

Multi-Crop Transformation Facility

2019 Cotton Breeders Tour
July 23

mayrafmolina@tamu.edu
marco.molina@tamu.edu



Multi-Crop Transformation Facility

Institute for Plant Genomics & Biotechnology



Multi-Crop
Transformation
Facility

TEXAS A&M
AGRILIFE
RESEARCH

Multi-Crop Transformation Facility

Institute for Plant Genomics & Biotechnology



External greenhouses



Second floor greenhouses



Greenhouse



Multi-Crop
Transformation
Facility

TEXAS A&M
AGRILIFE
RESEARCH

Multi-Crop Transformation Facility



Lab. Bench space



Lab. laminar flow



Media Prep Area



Growth Chambers



Multi-Crop Transformation Facility



MAYRA FAION MOLINA

Research Specialist IV, Multi-Crop
Transformation Facility Director
mayrafmolina@tamu.edu



MARCO MOLINA

Research Specialist III, Multi-Crop
Transformation Facility Co-Director
marco.molina@tamu.edu



MARCELA B.D. DELLA GRACIA

Research Associate



LINDSEY KATELYN BAILEY

Research Assistant

“ We provide high quality plant transformation services to the scientific community across Texas A&M AgriLife, TAMU, the Texas A&M University System, and external collaborators.”

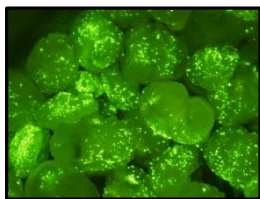


Multi-Crop
Transformation
Facility

TEXAS A&M
AGRILIFE
RESEARCH

Ongoing projects

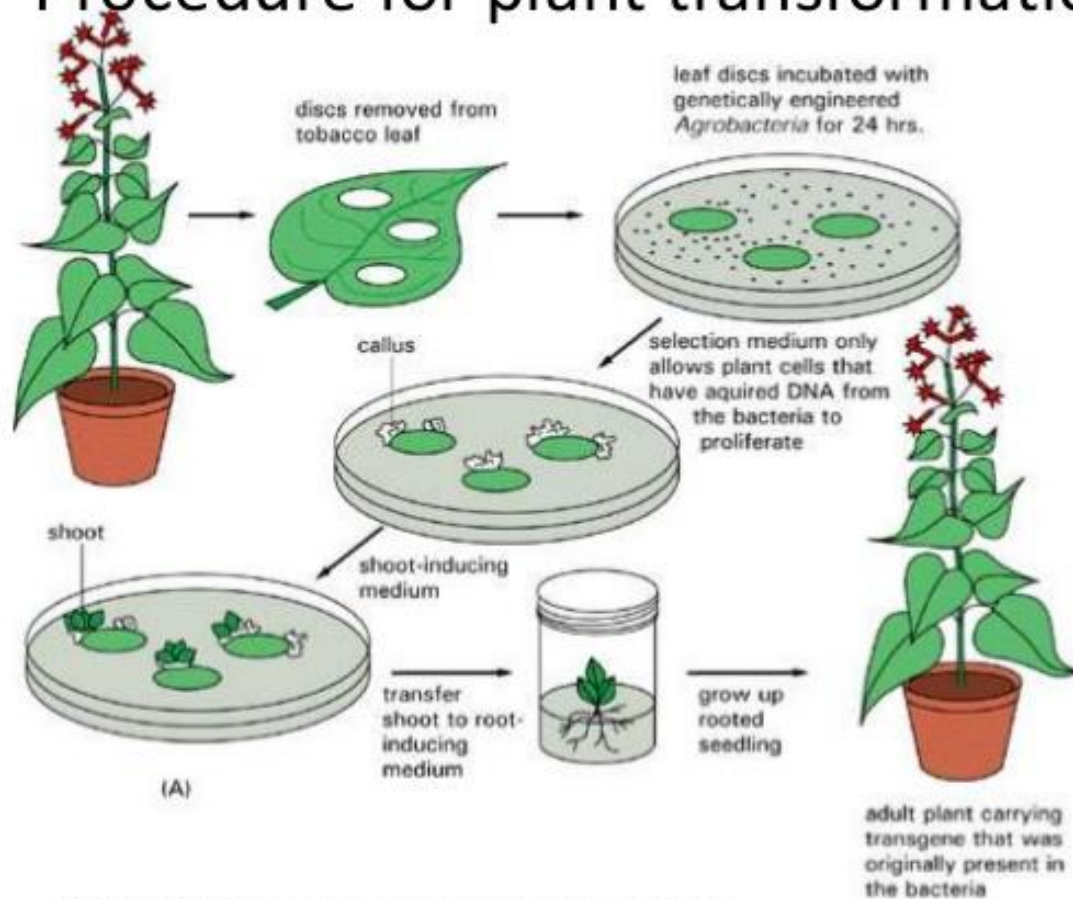
- ◉ AgriLife Seed Grant projects (15)
- ◉ AgriLife Commercial **Wheat** genotypes
- ◉ AgriLife Commercial **Peanuts** genotypes
- ◉ AgriLife Crop Improvement **Sorghum, Peanuts, Rice**
- ◉ AgriLife Dallas Center - **Potato**
- ◉ AgriLife Uvalde Center - **Onion**
- ◉ IPGB-MTF- **Cotton**
- ◉ X-Grant project (Wheat Rice and Cotton)
- ◉ Protocol development: **Amaranthus, Tomato, Cantaloupe, Coffee, Spinach**



