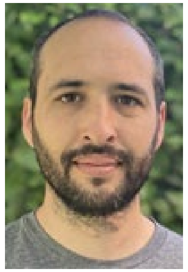
Today's featured speaker:



Juan Debernardi
Manager
UC Davis
Plant Transformation Facility

Dr. Debernardi will speak
about his research
and the Plant Transformation Facility,
as he takes over from long-time
manager David Tricoli.

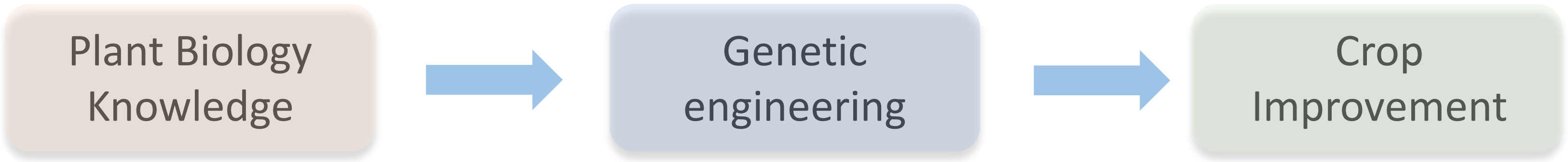
UC Davis Plant Transformation Facility

Supporting plant research community with improved plant transformation and genome editing technologies

Juan M Debernardi

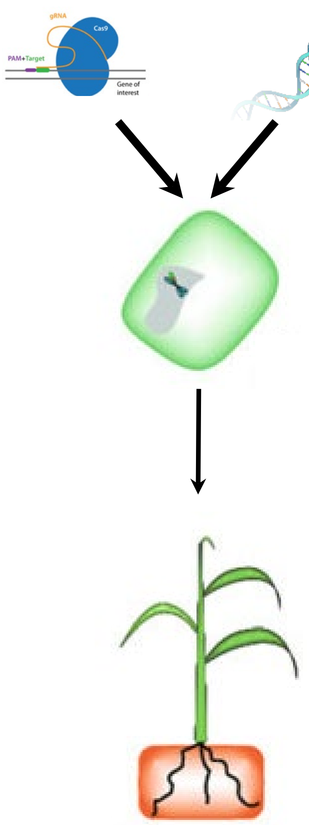
One UCDAVIS
PLANT TRANSFORMATION FACILITY
The Ralph M. Parsons Foundation

UCDAVIS
PLANT SCIENCES

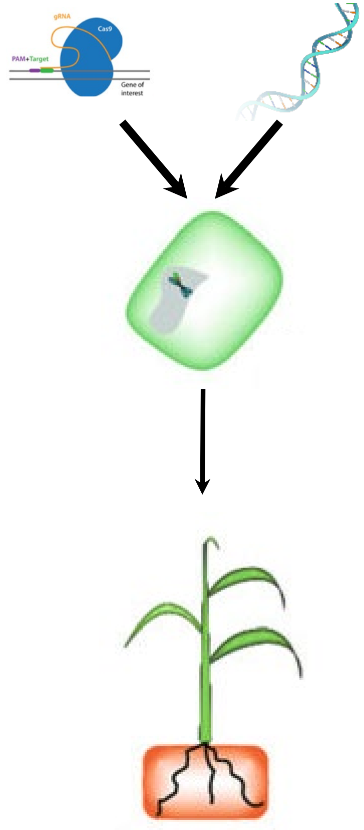

Plant Biology Knowledge
Genetic engineering
Crop Improvement

Plant Biology Knowledge → Genetic engineering → Crop Improvement

Plant **transformation** and **regeneration** are main bottlenecks in applying genetic engineering technologies to Crop Improvement.

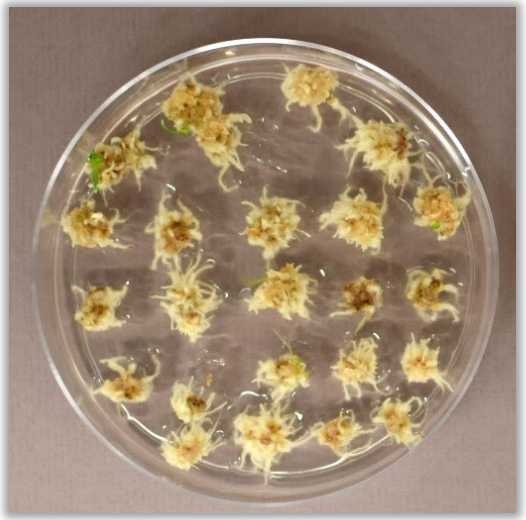


Plant Biology Knowledge → Genetic engineering → Crop Improvement



Plant **transformation** and **regeneration** are main bottlenecks in applying genetic engineering technologies to Crop Improvement.

- Low transformation ability of most crops.
- Limited number of genotypes that can be currently transformed.
- Intensive labor and space required to supply adequate tissue for transformation.



Typical wheat transformation (UCD)

Our mission:

To provide cost effective plant transformation and plant cell biology services for the plant research community.

Our team

Director
Abhaya Dandekar

Interim Manager
Juan M Debernardi

Staff Research Associates
David M Tricoli
Vanna Ebanez
Danielle Inchaurregui
Lucero Jimenez
Mariana Padilla
Alice Lutzenhiser
Mackenzie Ella Benson

https://ptf.ucdavis.edu/

University of California
The Ralph M. Parsons Foundation
Plant Transformation Facility

Our core activities

Plant Transformation

Genome editing

Plant Cell Biology

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Plant Transformation Facility

Our core activities

Plant Transformation
Genome editing
Plant Cell Biology

Our work..

Services

. Plant transformation
. Genome editing
. Plant cell biology

Research

Protocol and technology development

Innovation

Technology evaluation and optimization

University of California
The Ralph M. Parsons Foundation
Plant Transformation Facility

Our core activities

Plant Transformation
Genome editing
Plant Cell Biology

Our work..

Services
. *Plant transformation*
. *Genome editing*
. *Plant cell biology*

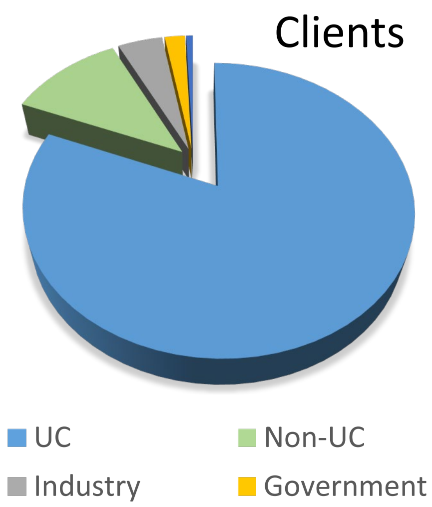
Research
Protocol and technology development

Innovation
Technology evaluation and optimization

Ways to interact with us..

Services agreements
Research agreements

Sponsored Research
Grants
Gifts



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Plant Transformation Facility

Our core activities

Plant Transformation
Genome editing
Plant Cell Biology

Our work..

Services
. Plant transformation
. Genome editing
. Plant cell biology

Research
Protocol and technology development

Innovation
Technology evaluation and optimization

Ways to interact with us..

Services agreements
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Services: Plant transformation and genome editing

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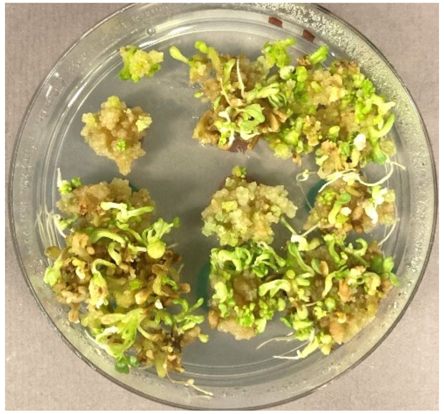
The Ralph M. Parsons Foundation
Plant Transformation Facility

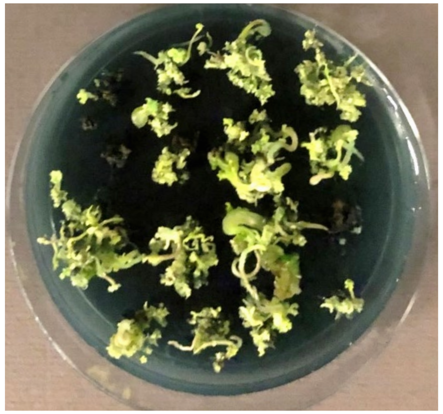
Services: Plant transformation and genome editing

Pepper

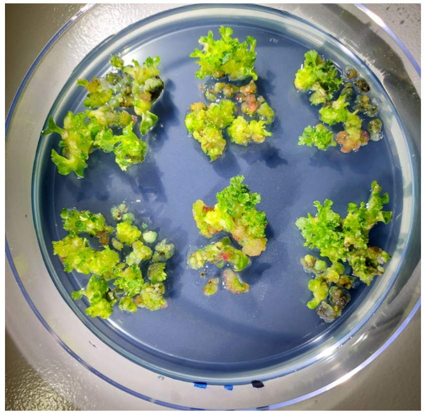
Alfalfa



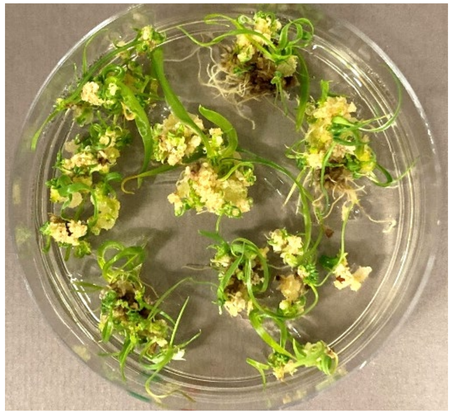
Grape



Strawberry



Wheat



Our most common crops

Tomato (most genotypes)	Alfalfa	Wheat (most genotypes)
Lettuce (most genotypes)	Potato	Rice
Grape	Pepper	Barley
Strawberry	Citrus	
Tobacco	Petunia	

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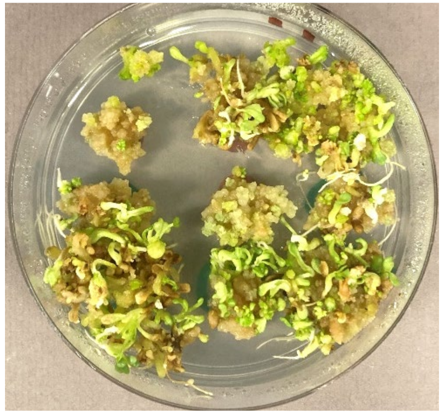
The Ralph M. Parsons Foundation
Plant Transformation Facility

Services: Plant transformation and genome editing

Pepper

Alfalfa



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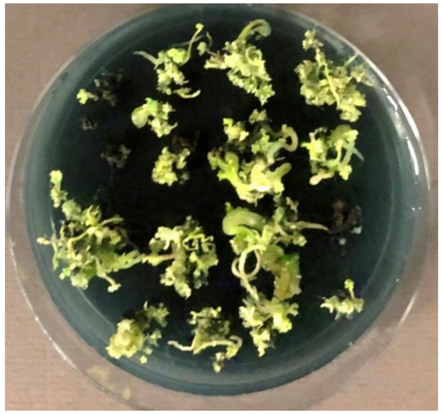

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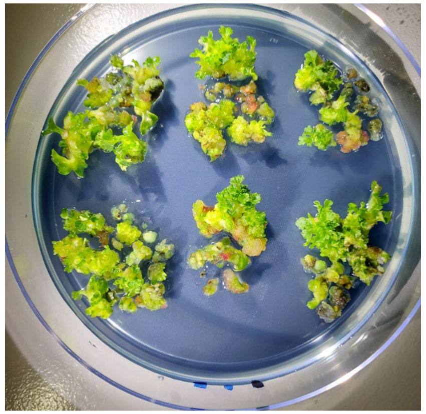
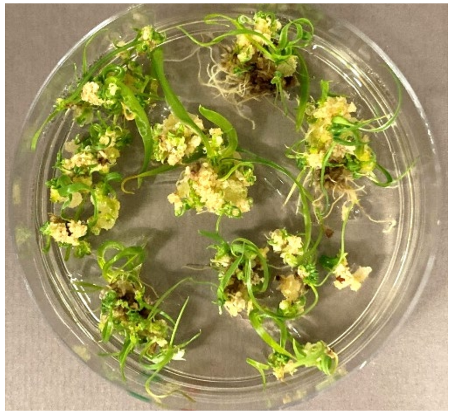


Wheat



Our most common crops

Tomato (most genotypes)
Lettuce (most genotypes)
Grape
Strawberry
Tobacco

Alfalfa
Potato
Pepper
Citrus
Petunia

Wheat (most genotypes)
Rice
Barley

Additional crops

Carrot
Cucumber
Ipomea
Melons
Mimulus

Rose
Truncatula A17
Walnut
Canola

https://ptf.ucdavis.edu/services

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Plant Transformation Facility

Services: Plant transformation and genome editing

Pepper

Order Online

Crop	Genotype	Selection System	# of Independent Events	UC Clients Cost	Non-UC Clients Cost*
Alfalfa - *Medicago sativa*	Regen	kanamycin	10	$1,050	$2,000
Canola	Wester	kanamycin	5	$1,050	Contact us
Citrus	Carrizo	kanamycin	5	$1,050	$2,000
Grape	Thompson Seedless	kanamycin	5	$2,010	$4,000
Lettuce - *Lactuca sativa*	most genotypes	kanamycin	10	$1,050	Contact us
Petunia	Mitchell diploid	kanamycin	10	$1,050	$2,000
Rice	Kitaake	hygromycin	10	$1,050	$2,000
Strawberry	Chandler Camarosa Camino Real	kanamycin	5	$1,050	$2,000
Tobacco	Benthamiana	kanamycin hygromycin glufosinate	10	$1,050	Contact us
Tobacco - *Nicotiana*	Sr1, Samsun, TI1347, Xanthi	kanamycin hygromycin glufosinate	10	$525	$1,050
Tomato - *S. lycopersicum*, *S.* habrochaites, *S. pimpinellifolium*,	Money maker+, T-5, UC 82B, VF 36, MicroTom, etc	kanamycin hygromycin glufosinate	10	$1,050	$2,000
Wheat***	Kronos, Fielder, **Contact us** for other genotypes	hygromycin	5	$1,050	$2,000
Barley***	Golden Promise, **Contact us** for other genotypes	hygromycin	5	$1,050	$2,000

Our most com

Tomato (most

Lettuce (most

Grape

Strawberry

Tobacco

s

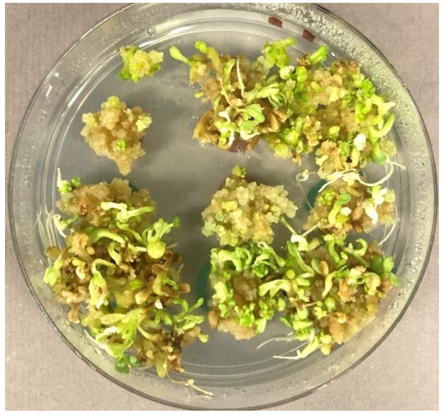
A17

Services: Plant transformation and genome editing*

University of California
The Ralph M. Parsons Foundation
Plant Transformation Facility

Pepper

Alfalfa



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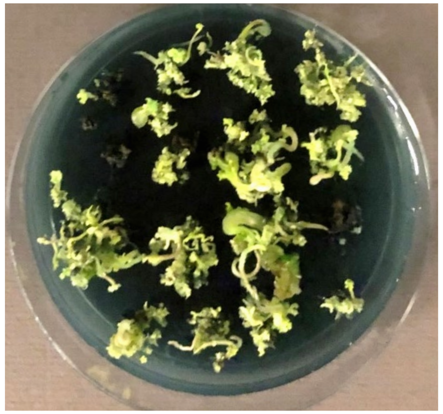

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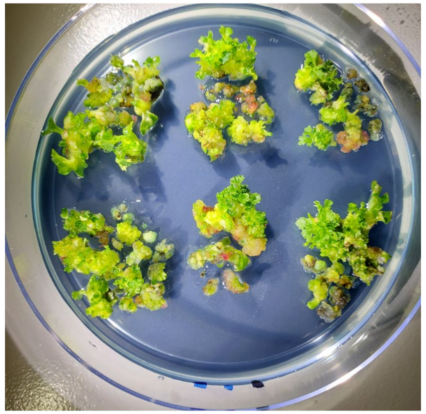

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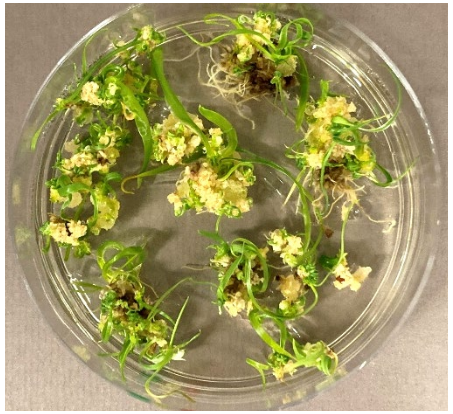

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Additional crops

Carrot
Cucumber
Ipomea
Melons
Mimulus

Rose
Truncatula A17
Walnut
Canola

***We offer different molecular services for CRISPR-related projects, including simple vector cloning, target gene sequencing and genotyping to events to determine edits**

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The Ralph M. Parsons Foundation
Plant Transformation Facility

Our core activities

Plant Transformation
Genome editing
Plant Cell Biology

Our work..

Services

. Plant transformation
. Genome editing
. Plant cell biology

Research

Protocol and technology development

Innovation

Technology evaluation and optimization

University of California
The Ralph M. Parsons Foundation
Plant Transformation Facility

<u>Our core activities</u>

Plant Transformation
Genome editing
Plant Cell Biology

Our work..

<u>Services</u>
. Plant transformation
. Genome editing
. Plant cell biology

<u>Research</u>
Protocol and technology development

<u>Innovation</u>
Technology evaluation and optimization

1. Protocol development and optimization
2. Transformation technology improvement
3. DNA-free editing platform for clonal crops

Research #1: Protocol development

Octoploid cultivars

Chandler

Steven Knapp & Mitchell Feldmann

University of California
The Ralph M. Parsons Foundation
Plant Transformation Facility

Research #1: Protocol development

Lucero Jimenez

Octoploid cultivars

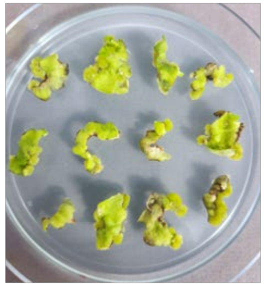
Chandler UCD Royal Royce*

*genome sequence available

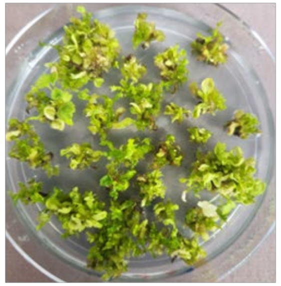
Steven Knapp & Mitchell Feldmann

Royal Royce transformation

Calli induction stage



Bud induction stage



Seedling rooting stage

≈ 8 months

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Plant Transformation Facility

Research #1: Protocol development

Lucero Jimenez

Octoploid cultivars

Chandler
Camarosa*
Camino Real
Fronteras
Monterey

UCD Royal Royce*
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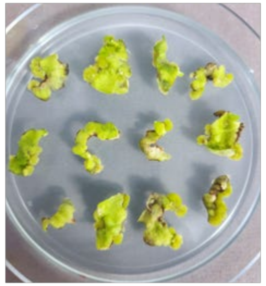

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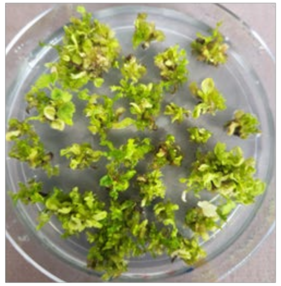
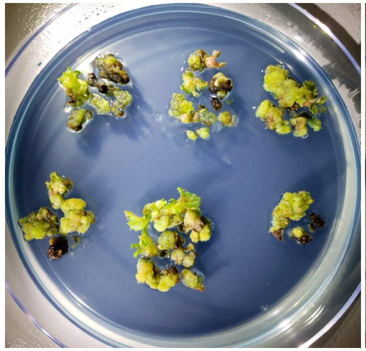


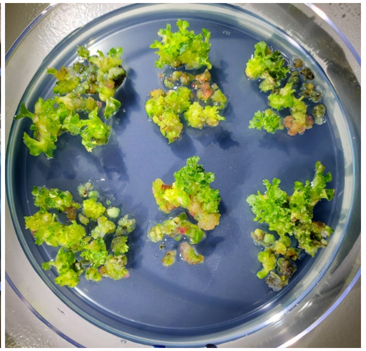
Seedling rooting stage

≈ 8 months

Camarosa



Camino Real



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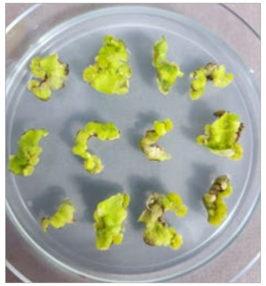

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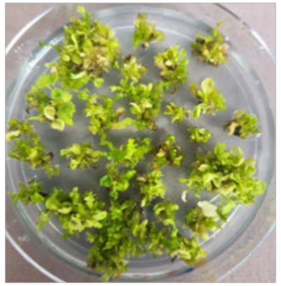

Seedling rooting stage

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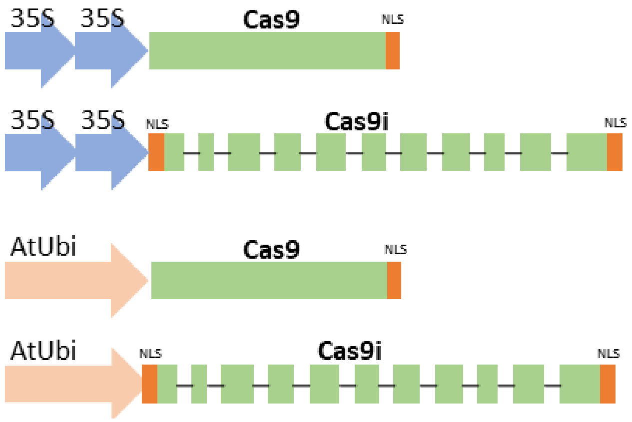
Test editing vectors



Research #1: Protocol development

Lucero Jimenez

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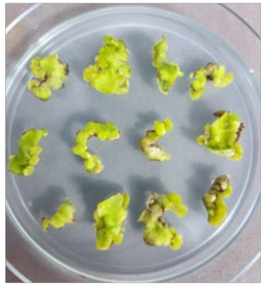

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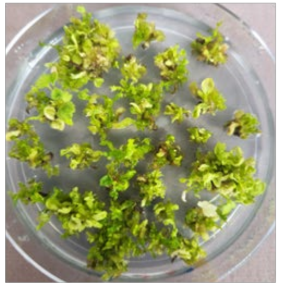

