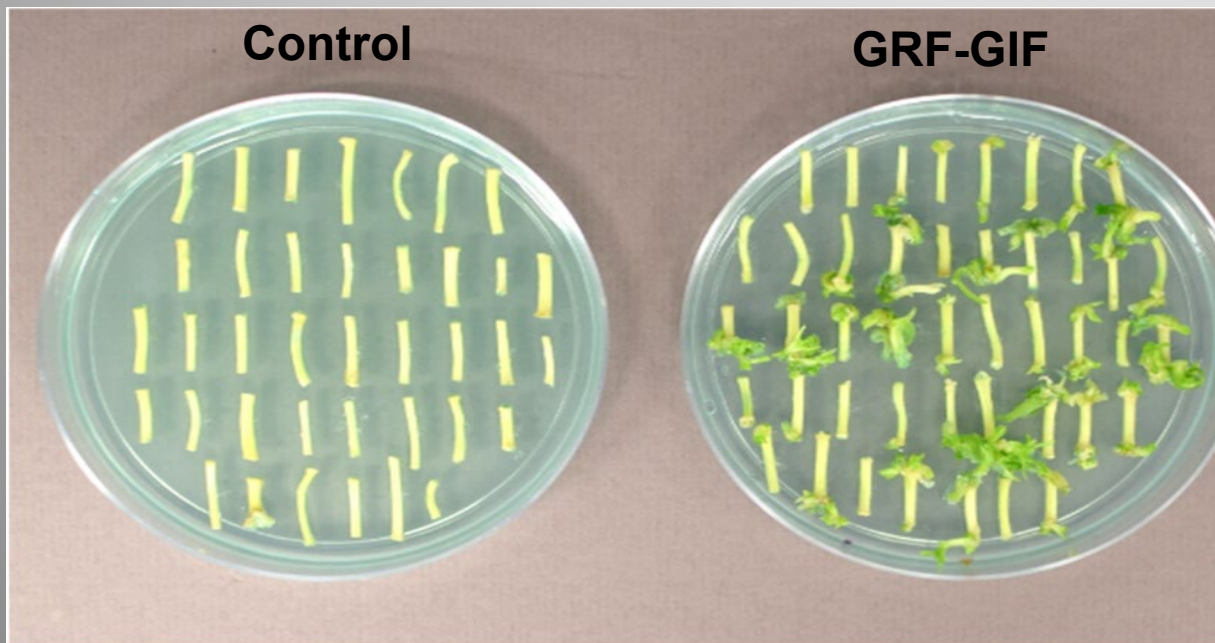
# GRF-GIF with much encouraging results in recent years



A chimeric protein comprised of *GROWTH-REGULATING FACTOR (GRF)* and *GRF-INTERACTING FACTOR (GIF)*

GRF & GIF interact with **chromatin remodeling** machinery and regulate transcription of meristem development genes

Nathan Ryan studied a wide variety of GRF-GIF homologs & sources, promoters, and miRNA sensitivities in poplar and eucalypts ([MS thesis 2022](#))

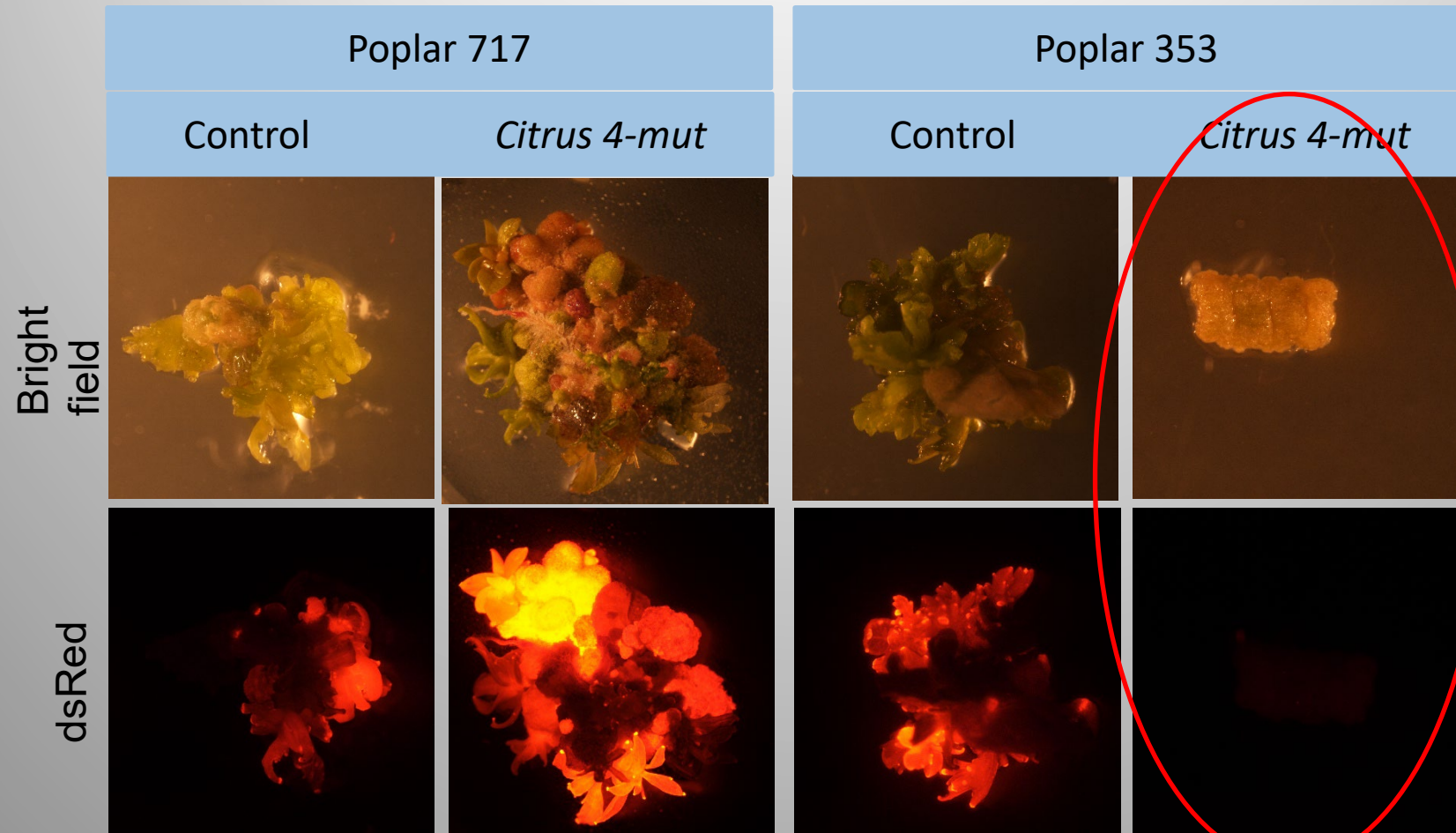


Citrus epicotyl explants; Debernardi et al., 2020



# Two poplar genotypes displayed very different callus responses to *Citrus 4-mut* GRF-GIF overexpression

717=*Populus tremula x alba* / 353 = *P. tremula x tremuloides*



# An ortholog of GRF-GIF from *Populus* doubled shoot regeneration in poplar 717 (single 35S promoter)

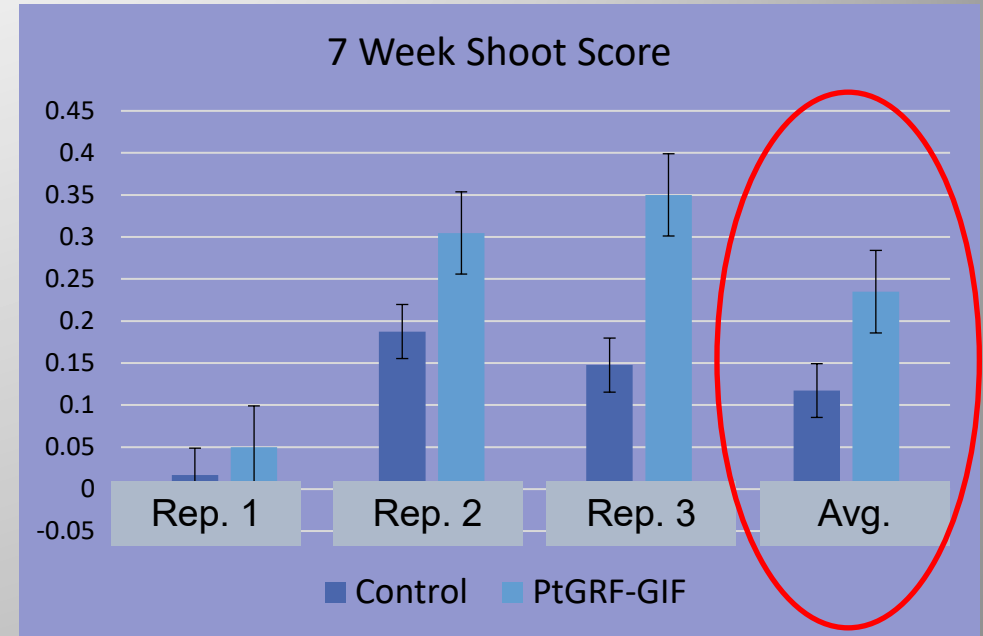
Control



1x35S::GRF4-GIF1



 = dsRed positive shoot

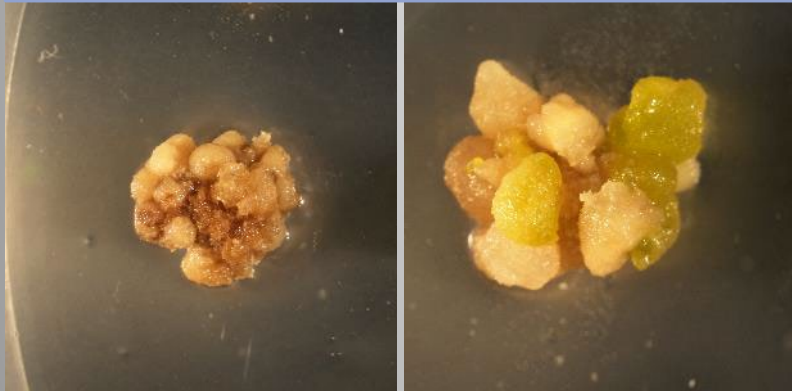


Error bars = SE

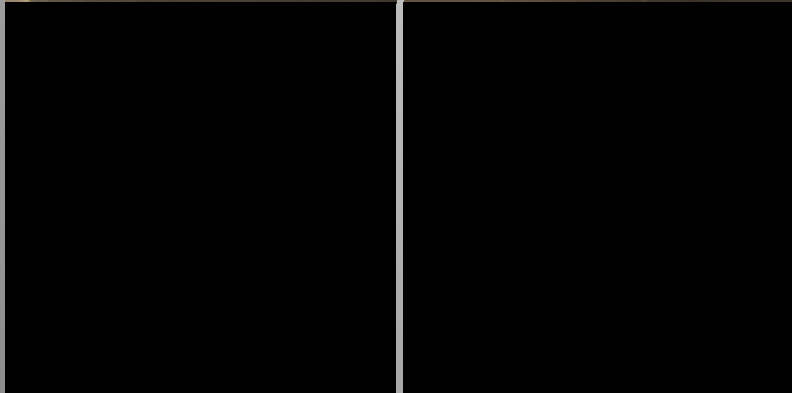
# *Populus* GRF-GIF also promoted shoot regeneration in recalcitrant *P. alba* clone '6K10'

Control (dsRed + *hpt*)

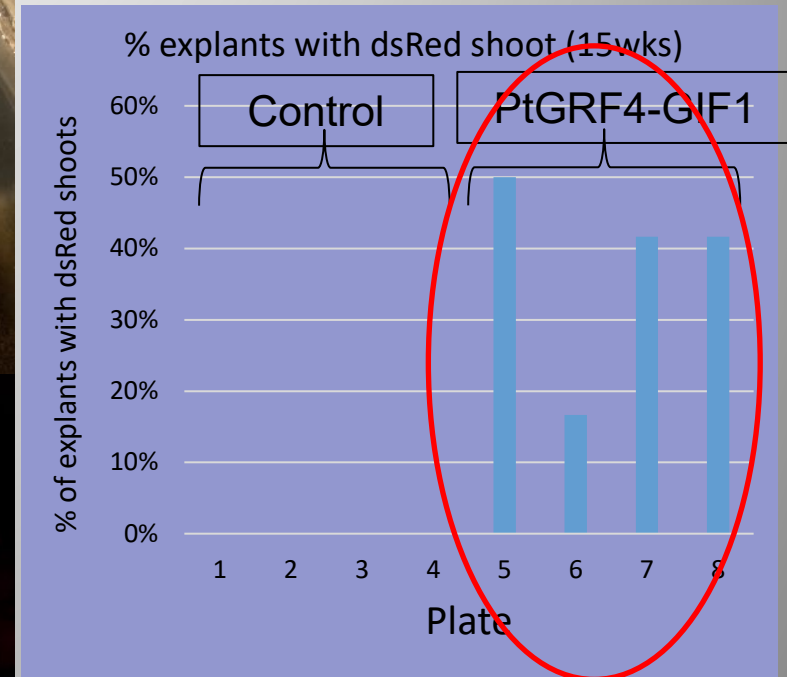
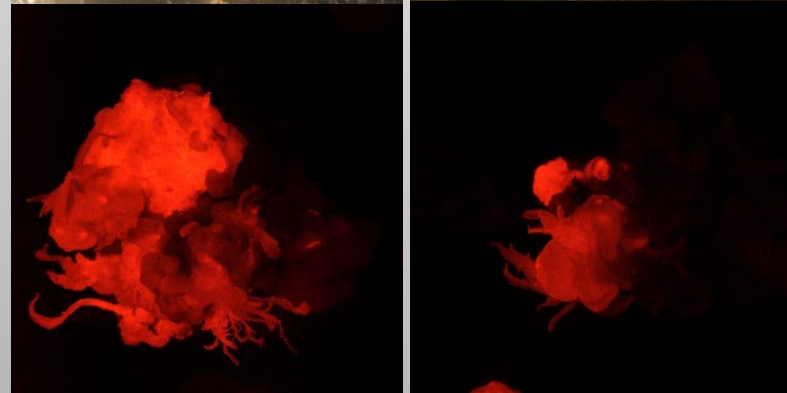
Bright field



dsRed



2x35S:PtGRF4-GIF1



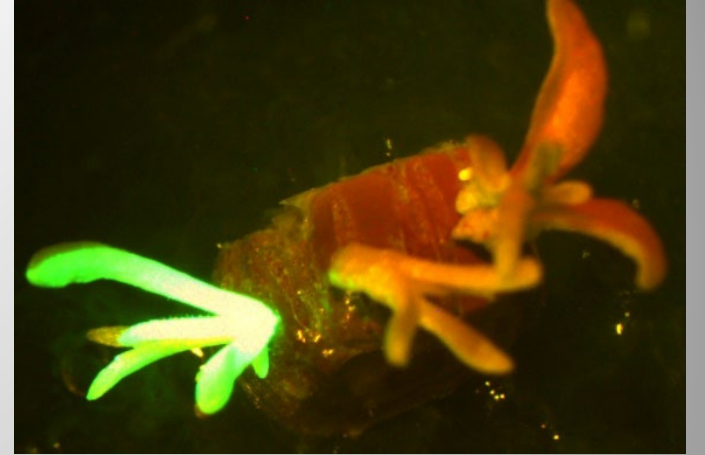


# GRF-GIF experience to date

- Gene source matters
- Degree of miRNA sensitivity matters
- Promoter matters
- Plant genotype matters in big way
- Induction of expression seems wise, but did not solve the genotype problem (glucocorticoid system)
- So far no general solutions to how to use it in poplar (or eucalypts) – today like one more medium/hormone customization tool
- The details
  - Ryan N.W.. 2022. Overexpression of the GROWTH REGULATING FACTOR 4-GRF-INTERACTING FACTOR 1 Transcription Factor Chimera Modifies Transformation and Regeneration Efficiency in Populus and Eucalyptus. Masters Thesis, Forest Ecosystems and Society. [Online](#) | Full Text: [PDF](#)