Multi-Crop Transformation Facility

General procedure Agrobacterium-mediated transformation

Procedure for plant transformation



- Agro-mediated
- Biolistic transformation

- Selectable marker

- Transformation Efficiency

- Molecular analysis

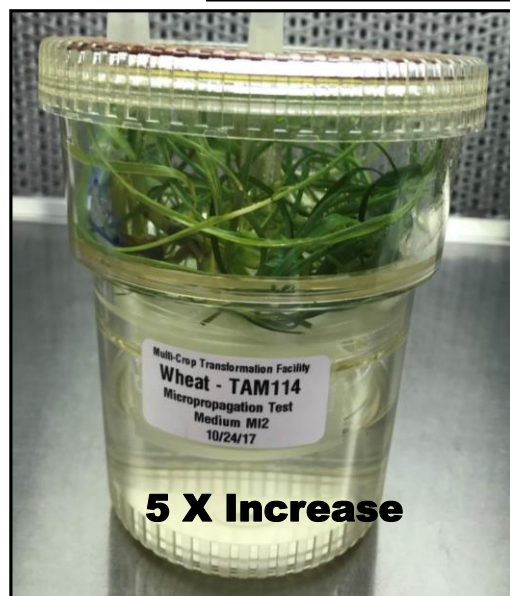
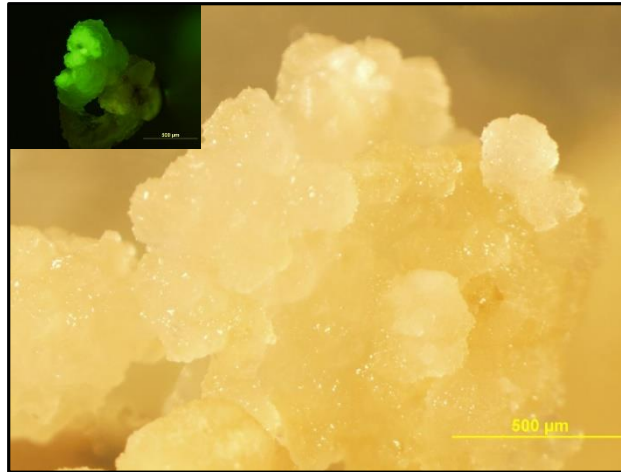
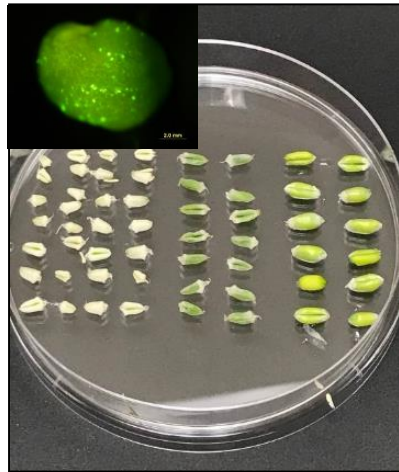
- Traceability

- Gene Editing/CRISPR efficiency

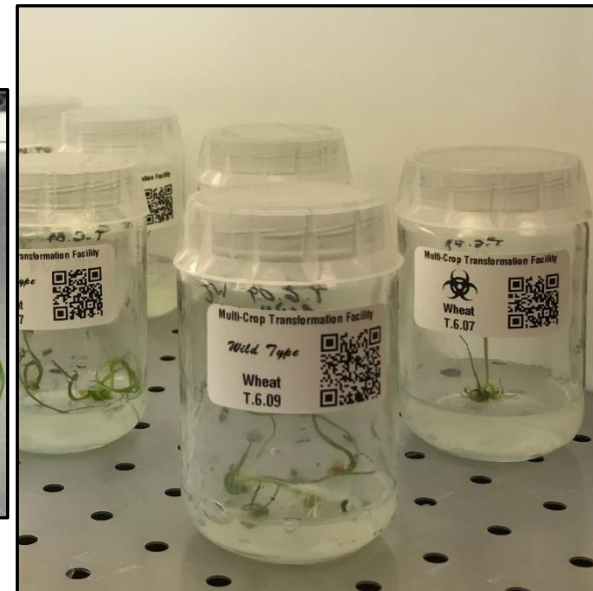
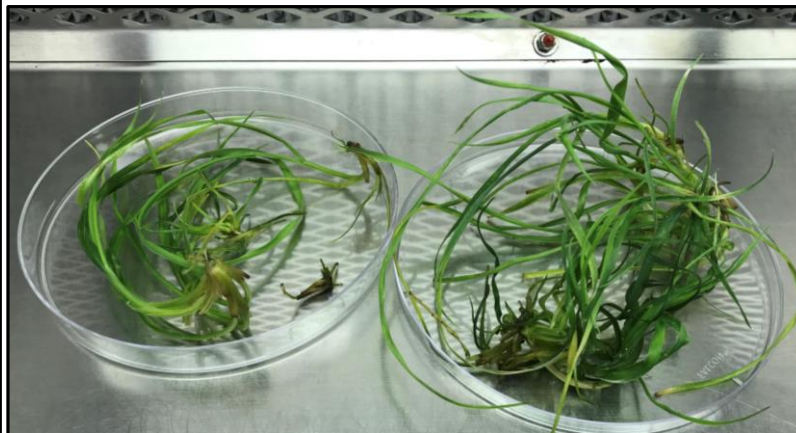
IMAGE: Mol bio of the cell by Albert (pg no:599)



