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Desde 1967 Ciencia para cultivar el cambio

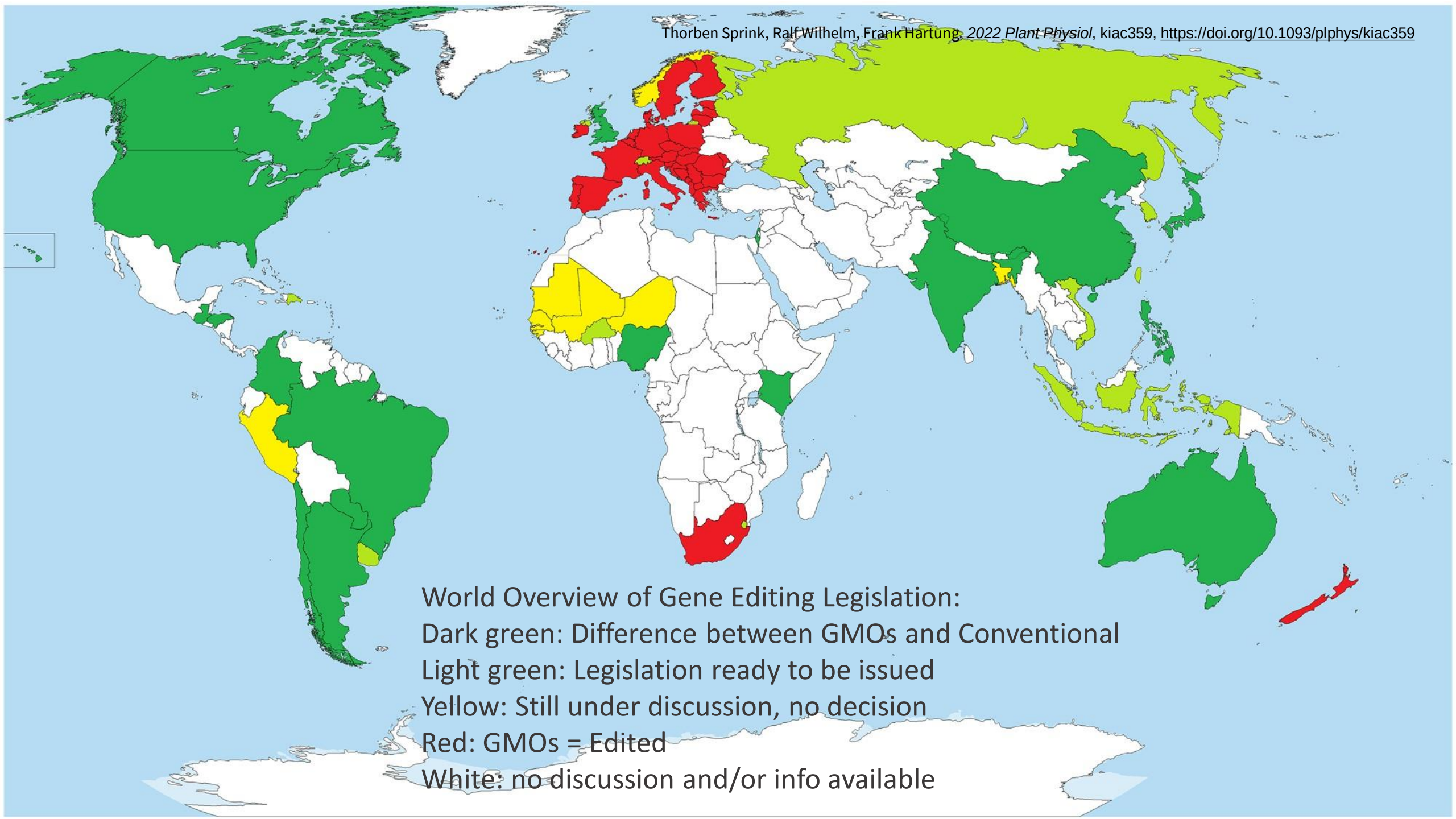
CGIAR

The Gene Editing Platform at The Alliance

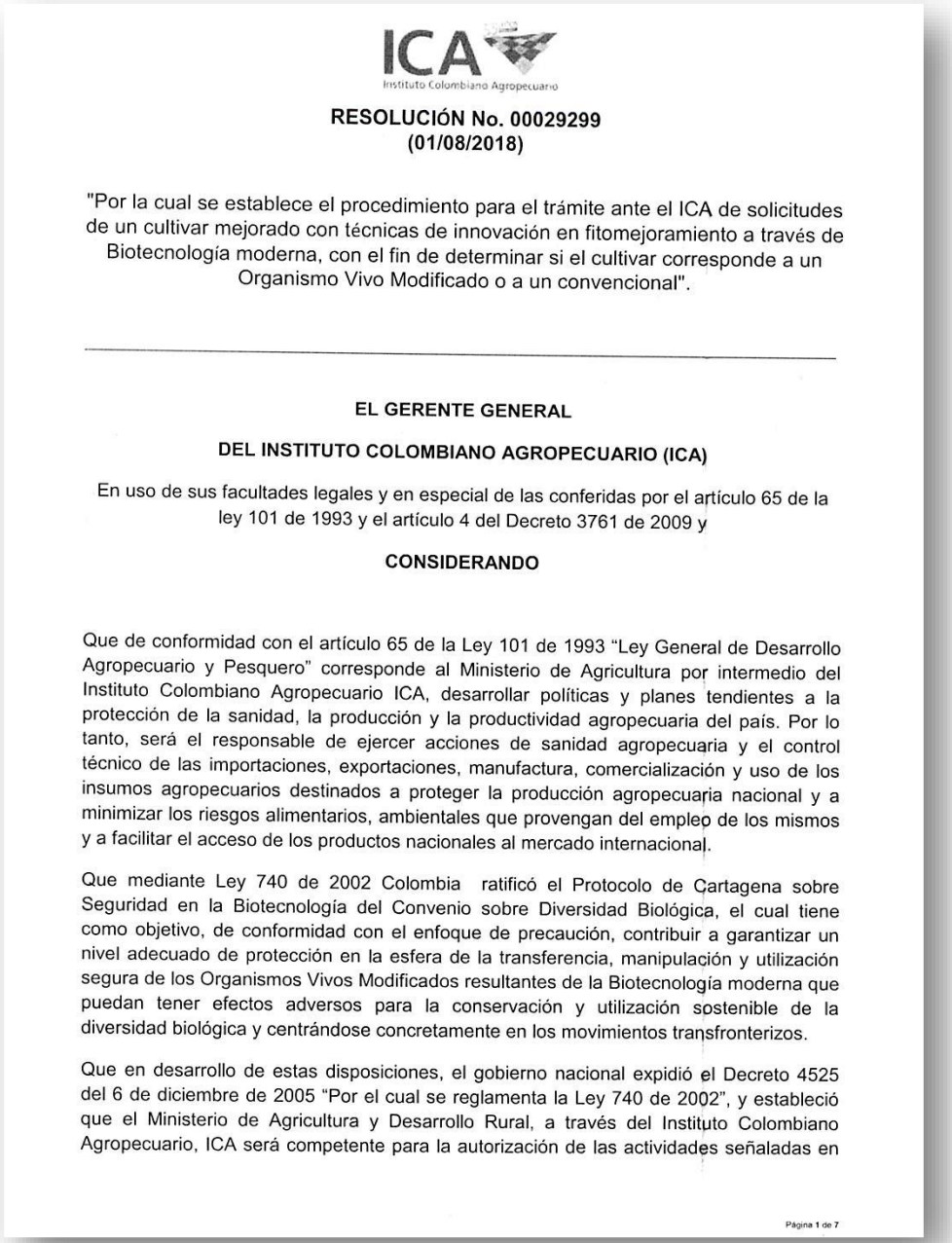
Gene Editing Platform –Cassava Team

Palmira, Valle del Cauca, 15-FEB-2023

Thorben Sprink, Ralf Wilhelm, Frank Hartung. *2022 Plant Physiol*, kiac359, https://doi.org/10.1093/plphys/kiac359



World Overview of Gene Editing Legislation:
Dark green: Difference between GMOs and Conventional
Light green: Legislation ready to be issued
Yellow: Still under discussion, no decision
Red: GMOs = Edited
White: no discussion and/or info available



ICA
Instituto Colombiano Agropecuario

RESOLUCIÓN No. 00029299
(01/08/2018)

"Por la cual se establece el procedimiento para el trámite ante el ICA de solicitudes de un cultivar mejorado con técnicas de innovación en fitomejoramiento a través de Biotecnología moderna, con el fin de determinar si el cultivar corresponde a un Organismo Vivo Modificado o a un convencional".

EL GERENTE GENERAL

DEL INSTITUTO COLOMBIANO AGROPECUARIO (ICA)

En uso de sus facultades legales y en especial de las conferidas por el artículo 65 de la ley 101 de 1993 y el artículo 4 del Decreto 3761 de 2009 y

CONSIDERANDO

Que de conformidad con el artículo 65 de la Ley 101 de 1993 "Ley General de Desarrollo Agropecuario y Pesquero" corresponde al Ministerio de Agricultura por intermedio del Instituto Colombiano Agropecuario ICA, desarrollar políticas y planes tendientes a la protección de