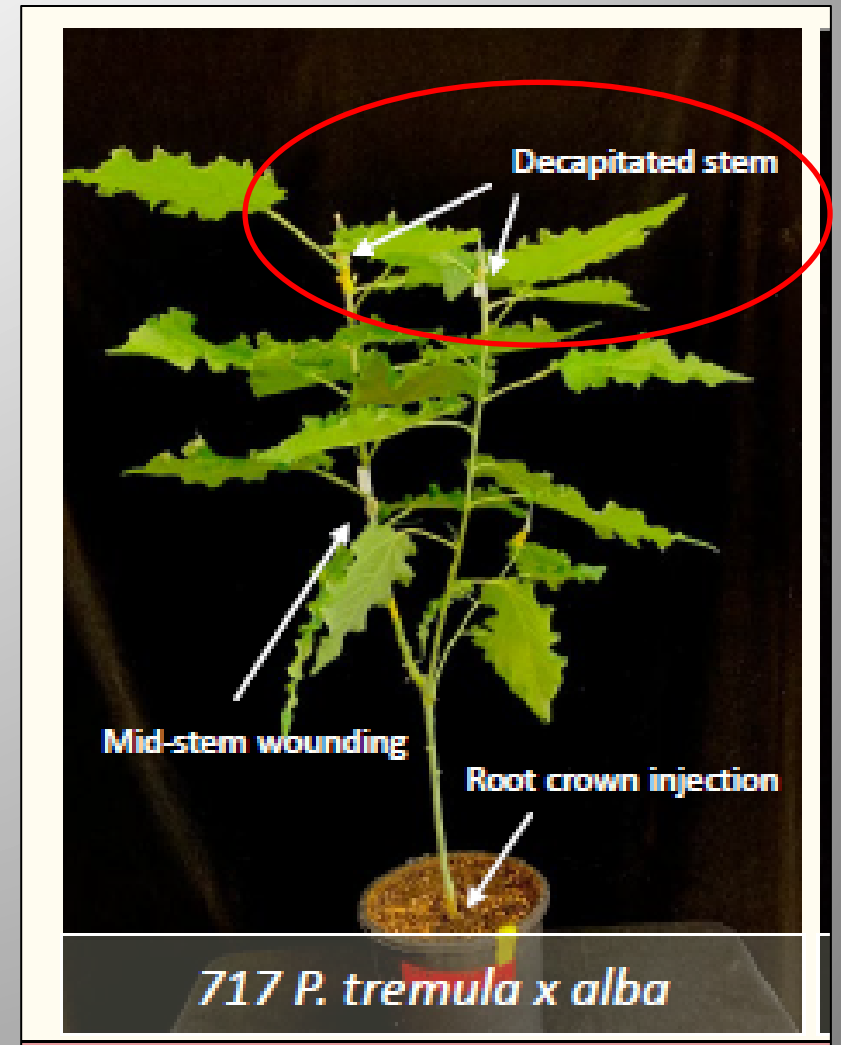
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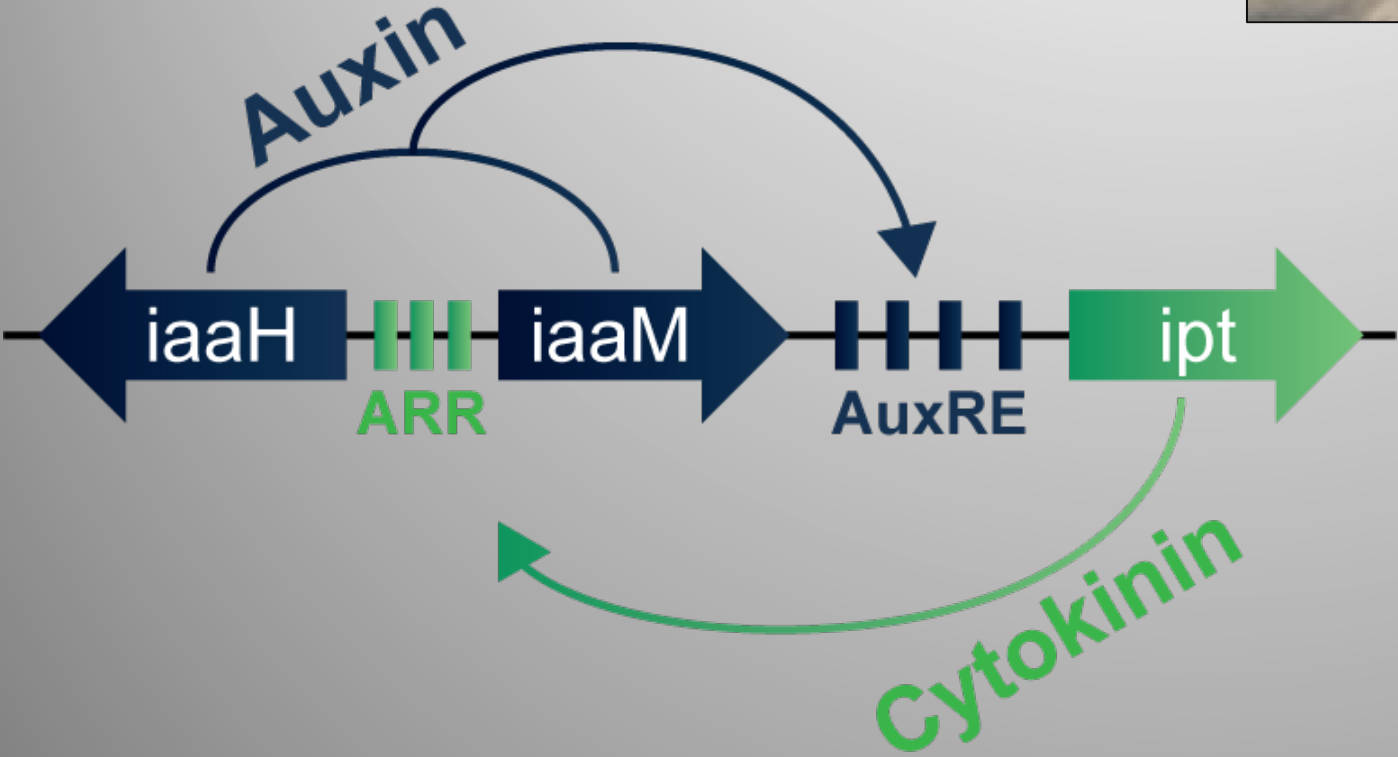


# *In planta* transformation of great interest

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

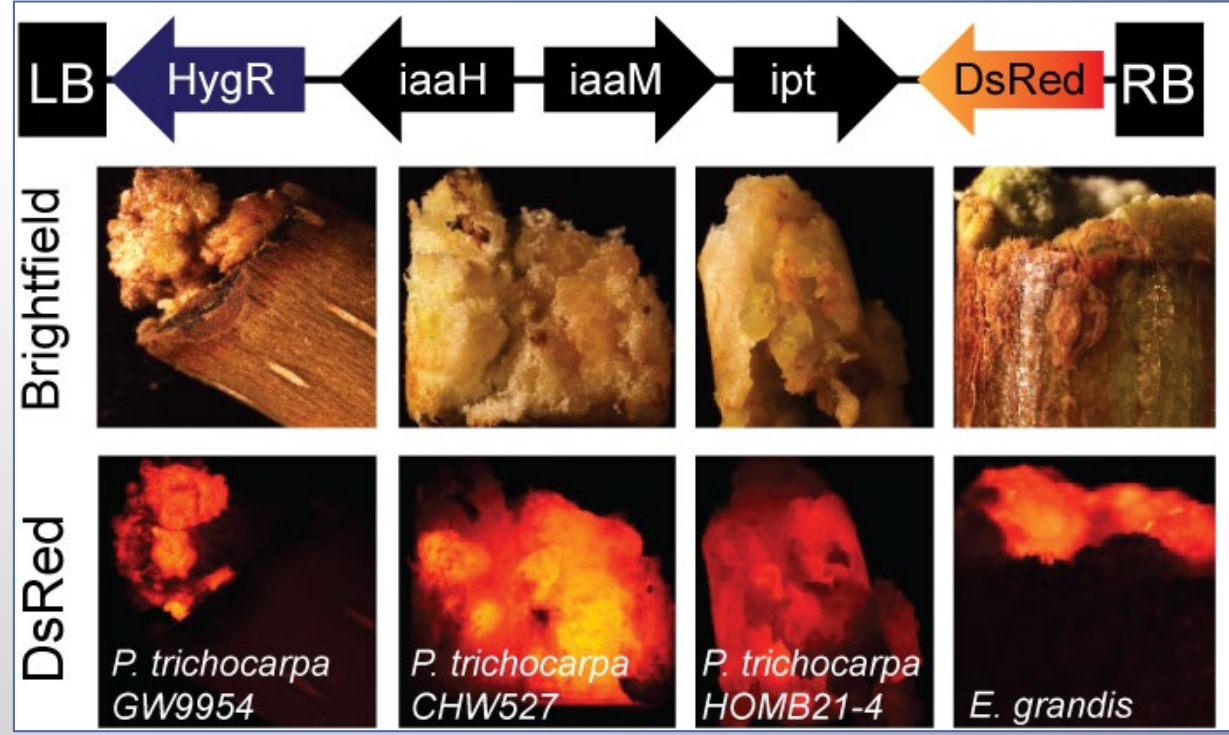
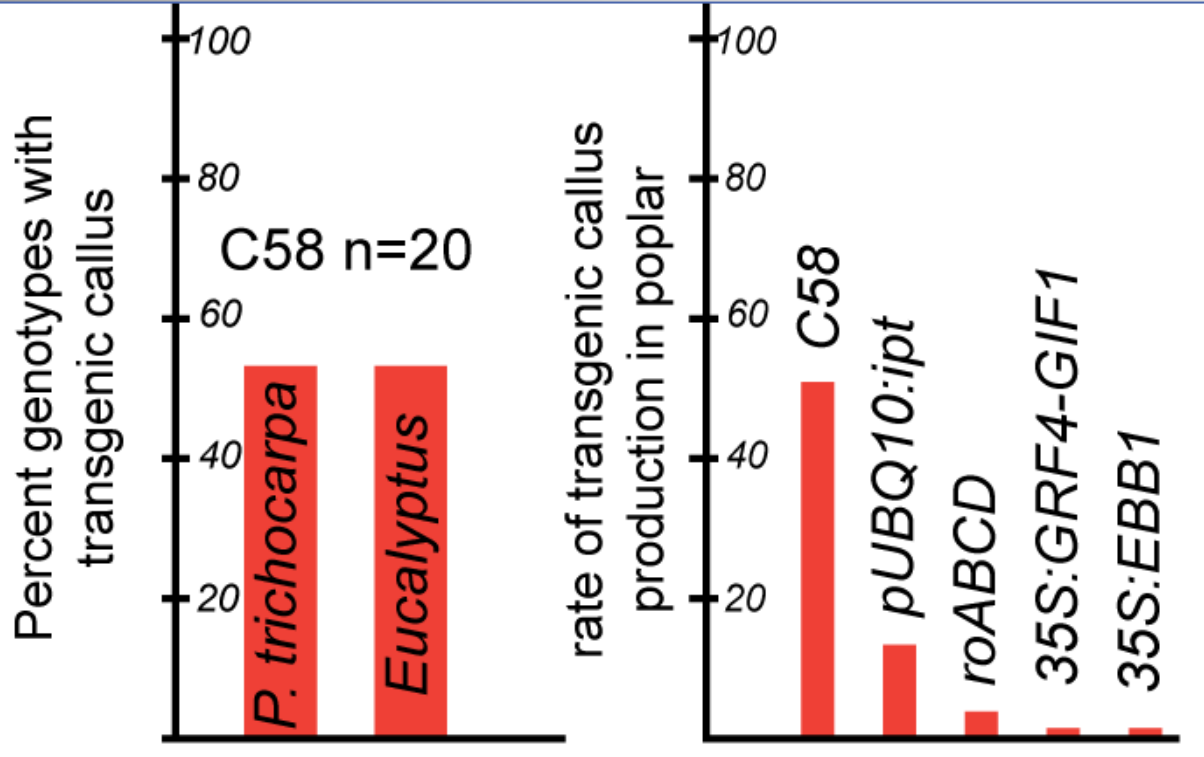


# Back to the future: *A. tumefaciens* DEV genes that promote gall development



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

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

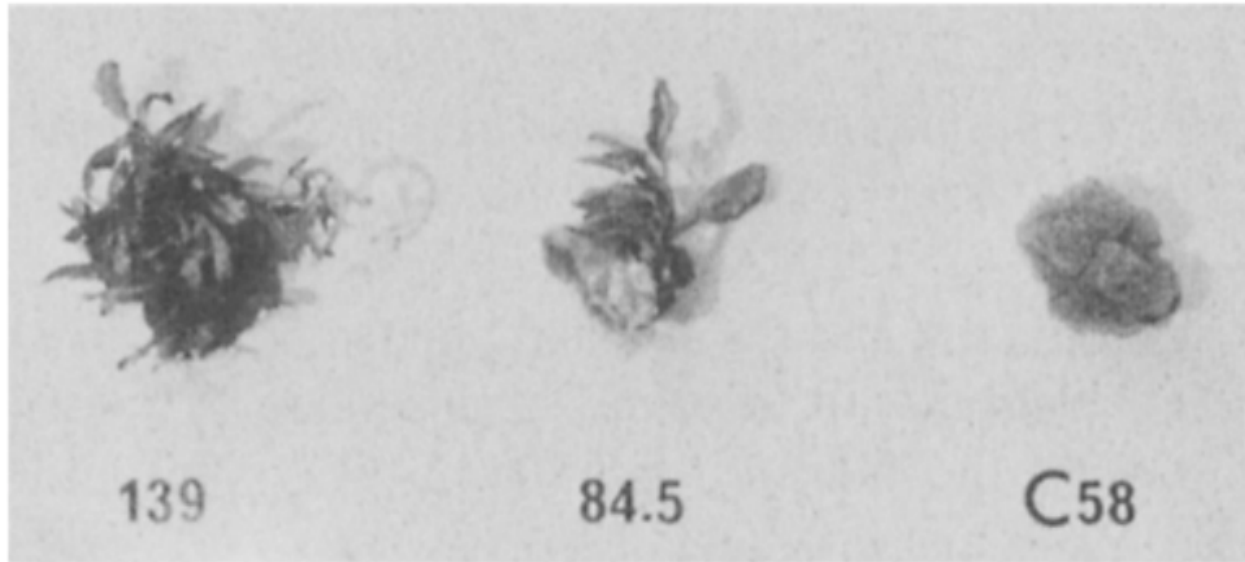


**But shoots could not be regenerated from transgenic galls**



# 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



*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.

*Plant Molecular Biology* 17: 441–452, 1991.  
© 1991 Kluwer Academic Publishers. Printed in Belgium.

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## An alternative approach for gene transfer in trees using wild-type *Agrobacterium* strains<sup>†</sup>

Ana Cristina Miranda Brasileiro<sup>1</sup>, Jean-Charles Leplé<sup>2</sup>, Joris Muzzin<sup>2,3</sup>, Dalila Ounnoughi<sup>2</sup>, Marie-France Michel<sup>2†</sup> and Lise Jouanin<sup>1\*</sup>

<sup>1</sup>Laboratoire de Biologie Cellulaire, INRA, route de Saint-Cyr, F-78026 Versailles Cedex, France (\* author for correspondence); <sup>2</sup>Station d'Amélioration des Arbres Forestiers, INRA, Ardon, F-45160 Olivet, France; <sup>3</sup>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

# Co-transformation is an old idea, either intentional or a limitation of the technology at the time

 © 1986 Nature Publishing Group <http://www.nature.com/naturebiotechnology>

## **CO-TRANSFORMATION OF UNLINKED FOREIGN GENES INTO PLANTS BY DIRECT GENE TRANSFER**

**R. J. Schocher, R. D. Shillito<sup>#</sup>, M. W. Saul, J. Paszkowski and I. Potrykus<sup>\*</sup>**

Friedrich Miescher-Institut, P.O. Box 2543, CH-4002 Basel, Switzerland. <sup>\*</sup>To whom reprint requests should be sent. <sup>#</sup>Present address: Ciba Geigy Corp., P.O. Box 12257, Research Triangle Park, Raleigh, NC 27709-2257.