©AgriLife commercial **Wheat** genotypes (3)



More vigorous plants



Challenges: Specific fresh embryo stage, laborious axis excision, switching to bombardment...

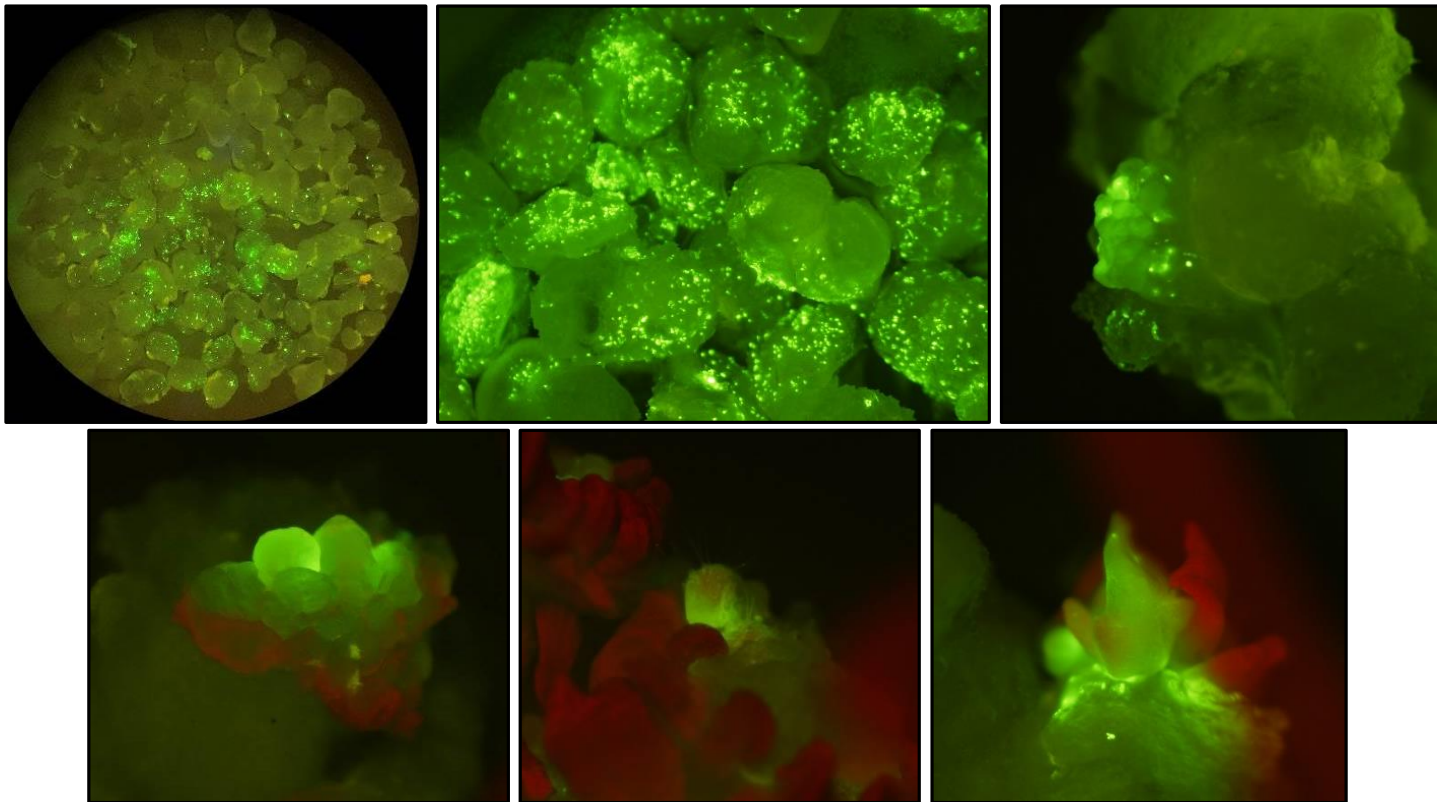


**Multi-Crop
Transformation
Facility**

**TEXAS A&M
AGRI LIFE
RESEARCH**

◎AgriLife - HR Wheat

✓ pPTN-EYFP bombardment optimization



✓ February 2019 update:

- 21 independent experiments
- 5207 immature embryos bombarded
- ~ 26,000 plants regenerated
- 5 different target constructs tested
- Under molecular analysis (bulk)