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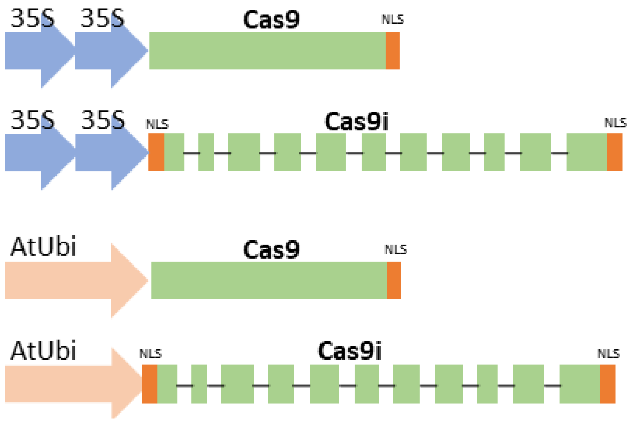
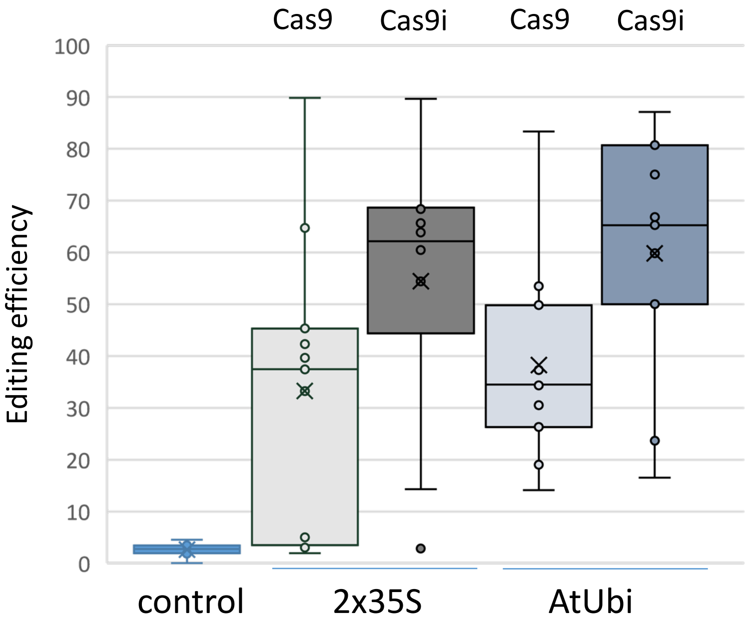
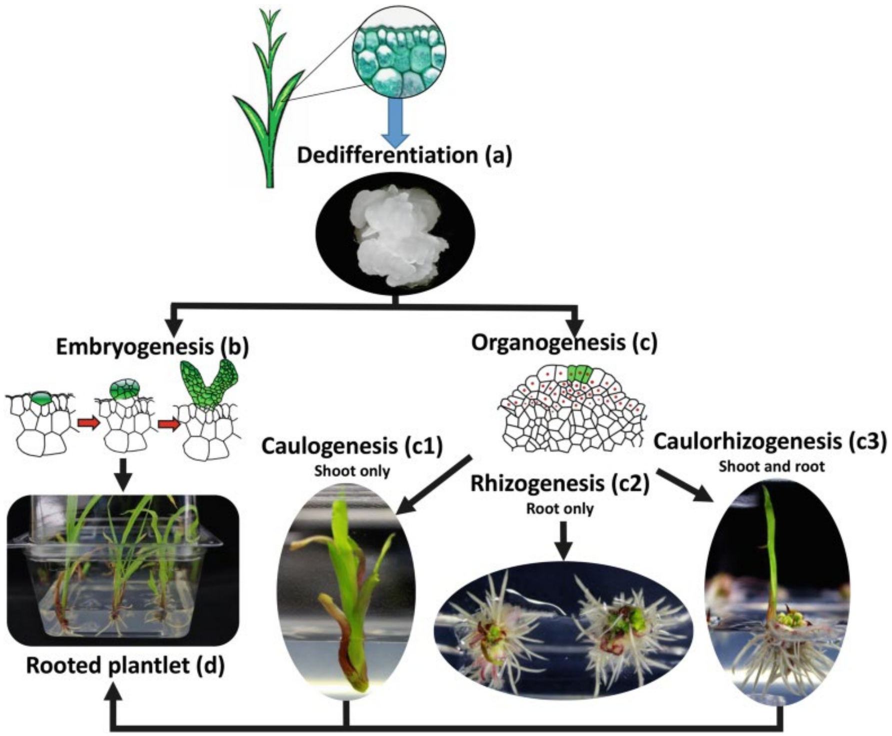




Research #2: Improving Plant Transformation Efficiency

Research #2: Improving Plant Transformation Efficiency

Plant regeneration problem/process	. Cell dedifferentiation . Cell proliferation . Acquisition of new fates



Samson Nalapalli, 2021

Research #2: Improving Plant Transformation Efficiency

Plant regeneration problem/process

. Cell dedifferentiation
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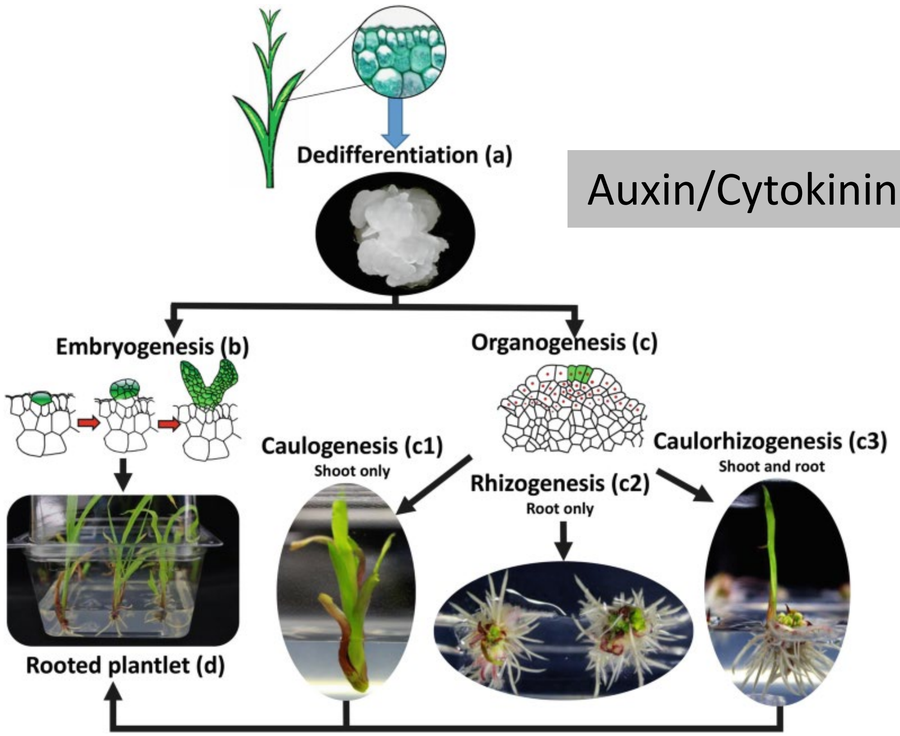
Auxin/Cytokinin



Samson Nalapalli, 2021

Research #2: Improving Plant Transformation Efficiency

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Plant Transformation Facility

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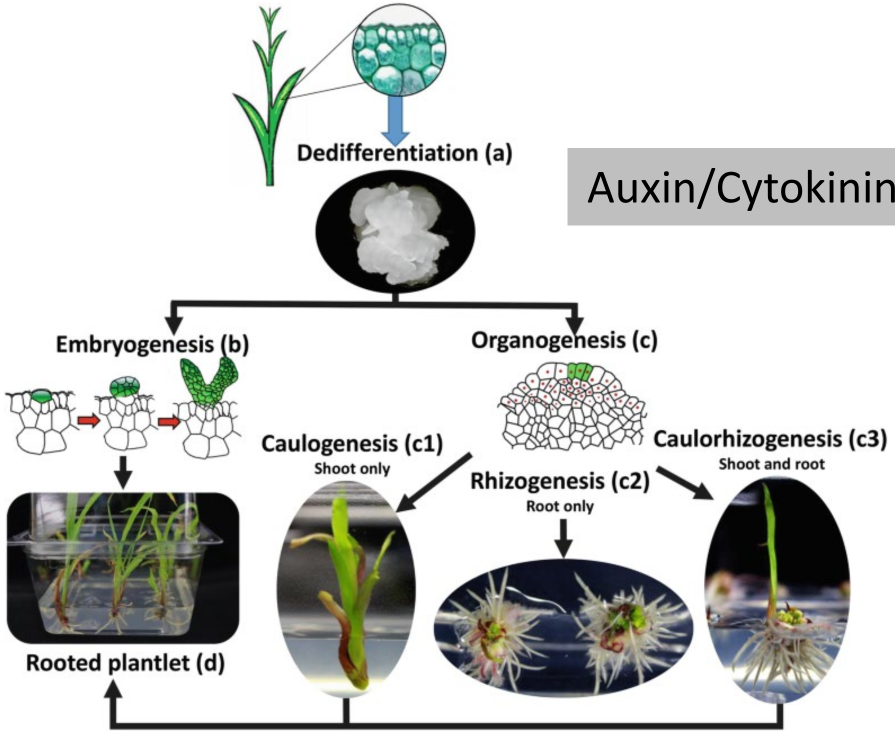
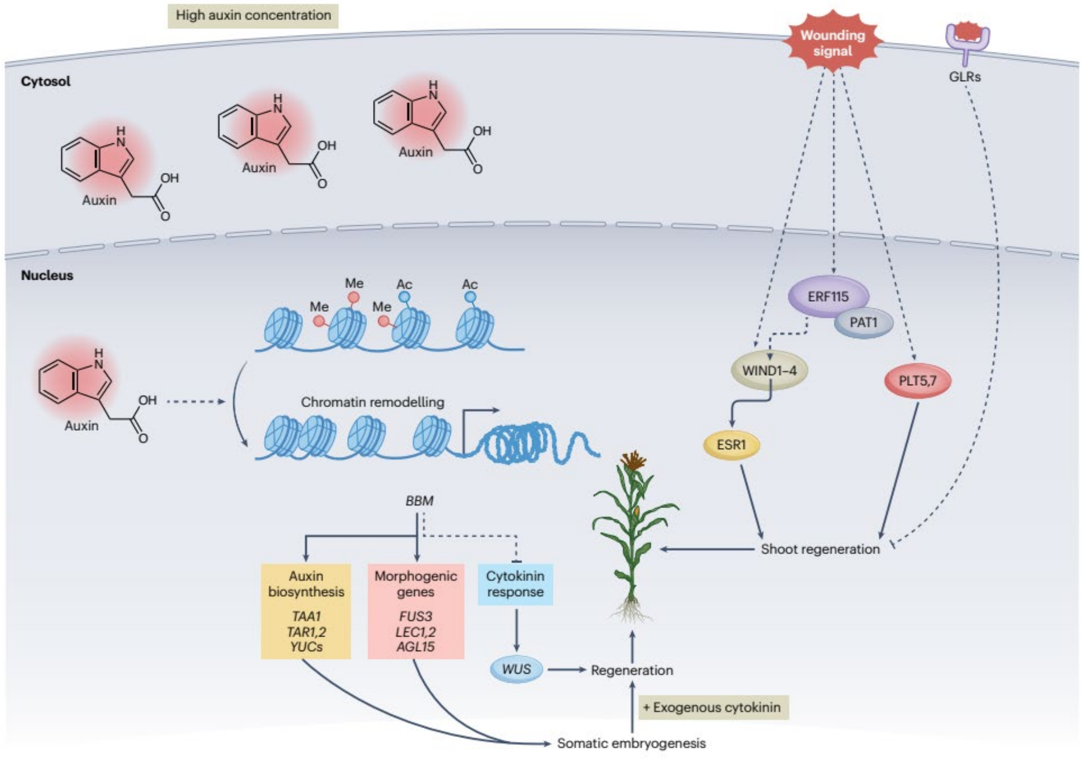


Samson Nalapalli, 2021

Plant regeneration pathways (Chen et al., 2022)



Research #2: Improving Plant Transformation Efficiency

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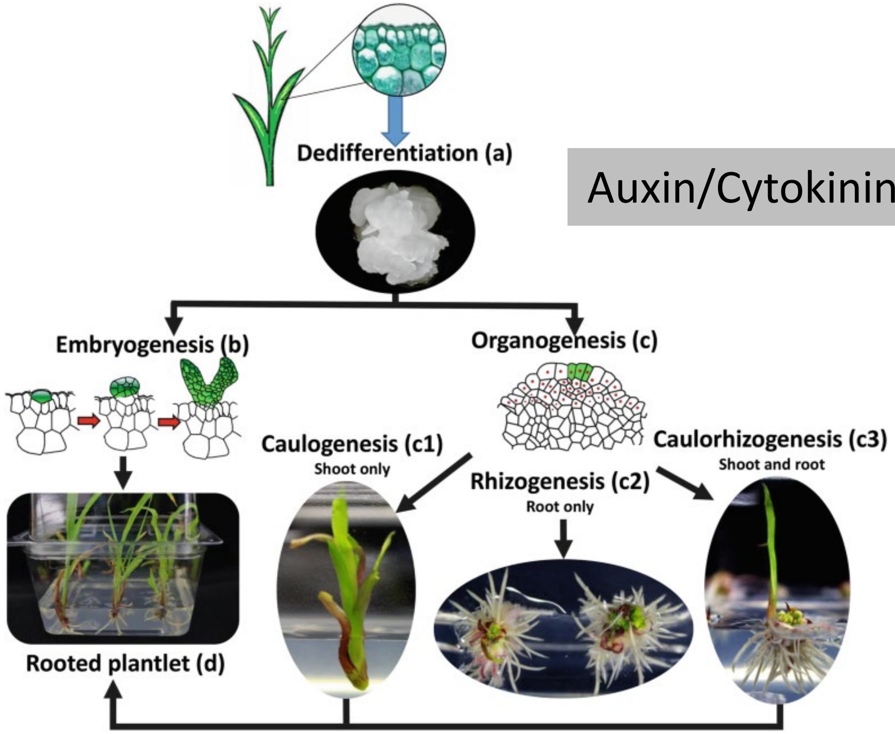

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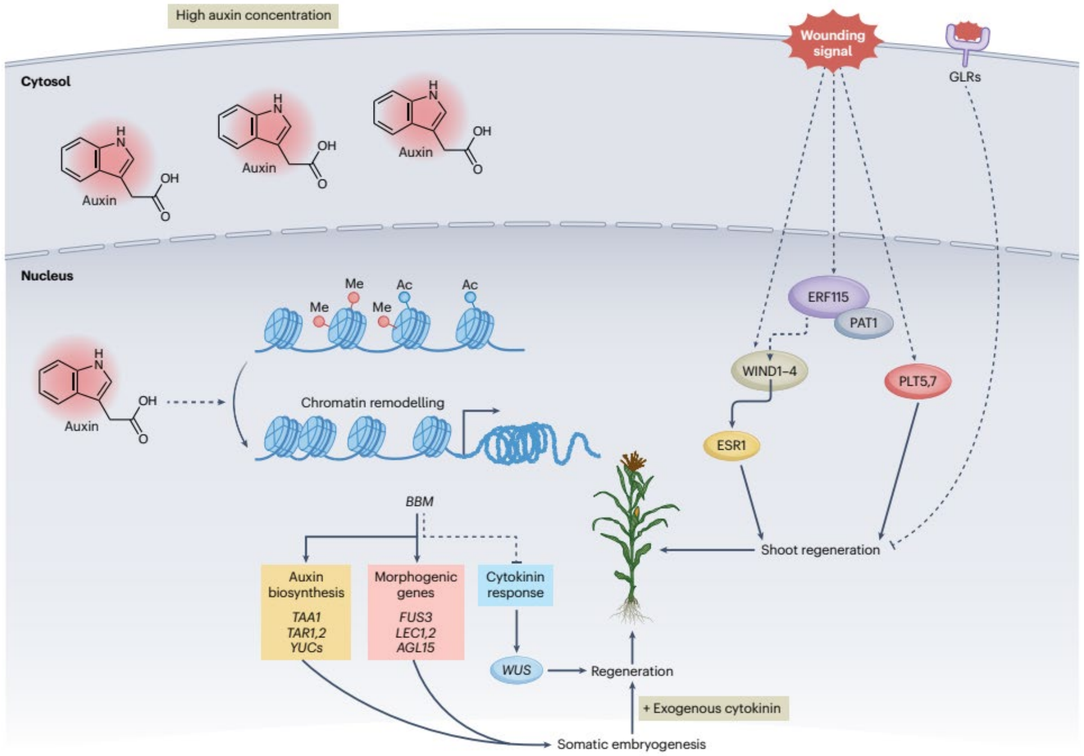
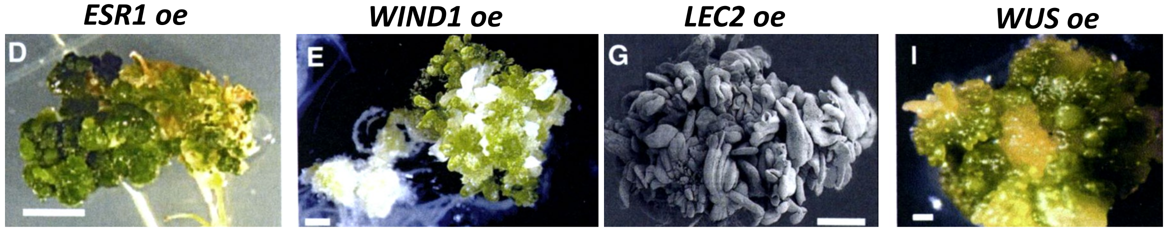




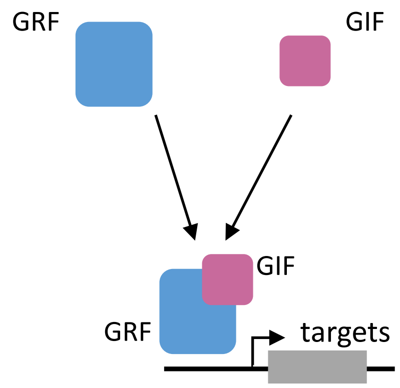
Adapted from Momoko Ikeuchi, 2013

Continued expression of these genes resulted in **developmental defects**.

Research #2: Improving Plant Transformation Efficiency

Dr. Palatnik Lab

*GROWTH REGULATING FACTOR (**GRF**)*
*GRF–INTERACTING FACTOR (**GIF**)*



Promote cell proliferation and control meristems homeostasis

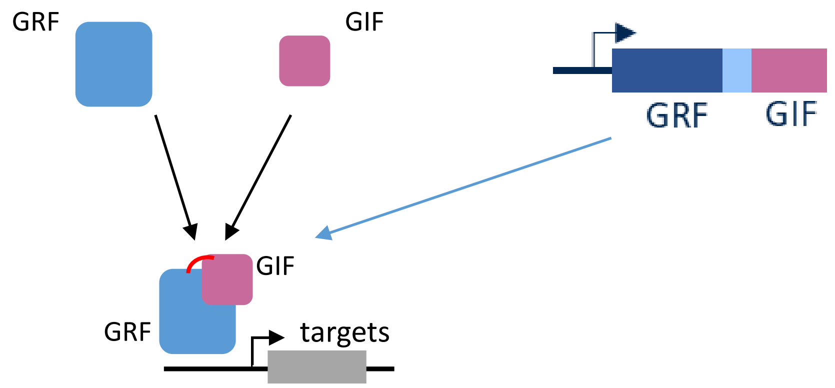
Wt *rGRF3*

Research #2: Improving Plant Transformation Efficiency

Dr. Palatnik Lab

GROWTH REGULATING FACTOR (***GRF***)
GRF–INTERACTING FACTOR (***GIF***)

A *GRF-GIF* chimera enhanced *GRF* activity and plant growth



Promote cell proliferation and control meristems homeostasis

Wt *rGRF3* *rGRF3-GIF1*

Research #2: Improving Plant Transformation Efficiency

Dr. Palatnik Lab

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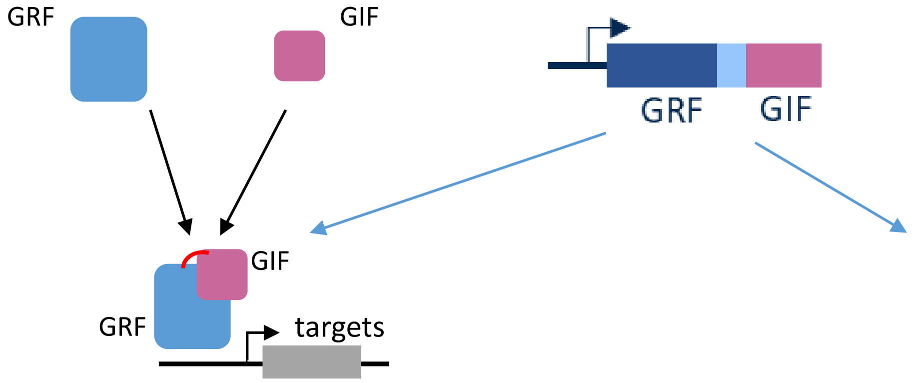

Promote cell proliferation and control meristems homeostasis

Wt *rGRF3* *rGRF3-GIF1*

Research #2: Improving Plant Transformation Efficiency

University of California
The Ralph M. Parsons Foundation
Plant Transformation Facility

Dr. Palatnik Lab

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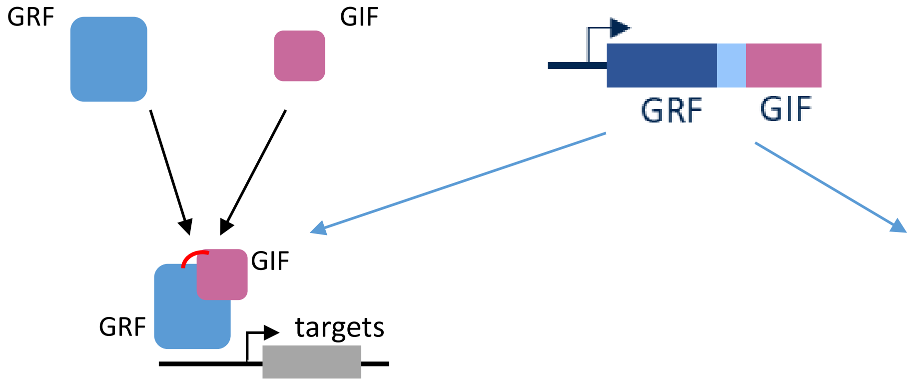
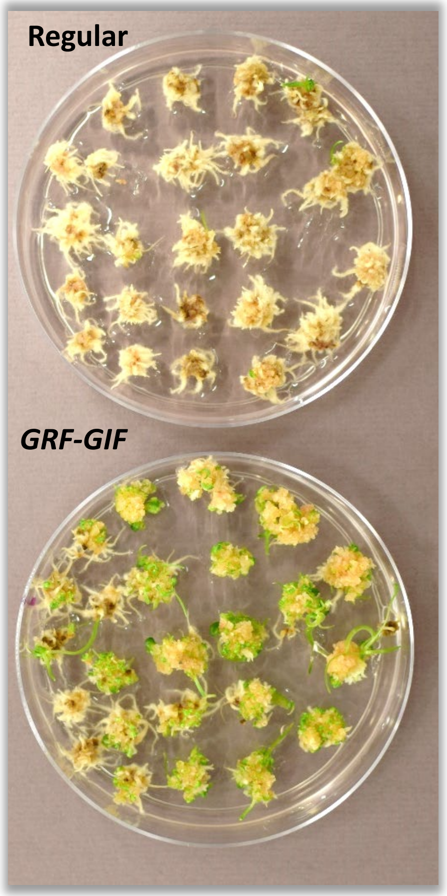
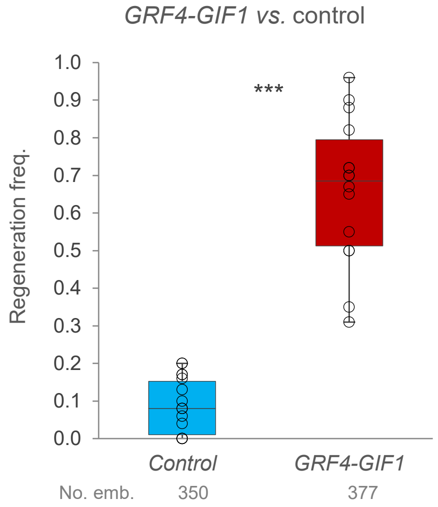