The EMBO Journal vol.4 no.2 pp.277–284, 1985

## **New cloning vehicles for transformation of higher plants**

**G.An<sup>1</sup>, B.D.Watson<sup>1,2</sup>, S.Stachel<sup>3,4</sup>, M.P.Gordon<sup>2</sup> and E.W.Nester<sup>3</sup>**

<sup>1</sup>Institute of Biological Chemistry, Washington State University, Pullman, WA 99164-6340, <sup>2</sup>Department of Biochemistry, and <sup>3</sup>Department of Microbiology and Immunology, University of Washington, Seattle, WA 98195, and <sup>4</sup>Department of Biochemistry and Biophysics, University of California, San Francisco, CA 94143, USA

**We demonstrate that DNA cloned into these vectors in *A. tumefaciens* can efficiently transform plants when in *trans* with a wild-type Ti plasmid which donates the functions necessary for DNA transfer and integration. We also show that only the right border of the T-DNA is necessary for DNA transformation.**

# The method reportedly worked to some degree in *Eucalyptus* and birch, using the wild strain

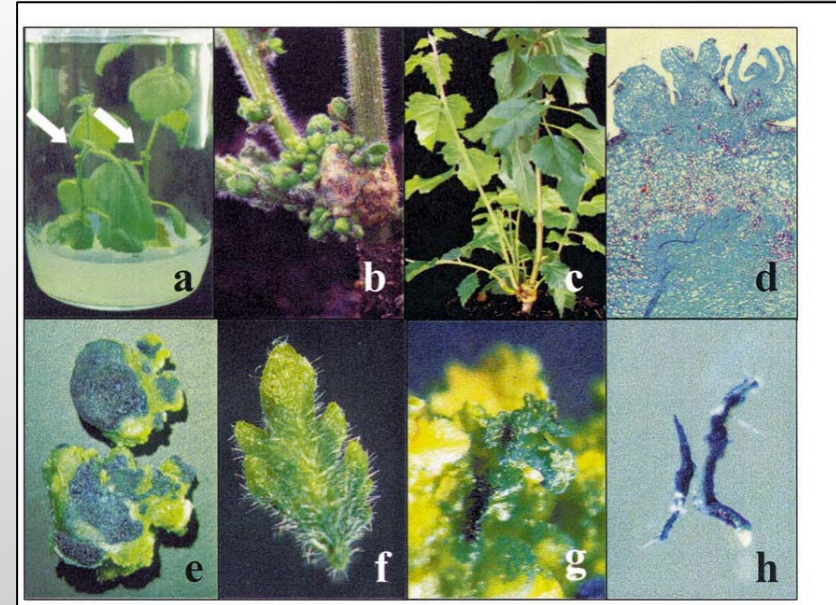
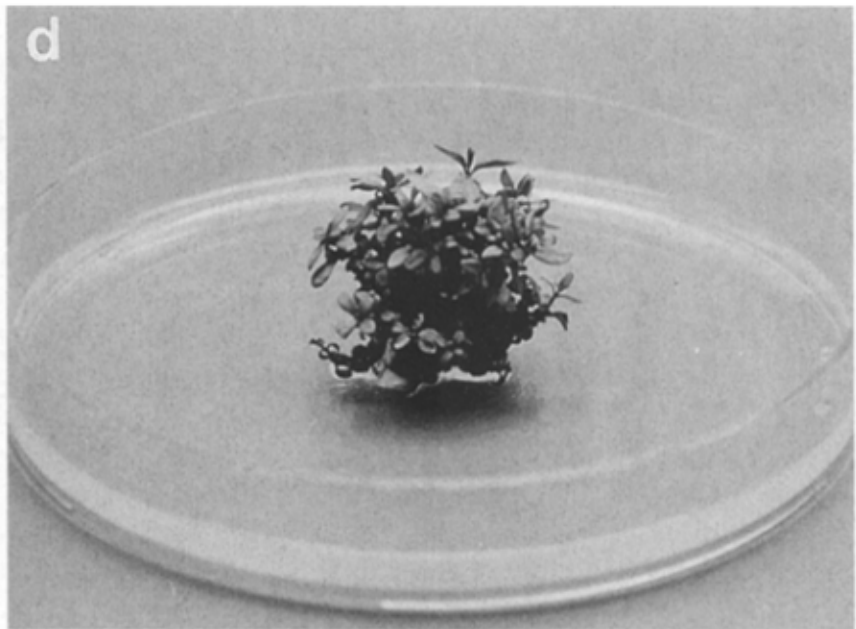
## *Agrobacterium* strain specificity and shooty tumour formation in eucalypt (*Eucalyptus grandis* × *E. urophylla*)

Luciana de Oliveira R. Machado<sup>1</sup>, Gisele M. de Andrade<sup>1</sup>, Luis Pedro Barrueto Cid<sup>1</sup>, Ricardo M. Penchel<sup>2</sup>, and Ana Cristina M. Brasileiro<sup>1</sup>

<sup>1</sup> Área de Biologia Celular, CENARGEN/EMBRAPA. C.P. 02372, 70.849-970 Brasília – DF, Brazil

<sup>2</sup> Aracruz Celulose S. A. Rua Prof. Lobo, 1128, 29.190-000 Aracruz – ES, Brazil

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.  
© 2002 Kluwer Academic Publishers. Printed in the Netherlands.

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## Applicability of the co-inoculation technique using *Agrobacterium tumefaciens* shooty-tumour strain 82.139 in silver birch

Tuija S. Aronen<sup>1</sup>, Juhani H. Häggman<sup>1</sup> & Hely M. Häggman<sup>1,2,\*</sup>

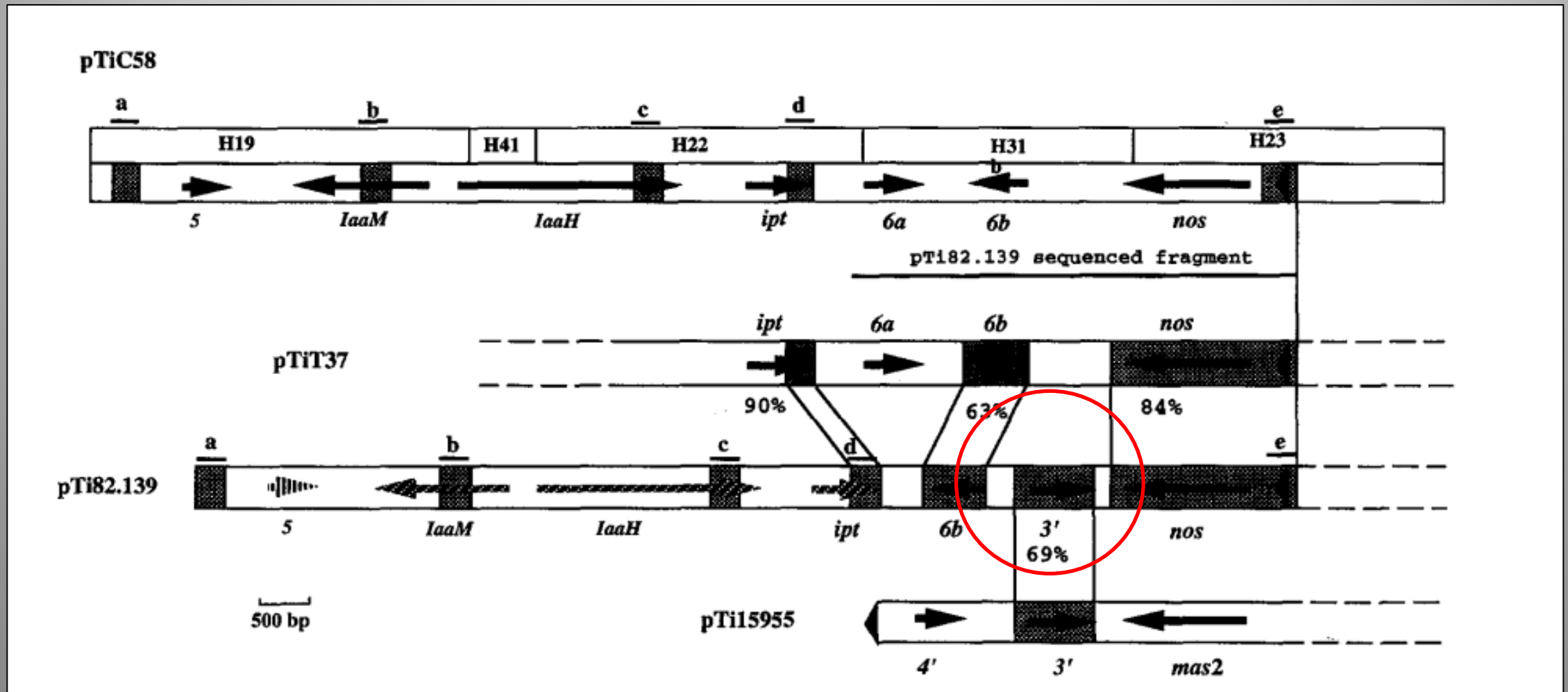
<sup>1</sup>Finnish Forest Research Institute, Punkaharju Research Station, Finlandiantie 18, FIN-58450 Punkaharju, Finland; <sup>2</sup>University of Oulu, Department of Biology, PO Box 3000, FIN-90014 Oulu, Finland (\*requests for offprints; Fax: +358-08-5531061; E-mail: hely.haggman@oulu.fi)

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

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



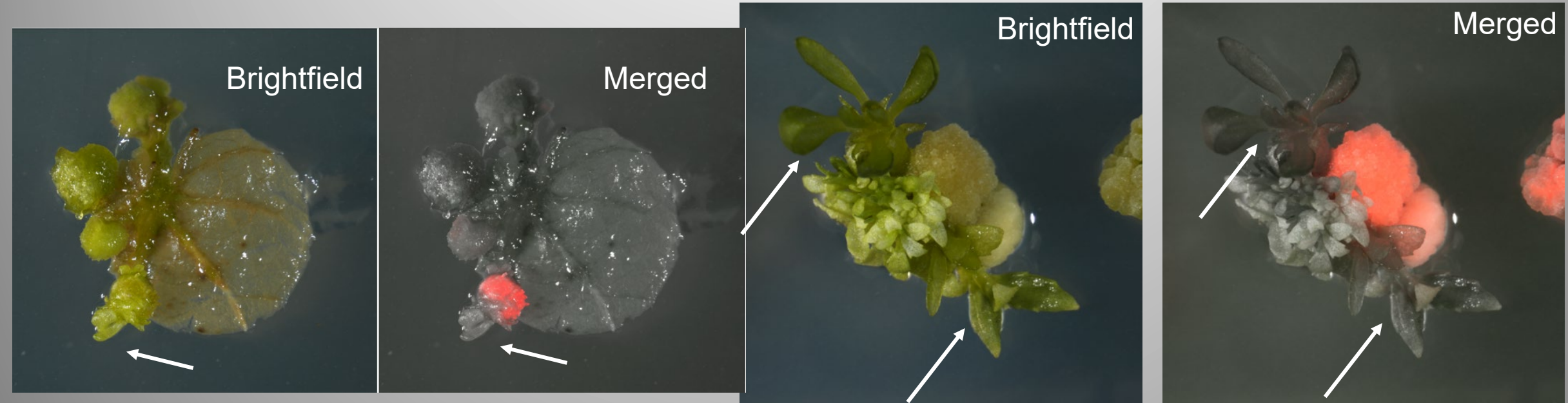
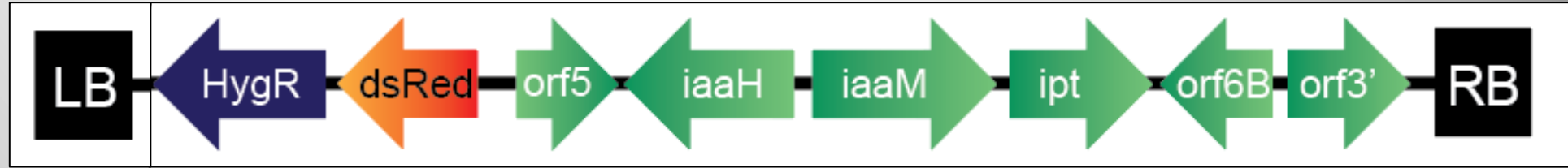
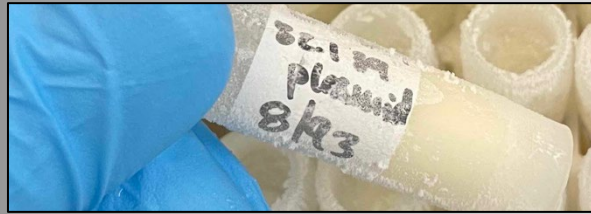
This strain has several genes added compared to C58 due to a recombination event, although expression of *iaa/ipt* could also be different



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 DEV genes prior to high throughput sequencing and advanced gene cloning systems

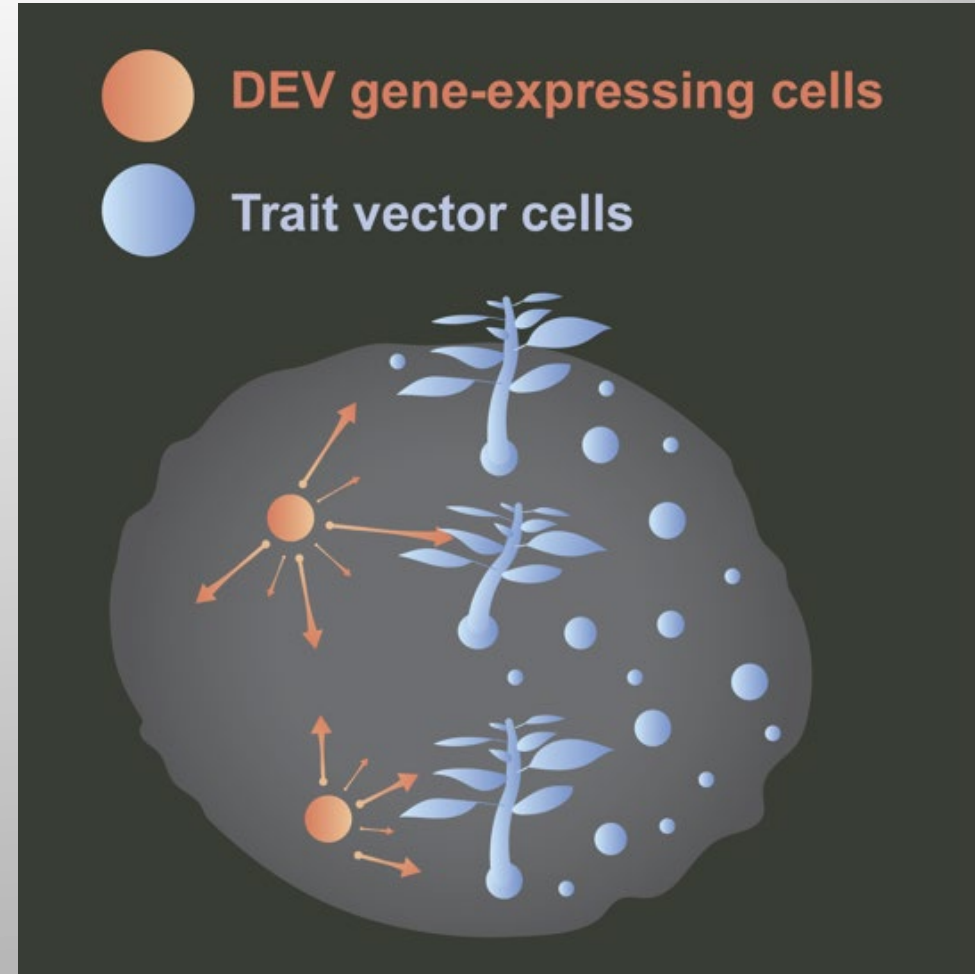
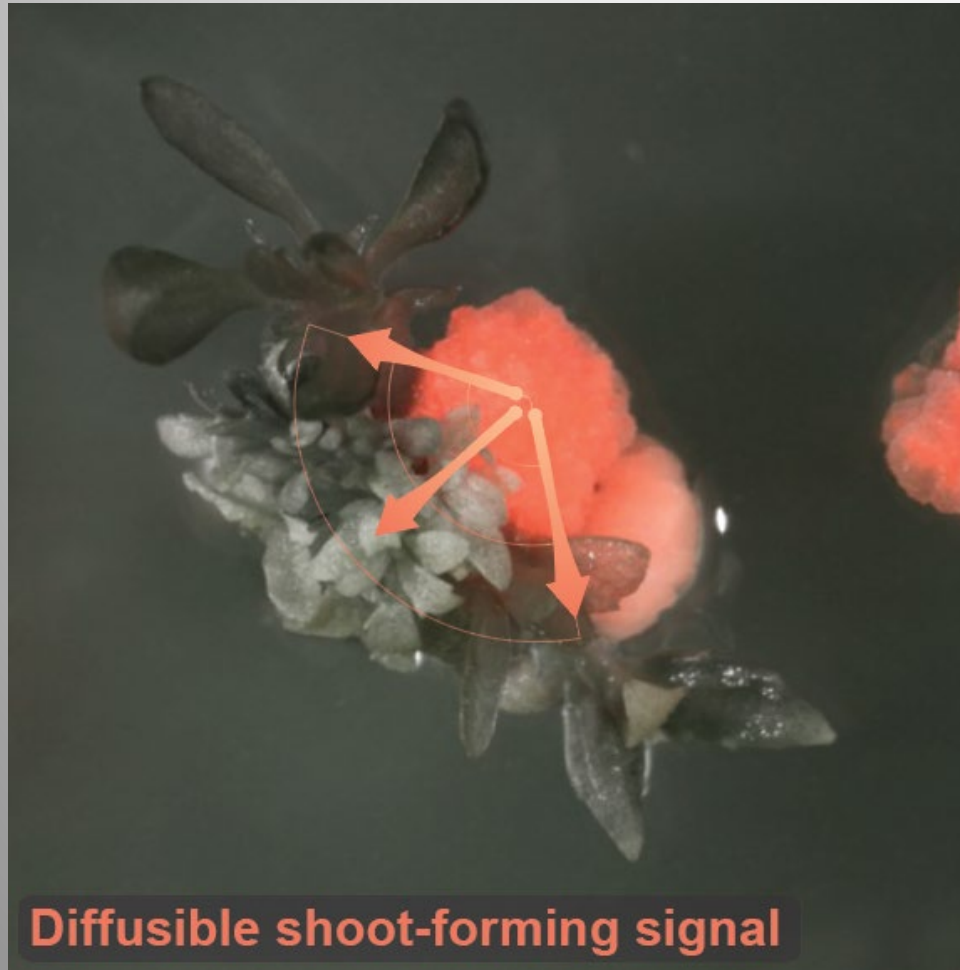


