Alfalfa to get back into planetary boundaries

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Technologies for alfalfa breeding and production

Julier B. & Noushahi H.

Parallel session 5

FROM 03 › TO 06 nov. 2025

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Genomic Editing for Alfalfa Improvement in Argentina

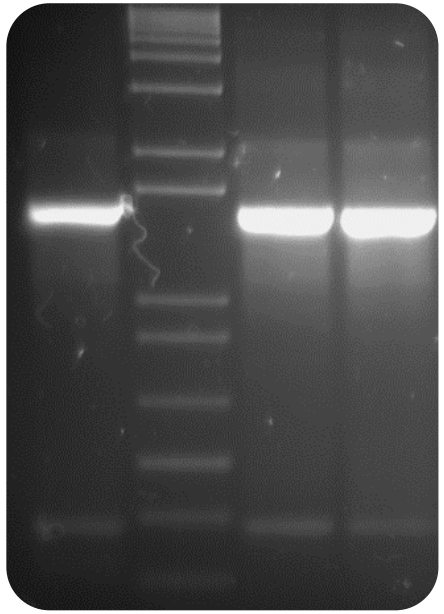
Gabriela Cynthia Soto

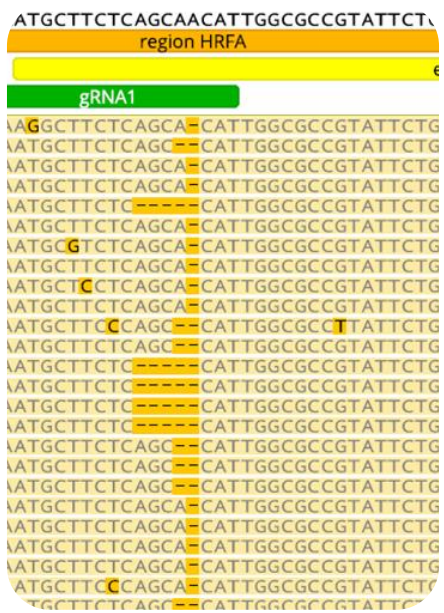
M. Stritzler; M. C. Gomez; M. E. Cardillo; L. Galindo Sotomonte; A. Ortíz; A. Cortez R. Frare; C. Jozefkowicz and C. Pascuan