Promote cell proliferation and control meristems homeostasis

Wt *rGRF3* *rGRF3-GIF1*

GRF-GIF increases transformation efficiency in **wheat**



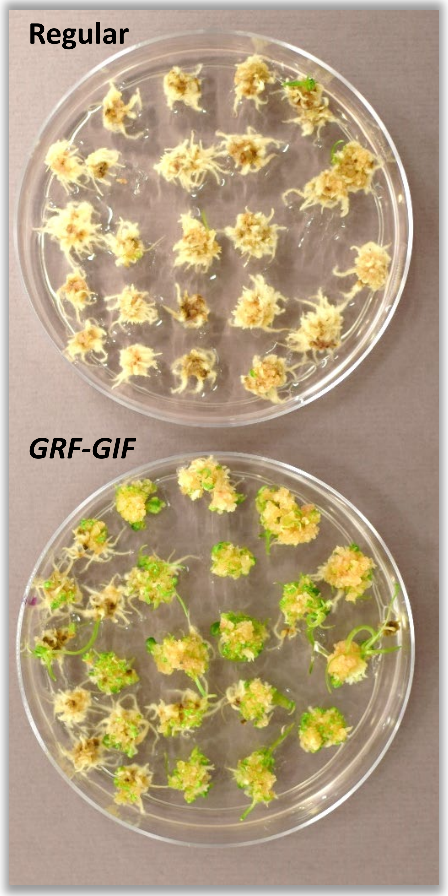


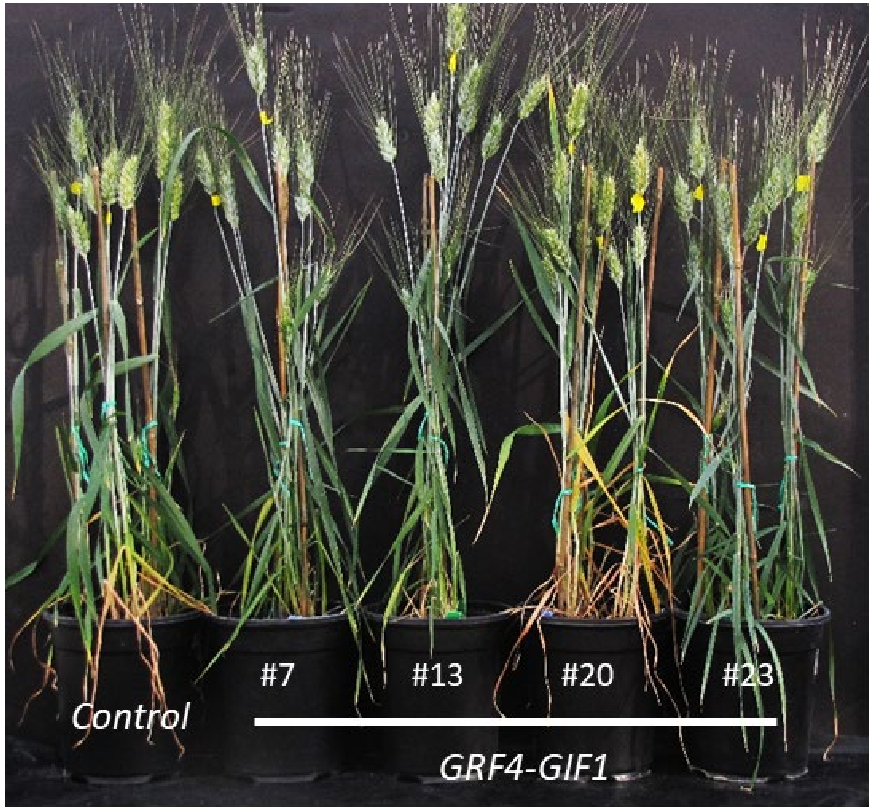
. Reduces the time of transformation process (from 90 to 55 days).

. Reduces constrains that limit transformation frequencies (plant conditions, embryo size, etc).

Research #2: Improving Plant Transformation Efficiency

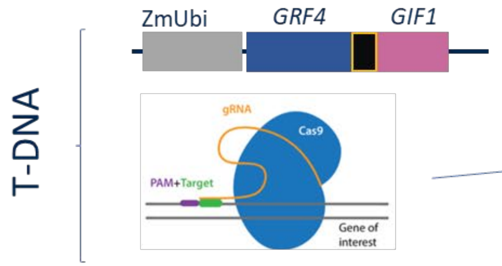
GRF-GIF increases transformation efficiency in wheat **without developmental defects**



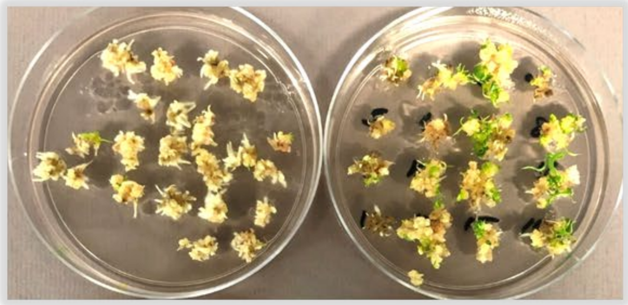


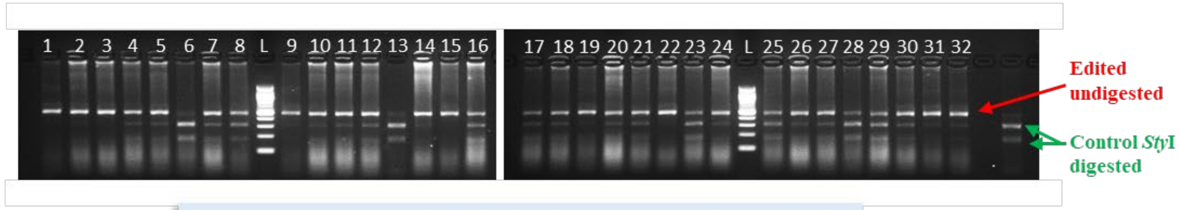
Research #2: Improving Plant Transformation Efficiency

GRF-GIF increases recovery of edited **wheat plants**

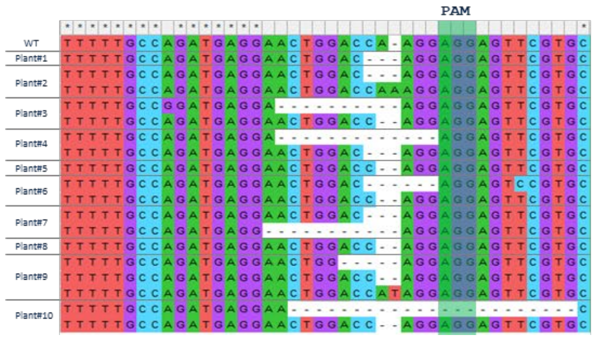


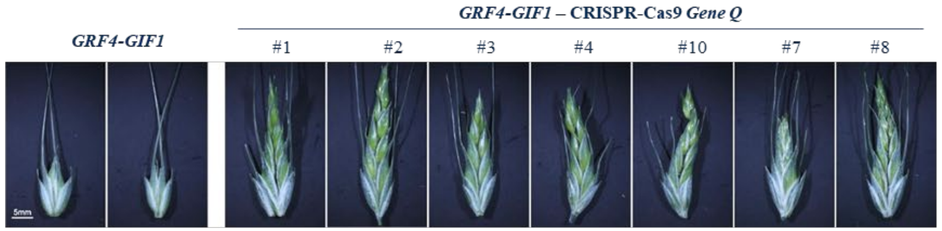
Control GRF4-GIF1 – CRISPR-Cas9 Gene Q





Frequency of regeneration 96%, frequency of editing 100%





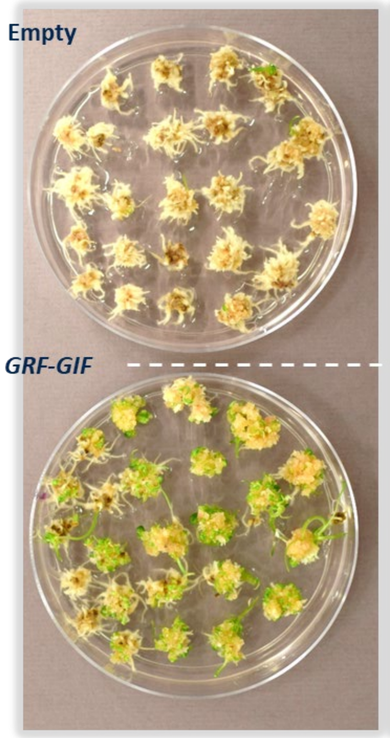
Research #2: Improving Plant Transformation Efficiency

GRF4-GIF1 expands the range of transformable genotypes

Kronos

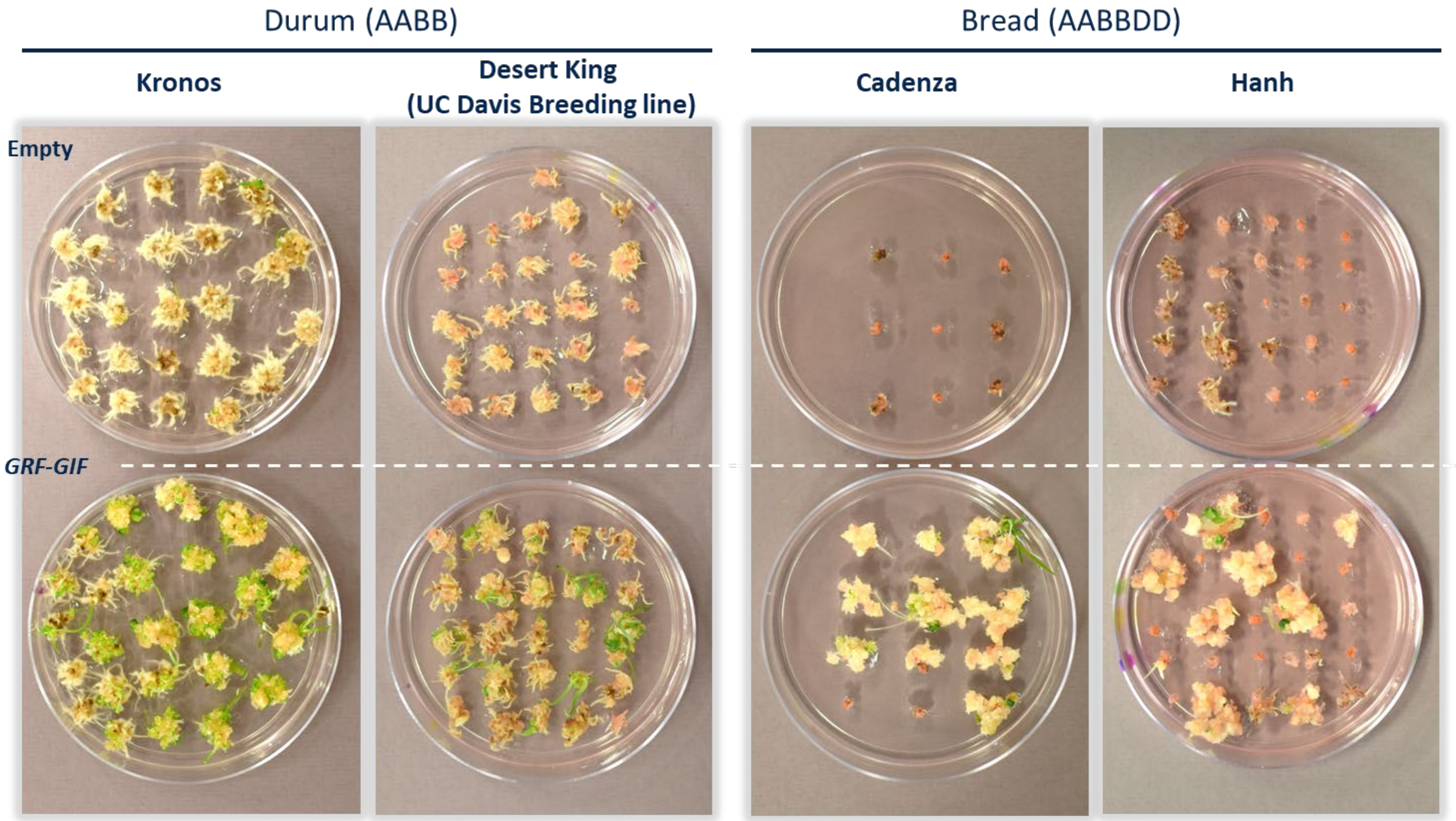
Empty

GRF-GIF



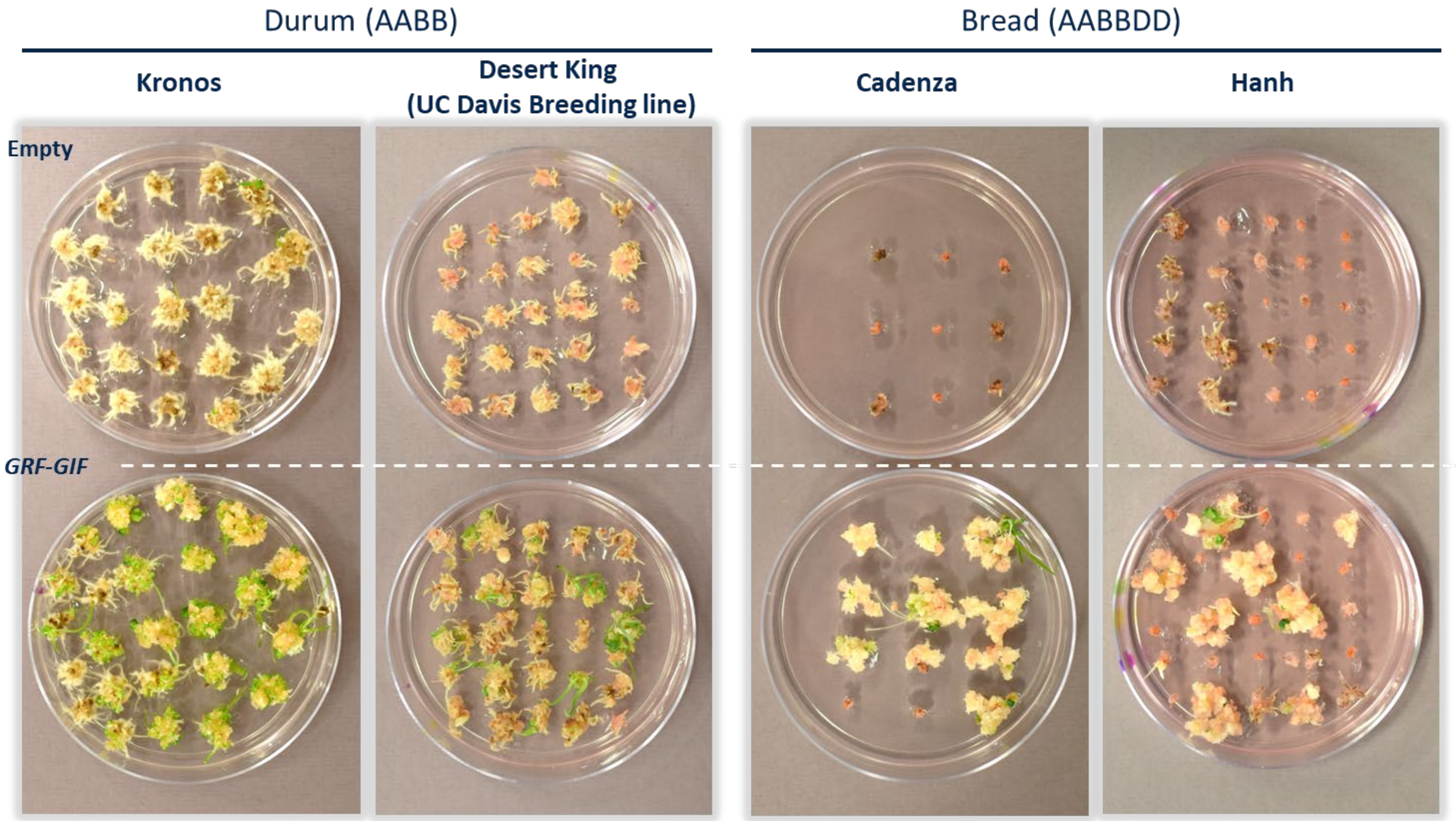
Research #2: Improving Plant Transformation Efficiency

GRF4-GIF1 expands the range of transformable genotypes



Research #2: Improving Plant Transformation Efficiency

GRF4-GIF1 expands the range of transformable genotypes



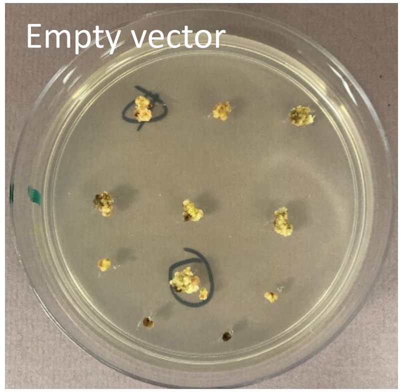
<u>Collaboration with CIMMYT</u>
(Biswal et al., 2023)
GRF4-GIF1 allows efficient and reproducible transformation of 6 elite/farmer preferred wheat varieties

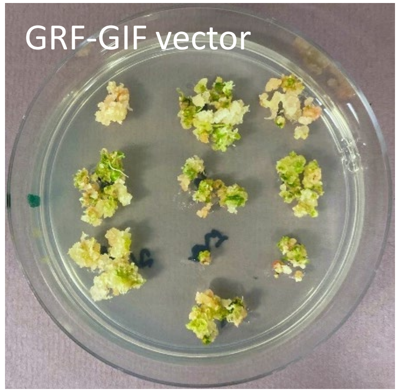
<u>Breeding programs</u>:
UC Davis
Idaho
Montana
Maryland

Research #2: Improving Plant Transformation Efficiency

GRF4-GIF1 improves transformation of barley

Golden Promise

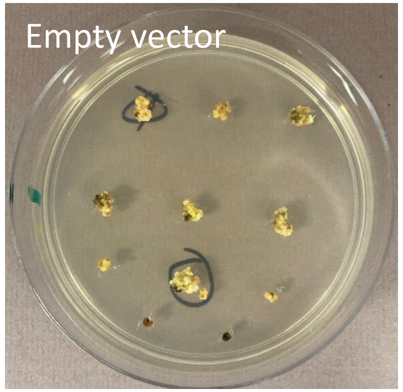


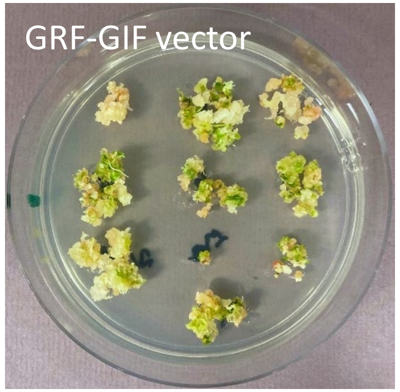


Research #2: Improving Plant Transformation Efficiency

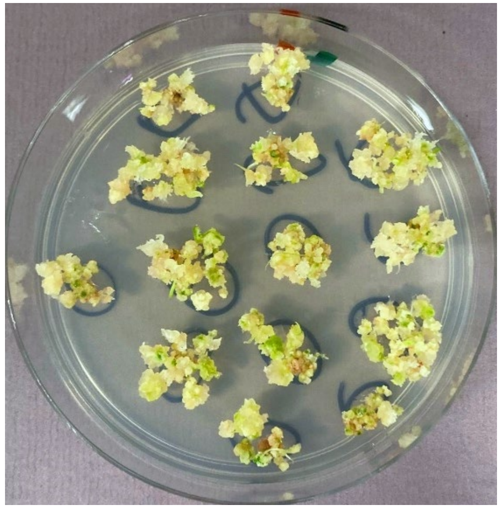
GRF4-GIF1 improves transformation of barley and expands the range of transformable genotypes

Golden Promise

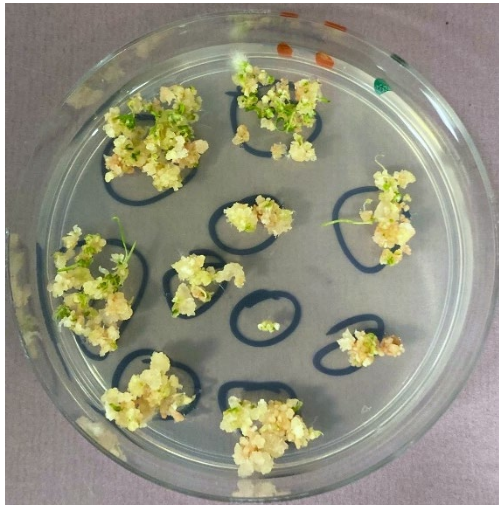




UC Davis breeding lines



UC Tahoe



UC Capay

Alicia del Blanco

Research #2: Improving Plant Transformation Efficiency

University of California
The Ralph M. Parsons Foundation
Plant Transformation Facility

Triticale

. Hybrid of wheat (Triticum) and rye (Secale)
AABBRR

. ≈40% increase in biomass (Davis)

. ≈50% increase in yield (Davis) – Larger Spikes

Joshua Hegarty

Research #2: Improving Plant Transformation Efficiency

University of California
The Ralph M. Parsons Foundation
Plant Transformation Facility

Triticale

Joshua Hegarty

. Hybrid of wheat (Triticum) and rye (Secale)
AABBRR

. ≈40% increase in biomass (Davis)

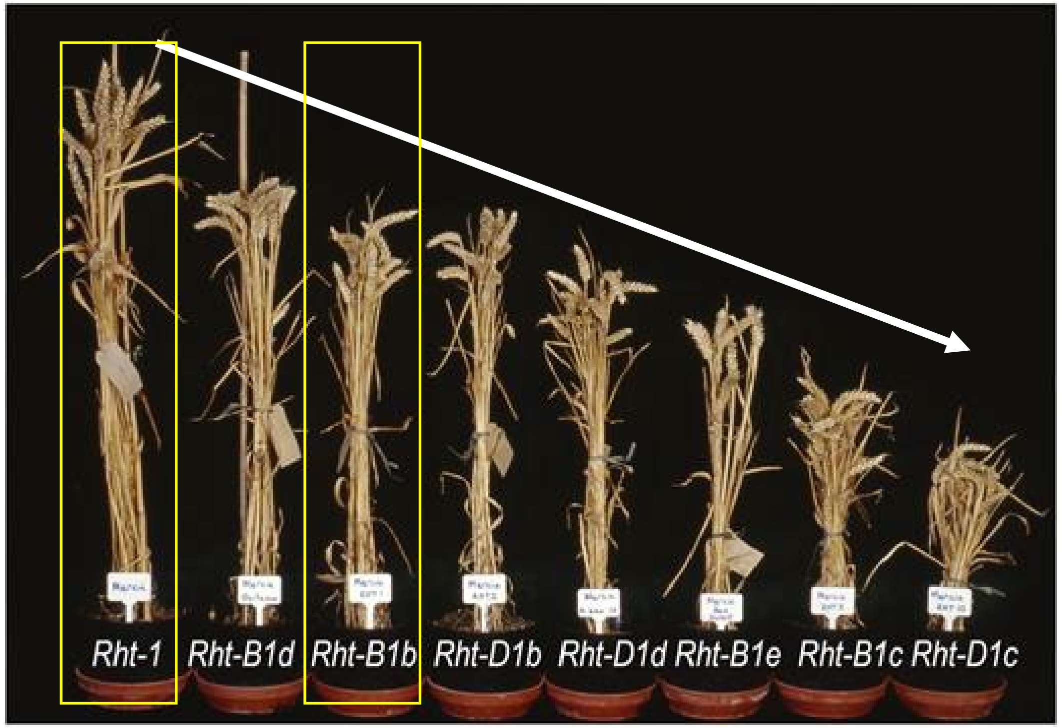
. ≈50% increase in yield (Davis) – Larger Spikes

. Lodging problems

“I would like to make the plants a little bit more compact”

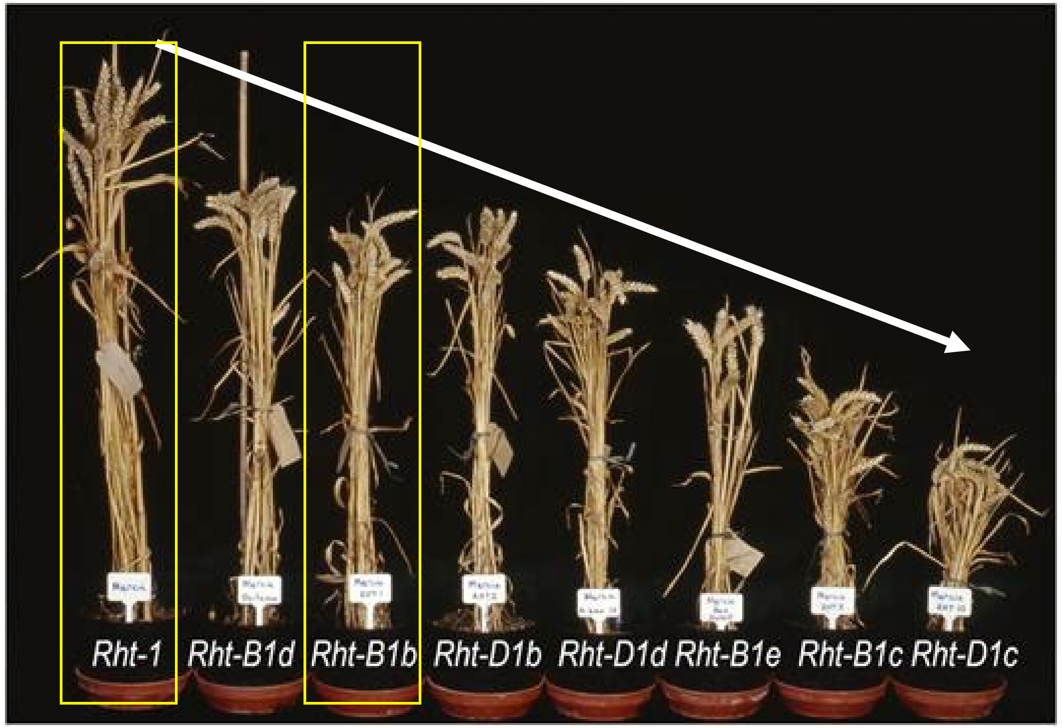
Research #2: Improving Plant Transformation Efficiency

Rht1: Dominant mutations in DELLA – "Green Revolution"



Research #2: Improving Plant Transformation Efficiency

Rht1: Dominant mutations in DELLA – "Green Revolution"



Rht1 mutant alleles are associated with negative pleotropics effects.