We cloned out the DEV genes from our resurrected clone in deep freeze, and added modern amenities like DsRed (called "S82")



The morphogenic properties of the intact strain were preserved: transgenic galls promoted altruistic regeneration of galls and shoots

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





# Altruistic methods regarded as a key option for DEV gene application



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

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\* Correspondence: william.gordon-kamm@pioneer.com; Tel.: 515-535-3243

Received: 23 November 2018; Accepted: 31 January 2019; Published: 11 February 2019

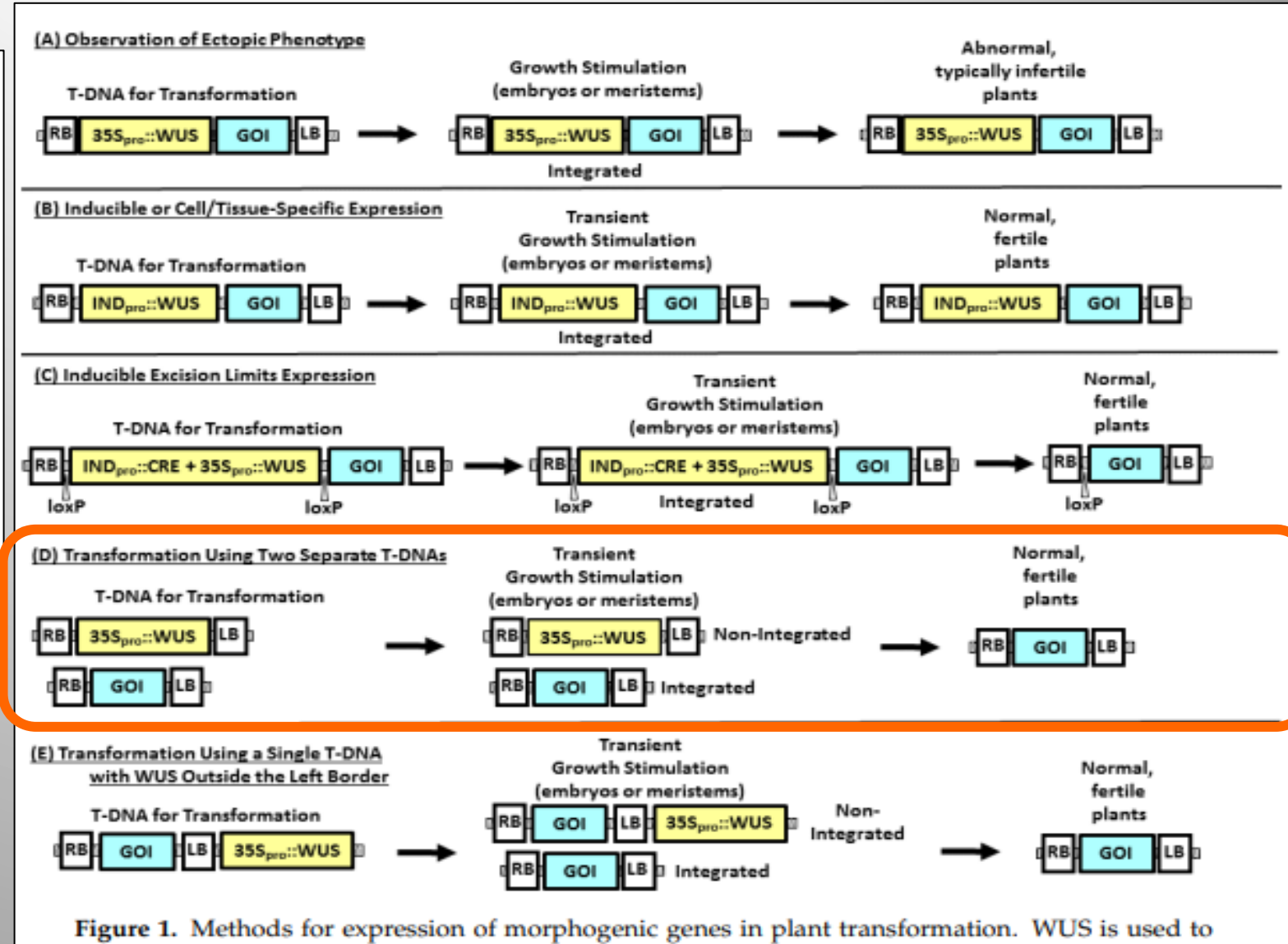


Figure 1. Methods for expression of morphogenic genes in plant transformation. WUS is used to

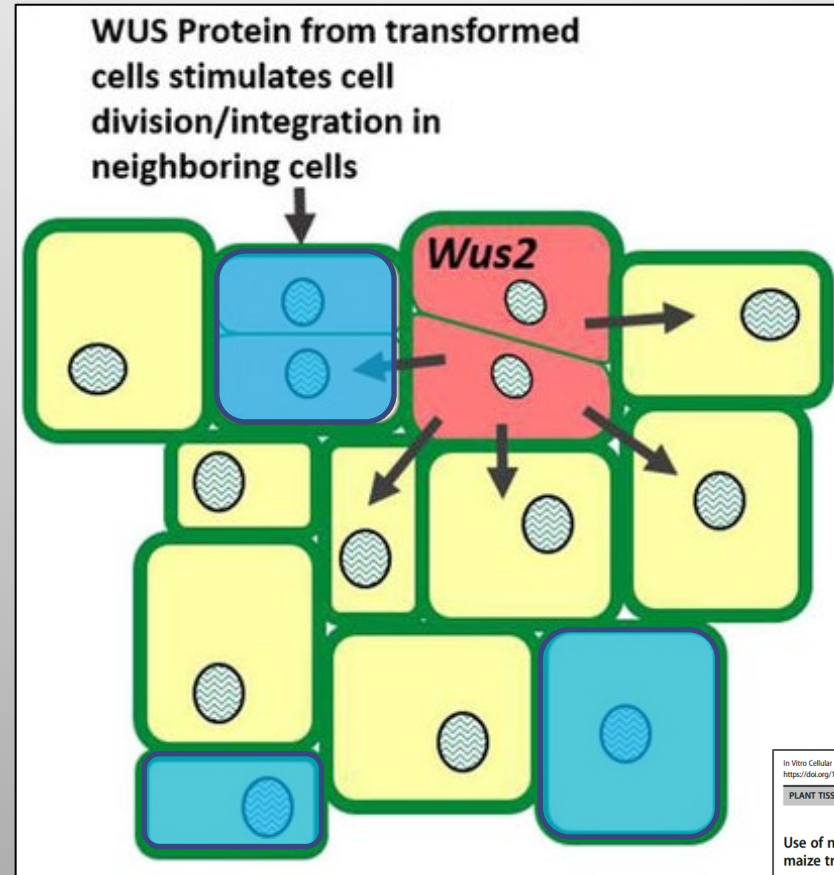
# “Altruistic” approach – minimizes pleiotropy, promotes diverse transcription factor/hormone gradients, and beneficial cell-cell interactions?

Mixed Agro in different ratios

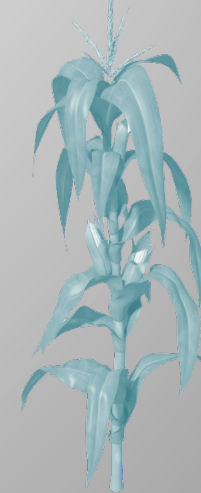
DEV gene Agro  
(like *WUS*)



Trait gene Agro



Plant with trait gene and no DEV genes



In Vitro Cellular & Developmental Biology - Plant  
<https://doi.org/10.1007/s11627-019-10042-2>

PLANT TISSUE CULTURE

Use of non-integrating *Zm-Wus2* vectors to enhance maize transformation

Non-integrating WUS2 enhances transformation

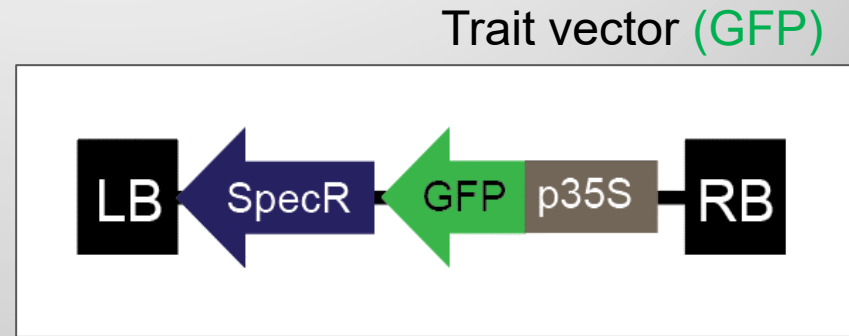
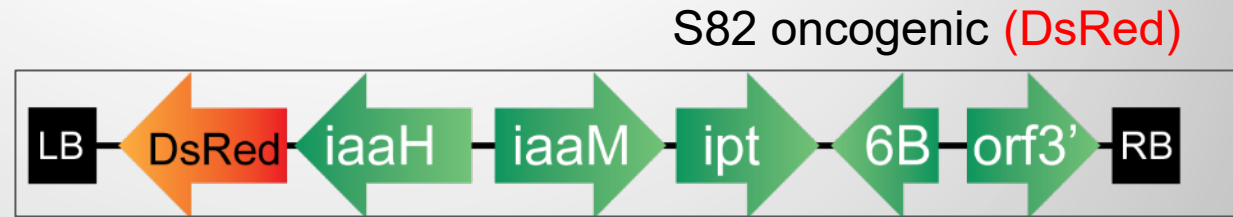
George Hoerster<sup>1</sup> · Ning Wang<sup>1</sup> · Larisa Ryan<sup>1</sup> · Emily Wu<sup>1</sup> · Ajith Anand<sup>1</sup> · Kevin McBride<sup>1</sup> · Keith Lowe<sup>1</sup> · Todd Jones<sup>1</sup> · Bill Gordon-Kamm<sup>1</sup>

Received: 23 May 2019 / Accepted: 9 December 2019 / Editor: Charles Armstrong  
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# Altruistic “S82” transformation in hybrid poplar