INTA

CONICET

FROM 03 › TO 06 nov. 2025

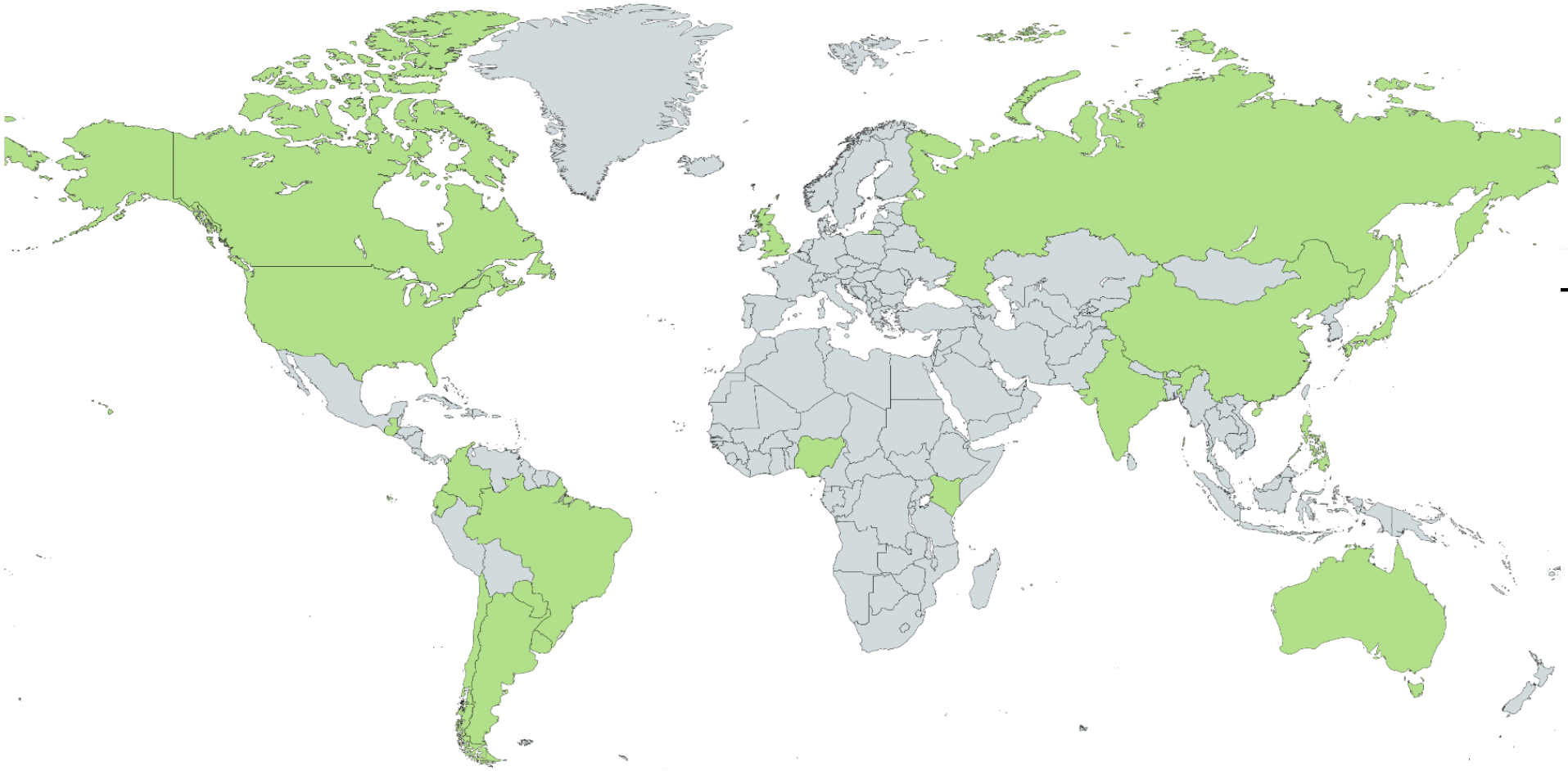




- Alfalfa genetic transformation
- Gene prospecting
- Development of experimental synthetic varieties

Biotechnology applied to alfalfa

Current status of genetically-edited organism acceptance



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NEWS | 11 February 2022

China's approval of gene-edited crops energizes researchers

Scientists say newly published guidelines will spur research into crops that have increased yields and greater resilience to climate change.

Countries with regulations allowing the commercialization of gene-edited organisms as traditional (non-transgenic), when the alteration is minimal and does not result in the net incorporation of foreign DNA, represent more than 70% of the world's population.

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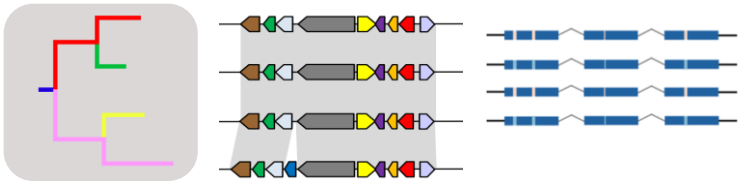
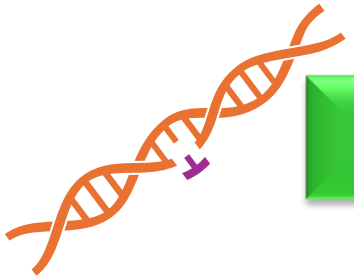
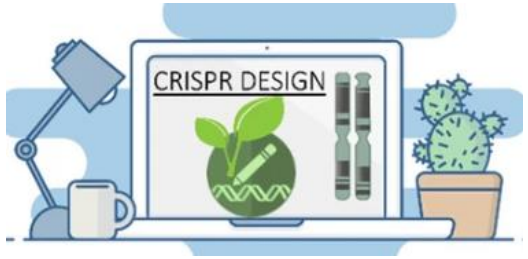
Alfalfa Gene Editing Pipeline