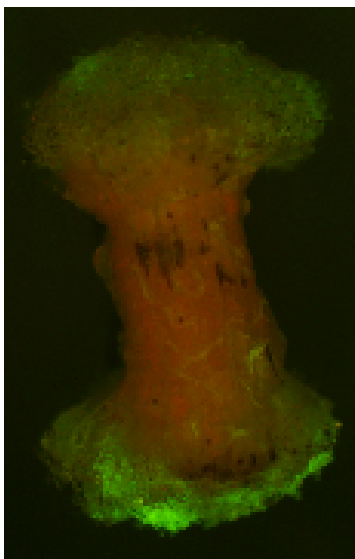
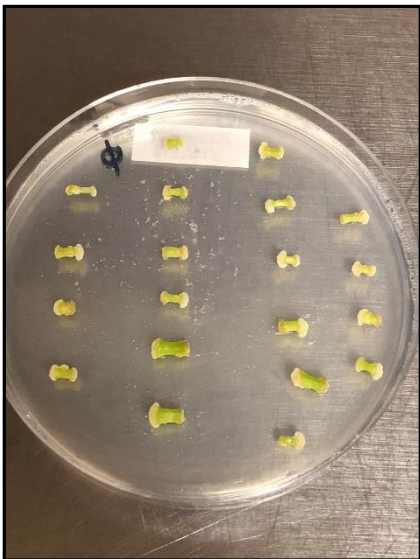


Multi-Crop
Transformation
Facility

TEXAS A&M
AGRI LIFE
RESEARCH

© AgriLife Dallas Center Potato

✓ Russet Norkotah, Blue Potato, Desiree, Kathadin, 24M28



Challenges: protocol development for every independent variety.

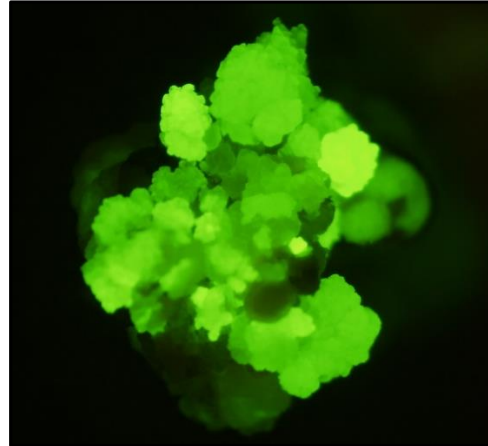
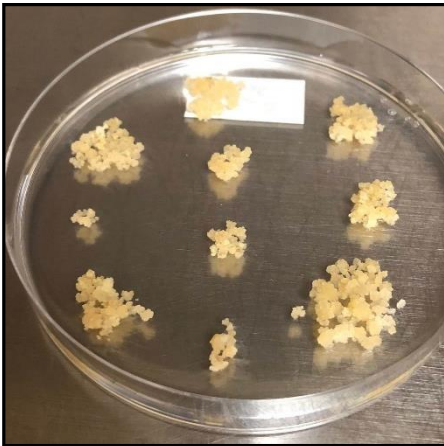


Multi-Crop
Transformation
Facility

TEXAS A&M
AGRI LIFE
RESEARCH

◎ AgriLife Crop Improvement (Sorghum, Peanuts, **Rice**)

- ✓ Rice Presidio line >>>> **Tissue culture Protocol validated**



Challenges and next steps: Specific fresh embryo stage, laborious embryo excision, Mature embryo protocol.



Multi-Crop
Transformation
Facility

TEXAS A&M
AGRI LIFE
RESEARCH

◉ AgriLife Crop Improvement (**Sorghum**, Peanuts, Rice)