Rht-1 *Rht-B1b*

***Rht-B1b* reduces coleoptile and leaf length**

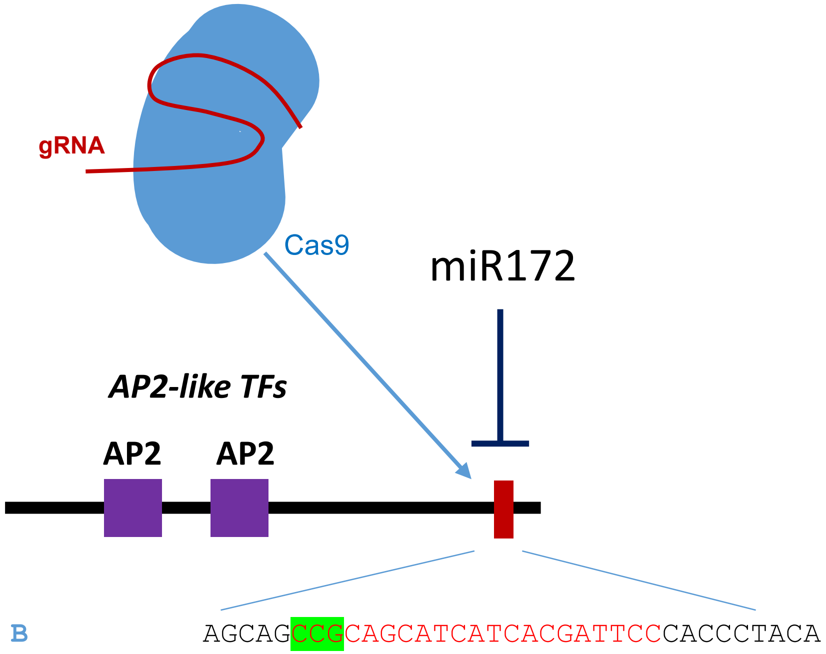
Shorter seedlings limits the use of Rht1 mutant alleles in semiarid regions.

Novel genetic resources to control plant height in wheat and triticale

Research #2: Improving Plant Transformation Efficiency

Novel genetic resources to control plant height in wheat and triticale

Chaozhong Zhang



Durum wheat

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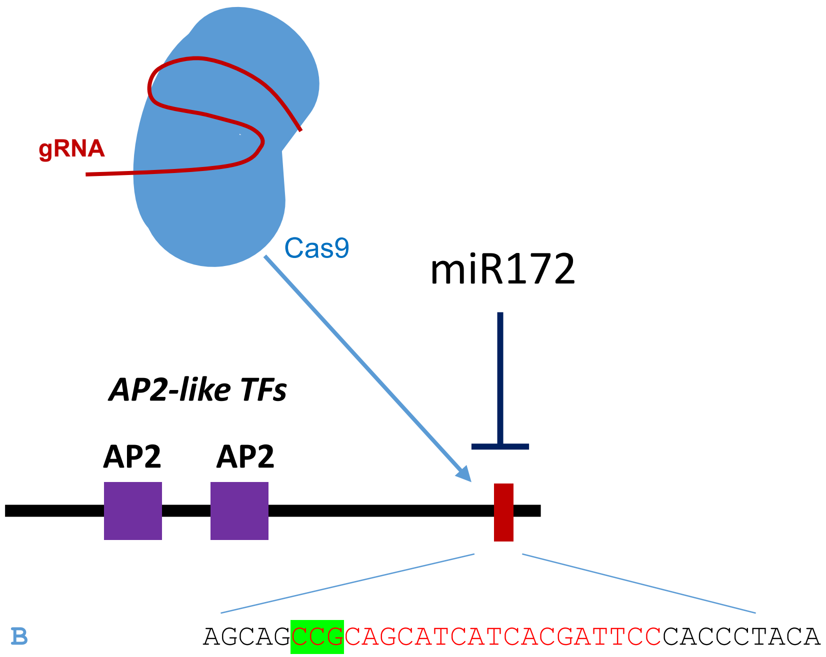
Plant Transformation Facility

Novel genetic resources to control plant height in wheat and triticale

Chaozhong Zhang



Durum wheat

Research #2: Improving Plant Transformation Efficiency

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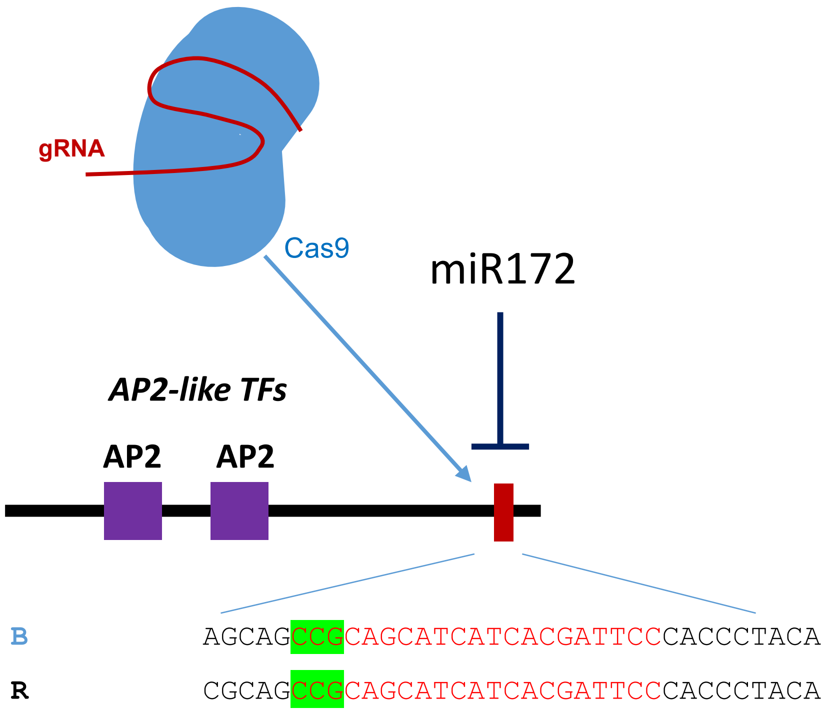
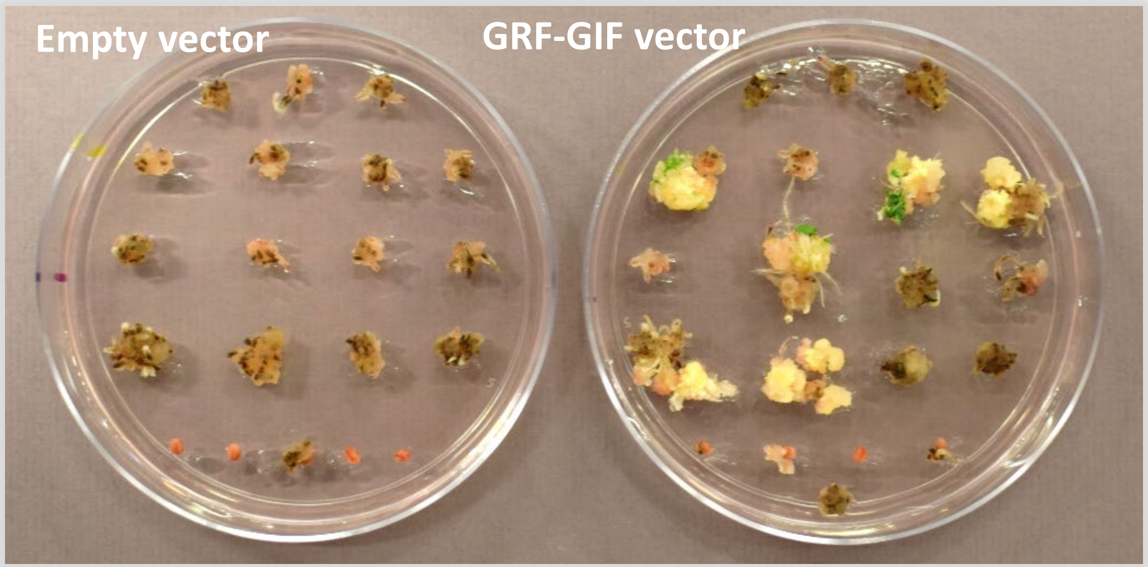
Novel genetic resources to control plant height in wheat and triticale



Editing *AP2-like* in Triticale (AABBRR)

Improved triticale transformation



Research #2: Improving Plant Transformation Efficiency

Triticale

Wt **CRISPR lines**

genomes
B
R

Research #2: Improving Plant Transformation Efficiency

Triticale

Wt — CRISPR lines

genomes
B
R

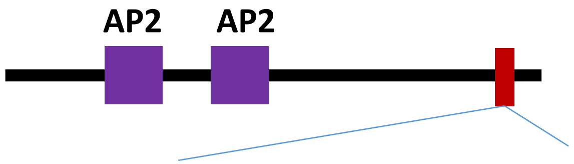
Interaction with miR172

Plant height

Quantitative control of plant height:

. Type of mutation

AP2-like TFs




```
Wt           CGCAGCCGCAG-CATCATCACGATTCCCACCCTACA    -33.0 Kcal/mol
Mutant +1    CGCAGCCGCAGTCATCATCACGATTCCCACCCTACA    -29.2 kcal/mol
Mutant -1    CGCAGCCGCAG--ATCATCACGATTCCCACCCTACA    -26.9 kcal/mol
```

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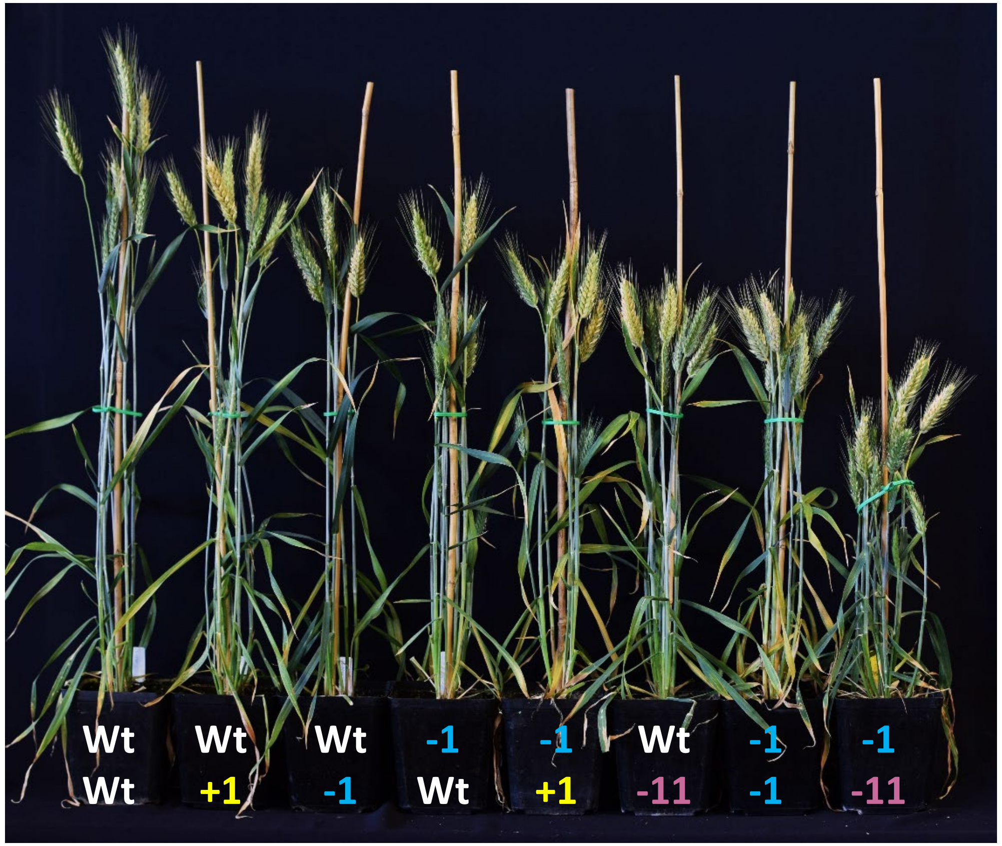
Research #2: Improving Plant Transformation Efficiency

Triticale

Wt **CRISPR lines**



genomes
B
R

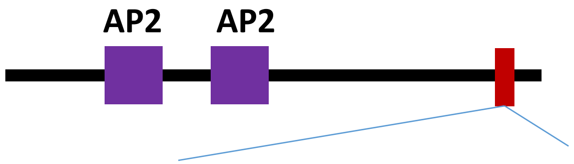
Interaction with miR172

Plant height

Quantitative control of plant height:

. Type of mutation
. Dosage of mutations
. Combination of mutations

AP2-like TFs



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Research #2: Improving Plant Transformation Efficiency

Triticale

Joshua Hegarty

“I would like to make the plants a little bit more compact”

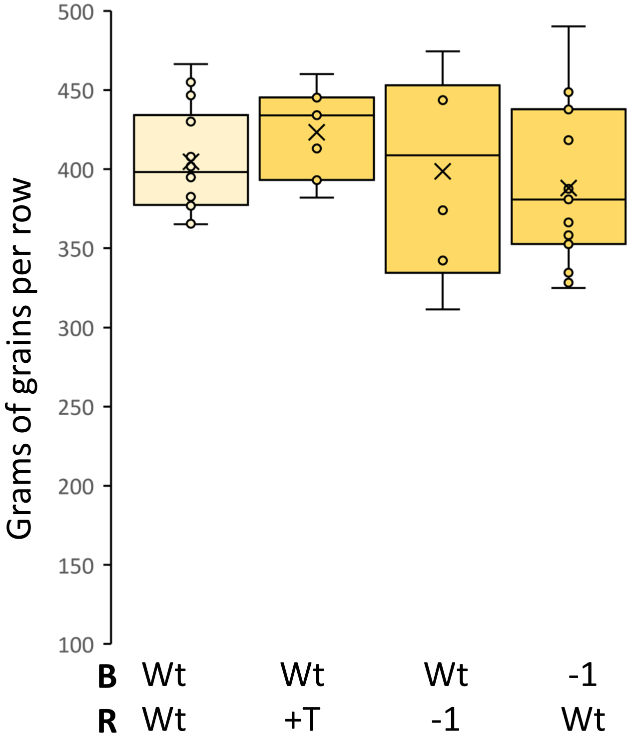
Research #2: Improving Plant Transformation Efficiency

Triticale

Joshua Hegarty

“I would like to make the plants a little bit more compact”

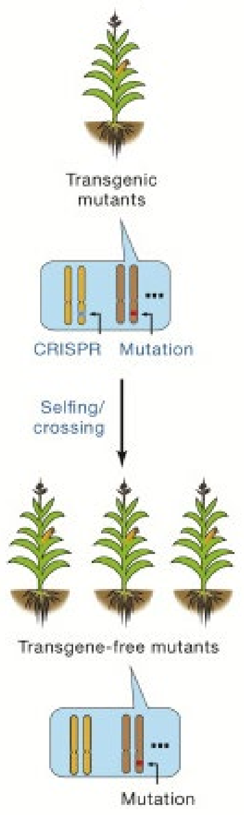
No yield penalty



Research #3: Genome editing in clonal crops

Research #3: Genome editing in clonal crops

Transgene-free mutants in seed crops

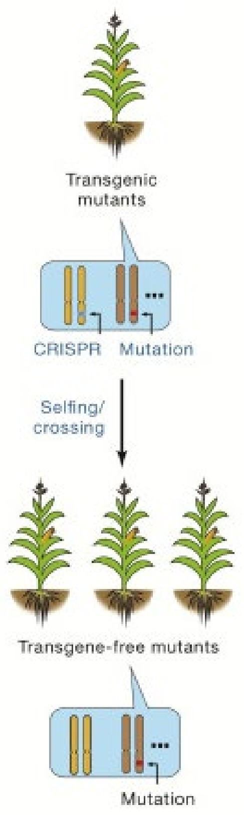


Gao 2021

Research #3: Genome editing in clonal crops

In clonal crops it is not possible to use breeding to eliminate CRISPR sequences and maintain the fidelity of clonal germplasms

Transgene-free mutants in seed crops

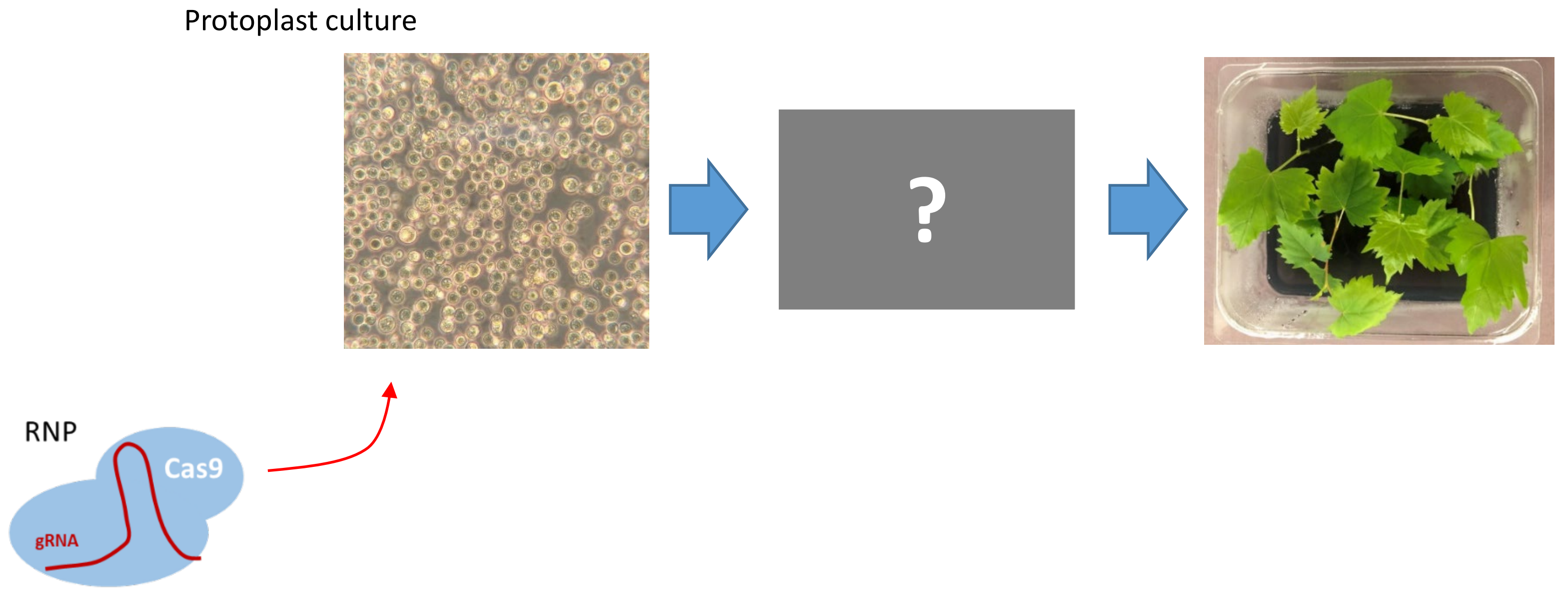


Gao 2021

Research #3: Genome editing in clonal crops

In clonal crops it is not possible to use breeding to eliminate CRISPR sequences and maintain the fidelity of clonal germplasms

Protoplast culture provides one of the best avenues for producing transgene-free gene edited plants



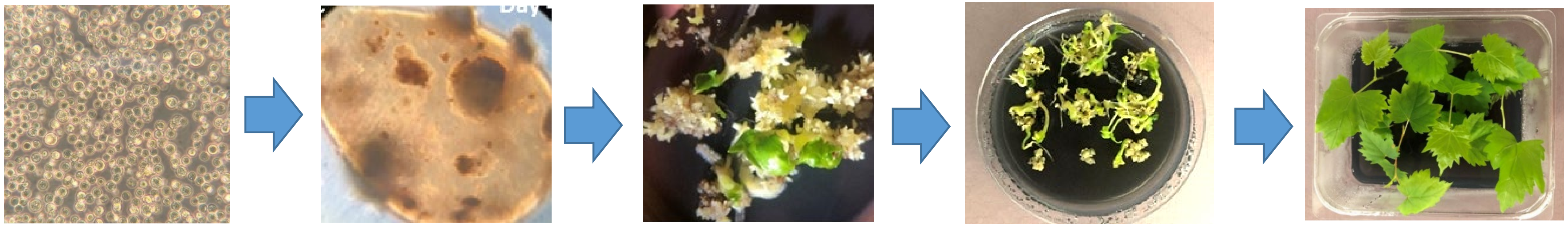
Research #3: Genome editing in clonal crops