Target Genes Selection
Assignment of Orthologs

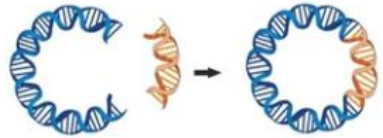




sgRNA design
multiplex

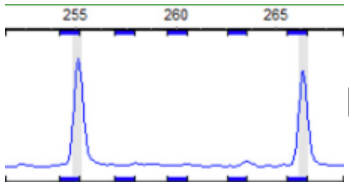


Cas9/sgRNA Assembly
(Binary vectors: *in vivo* assembly)



Delivery to the plant cell
Genetic transformation via
Agrobacterium tumefaciens

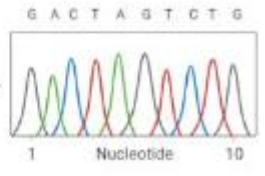
Screening



HRFA

Genotyping

NGS



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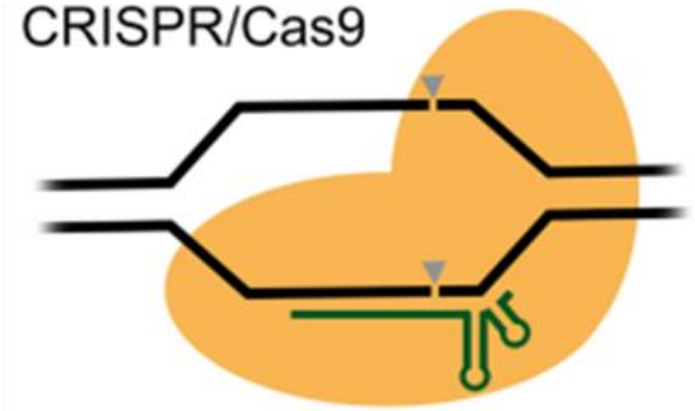
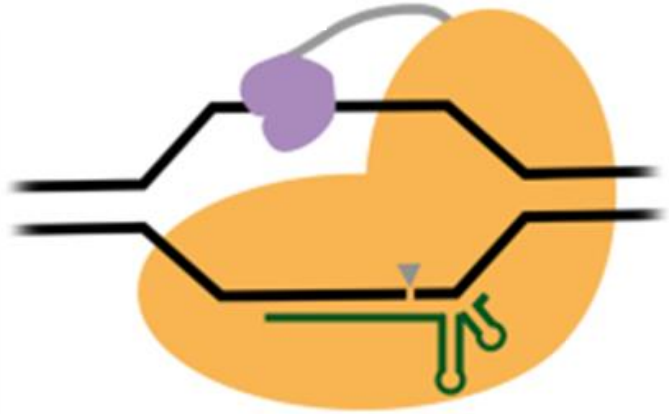
CRISPR/Cas9 Systems