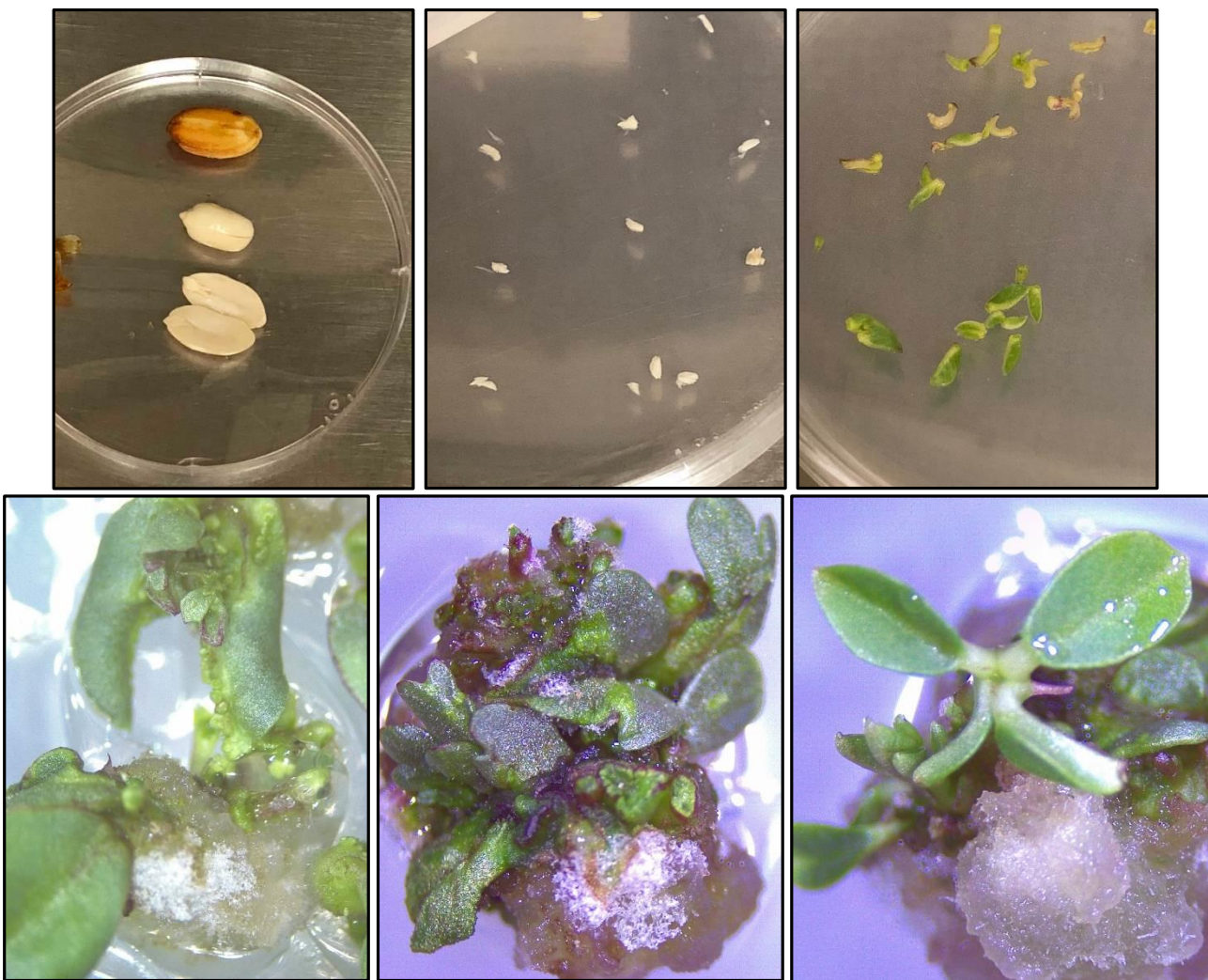
✓ RTx430 + 11 varieties – **Tissue culture Protocol validated**



Challenges: Specific fresh embryo stage, laborious embryo excision, protocol for every independent line and Development of *Agrobacterium*-mediated transformation.

©AgriLife Crop Improvement (Sorghum, **Peanuts**, Rice)

✓ Schubert >>> **Tissue culture Protocol validated**



Challenge and next step: Development of
Agrobacterium-mediated transformation

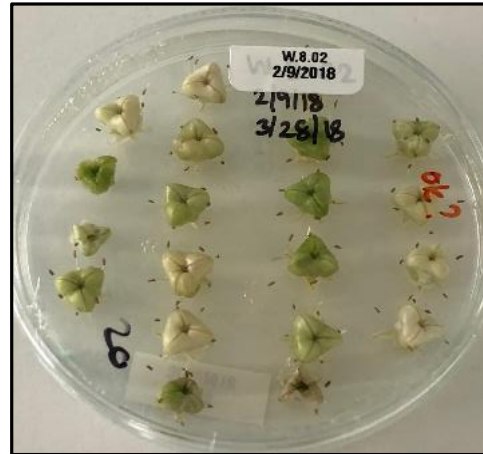
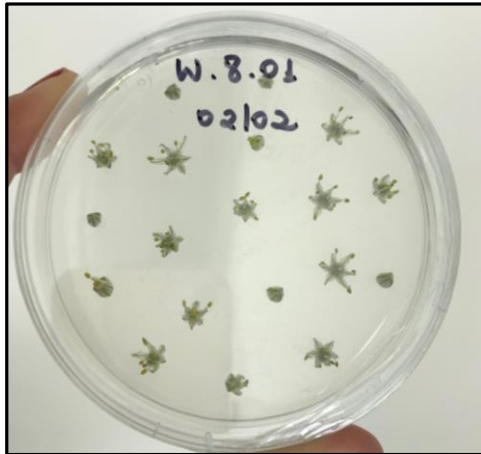


Multi-Crop
Transformation
Facility

TEXAS A&M
AGRI LIFE
RESEARCH

◉ AgriLife Uvalde Center - **Onion**

✓ Double Haploid



Challenges: Extremely low efficiency method;
genotype specificity; Ongoing protocol validation.