## 4 transformations

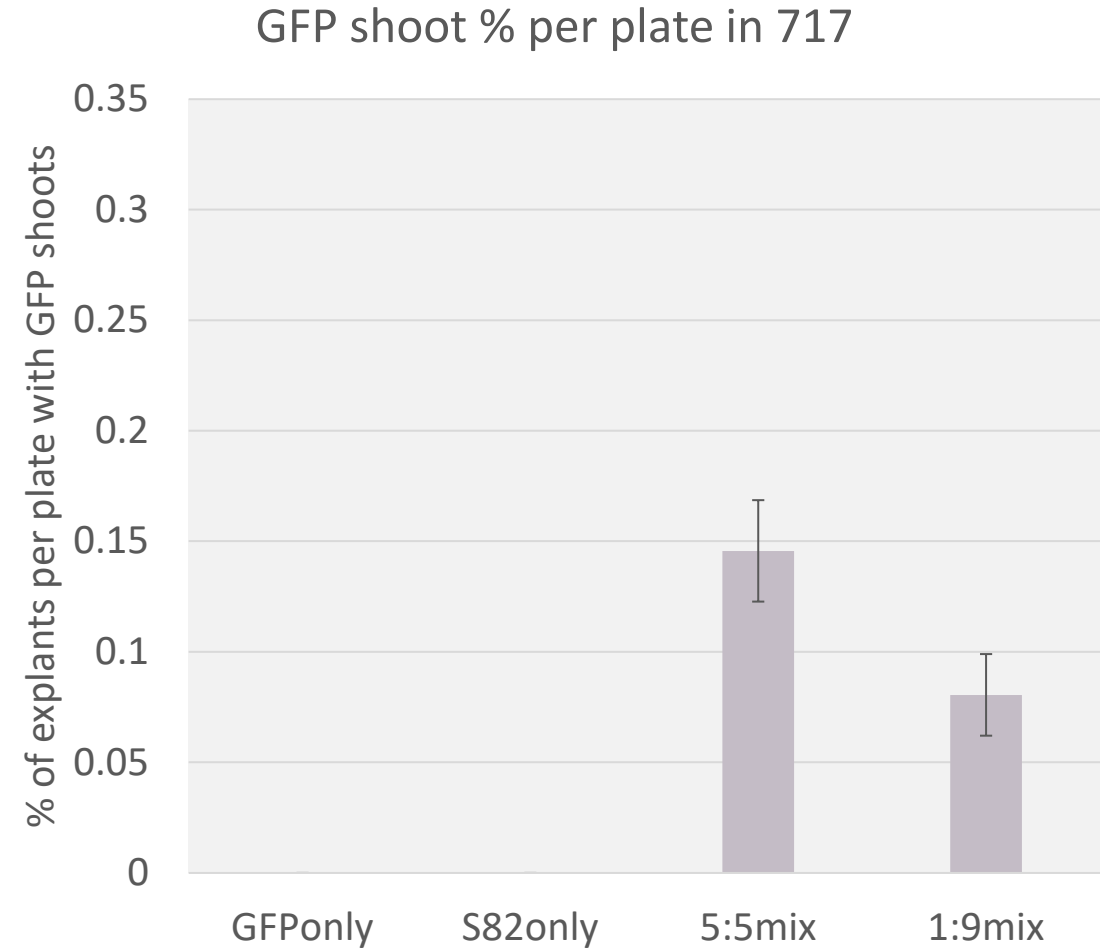
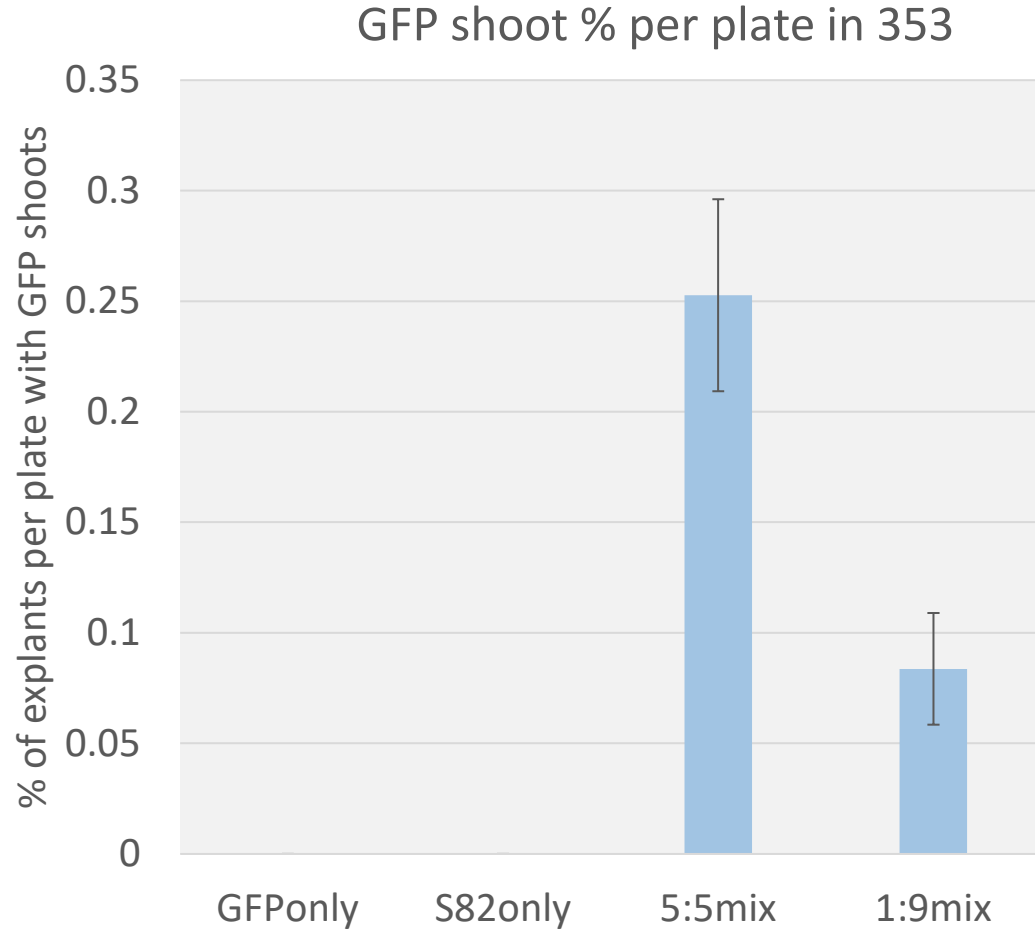
- 100% S82
- 50% S82 / 50% Trait-GFP
- 10% S82 / 90% Trait-GFP
- 100% Trait-GFP



**No hormones to induce regeneration**

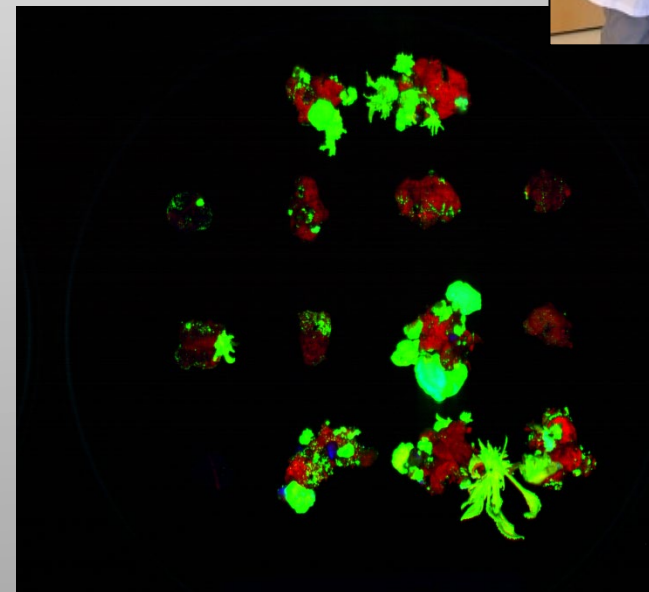
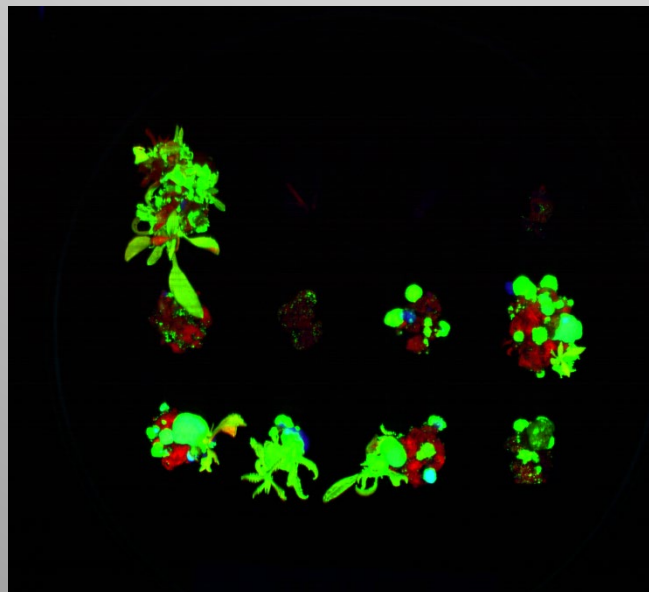
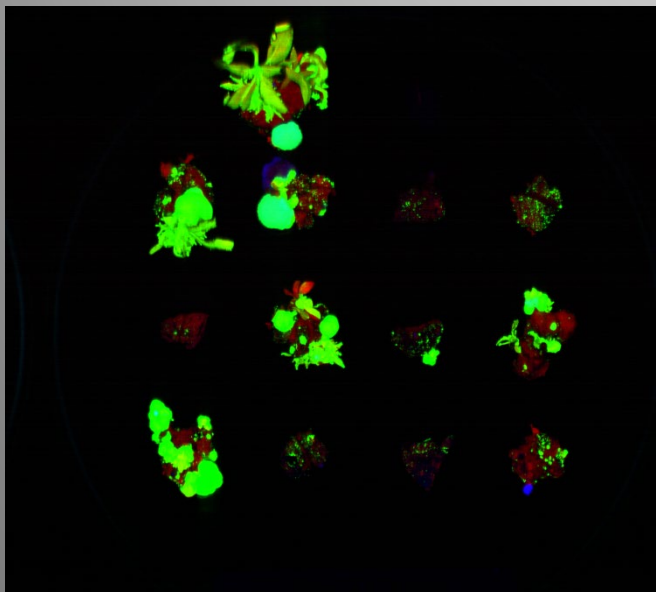
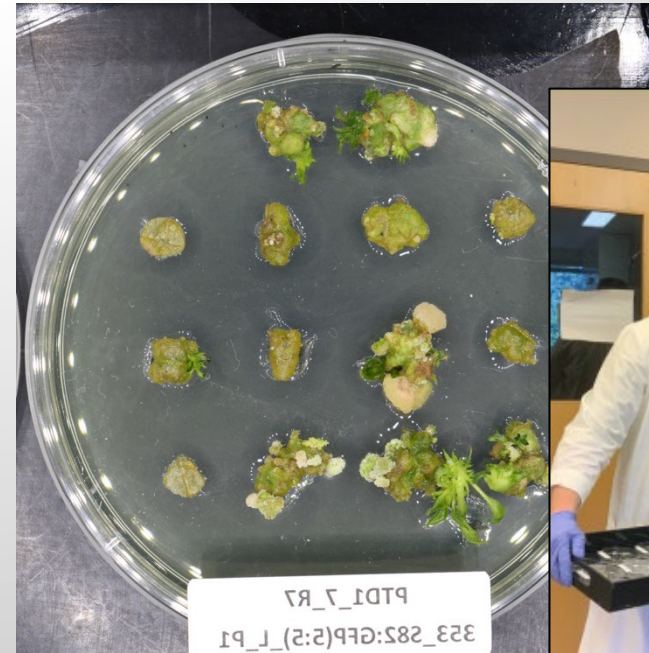
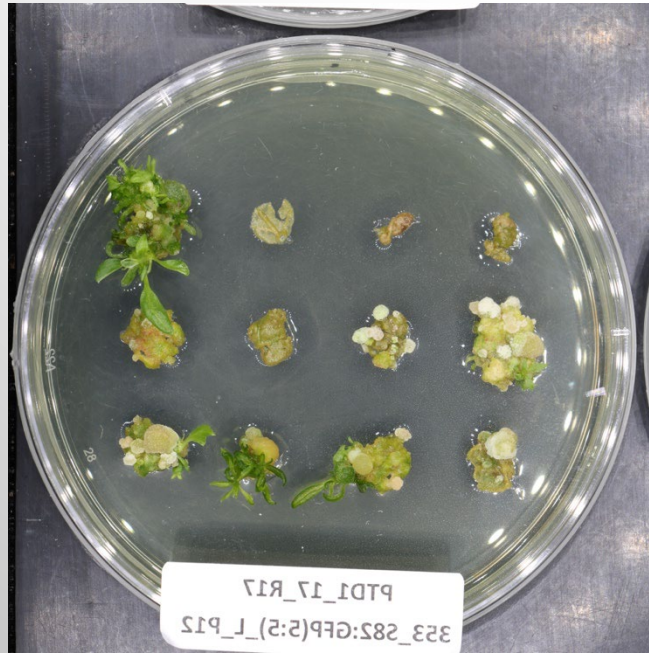
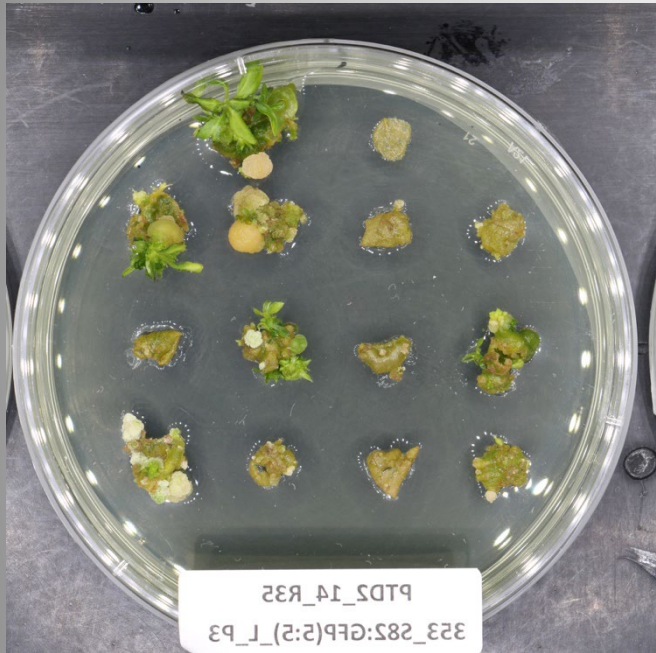
**Only spec selection**