David Tricoli

In clonal crops it is not possible to use breeding to eliminate CRISPR sequences and maintain the fidelity of clonal germplasms

Protoplast culture provides one of the best avenues for producing transgene-free gene edited plants

Protoplast culture



≈ 6 months

A Protoplast-based Gene Editing protocol for *Vitis* species

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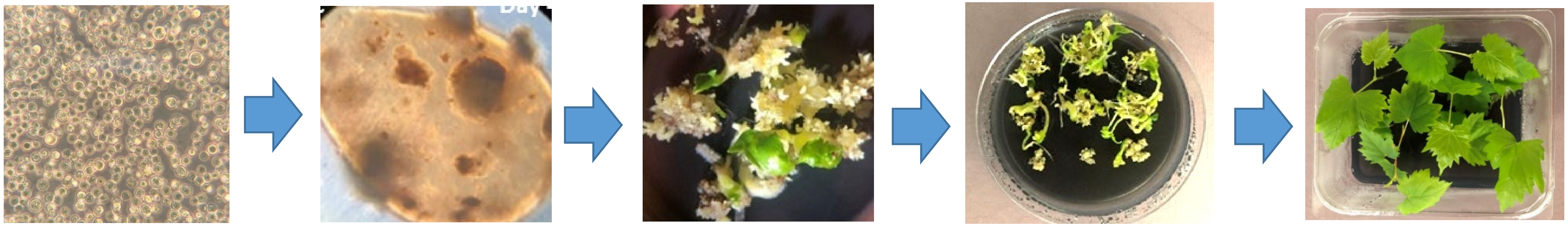
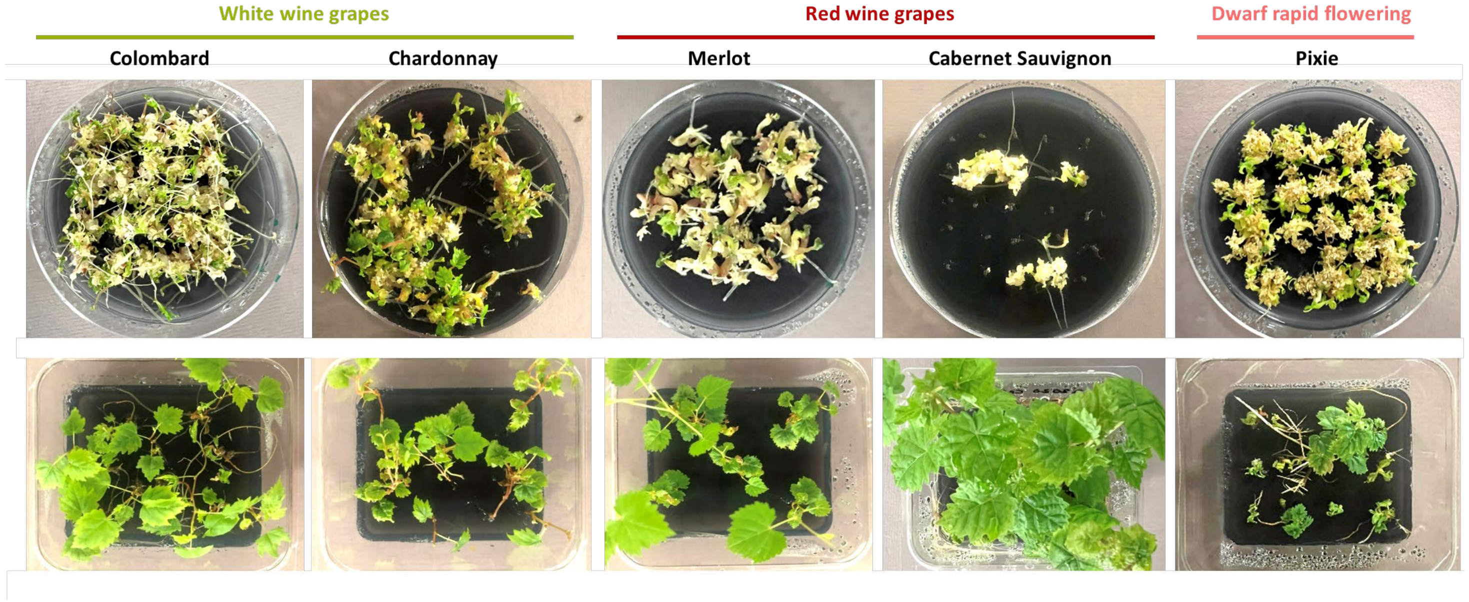
Research #3: Genome editing in clonal crops

David Tricoli

Protoplast culture



Our grape protoplast regeneration protocol works in multiple cultivars



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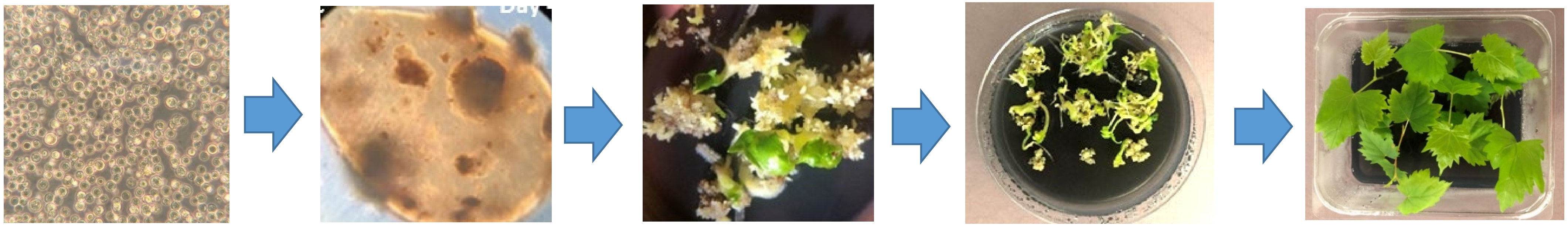
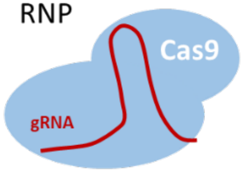
Research #3: Genome editing in clonal crops

David Tricoli

Protoplast culture



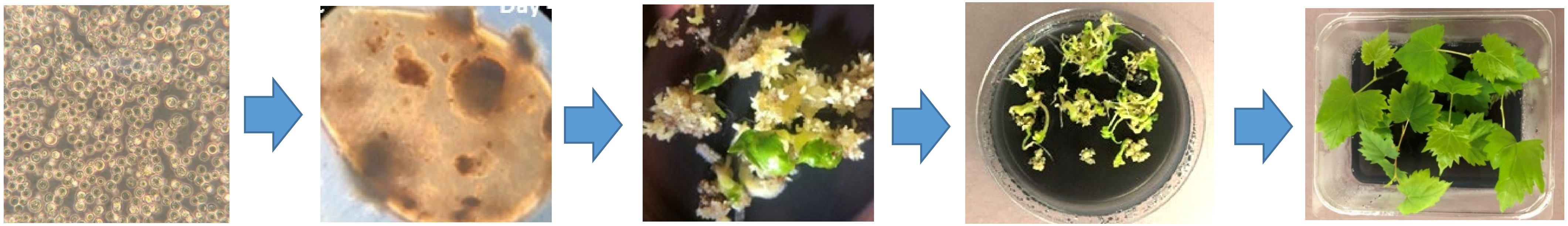
RNP

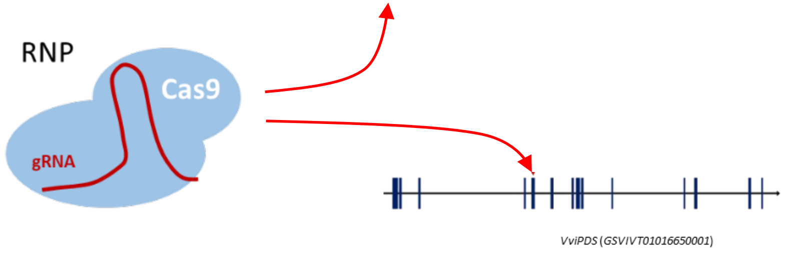


Research #3: Genome editing in clonal crops

David Tricoli

Protoplast culture






```
Wt            AGCCAGGG-GAATTCAGCCGATTTGA

Thompson Seedless
#1            AGCCAGGG---ATTCAGCCGATTTGA
              AGCCAGGG-----TCAGCCGATTTGA
              AGCCAGGGTGAATTCAGCCGATTTGA

Colombard
#8,32,34      AGCCAGGGTGAATTCAGCCGATTTGA
              AGCCAGGGGGAATTCAGCCGATTTGA

V. arizonica
#2            AGCCAGGG---ATTCAGCCGATTTGA
              del 70bp
```

Thompson Seedless | **Colombard** | ***V. arizonica***

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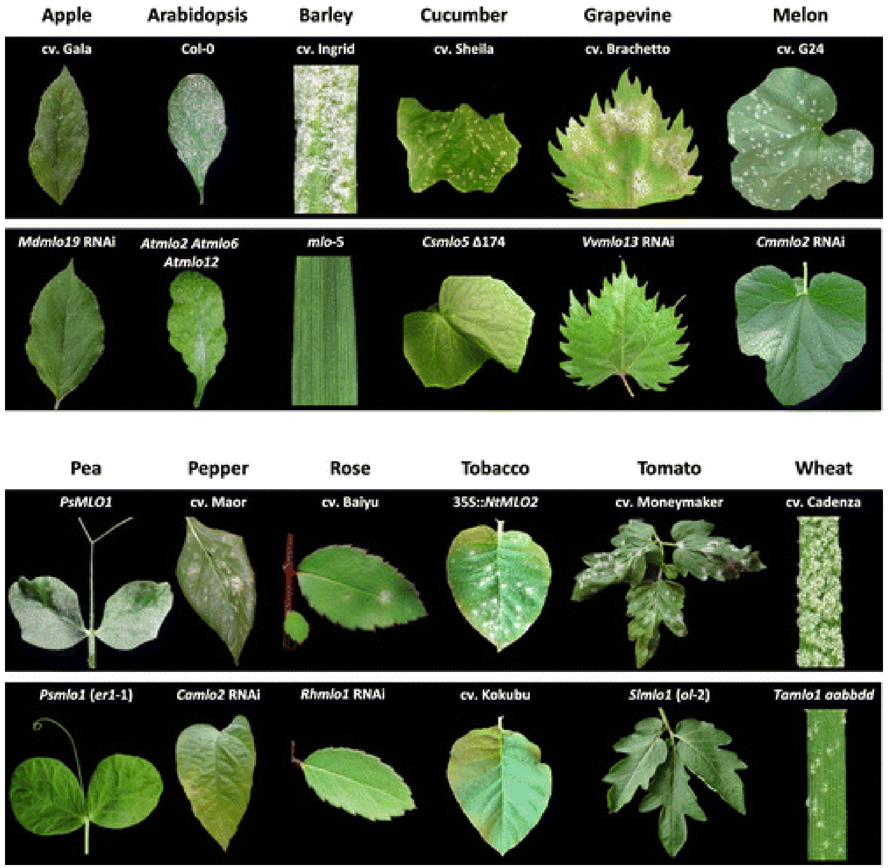
Research #3: Genome editing in clonal crops

Protoplast-Mediated Gene Editing for Disease Resistance

Loss-of-function of the *Mildew resistance locus o* (*Mlo*) gene
broad-spectrum resistance to powdery mildew

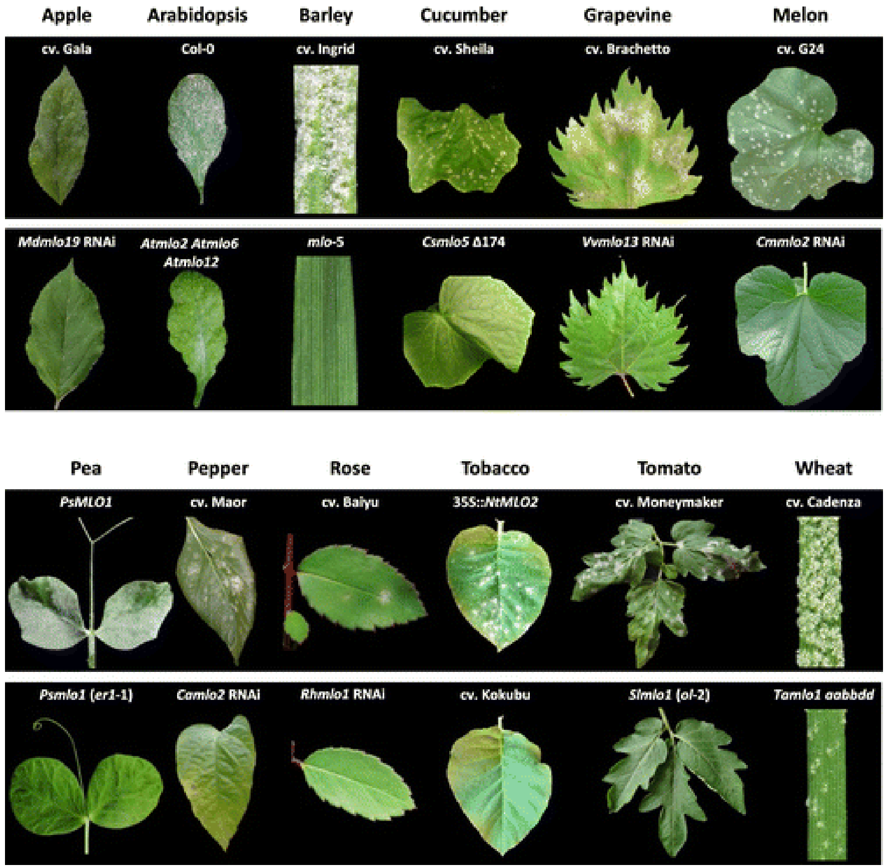


Stefan Kusch and Ralph Panstruga 2017

Research #3: Genome editing in clonal crops

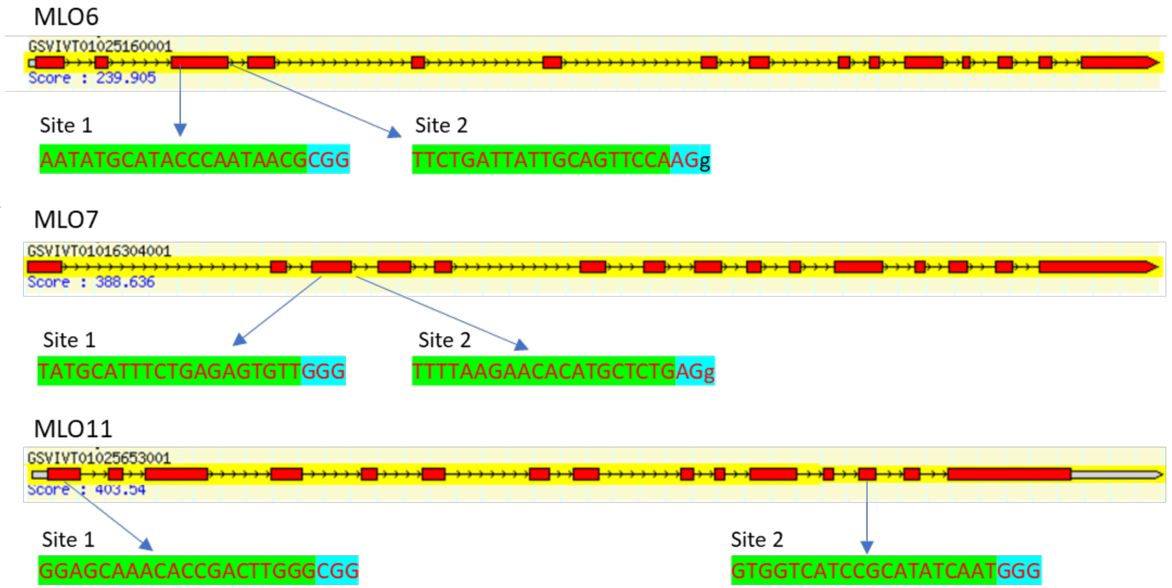
Protoplast-Mediated Gene Editing for Disease Resistance

Loss-of-function of the *Mildew resistance locus o* (*Mlo*) gene broad-spectrum resistance to powdery mildew



Stefan Kusch and Ralph Panstruga 2017

3 *MLO* genes were associated with PM resistance in grape



Pessina et al., 2016
Wan et al., 2020

Research #3: Genome editing in clonal crops

Protoplast-Mediated Gene Editing for Disease Resistance

A MLO-edited grape population!

Collaboration with Dario Cantu Lab (UC Davis)

Genotypes	Line	MLO6	MLO7	MLO11
1	40-1	TA>AT het	insT hom	insT hom
	39-2	TA>AT het	insT hom	insT hom
	39-3	TA>AT het	insT hom	insT hom
	39-4	TA>AT het	insT hom	insT hom
2	93-2	del1 het	insT hom	insT hom
3	39-1	del1 het	insT het	del1 hom
4	41-1	del1 het	insT het	insT het
5	32-1	del1 hom	insT hom	insT/del2
	117	del1 hom	insT hom	insT/del2
6	112	del1 hom	insT hom	insT/del1
7	3--1	del1 hom	insT/large del	insT hom
	103	del1 hom	insT/large del	insT hom
8	107	insT het	insT het	del1 het
9	48-1	large del	insT/large del	insT het
10	24-1	insA/large del	insT hom	insT hom
11	44-1	wt	insT het	insA hom
	45-1	wt	insT het	insA hom
	119	wt	insT het	insA hom
12	23-1	wt	insT hom	del9/del1/insA
	23-2	wt	insT hom	del9/del1/insA
13	87-2	wt	wt	insT/TT
	92-1	wt	wt	insT/TT
14	106	wt	wt	insT het
15	118	wt	wt	insA het
16	29-1	wt	insT het	wt
	29-2	wt	insT het	wt

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Plant Transformation Facility

Thank you!

https://ptf.ucdavis.edu/

Our team

Director
Abhaya Dandekar

Interim Manager
Juan M Debernardi

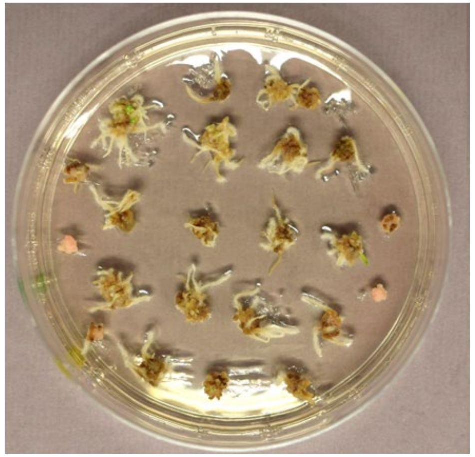
Staff Research Associates
David M Tricoli
Vanna Ebanez
Danielle Inchaurregui
Lucero Jimenez
Mariana Padilla
Alice Lutzenhiser
Mackenzie Ella Benson

Collaborators
Jorge Dubcovsky, Alicia del Blanco, Josh Hegarty (UC Davis)
Javier Palatnik (IBR, Argentina)
Steven Knapp & Mitchel Feldmann (UC Davis)
Patrick Brown (UC Davis)
Dandekar Lab (UC Davis)
Christine Diepenbrock (UC Davis)
Richard Michelmore Lab (UC Davis)
Barbara Blanco (UC Davis)
Dario Cantu (UC Davis)
Laurens Pauwels (VIB, Belgium)
Sadiye Hayta and Mark Smedley (JIC, UK)
Andrea Gallavotti (Rutgers, The State University of New Jersey)

Research: Improving Plant Transformation Efficiency

Transformation with “regular” vectors?

Regular vector (+Hyg)

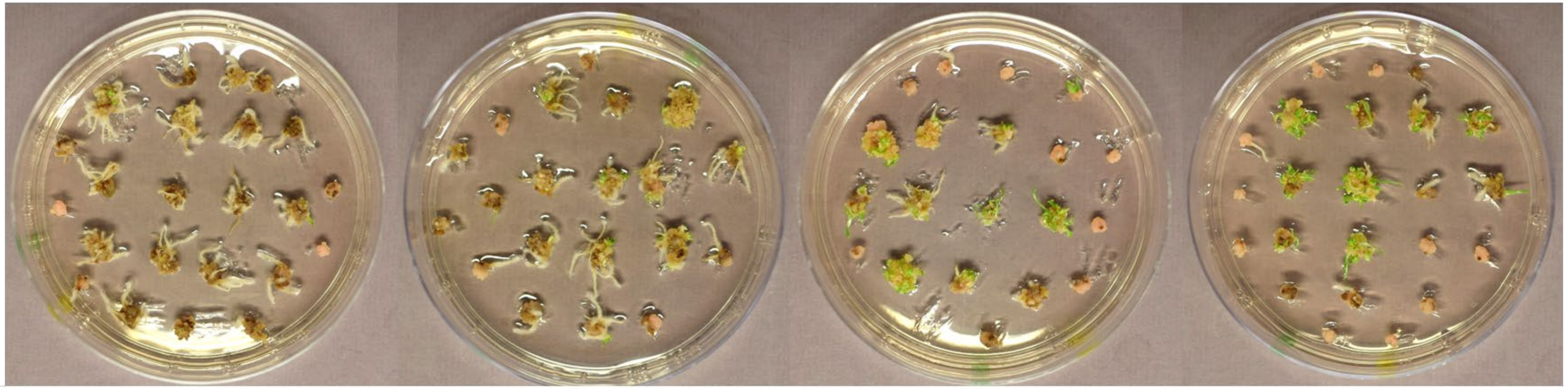


Research: Improving Plant Transformation Efficiency

Co-transformation with *GRF4-GIF1* chimera allow to recover high frequency of transgenic events using regular vectors

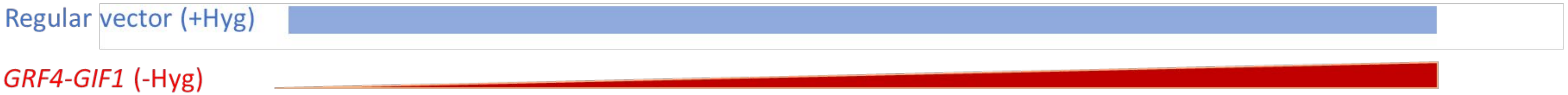
Regular vector (+Hyg)