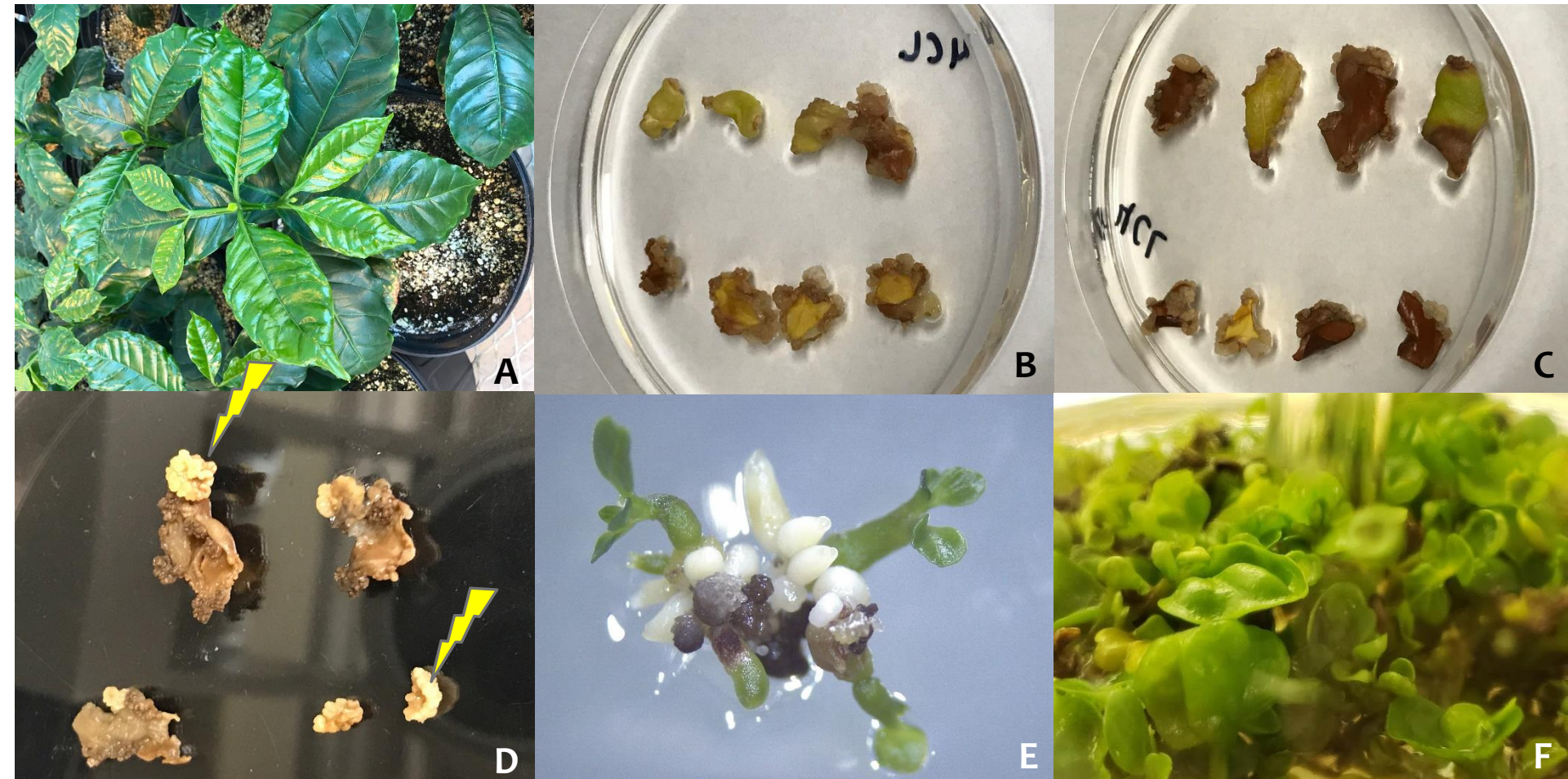


Multi-Crop
Transformation
Facility

TEXAS A&M
AGRI LIFE
RESEARCH

Multi-Crop Transformation Facility

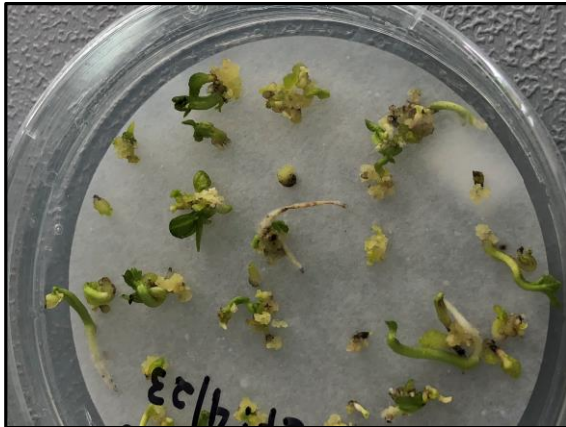
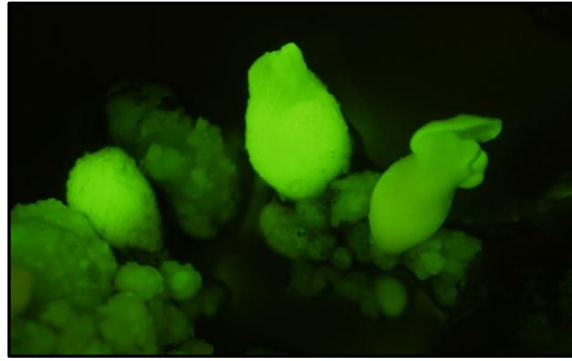
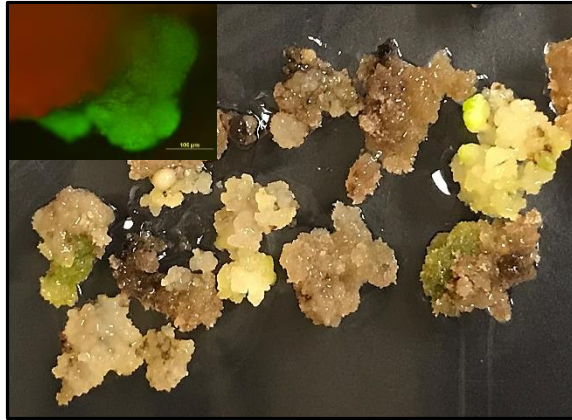
Coffee