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





# Hyperspectral imaging showed transgenic shoot regeneration

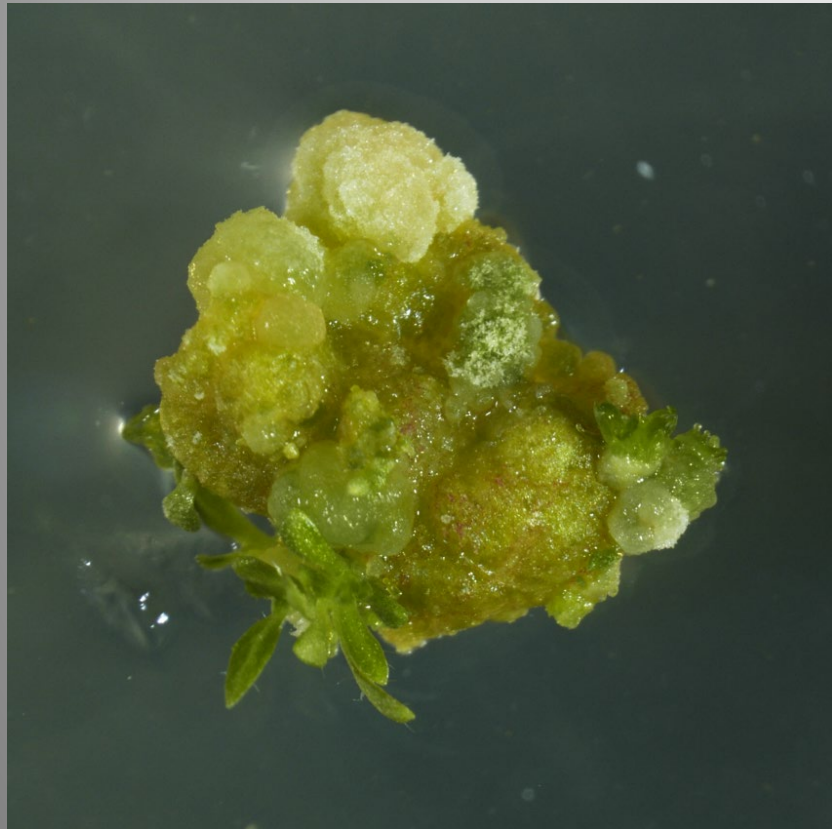


**Green** =  
GFP

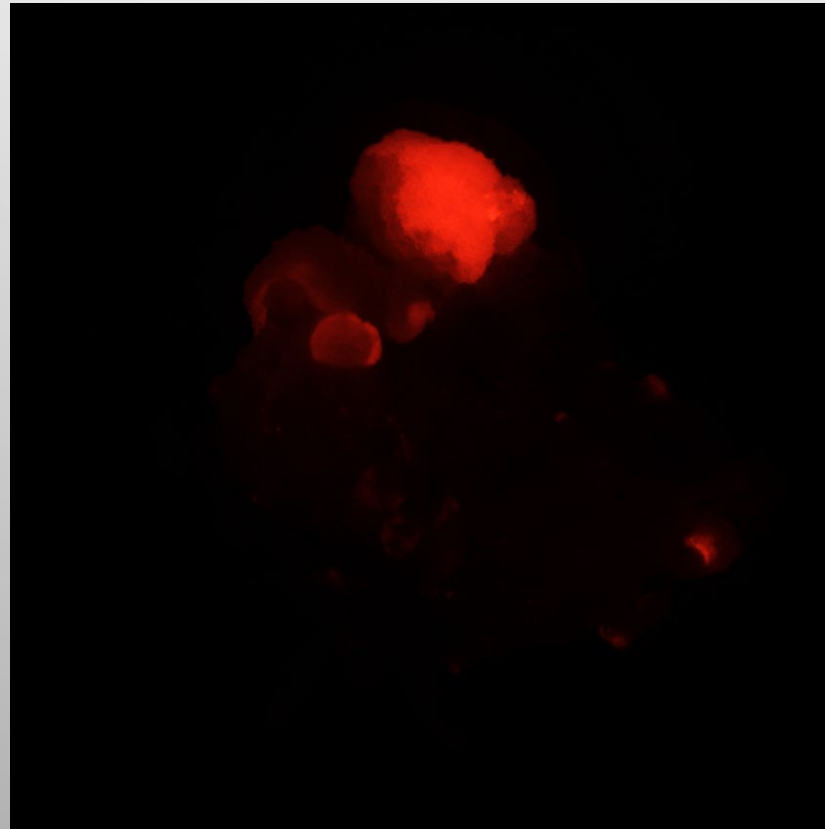
**Red** =  
Chlorophyll

# A closer look: 5:5 mix at week 6

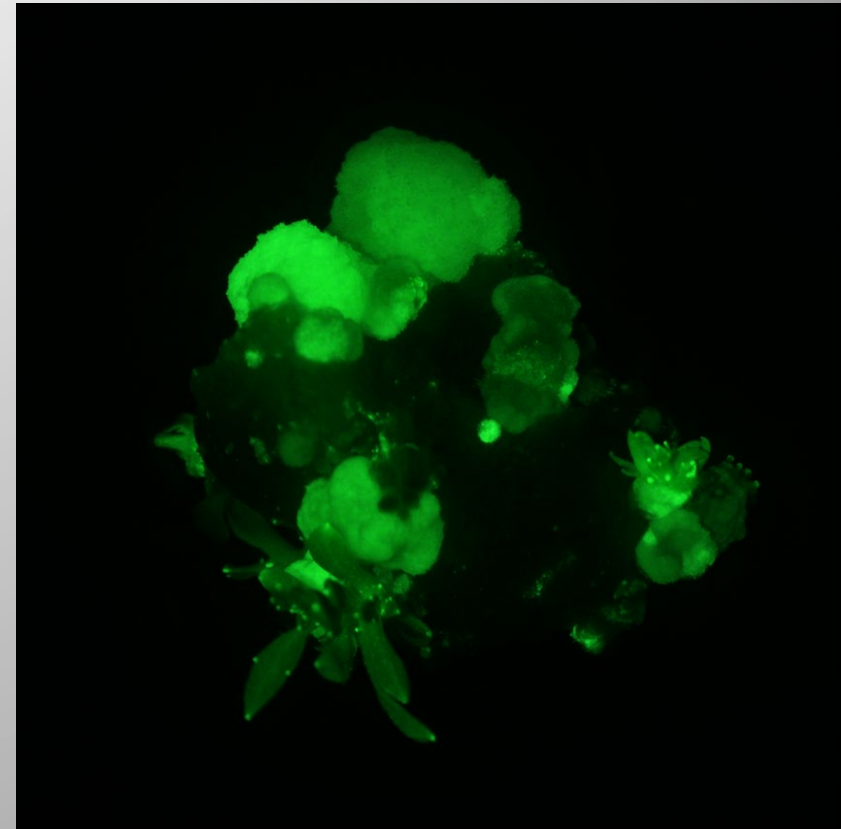
Bright-field

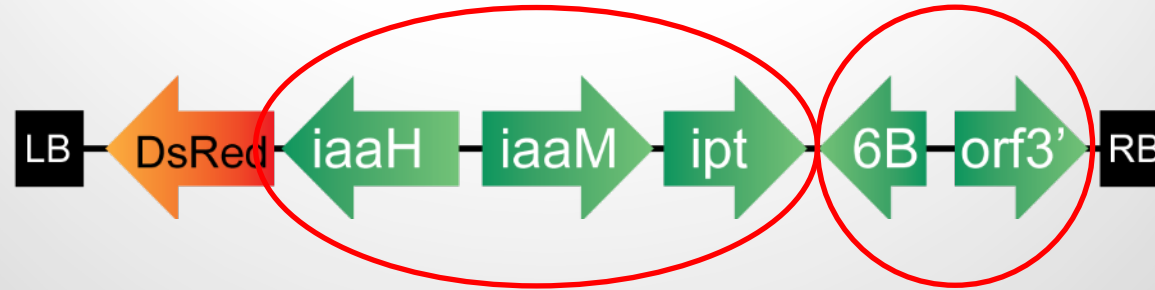


DsRed



GFP





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

Is there novel *iaa/ipt* expression in this strain?

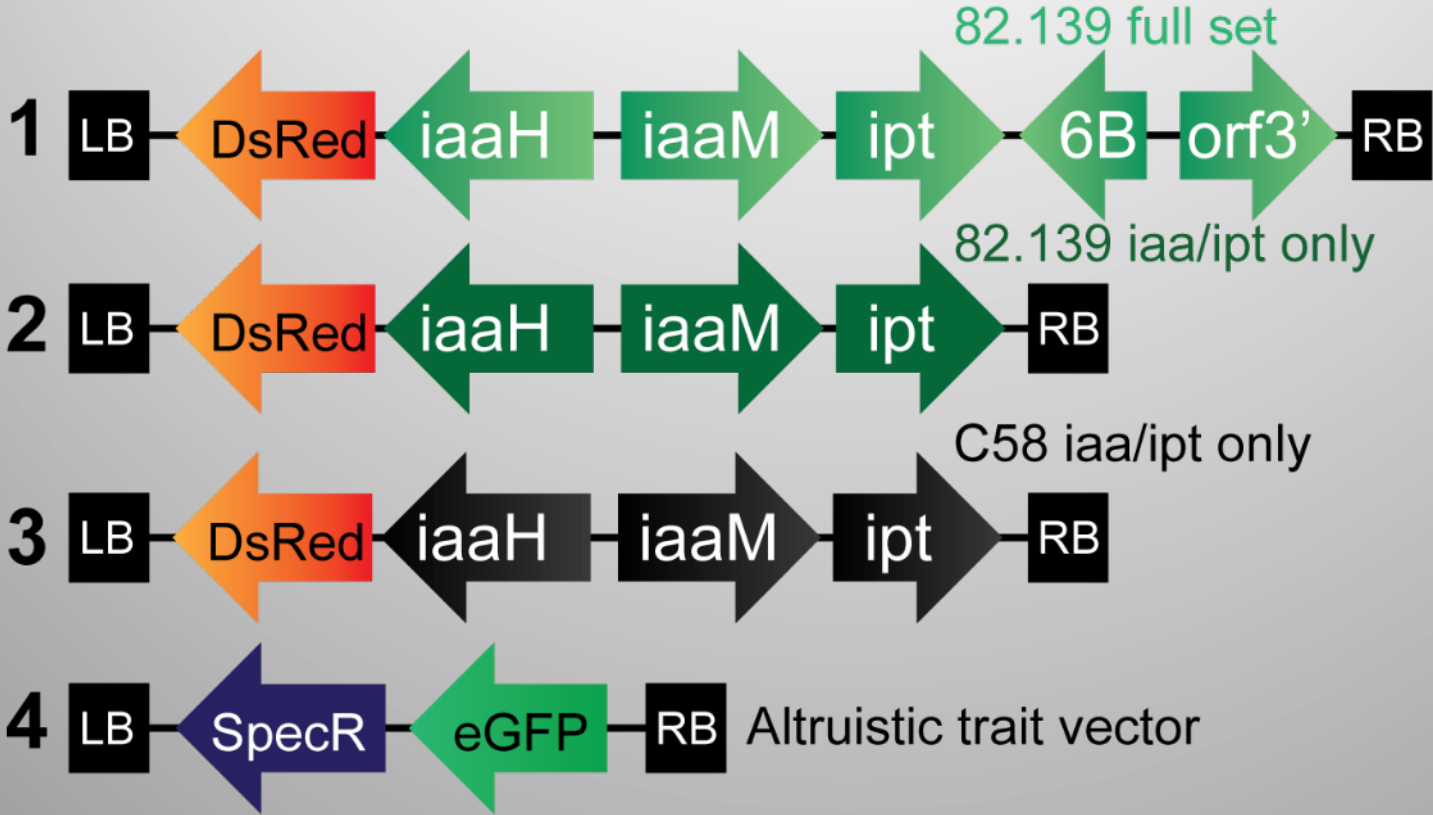
Or are the novel genes there most important?

# Experimental setup

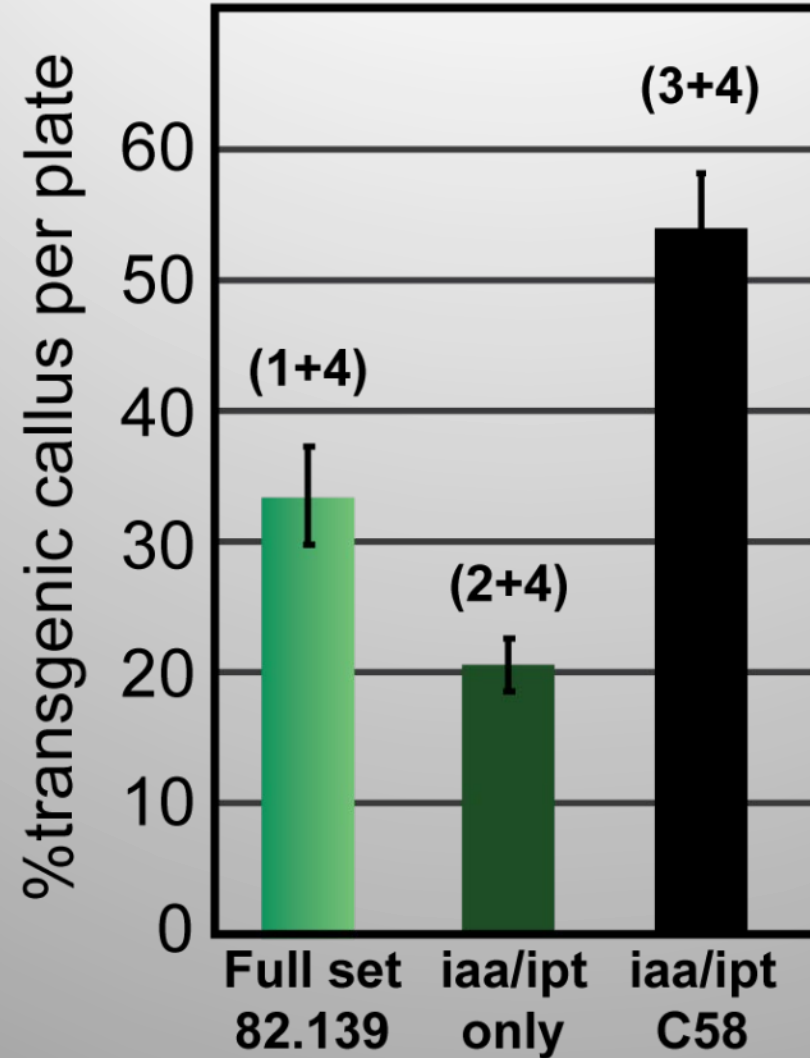
- 3 constructs
  - C58 (just *iaa* and *ipt* genes)
  - S82 (all six cloned genes)
  - S82 (just *iaa* and *ipt* genes)
- All constructs mixed 5:5 with SpecR GFP binary vector
- 1 week rest after co-culture without spectinomycin, 6 weeks on MS media without hormones but with spectinomycin

# Four vectors used in combination: 1-3 x 4

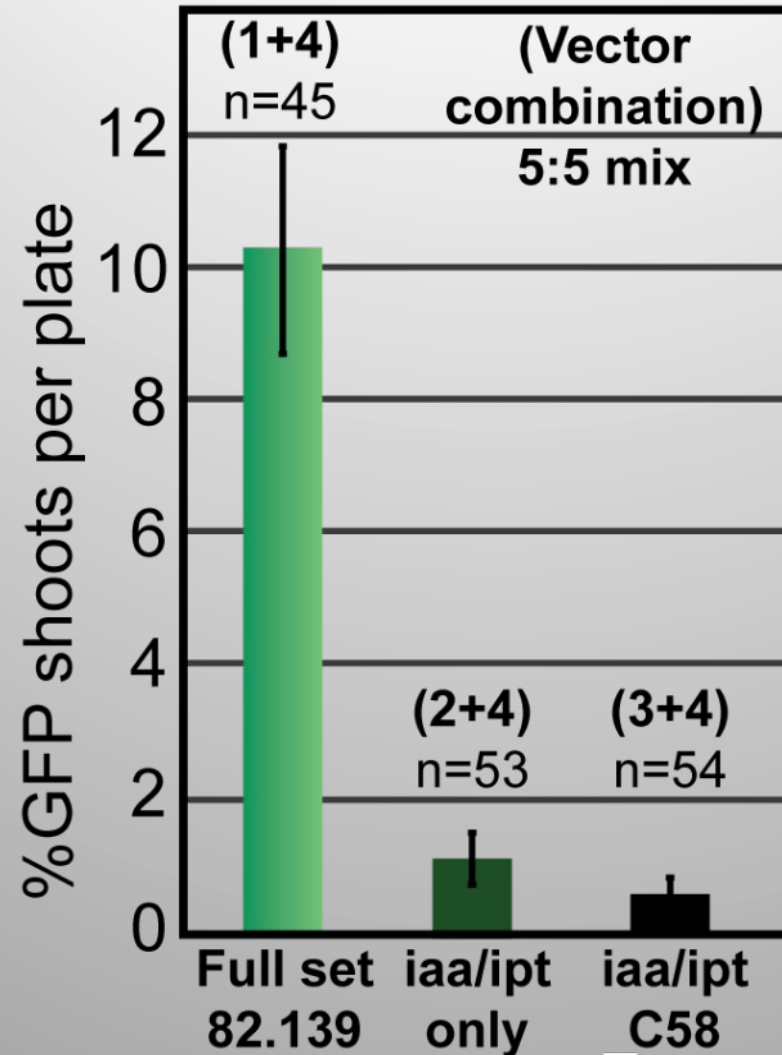
## Vectors used



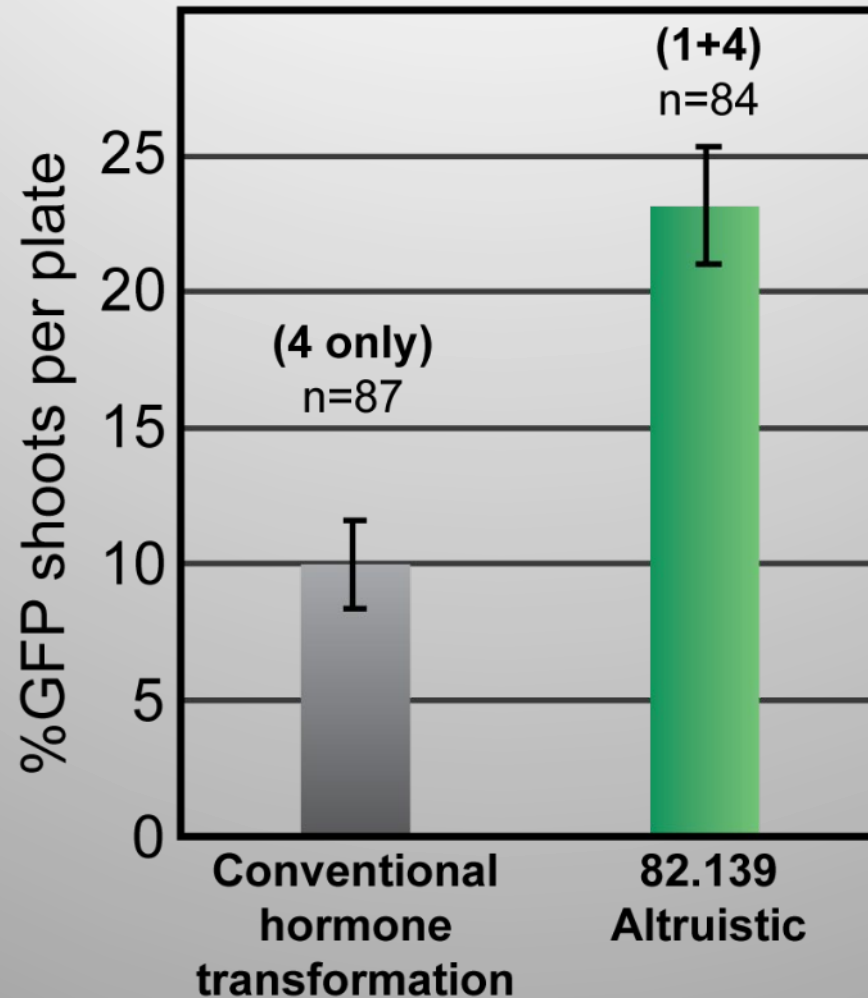
C58 *iaa/ipt* genes were best at inducing transgenic callus