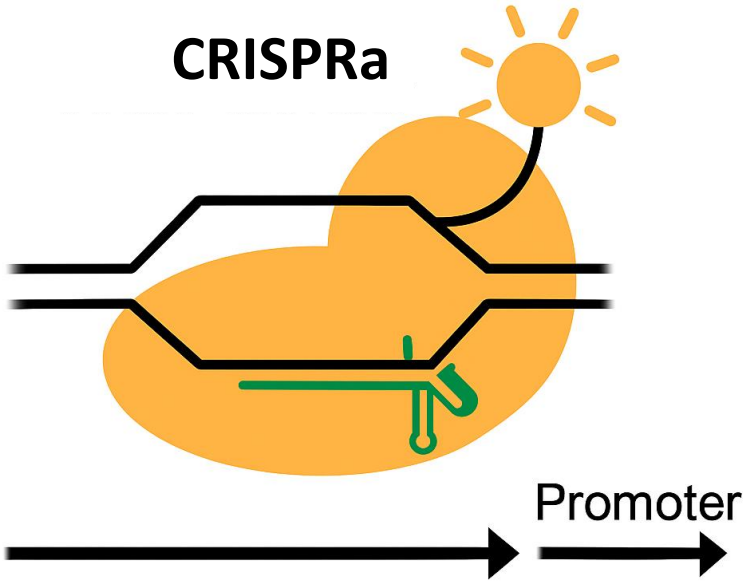
Knock Out

CRISPR/dCas9-BE



Point mutation

CRISPRa



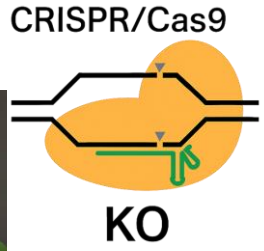
- CRISPR–dCas9-VP64
- CRISPR–dCas9-TET1

Simplicity
Precision
Versatility
No DNA traces

Alfalfa CRONOS

Variety characteristics:

- Delayed flowering
- Higher total biomass
- Improved digestibility and quality



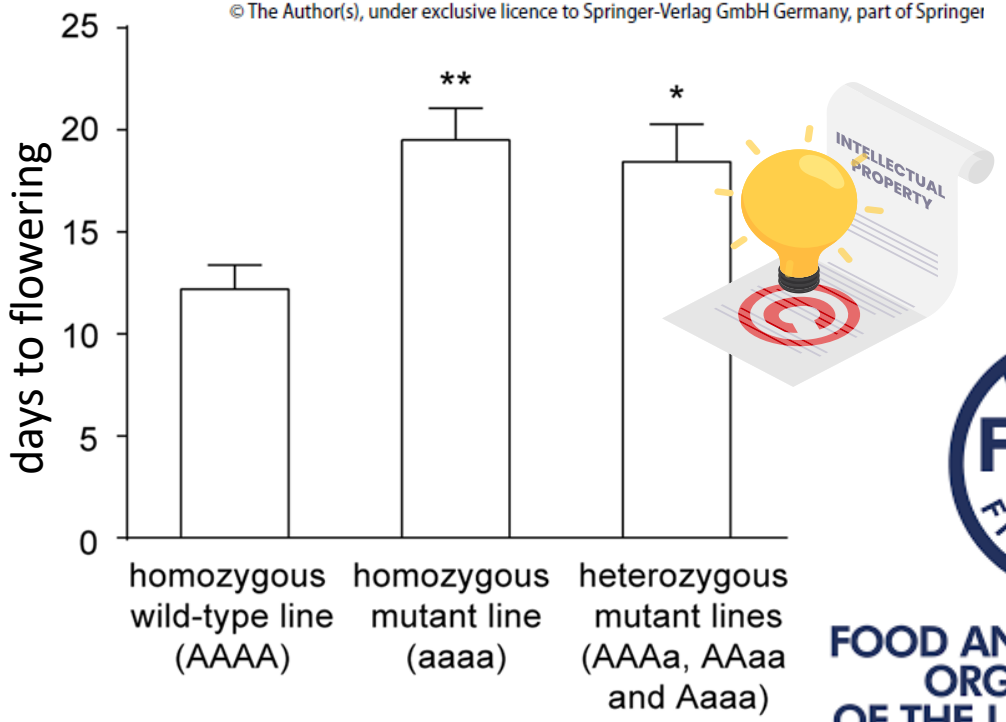
Plant Cell Reports
https://doi.org/10.1007/s00299-023-02997-9

FOCUS ARTICLE

CRISPR/Cas9-mediated knockout of a polyester synthase-like gene delays flowering time in alfalfa

Luisa Galindo-Sotomonte[1,2] · Cintia Jozefkowicz[1,2] · Cristina Gómez[1,2] · Margarita Stritzler[1,2] · Romina Frare[1,2] · Emilia Bottero[1,2] · Hiromi Tajima[3] · Eduardo Blumwald[3] · Nicolas Ayub[1,2] · Gabriela Soto[1,2]