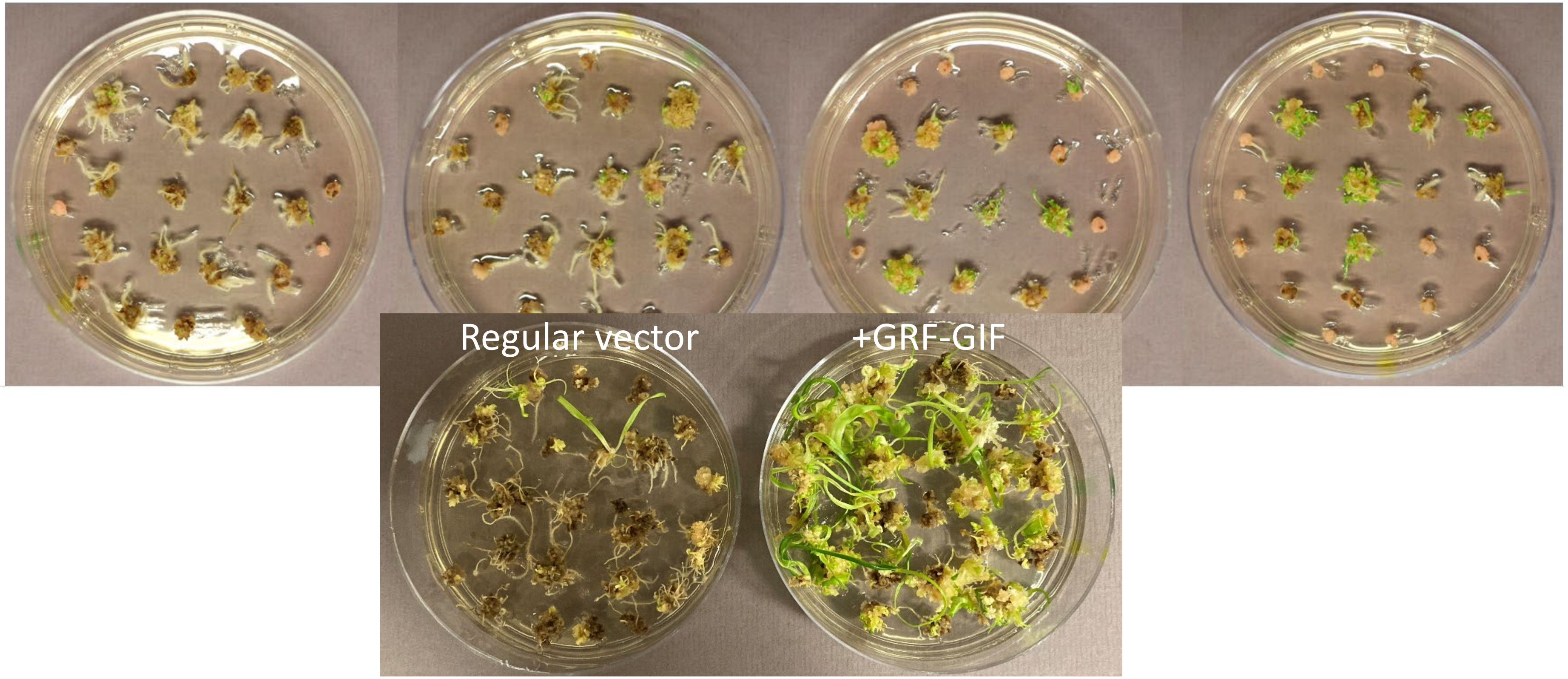
GRF4-GIF1 (-Hyg)



Research: Improving Plant Transformation Efficiency

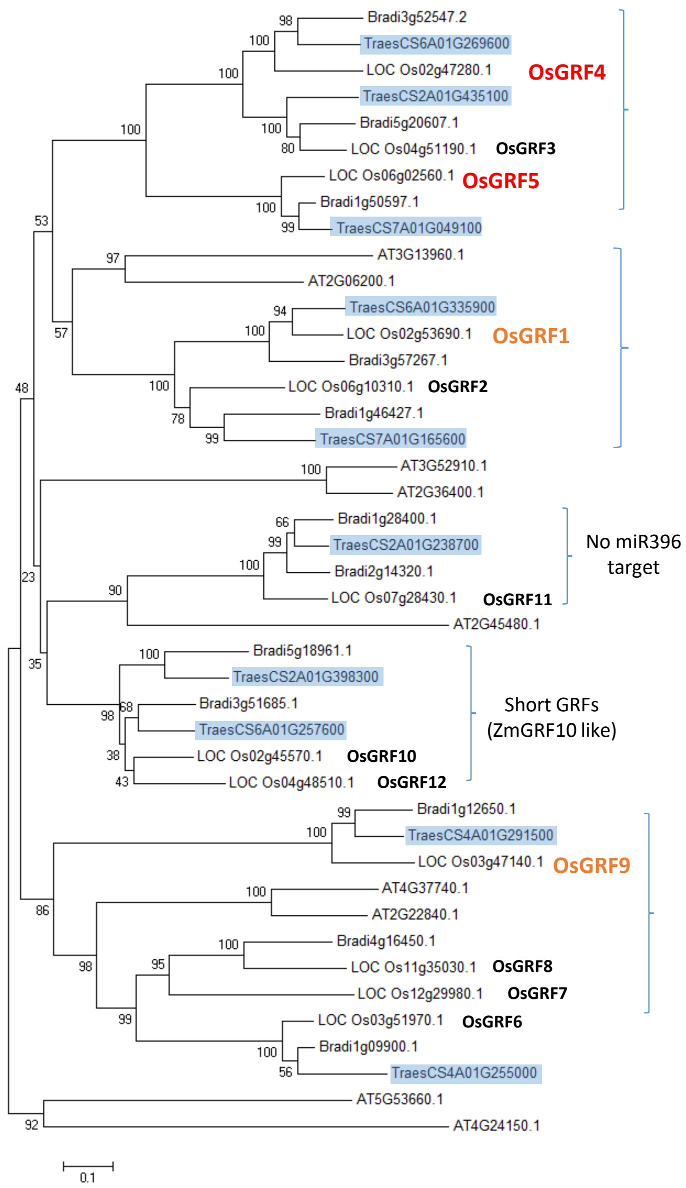
Co-transformation with *GRF4-GIF1* chimera allow to recover high frequency of transgenic events using regular vectors



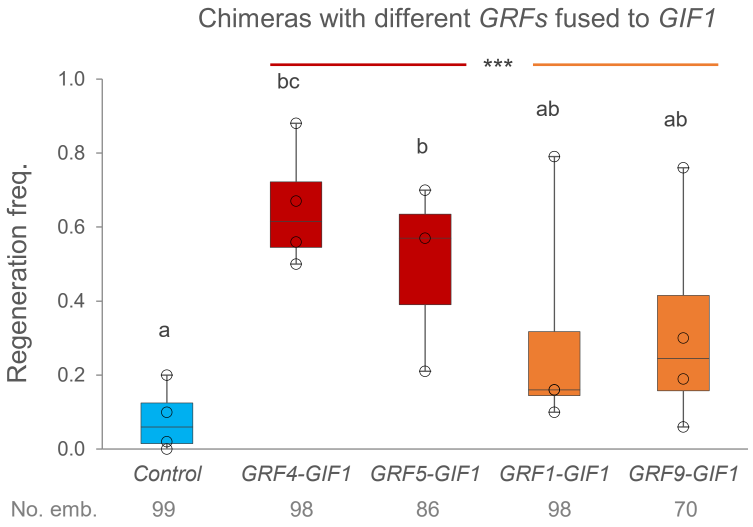


Plant Transformation Facility service

GRF4 produces the more robust response, but other *GRFs* also promote regeneration

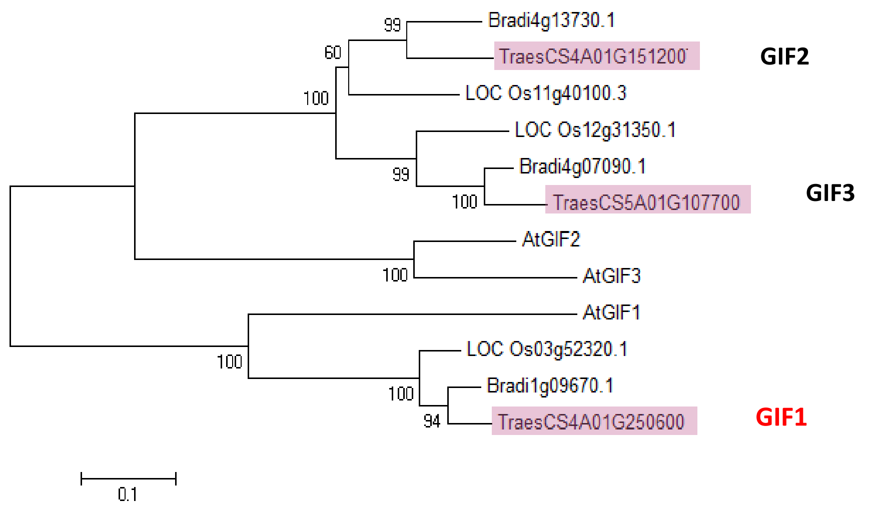


The *GRF-GIF* chimeras did not generate deleterious phenotypes and produced seeds

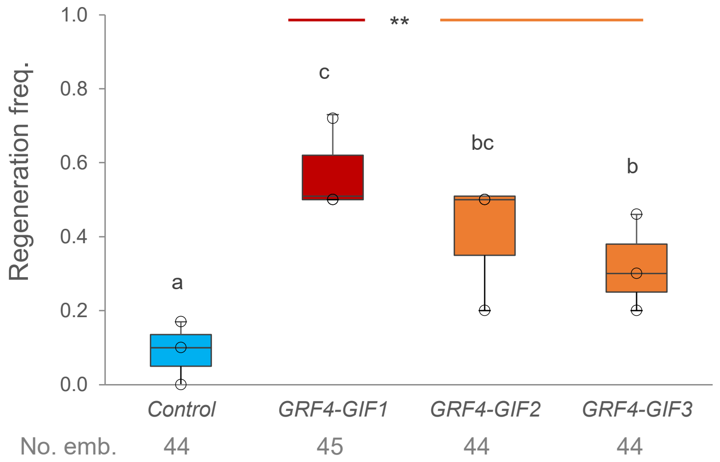


Control *GRF4-GIF1* *GRF5-GIF1* *GRF1-GIF1* *GRF9-GIF1*

GIF1 produces the most robust response, but *GIF2* and *GIF3* also promote regeneration

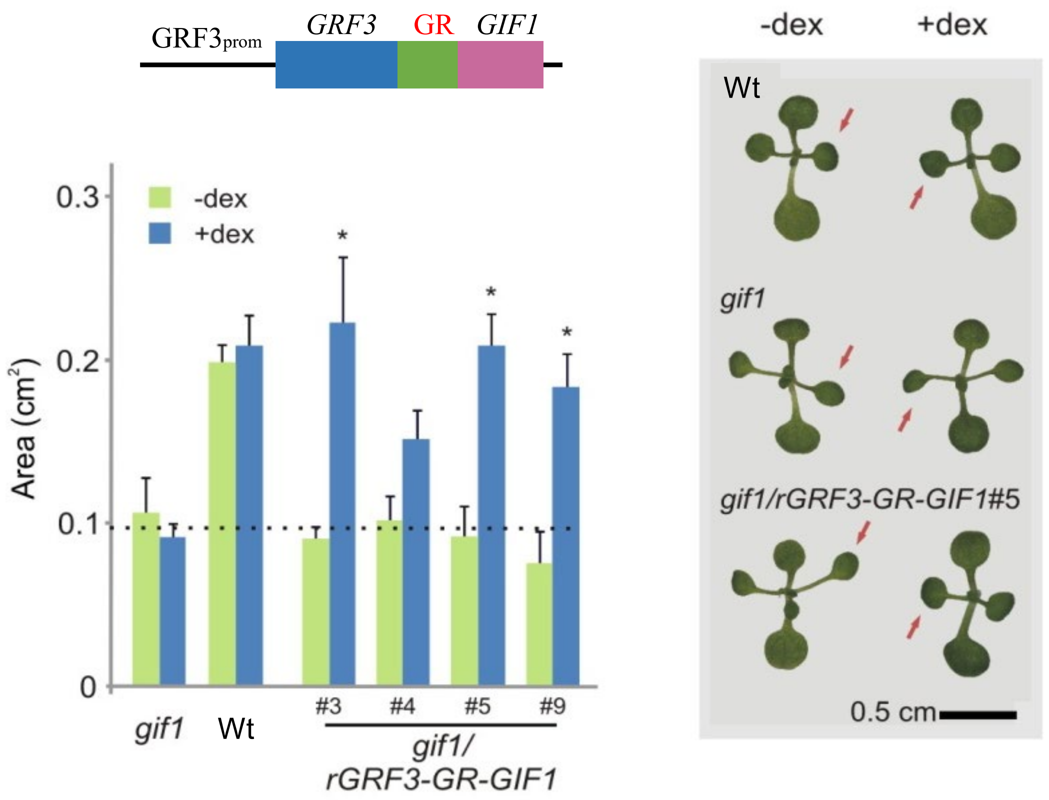


% regenerating embryos	Avg. 3 exp.
Empty	9 %
ZmUbi GRF4 GIF1	57 %
ZmUbi GRF4 GIF2	40 %
ZmUbi GRF4 GIF3	32 %

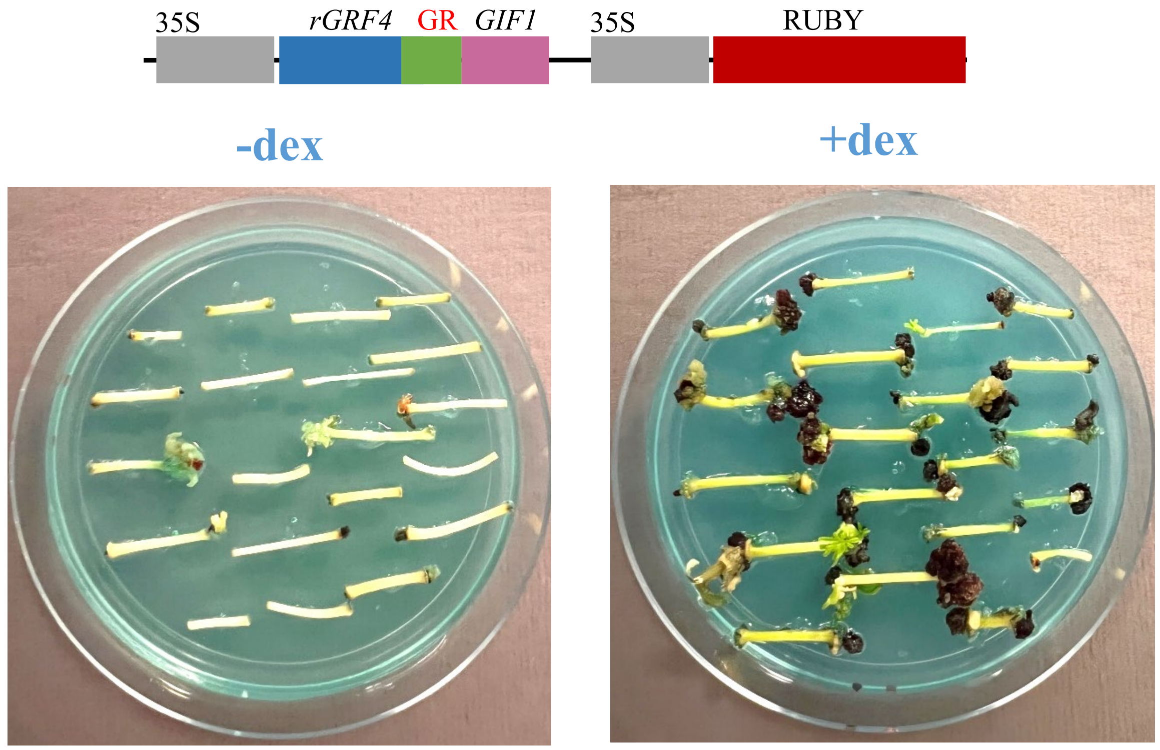


GRF-GR-GIF can be induced by addition of dexamethasone

Arabidopsis thaliana



Citrus



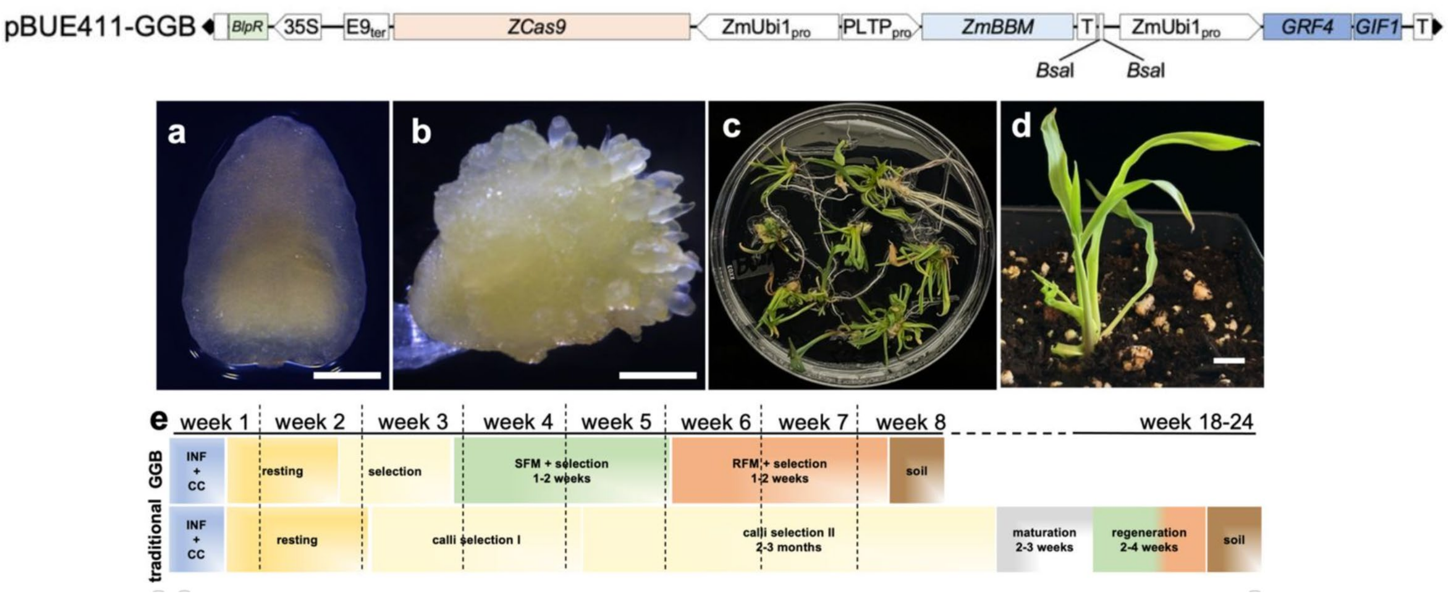
Synergy with other morphogenic regulators: GRF4-GIF1 + BBM increase transformation frequency in maize

Work done in collaboration with Andrea Gallavotti lab

The combination of morphogenic regulators BABY BOOM and GRF-GIF improves maize transformation efficiency

Zongliang Chen, Juan M. Debernardi, Jorge Dubcovsky, Andrea Gallavotti

doi: https://doi.org/10.1101/2022.09.02.506370



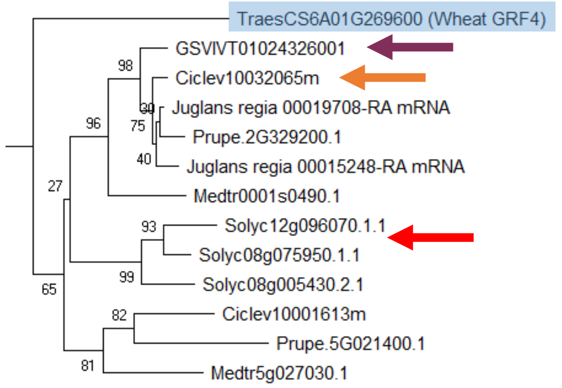
date	background	construct	# embryos	# positive plants	efficiency (%)	average efficiency
2/24/22	Hi-II	pBUE411_B	46	2	4.3	**(9/188)*100=4.8**
2/22/22	Hi-II	pBUE411_GG	95	6	6.3	
2/24/22	Hi-II	pBUE411_GG	47	1	2.1	
4/3/21	Hi-II	pBUE411_GGB	110	137	124.5	**(408/1113)*100=36.7**
2/22/22	Hi-II	pBUE411_GGB	28	9	32.1	
2/24/22	Hi-II	pBUE411_GGB	45	8	17.8	
8/10/21	Hi-II	pBUE411_GGB_AB^{CR}	178	72	40.4	
8/11/21	Hi-II	pBUE411_GGB_AB^{CR}	266	89	33.4	
2/14/22	Hi-II	pBUE411_GGB_CD^{CR}	102	32	31.4	
2/14/22	Hi-II	pBUE411_GGB_C^{CR}	55	14	25.4	
4/1/22	B104	pBUE411_GGB	36	10	27.8	**(363/1392)*100=26.1**
1/18/22	B104	pBUE411_GGB_E^{CR}	16	5	31.3	
1/18/22	B104	pBUE411_GGB_CD^{CR}	16	0	0	
4/1/22	B104	pBUE411_GGB_CD^{CR}	35	10	28.6	
4/1/22	B104	pBUE411_GGB_IJ^{CR}	38	6	15.8	
4/1/22	B104	pBUE411_GGB_KL^{CR}	53	0	0	
4/1/22	B104	pBUE411_GGB_FGH^{CR}	38	2	5.3	
5/6/22	B104	pBUE411_GGB_M	102	39	38.2	
5/6/22	B104	pBUE411_GGB_N	107	38	35.5	
5/16/22	B104	pBUE411_GGB_O^{CR}	46	7	15.2	
5/16/22	B104	pBUE411_GGB_P^{CR}	38	3	7.9	
5/16/22	B104	pBUE411_GGB_Q^{CR}	56	7	12.5	
5/20/22	B104	pBUE411_GGB_R^{CR}	34	7	20.6	
5/20/22	B104	pBUE411_GGB_C^{CR}	36	11	30.6	
5/25/22	B104	pBUE411_GGB_O^{CR}	69	9	13	
5/25/22	B104	pBUE411_GGB_P^{CR}	65	13	20	
5/25/22	B104	pBUE411_GGB_Q^{CR}	61	14	23	
5/25/22	B104	pBUE411_GGB_S^{CR}	61	9	14.8	
5/25/22	B104	pBUE411_GGB_R^{CR}	90	9	10	
5/25/22	B104	pBUE411_GGB_KL^{CR}	101	9	8.9	
5/27/22	B104	pBUE411_GGB_C^{CR}	105	59	56.2	
5/27/22	B104	pBUE411_GGB_T^{CR}	107	86	80.4	
6/3/22	B104	pBUE411_GGB_U^{CR}	82	10	12.2	

Background	Efficiency (%)
Hi-II	4.8 vs 36.7
B104	3.5 vs 26.1

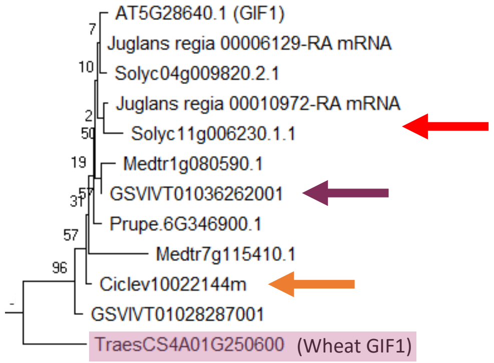
Preliminary results in dicot species using the *GRF-GIF* technology