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

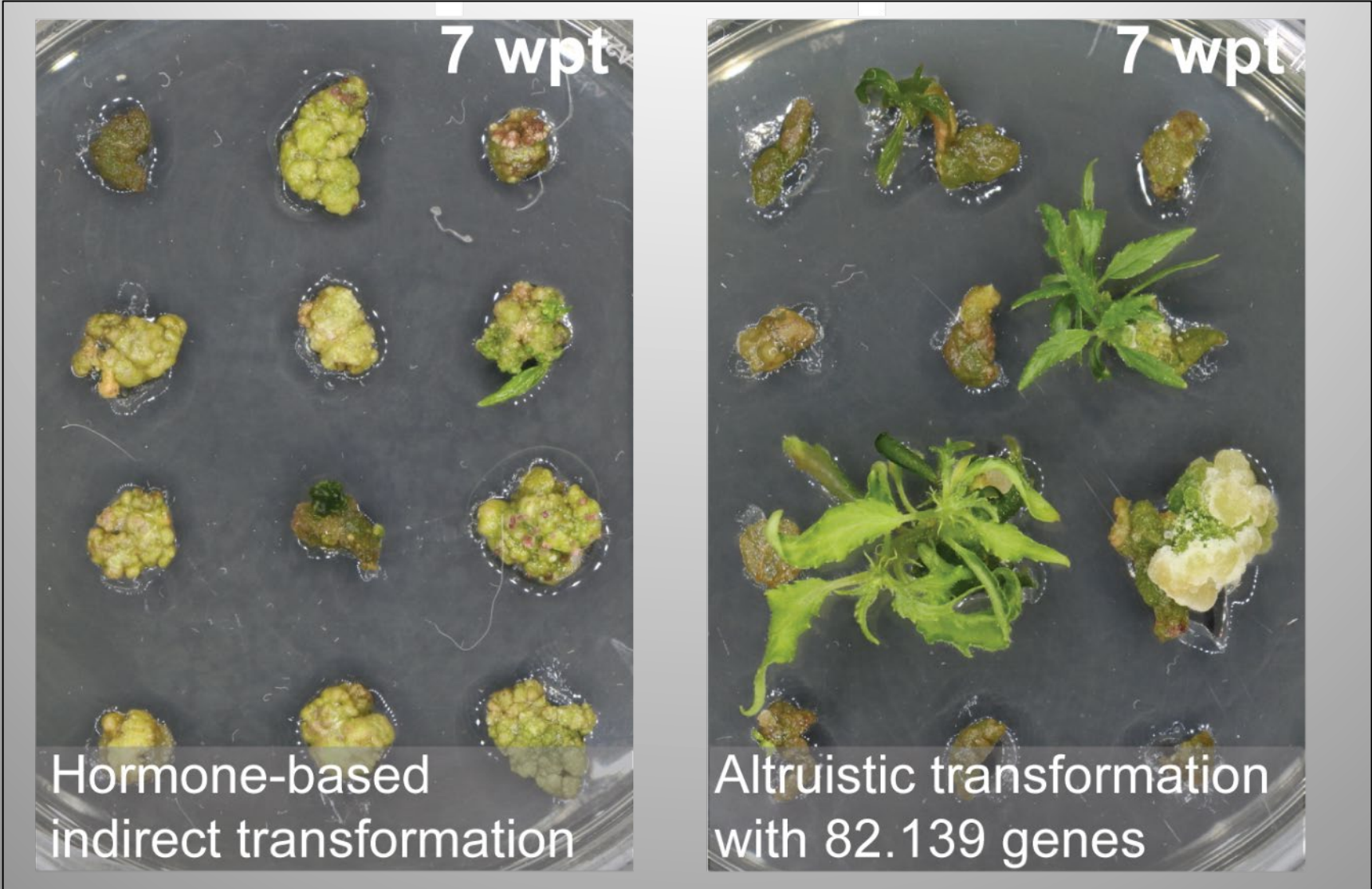


82.139 altruistic transformation was superior to routine hormone-based indirect transformation





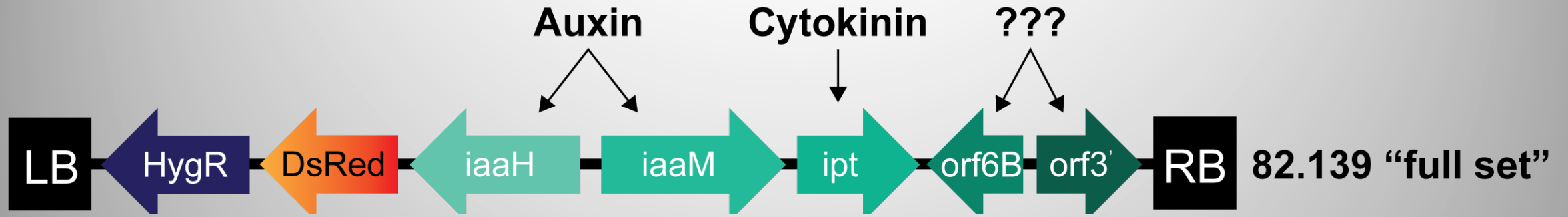
82.139 altruistic method also significantly faster, shortening time to propagation by half



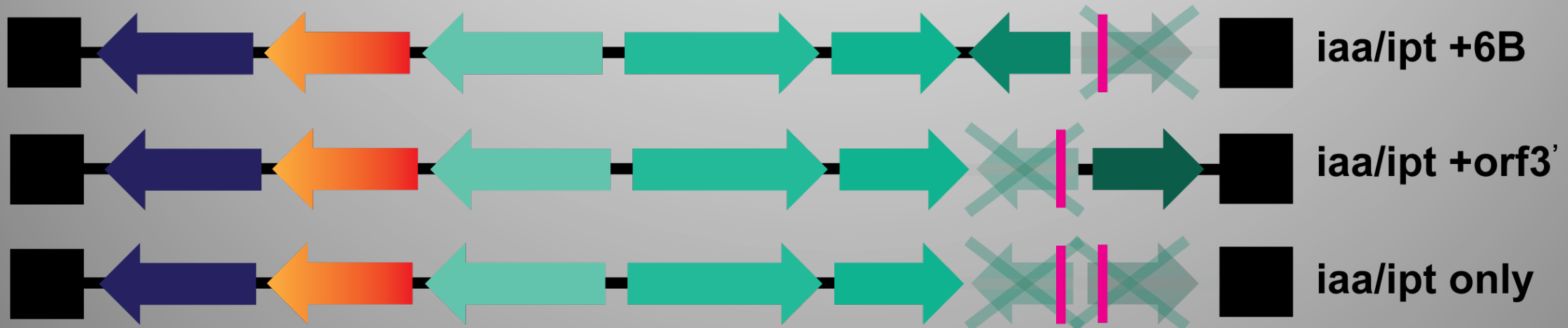
# Next steps for making altruistic transformation with 82.139 a useful tool

- Delivery of the 82.139 DEV genes is presently in our vir-plasmid-based GAENTRY strain (ARS Albany, J. Thomson)
  - This strain is aggressive and not an auxotroph
- Mobilized the genes into binary-compatible vectors
  - Different altruistic ratios appear needed – work ongoing
- Will move into auxotrophic Agro strains
- Begun further testing to identify which of the new morphogenic genes are most critical
- Testing in a variety of genotypes and species

# Use of stop codon mutations to study roles of other genes in contributing to diffusible shoot-inducing signals



 Premature stop codons introduced



# Are there other useful DEV genes/promoters from Agro? Its 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\*

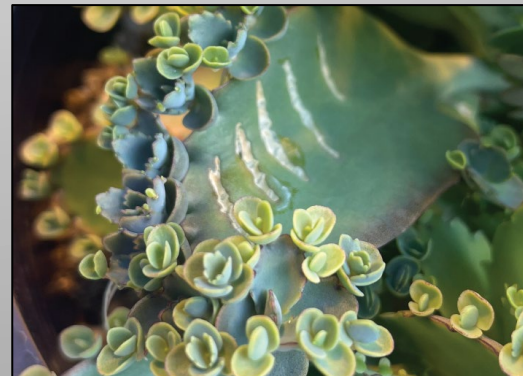
**INTRODUCTION:** Plasmids are autonomously replicating, nonessential DNA molecules that accelerate the evolution of many important bacterial-driven processes. For example, plasmids spread antibiotic resistance genes, which

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-




# Grants 4Ag

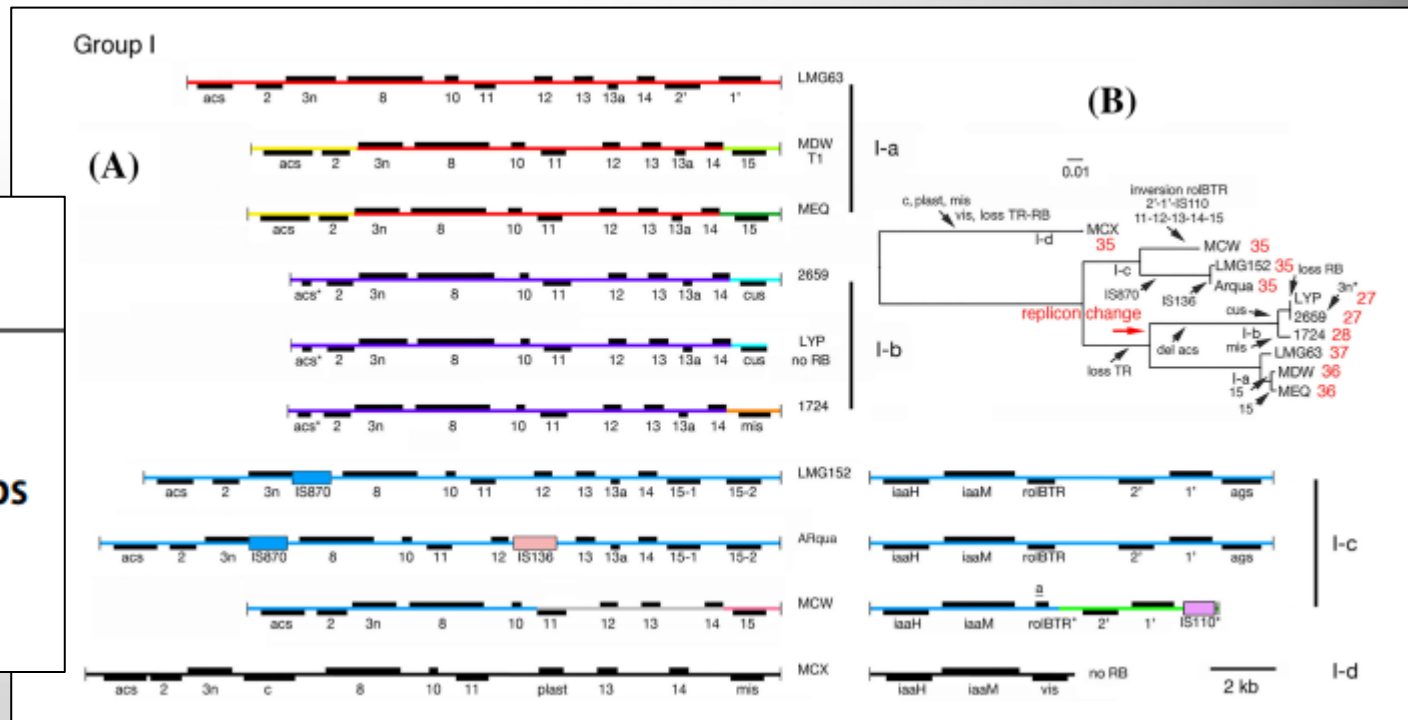


# Much to explore...

Plant Molecular Biology (2021) 106:239–258  
<https://doi.org/10.1007/s11103-021-01140-0>

## T-DNA regions from 350 *Agrobacterium* genomes: maps and phylogeny

Léon Otten<sup>1</sup> 



“...due to increased whole genome sequencing efforts, about 400 *Agrobacterium* sequences have now become available, 350 of which contain T-DNA regions. Detailed analysis identified 92 different T-DNA regions and several new T-DNA genes...”

# Synthetic biology a further powerful means to tune Agro DEV genes for particular hosts and transformation systems?



Cold  
Spring  
Harbor  
Laboratory

bioRxiv

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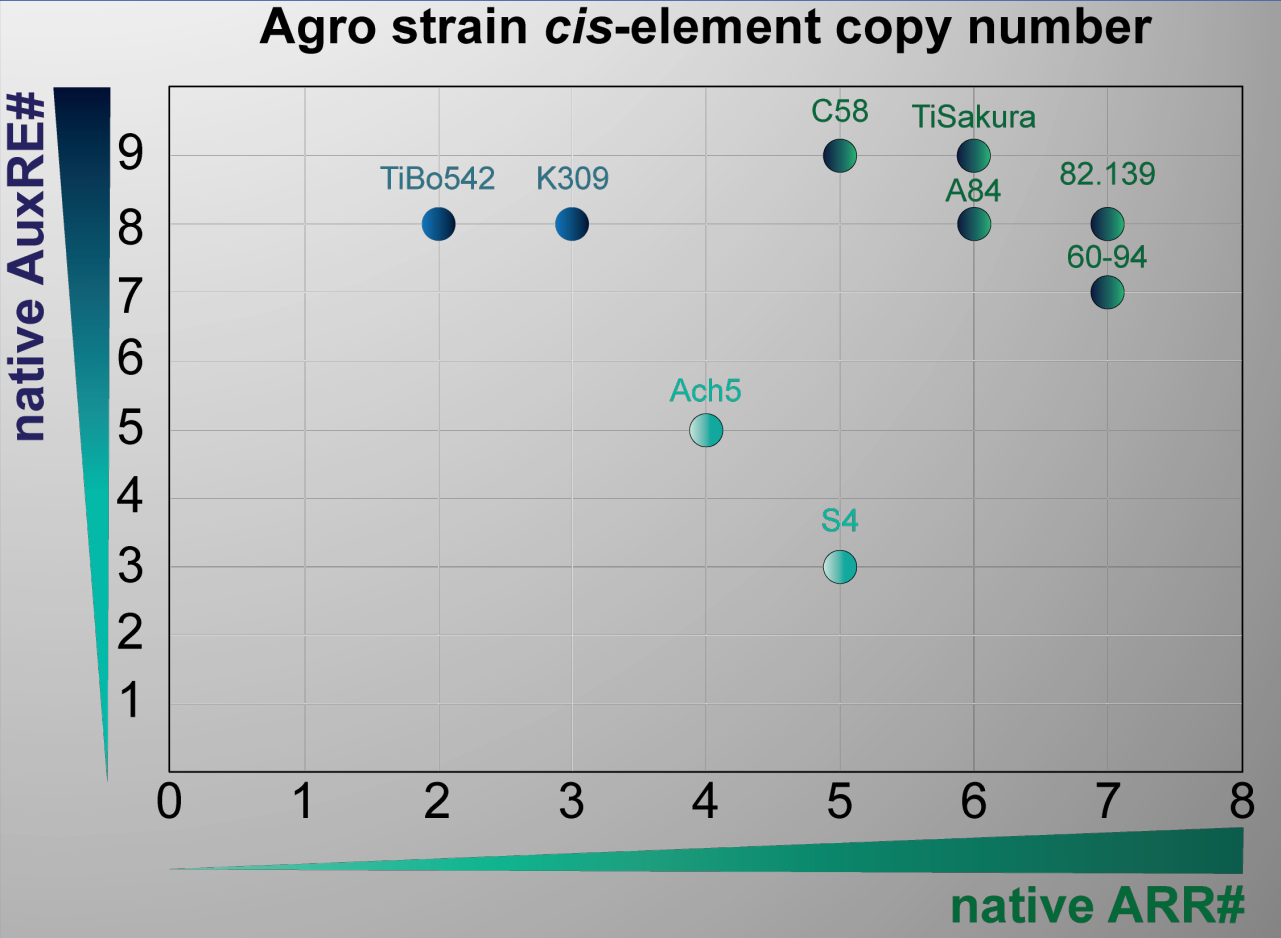
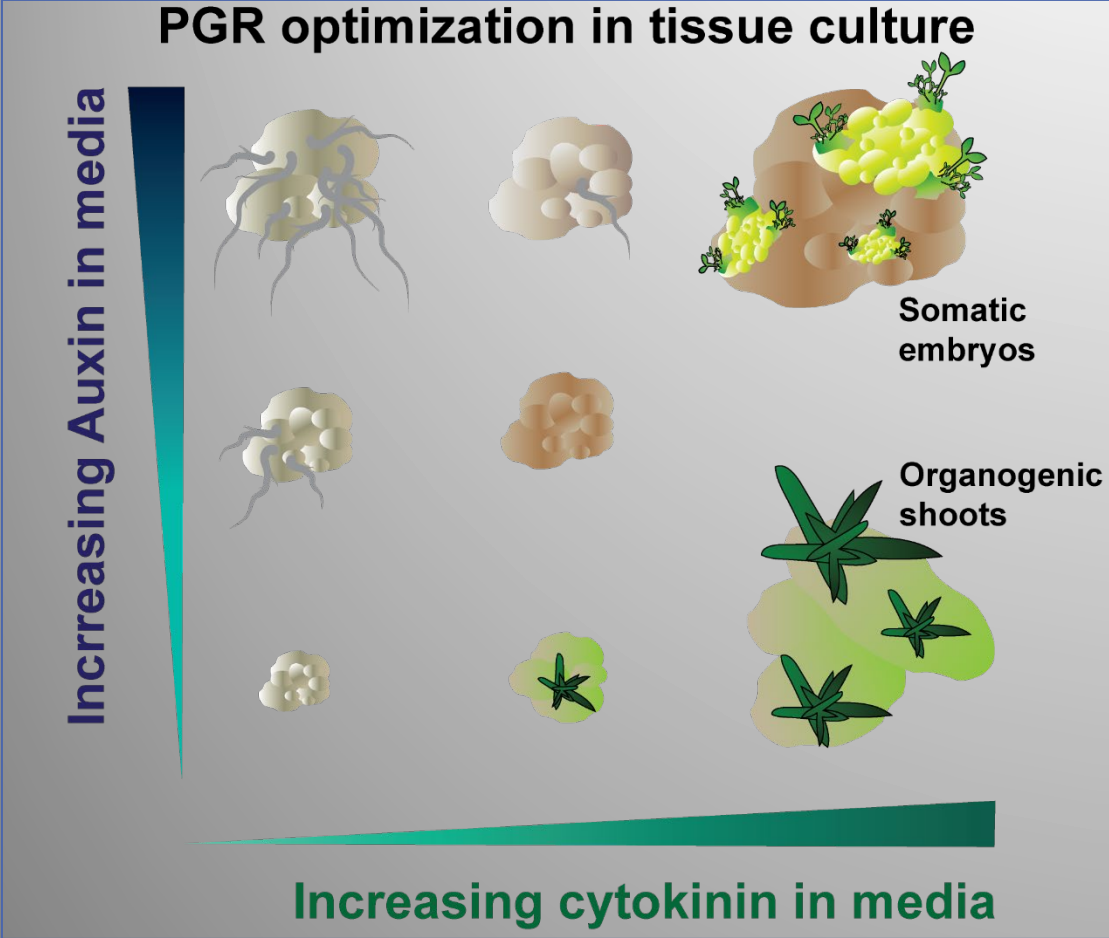
## Genetically refactored *Agrobacterium*-mediated transformation