Received: 10 January 2023 / Accepted: 2 February 2023



FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS

https://www.fao.org/3/cc5136en/cc5136en.pdf

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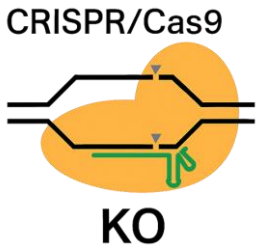
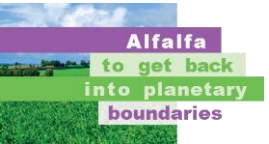
Alfalfa COMPACTA

Variety characteristics:

- Compact growth, anti-lodging
- For direct grazing
- High-quality hay





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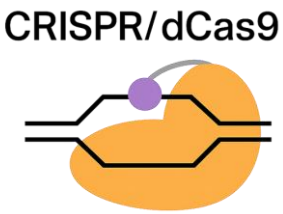
Alfalfa tolerant to ALS inhibitors



Variety characteristics:

- First alfalfa edited using the Base Editing system.
- Tolerant to IMI and Sus.

Plant Cell Reports
https://doi.org/10.1007/s00299-021-02827-w

FOCUS ARTICLE

Generation of a multi-herbicide-tolerant alfalfa by using base editing

Emilia Bottero[1,2] · Cristina Gómez[1,2] · Margarita Stritzler[1,2] · Hiromi Tajima[3] · Romina Frare[1,2] · Cecilia Pascuan[1,2] · Eduardo Blumwald[3] · Nicolás Ayub[1,2] · Gabriela Soto[1,2]

Received: 20 November 2021 / Accepted: 17 December 2021

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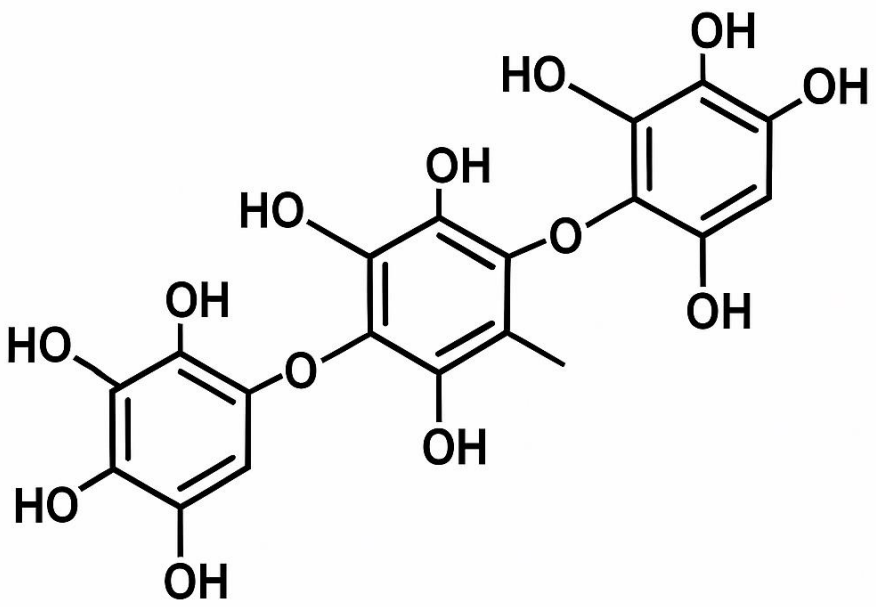
Anti boat Strategy

Condensed Tannins



How Proant

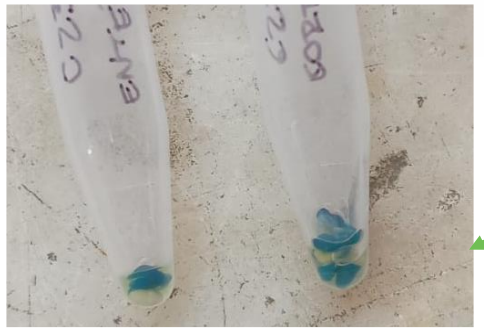
Wit

Tra

on switch PAs

PAR

TT8/bHLH
WD40
MYB5/14



DMACA staining of PA
seeds and leaves

Myb2
UGTs
LAP1

Phenylalanine
PAL
Cinnamate
C4H
p-Coumarate
4CL
p-Coumarate-CoA + 3 maalonyl-CoA
CHS
Flavanones
CHI
Flavonones
F3H
Dihydroflavonols
DFR
Leucoanthocyanidids