Citrus, Pepper and Tomato: organogenic process

GRF homologues

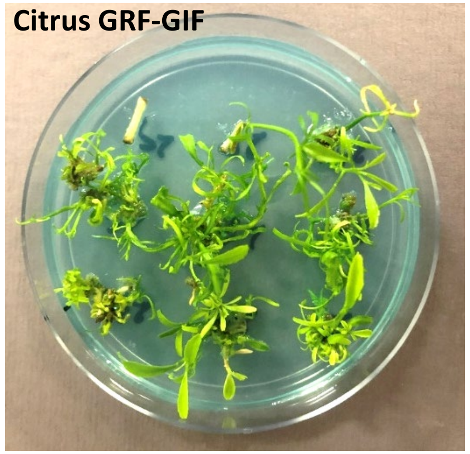


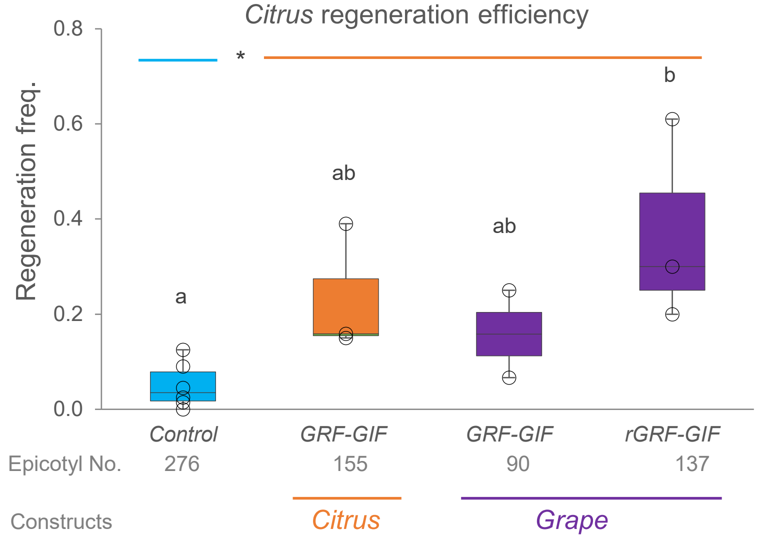
GIF homologues

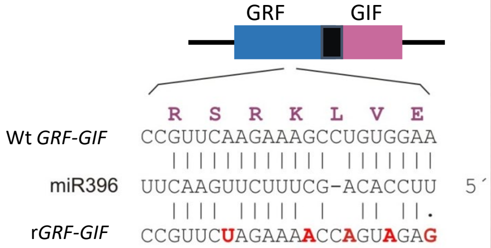


GRF-GIF induced a higher frequency of regenerated shoots, either using citrus genes or a heterologous *GRF-GIF* chimera from grape

Citrus (Carrizo)

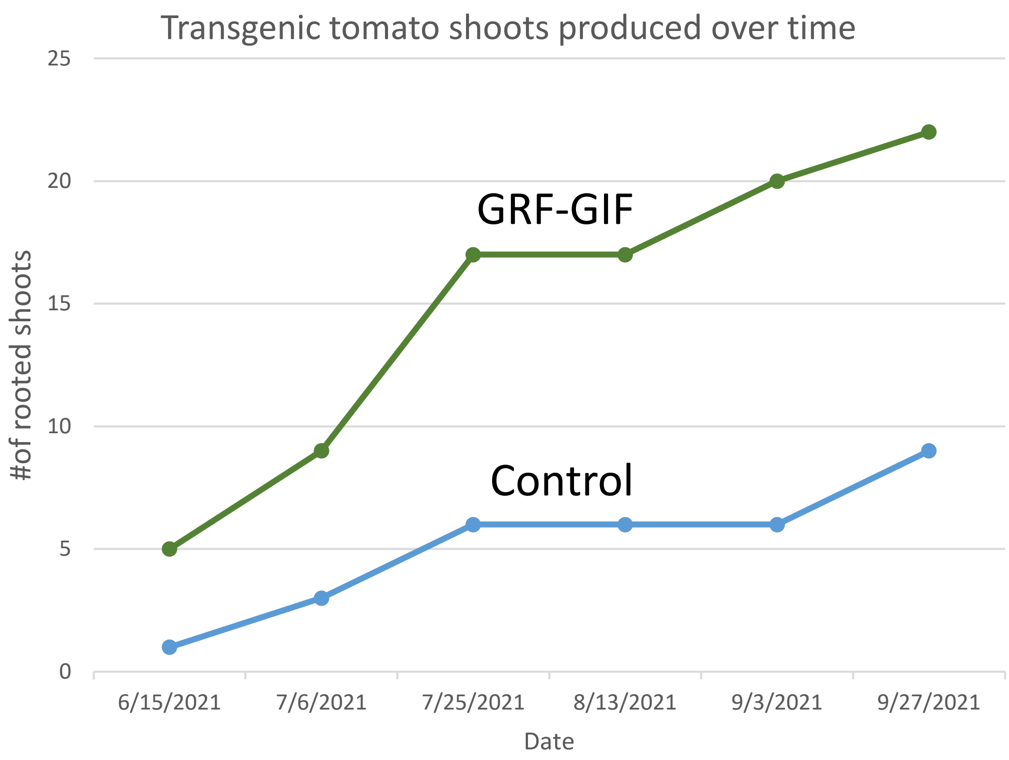






Preliminary results in dicot species using the *GRF-GIF* technology

GRF-GIF induces a higher frequency of regenerated shoots and the regenerated plants have **a normal phenotype**



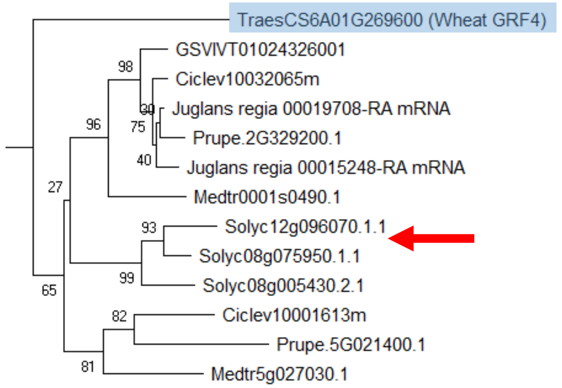
GRF-GIF Control

Preliminary results in dicot species using the *GRF-GIF* technology

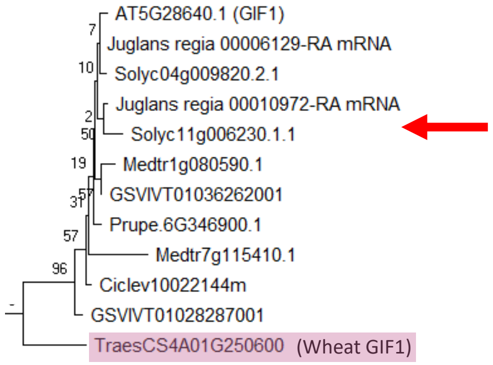
Pepper: organogenic process

Citrus: organogenic process

GRF homologues



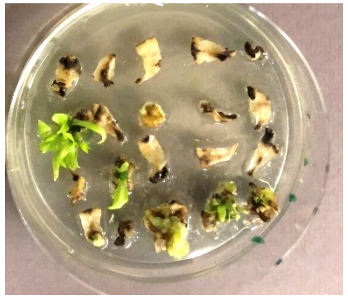
GIF homologues



On-going experiments: *GRF-GIF* induced a higher frequency of regenerated shoots

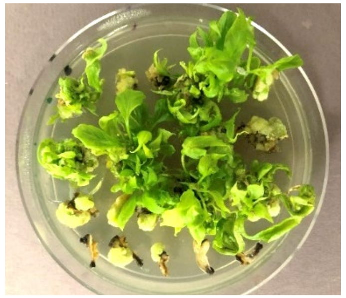
Pepper (R&C Cayenne)

Empty vector

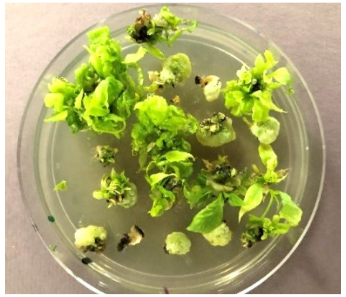


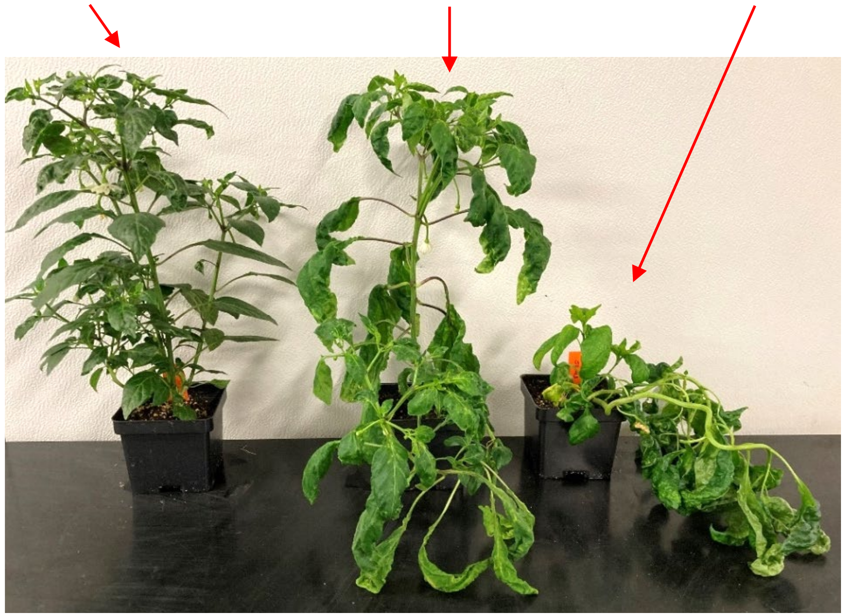
Tomato *GRF(#8)-GIF*

Tomato r*GRF(#8)-GIF*



Tomato t*GRF(#12)-GIF*

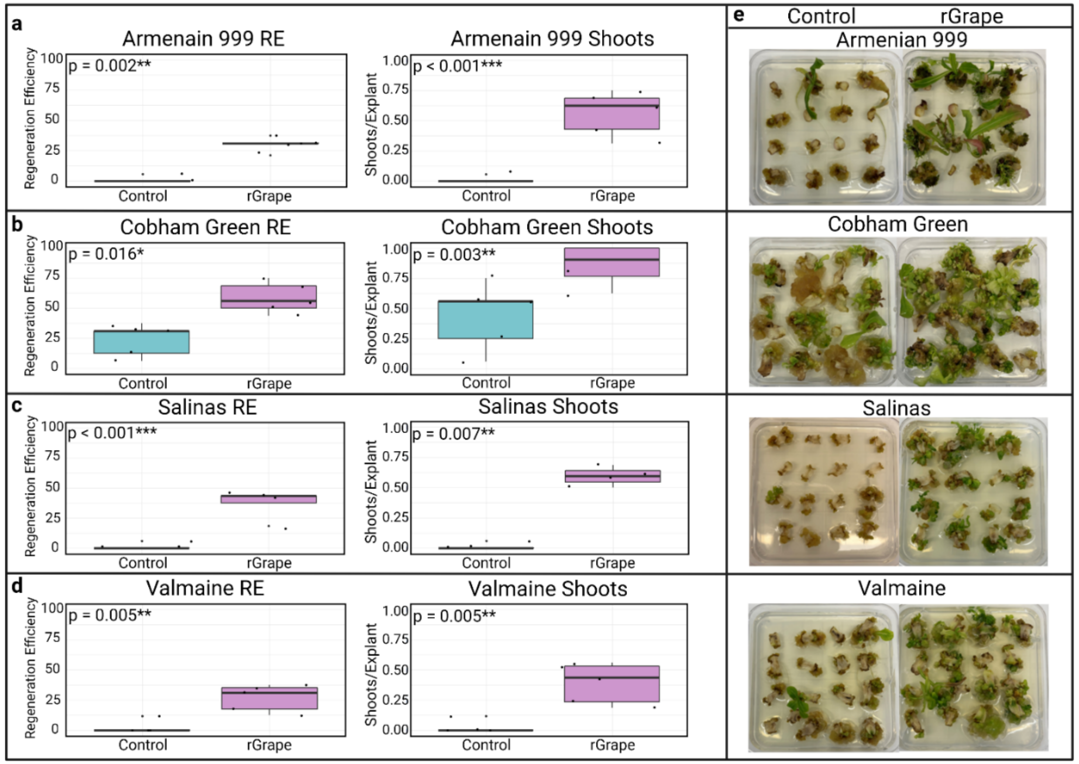




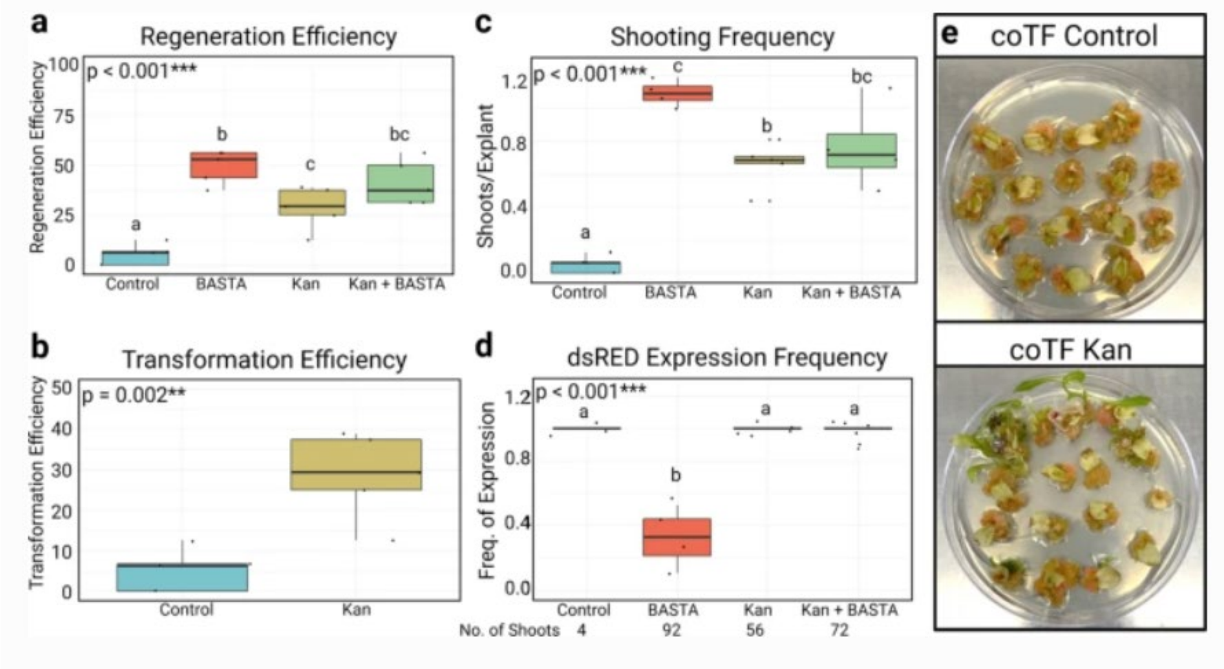
Preliminary results in dicot species using the *GRF-GIF* technology

GRF-GIF chimeric proteins enhance in vitro regeneration and Agrobacterium-mediated transformation efficiencies of lettuce (Lactuca spp.)

rGRF4–GIF1 increased the regeneration of all cultivars



Co-transformation with *GRF–GIF* boosted regeneration efficiency and shooting frequency



Bull et al., 2023

Preliminary results in dicot species using the *GRF-GIF* technology

Plant Biotechnology Journal

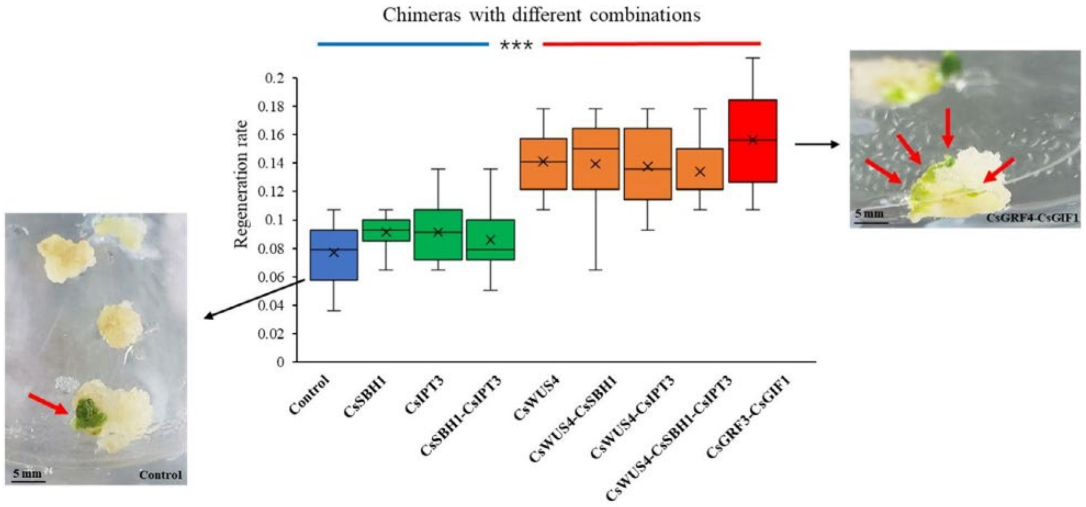
Plant Biotechnology Journal (2021) **19**, pp. 1979–1987

doi: 10.1111/pbi.13611

Establishment of an *Agrobacterium*-mediated genetic transformation and CRISPR/Cas9-mediated targeted mutagenesis in Hemp (*Cannabis Sativa* L.)

Xiaoyu Zhang[1,†], Gencheng Xu[1,†], Chaohua Cheng[1,†], Lei Lei[2], Jian Sun[3], Ying Xu[1], Canhui Deng[1], Zhigang Dai[1], Zemao Yang[1], Xiaojun Chen[1], Chan Liu[1], Qing Tang[1,*] and Jianguang Su[1,*]

Stable transformation and genome editing in Hemp



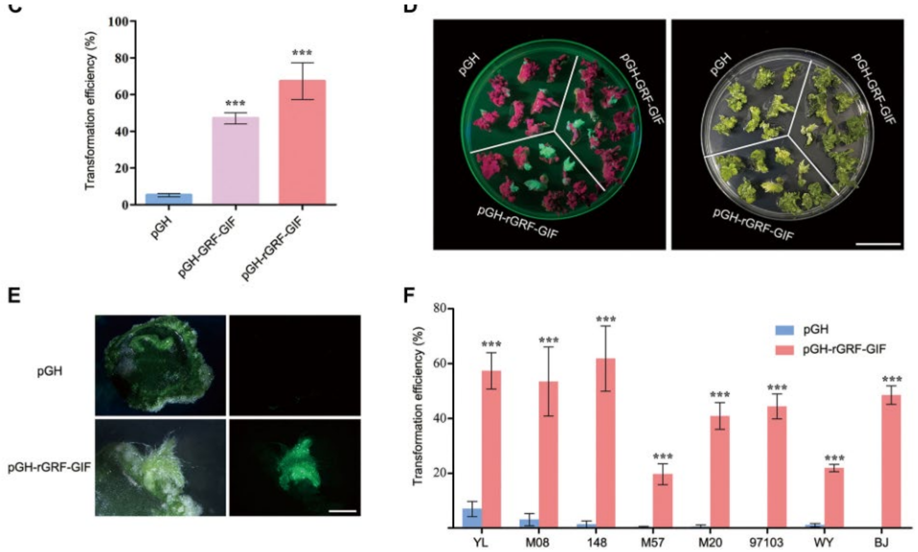
JIPB Journal of Integrative Plant Biology

Breakthrough Report

https://doi.org/10.1111/jipb.13199

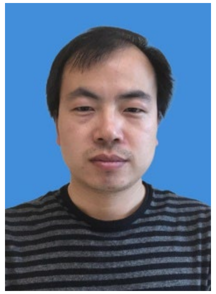
Highly efficient, genotype-independent transformation and gene editing in watermelon (*Citrullus lanatus*) using a chimeric *ClGRF4-GIF1* gene

Qin Feng[†], Ling Xiao[†], Yizhen He, Man Liu, Jiafa Wang, Shujuan Tian, Xian Zhang and Li Yuan*



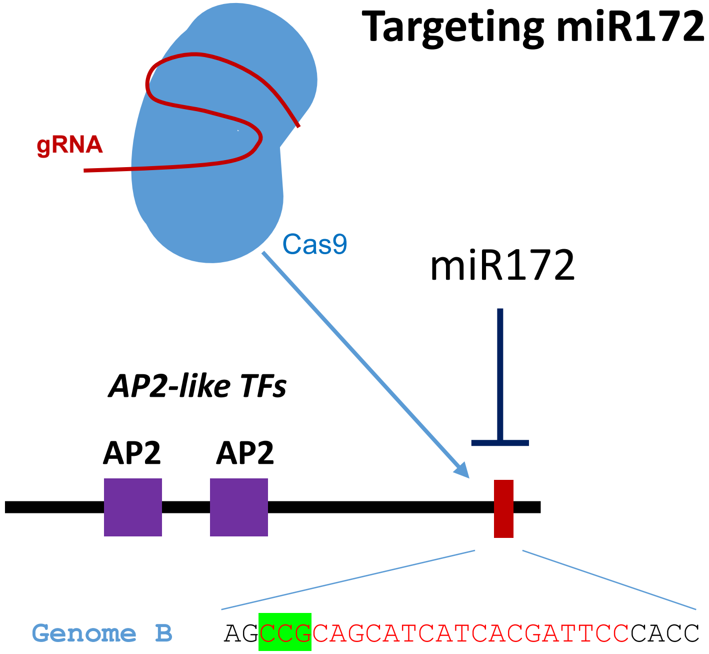
Novel genetic resources to control plant height in wheat and triticale

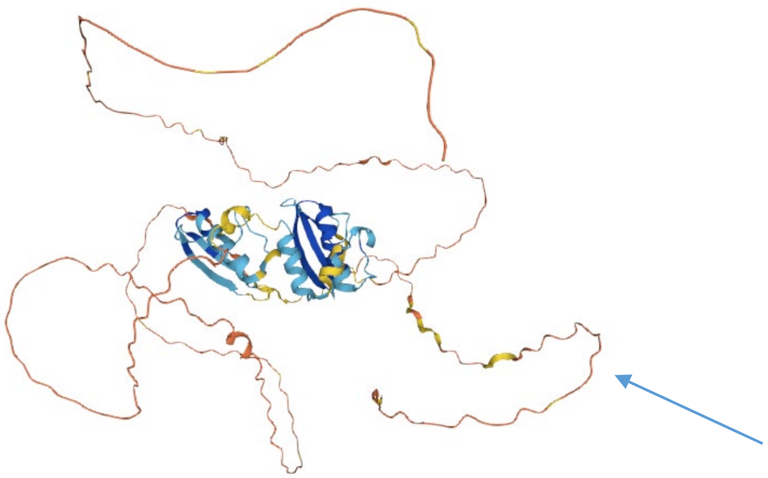
Chaozhong Zhang



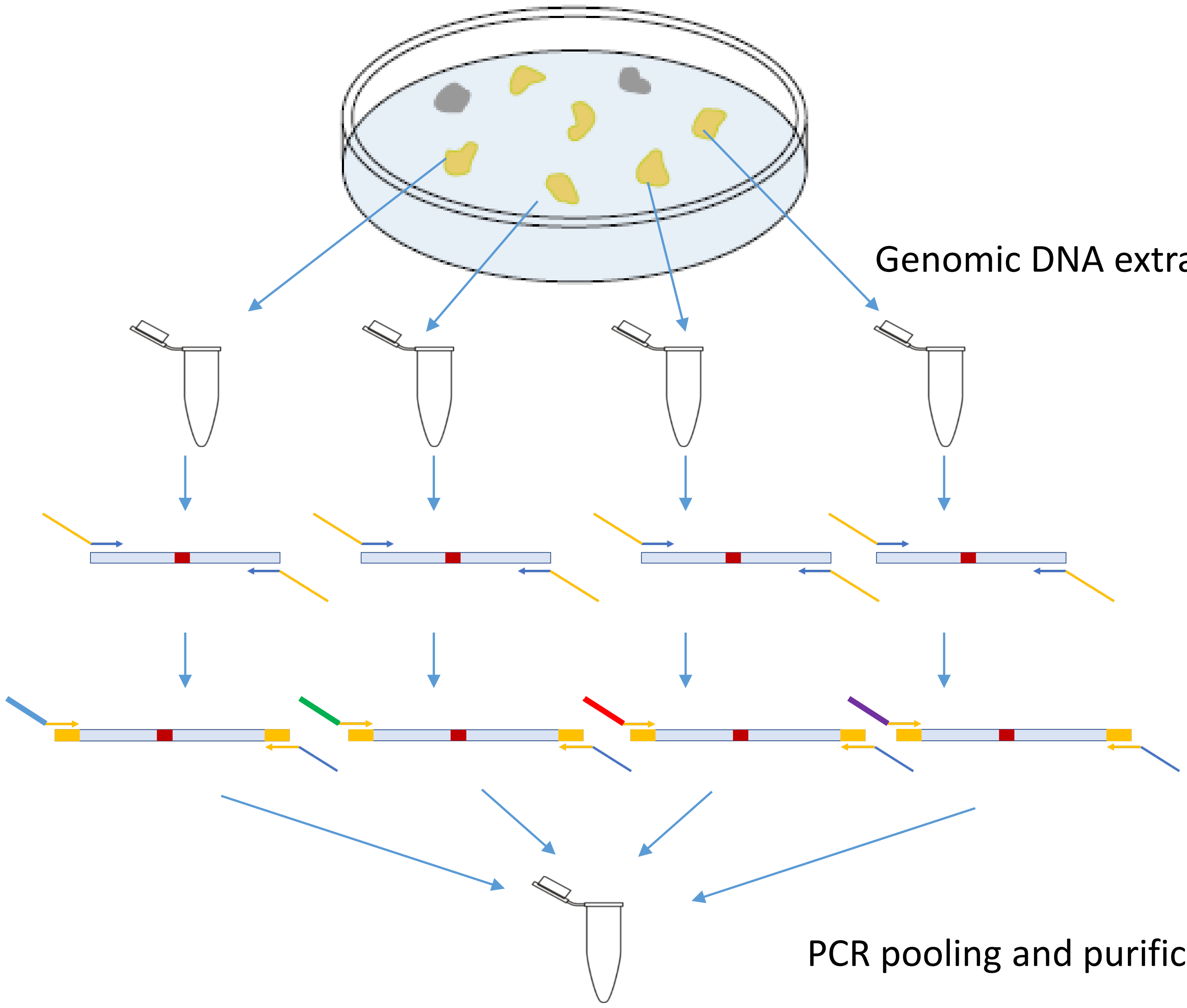
The miR172-*AP2-like* module controls internode elongation