Mitchell G. Thompson, Liam D. Kirkpatrick, Gina M. Geiselman, Lucas M. Waldburger, Allison N. Pearson, Matthew Szarzanowicz, Khanh M. Vuu, Kasey Markel, Niklas F. C. Hummel, Dennis D. Suazo, Claudine Tahmin, Ruoming Cui, Shuying Liu, Jasmine Cevallos, Hamreet Pannu, Di Liu, Jennifer W. Gin, Yan Chen, Christopher J. Petzold, John M. Gladden, Jay D. Keasling, Jeff H. Chang, Alexandra J. Weisberg, Patrick M. Shih

doi: <https://doi.org/10.1101/2023.10.13.561914>

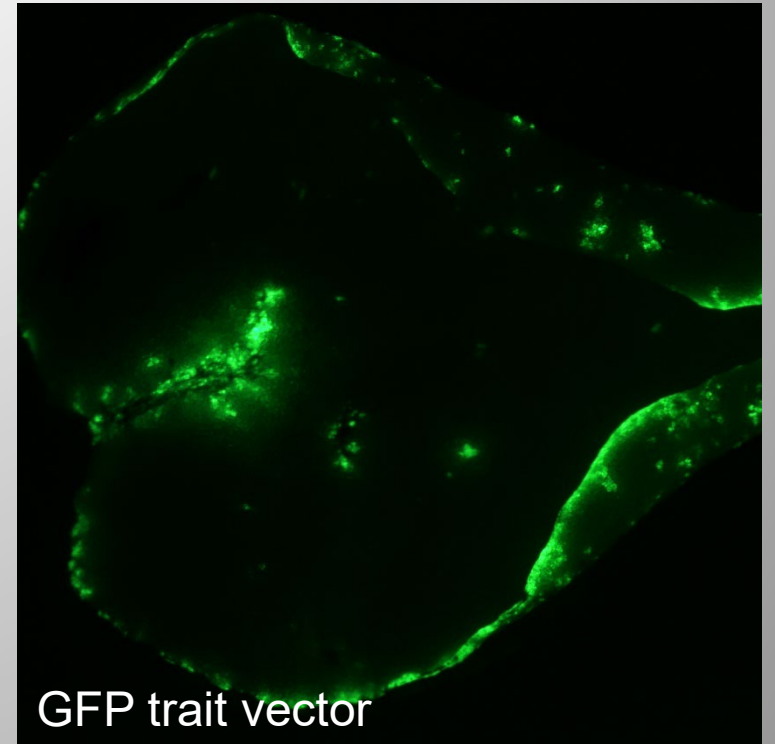
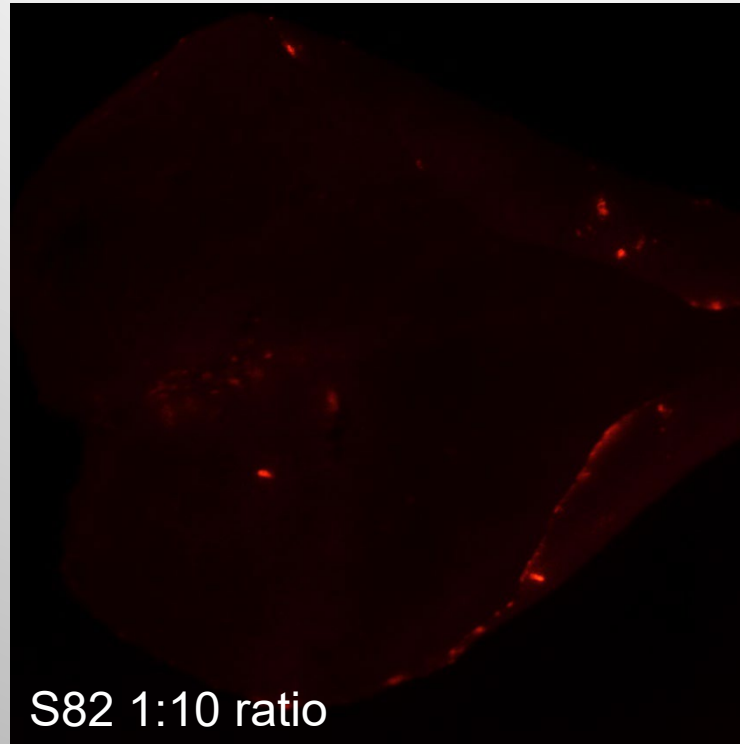
This article is a preprint and has not been certified by peer review [what does this mean?].

# From an engineering perspective, diversity informs DEV gene “tuning” for different species/genotypes



# We are beginning to test in other dicot species

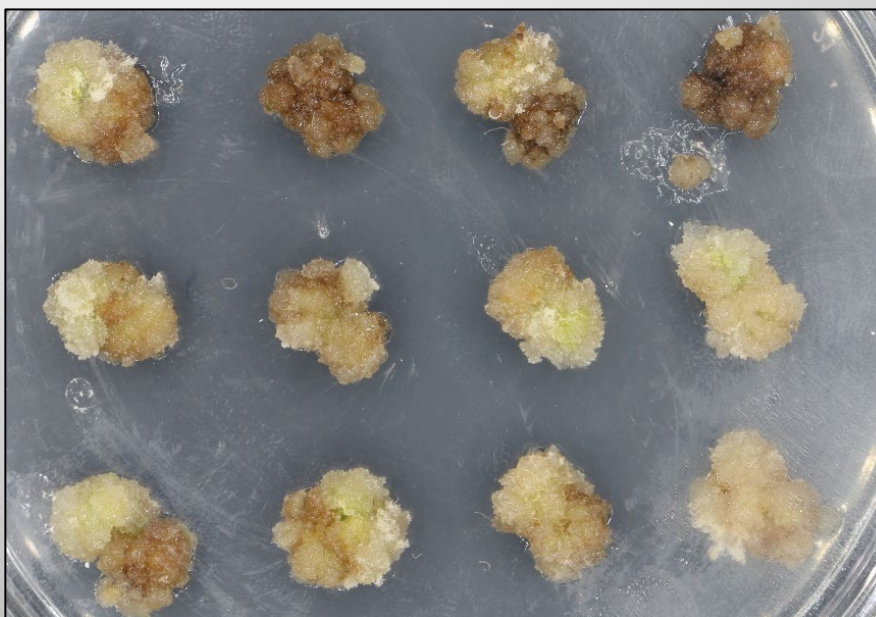
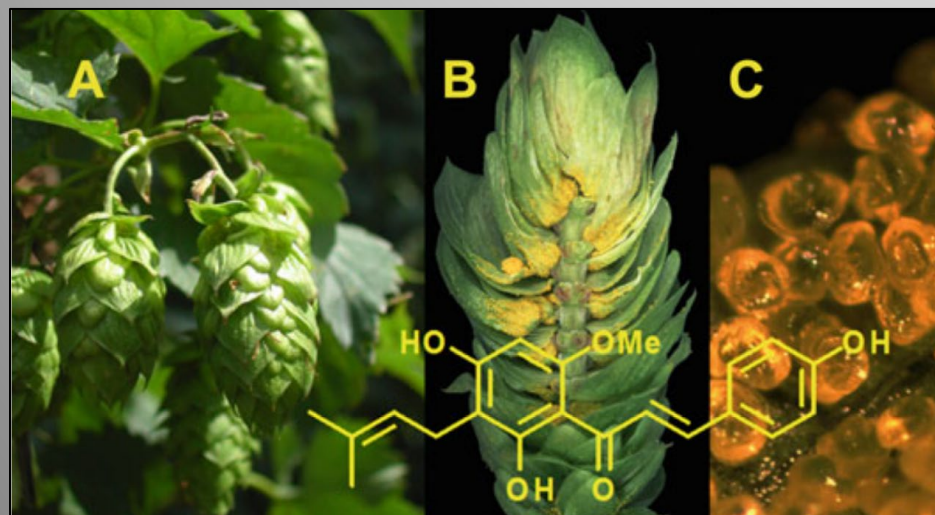
Hop, lettuce, tobacco, tomato at present



Early expression in lettuce



# Hops a major new focus, very tough to transform



Chris Willig  
Postdoc



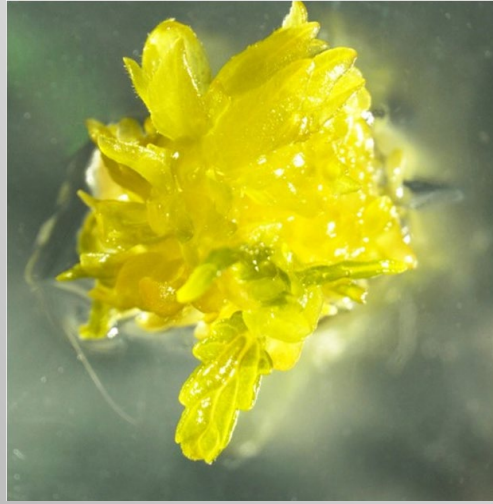
Michele Wiseman  
Botany



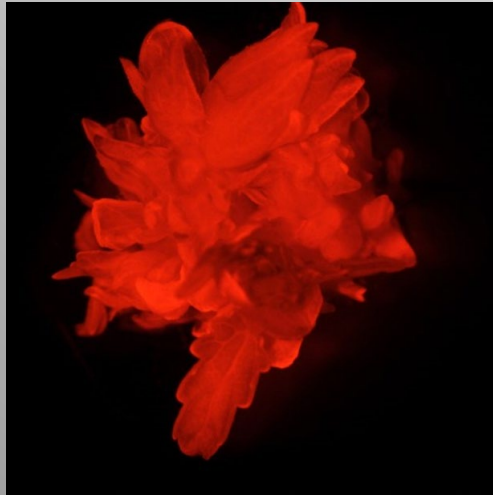
David Gent  
ARS Corvallis

# We have had success with the altruistic approach in two hop varieties

Bright field



DsRed as trait vector



# Going forward

- Woody species, mature-clonal tissues, are tough, slow and highly diverse – DEV genes not the miracles we hoped for  
*(At least not yet!)*
- Shooty Agro DEV genes, delivered altruistically, promising transformation approach – also being tested *in planta*
- Hop: First promising results in application to other difficult plant species
- Agrobacterium DEV gene diversity is great, worth exploration and tuning – 82.139 the tip of an iceberg of possibilities?

# Thanks to our funders and collaborators

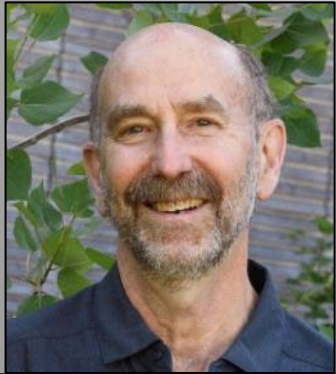


## GREAT TREES Consortium

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



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



**Steve Strauss**  
Director TBGRC, Professor



**Cathleen Ma**  
Transformation &  
Greenhouse  
Experiments



**Lisa Hargest**  
Biotechnology  
Program  
Manager



**Greg  
Goralogia**  
Postdoc,  
Flowering &  
Gene Editing



**Victoria Conrad**  
Undergraduate  
Gene Editing



**Kate Peremyslova**  
GWAS,  
Transformation  
Experiments



**Chaney Hart**  
PhD Fellow  
Ecophysiology/genetics



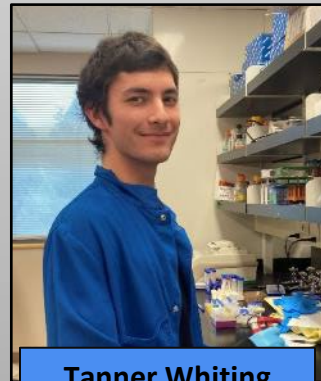
**Michael Gordon**  
PhD Candidate, HIGS



**Michael Nagle**  
Postdoc: GWAS,  
Phenomic systems



**Chris Willig**  
Postdoc: Hop  
transformation



**Tanner Whiting**  
Undergraduate  
Hop transformation



**Anthony Marroquin**  
Greenhouse Manager



**Xavier Tacker**  
Undergraduate  
Researcher

# New Phytologist – new section on “transformative plant biotechnology”

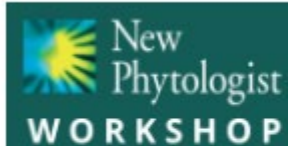
- **Transformative Plant Biotechnology:** plant bioengineering, plant biotechnology, plant gene editing, genetic manipulation or engineering of plant biosynthetic pathways or regulatory circuits or signalling cascades, plant synthetic biology.



*Editorial*

## Introducing Transformative Plant Biotechnology

In the first issue of *New Phytologist*, in 1902, the founding editor, Sir Arthur Tansley told his readers that “Topics are constantly arising on which ... discussion would be valuable not only to the one or



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New Phytologist Workshop: introducing Transformative Plant Biotechnology

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Edinburgh (National Museum of Scotland auditorium)

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