Flavonols

bHLH-MYB-WD0-regulatory complex

LAR
ANS
Catechin
Anthocyannidins
Epitactechin
UGT72L1
Epica, gluc
OMT
UFGT
ACT

Proanthocyanidins (PAs)
(condensed tannins)

Anthocyanins

TT8/bHLH
WD40
MYB5/14

ıe Rumen

escapes
ırmally

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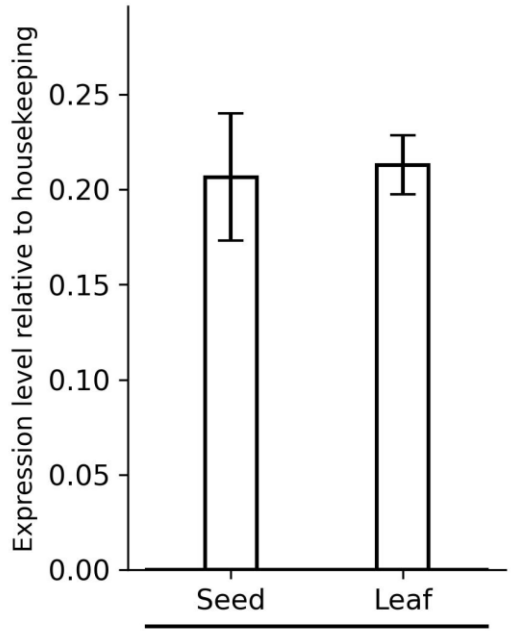
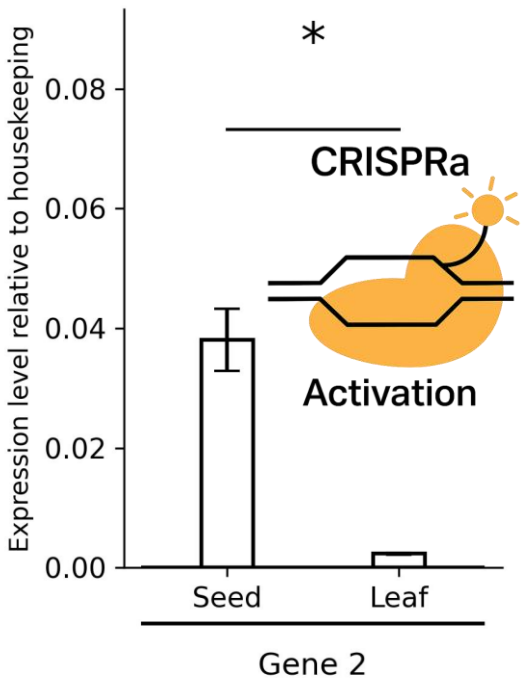
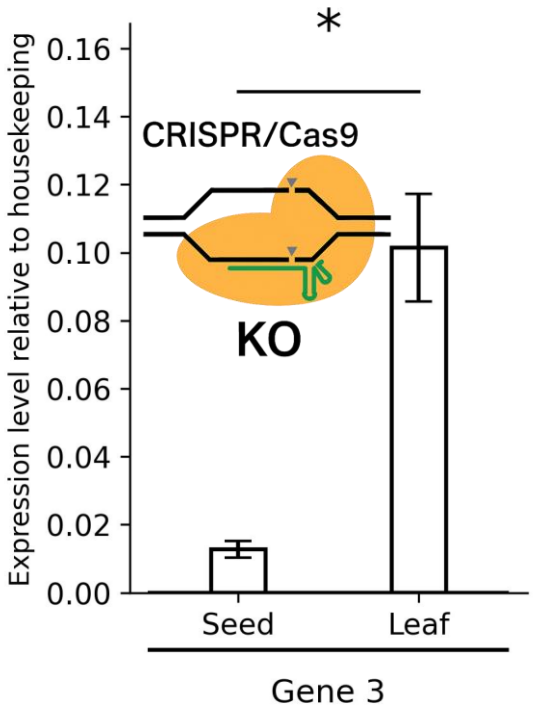
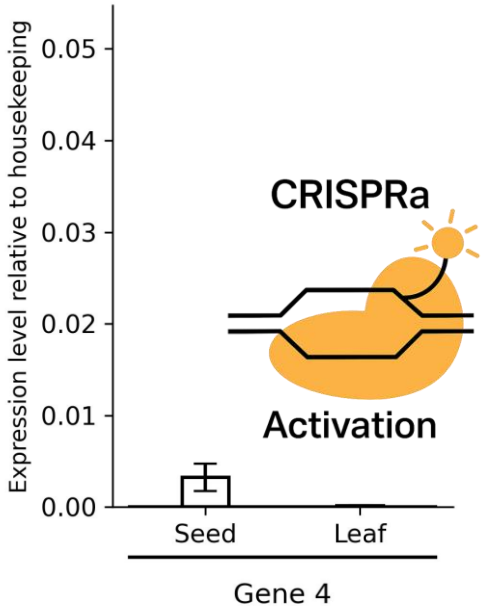
Anti boat Strategy