Figures: (A) Initial explant; (B and C) calli induction after 4 weeks; (D) beginning of embryogenic calli formation; (E) Embryogenic calli germination; (F) Plant Elongation

© MTF Cotton

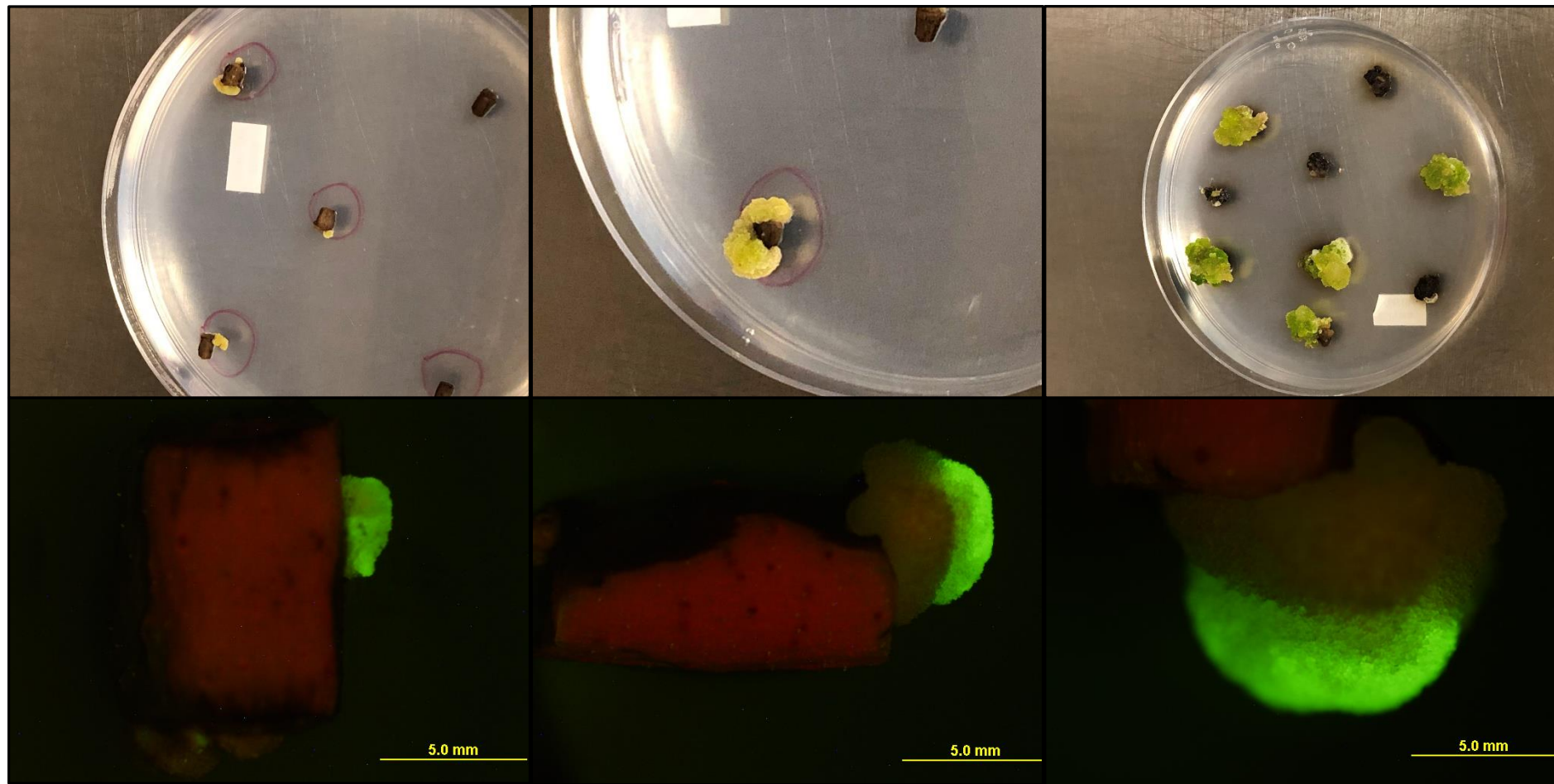
✓ Gene Editing - Coker312



February 2019 update: **100+ regenerated** plants delivered to Dr. Libo Shan lab., with two different constructs.