Targeting miR172 target site by CRISPR-Cas9 + GRF-GIF





Gene editing – genotyping by amplicon sequencing



Genomic DNA extraction

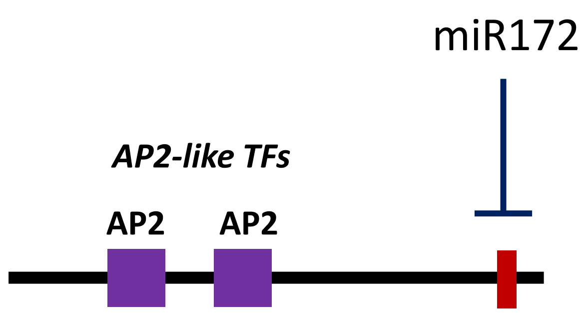
PCR with primers flanking the targeted region

2nd PCR to add different barcodes to each sample

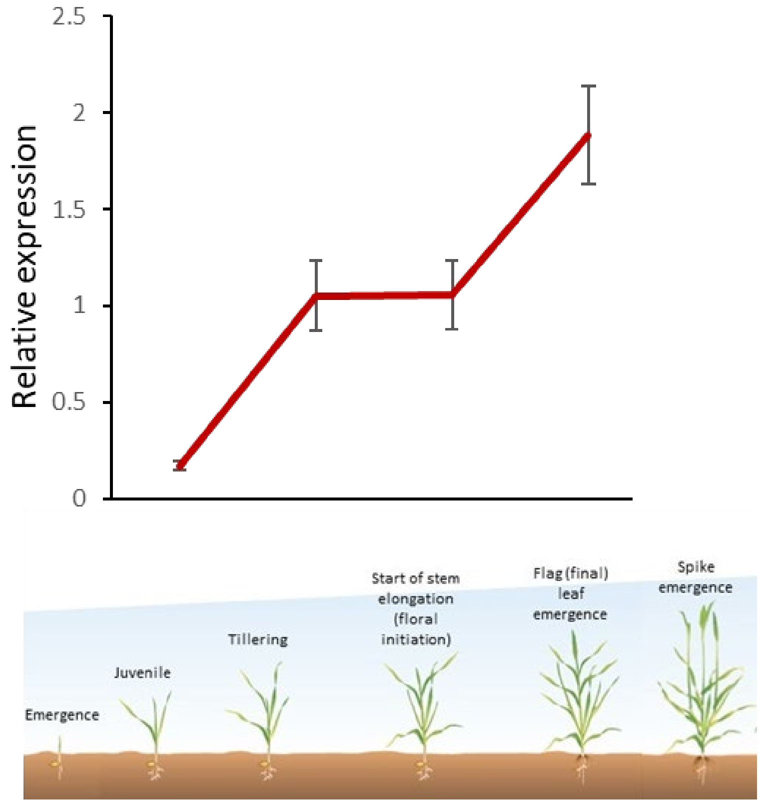
PCR pooling and purification. -------> sequencing

The miR172-*AP2-like* module controls flowering transition

miR172 repress *AP2-like* TFs at post-transcriptional level



miR172 is induced during reproductive transition



Debernardi et al., 2017
Debernardi et al., 2022

GRF gene family

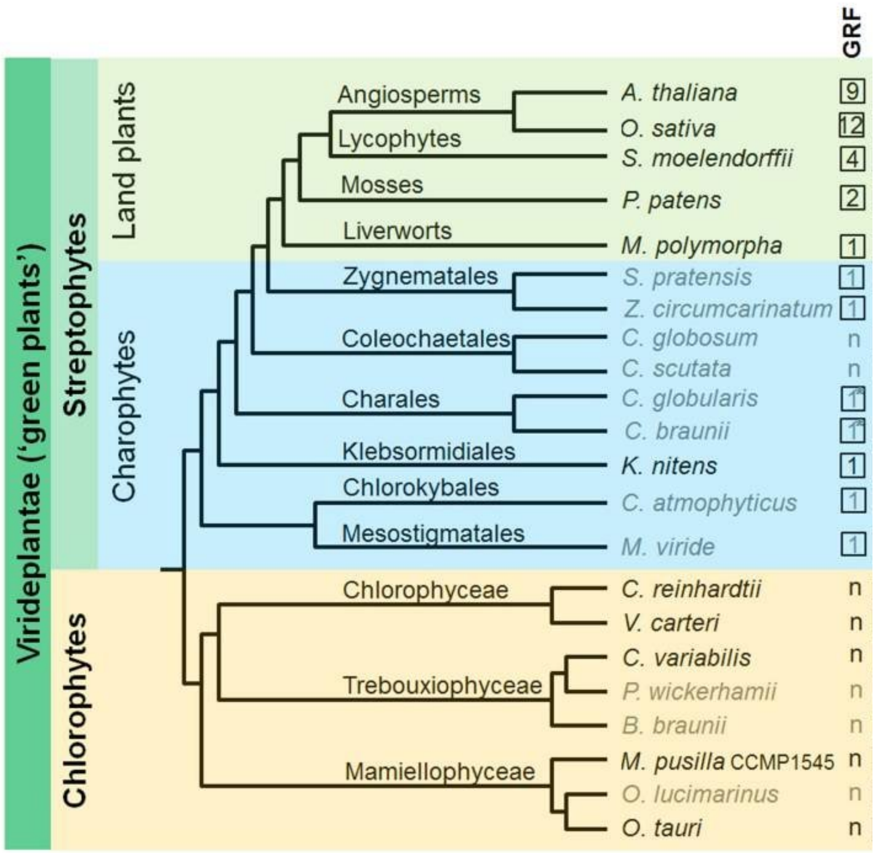
<u>*GROWTH REGULATING FACTOR (GRF)* family</u>

- Plant specific transcription factor family
- Highly conserved in land plants (dicots, monocots, gymnosperms and moss).

Plant Physiology, March 2000, Vol. 122, pp. 695–704, www.plantphysiol.org © 2000 American Society of Plant Physiologists

A Novel Gibberellin-Induced Gene from Rice and Its Potential Regulatory Role in Stem Growth[1]

Esther van der Knaap[2], Jeong Hoe Kim, and Hans Kende*
Michigan State University-Department of Energy Plant Research Laboratory, Michigan State University, East Lansing, Michigan 48824–1312

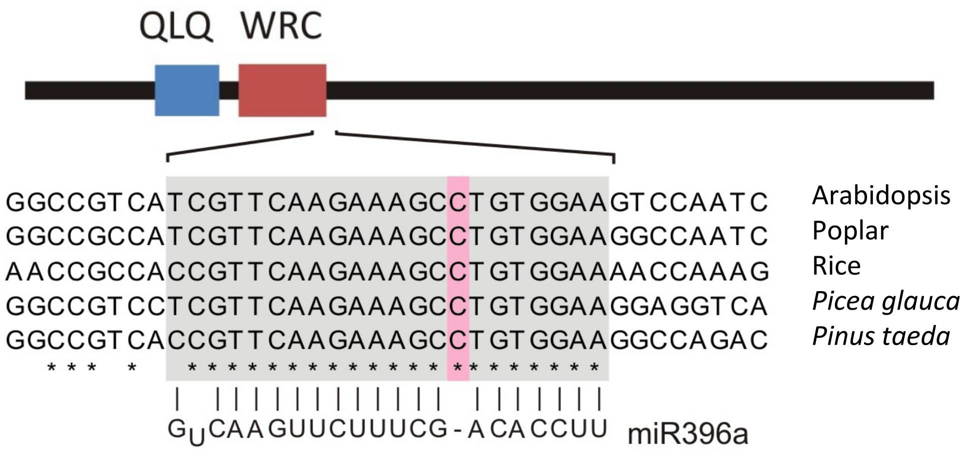


Jeong Hoe Kim 2019

GRF gene family

GROWTH REGULATING FACTOR (GRF) family

- Plant specific transcription factor family
- Highly conserved in land plants (dicots, monocots, gymnosperms and moss).
- Defined by QLQ and WRC domains, which mediate protein-protein and protein-DNA interactions, respectively.
- Targets of microRNA miR396

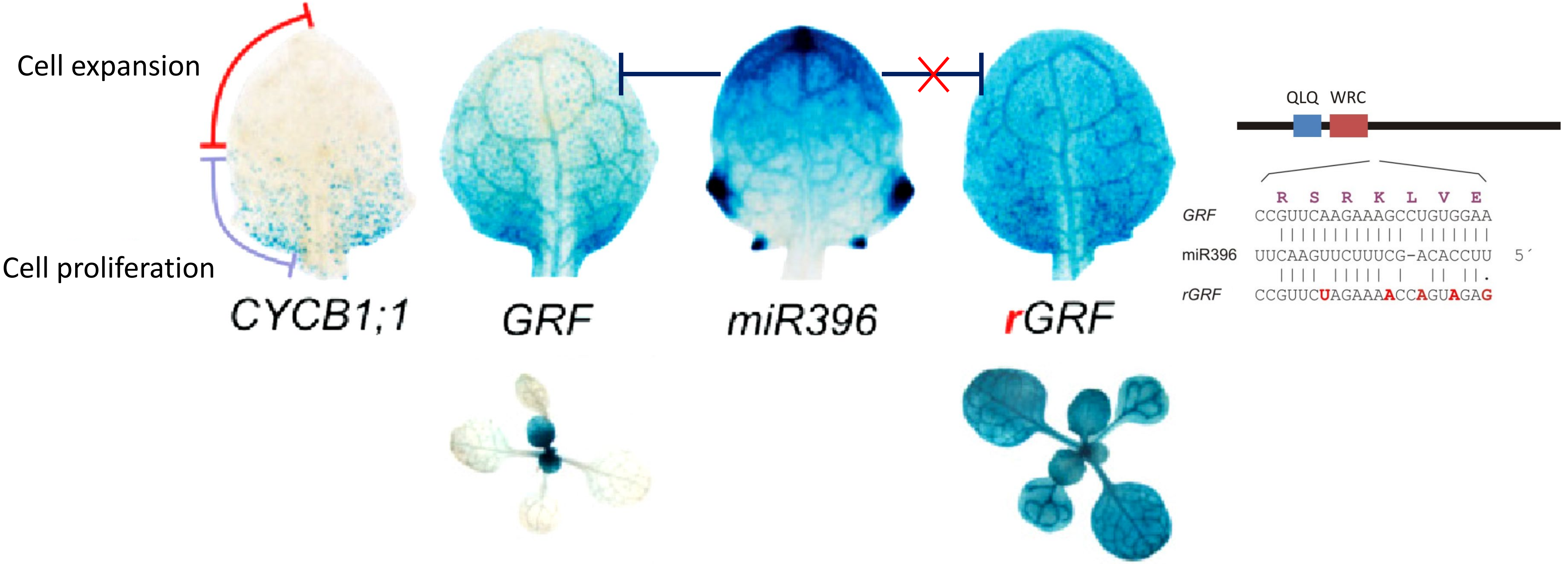


Rodriguez et al., 2010; Debernardi et al., 2012

GRFs are expressed in meristems and in tissues enriched in cell proliferation

Arabidopsis thaliana (leaf primordia)

miR396 restricts *GRF* expression to proliferative tissues

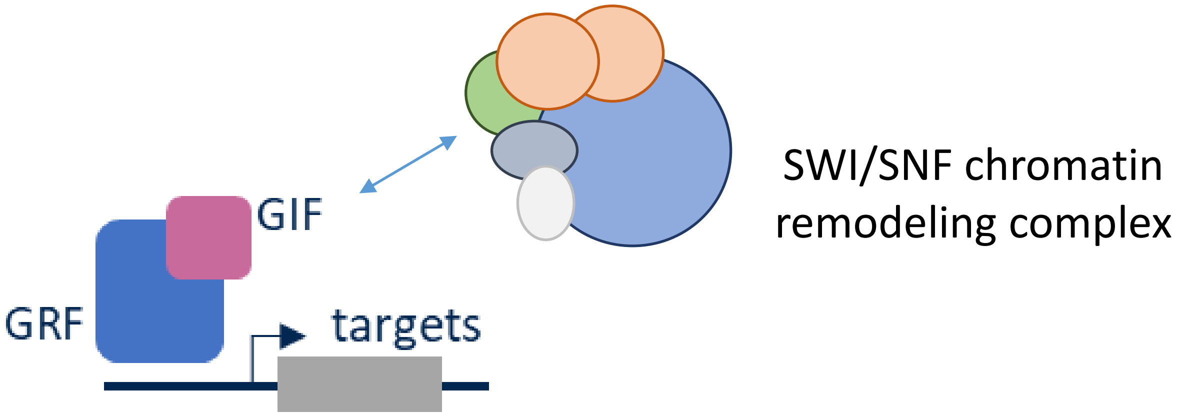


Rodriguez et al., 2010; Debernardi et al., 2012

GRFs interact with GIF proteins

<u>*GRF–INTERACTING FACTOR (GIF)* family</u>

- Do not have a DNA binding domain
- SNH domain mediates interaction with GRFs and with chromatin remodeling complexes *in vivo*



Kim & Kende 2004; Debernardi et al., 2014; Vercruyssen et al., 2014; Guo et al., 2022

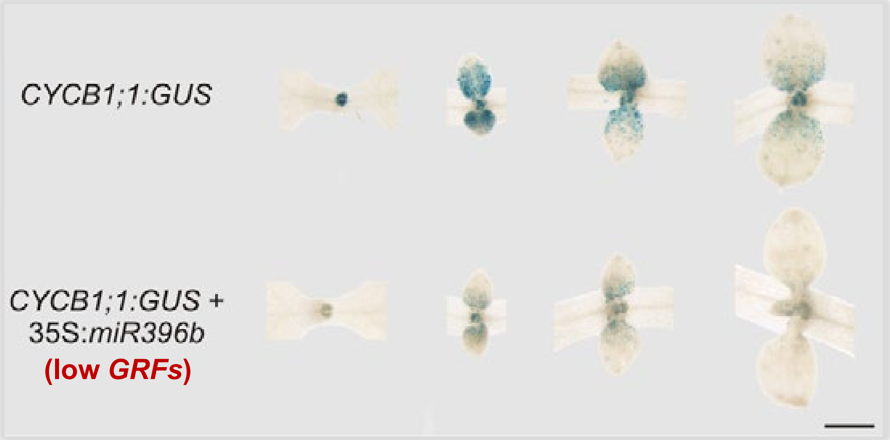
GRF and *GIF* control cell proliferation and meristems homeostasis

Arabidopsis thaliana

Leaf size

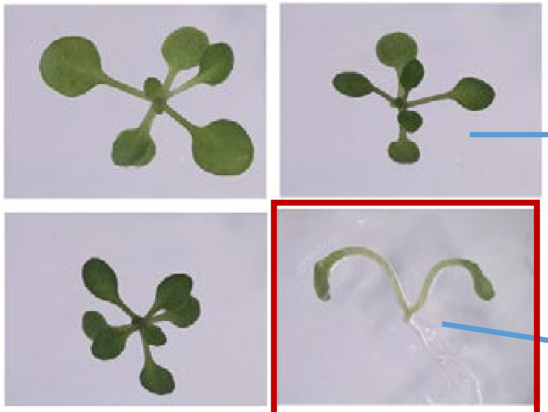
(low *GRFs*)

Wt | 35S:*MIR396b* | *gif1*

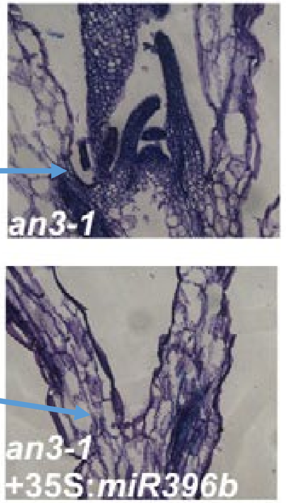


Root and shoot meristem size and homeostasis

Wt | *gif1*

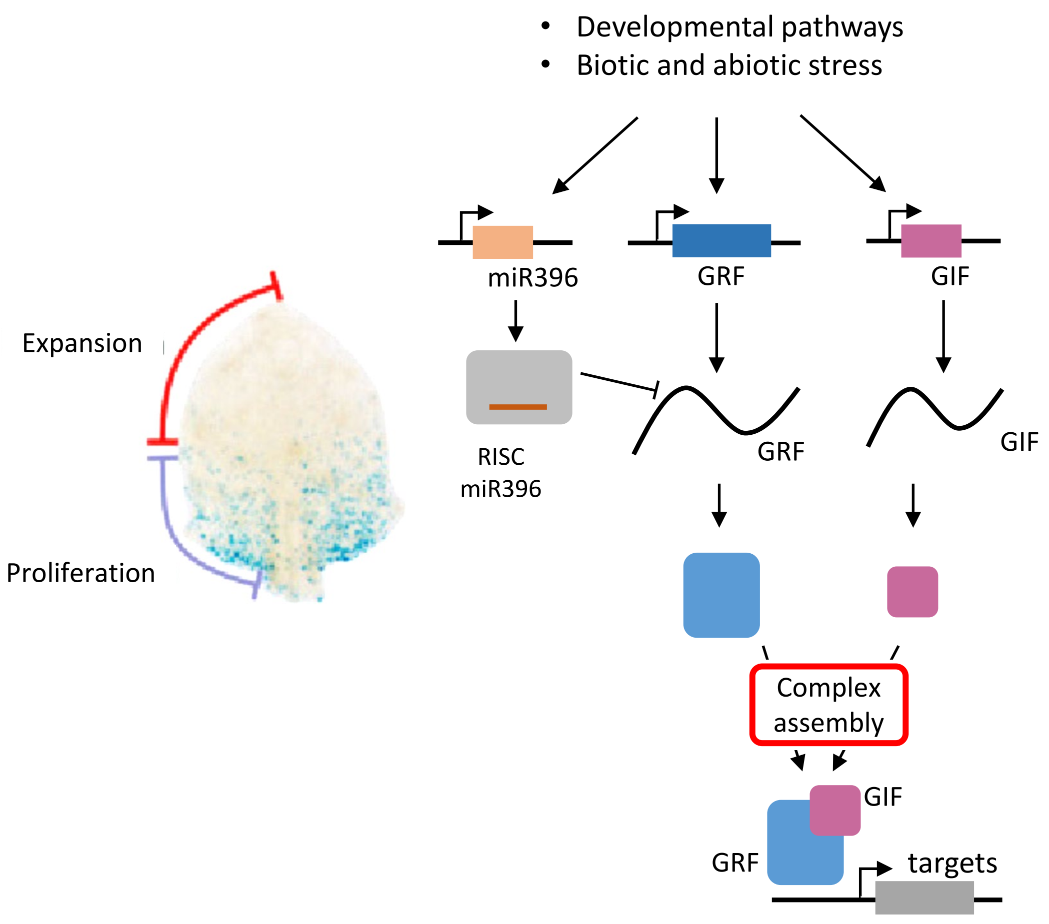


35S:miR396 | *gif1* x *35S:miR396*

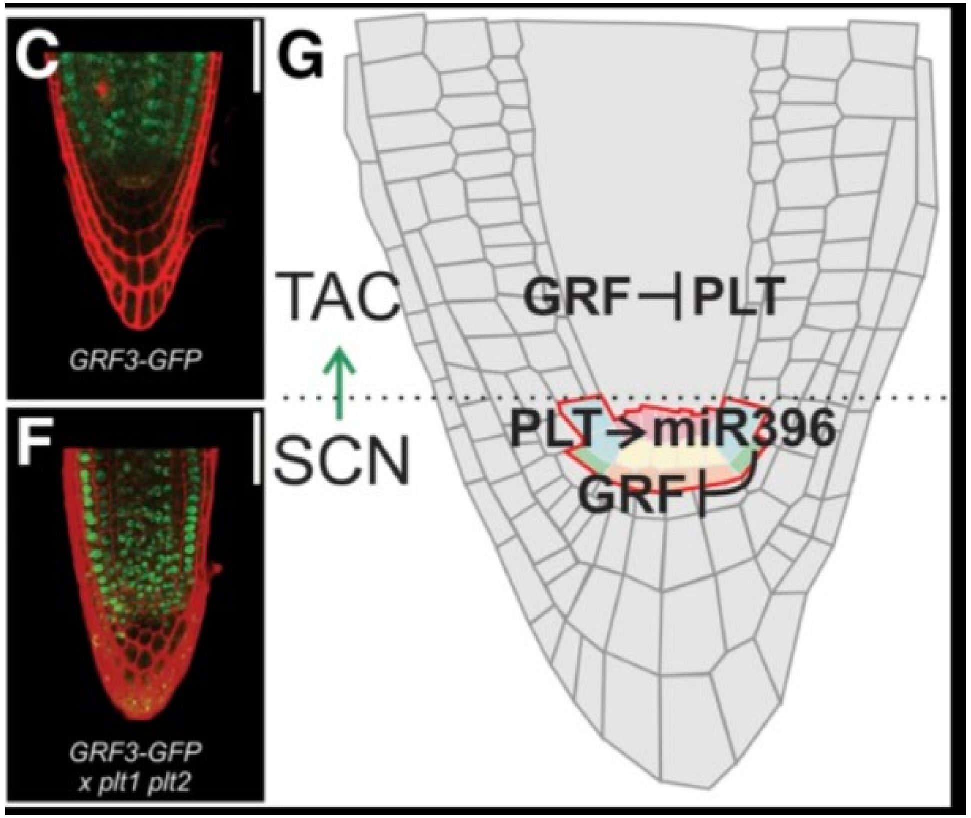


Rodriguez et al., 2010, 2015;
Ercoli et al., 2018

The miR396–*GRF/GIF* regulatory network on meristem homeostasis and organization



- Meristem homeostasis
- Plant growth (cell proliferation / cell expansion)



Liebsch & Palatnik 2020

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