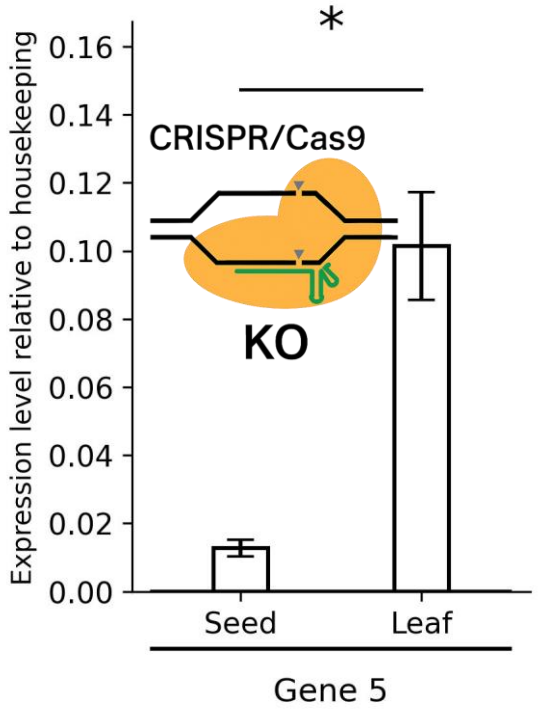
RT-qPCR and RNA-seq of PA biosynthetic genes and their positive and negative regulators.