◎ MTF Cotton

✓ **CA4002 – Use of developmental genes and protocol validation**



Ongoing collaboration with Dr. Libo Shan and Dr. Ping He



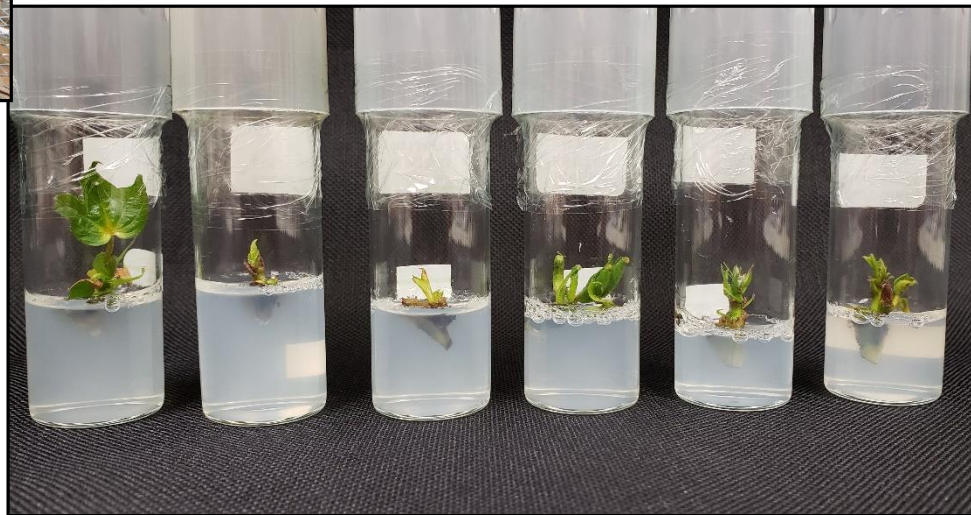
Multi-Crop
Transformation
Facility

TEXAS A&M
AGRILIFE
RESEARCH

◎ MTF Cotton

✓ Cotton in vitro cultivation/propagation

- TM-1 > barbadense > tomentosum > mustelinum



Multi-Crop
Transformation
Facility

Ongoing collaboration with Dr. David Stelly and Dr. Robert Vaughn

TEXAS A&M
AGRILIFE
RESEARCH

Ongoing Projects – Summary

	Tissue culture protocol validation		Agro-Mediated Transformation Protocol Validation		Biolistic Transformation Protocol Validation		CRISPR Protocol validation
Sorghum	✓	12	✓	1	-		Ongoing
Cotton	✓	1	✓	1	-		✓
Sugarcane	✓	2	✓	2	✓		-
Coffee	✓	1	-	-	-		-
Tobacco	✓	1	-	-	-		-
Wheat	✓	3	✓	1	✓		✓
Potato	✓	4	✓	3	-		✓
Onion	✓	1	-	-	-		-
Rice	✓	1	✓	1	✓		✓
Peanuts	✓	1	Ongoing	-	-		-
Melon	✓	1	✓	1	-		Ongoing
Spinach	Ongoing	1	Ongoing	-	-		-
Tomato	✓	2	Ongoing	-	-		-
Amaranthus	✓	1	Ongoing	-	-		-



USA Plant Transformation Facilities



<http://twiggy.txgen.tamu.edu/crop>



<https://cropinnovation.cals.wisc.edu/pricing-2/>



<https://web.uri.edu/pbl/plant-transformation/>



<https://btscience.org/our-research/research-facilities/biotechnology-center/>



<https://www.biotech.iastate.edu/biotechnology-service-facilities/plant-transformation-facility/>



<https://www.canr.msu.edu/ptc/>



<https://plantsciencesweb.missouri.edu/muptcf/>



<https://sips.cals.cornell.edu/research/plant-transformation-facility/services-and-pricing/>



<https://ptrc.ucr.edu/hours2.html>



<https://crec.ifas.ufl.edu/facilities/transformation-lab/transgenic-citrus-order-form/>



<https://biotech.unl.edu/plant-transformation#tab2>



<https://ptf.ucdavis.edu/services>



**Multi-Crop
Transformation
Facility**



Multi-Crop Transformation Facility



MAYRA FAION MOLINA

Research Specialist IV, Multi-Crop
Transformation Facility Director
mayrafmolina@tamu.edu



MARCO MOLINA

Research Specialist III, Multi-Crop
Transformation Facility Co-Director
marco.molina@tamu.edu



MARCELA B.D. DELLA GRACIA

Research Associate



LINDSEY KATELYN BAILEY

Research Assistant

We provide high quality plant transformation services to the scientific community across Texas A&M AgriLife, TAMU, the Texas A&M University System, and external collaborators.



**Multi-Crop
Transformation
Facility**

**TEXAS A&M
AGRI LIFE
RESEARCH**