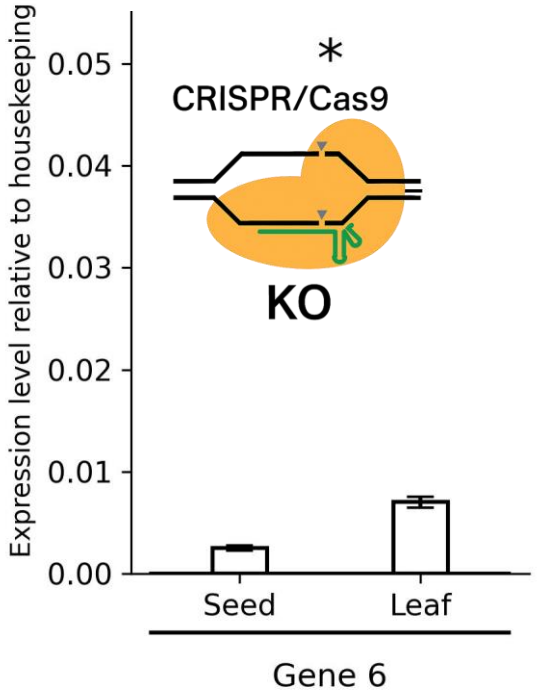


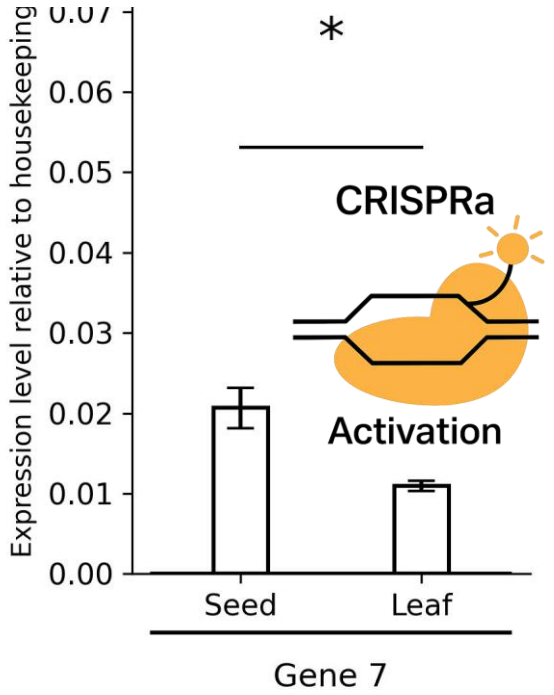












Perspectives and next goals:

- Complete the ongoing pre-varieties — CRONOS, COMPACTA, and ALS-tolerant lines;
- Enhance productivity through the manipulation of SPL13-type transcription factors and
- Develop drought tolerance by targeting various genes identified and characterized by our group and collaborators

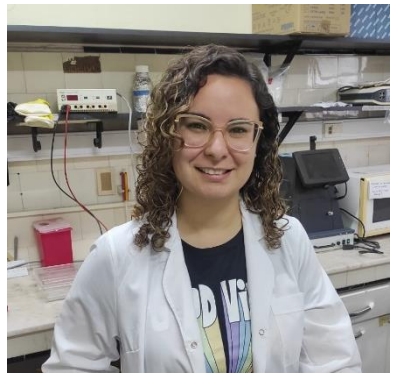
OUR TEAM

Researchers INTA and CONICET

Dra. Cecilia Pascuan
INTA-CONICET

Dra. Cintia Jozefkowicz
CONICET

Dra. Margarita Stritzler
CONICET



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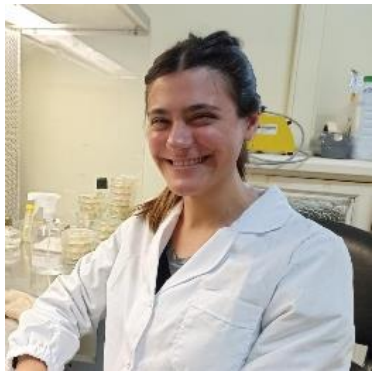
INTA

phD students

Lic. Luisa Galindo



Lic. Maru Cardillo

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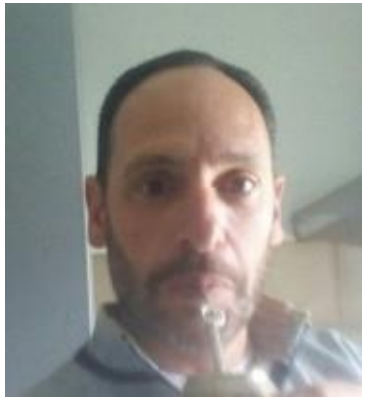
student

Tomás Blanzaco

Lab

Carmen Soria
INTA

greenhouse



Guillermo Piparola
INTA

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Thanks

INTA conventional alfalfa breeding program & evaluation network

Ed Blumwald´s Lab Davies-USA

Raquel Chan´s Lab IAL-CONICET

Francesco Paolocci´s Lab Perugia-Italy

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TOGETHER
EVERYONE
ACHIEVES
MORE

Thanks

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