la sanidad, la producción y la productividad agropecuaria del país. Por lo tanto, será el responsable de ejercer acciones de sanidad agropecuaria y el control técnico de las importaciones, exportaciones, manufactura, comercialización y uso de los insumos agropecuarios destinados a proteger la producción agropecuaria nacional y a minimizar los riesgos alimentarios, ambientales que provengan del empleo de los mismos y a facilitar el acceso de los productos nacionales al mercado internacional.

Que mediante Ley 740 de 2002 Colombia ratificó el Protocolo de Cartagena sobre Seguridad en la Biotecnología del Convenio sobre Diversidad Biológica, el cual tiene como objetivo, de conformidad con el enfoque de precaución, contribuir a garantizar un nivel adecuado de protección en la esfera de la transferencia, manipulación y utilización segura de los Organismos Vivos Modificados resultantes de la Biotecnología moderna que puedan tener efectos adversos para la conservación y utilización sostenible de la diversidad biológica y centrándose concretamente en los movimientos transfronterizos.

Que en desarrollo de estas disposiciones, el gobierno nacional expidió el Decreto 4525 del 6 de diciembre de 2005 "Por el cual se reglamenta la Ley 740 de 2002", y estableció que el Ministerio de Agricultura y Desarrollo Rural, a través del Instituto Colombiano Agropecuario, ICA será competente para la autorización de las actividades señaladas en

Página 1 de 7

Legislation in Colombia since August 2018

- ICA Resolution # 00029299
- Waxy corn, and *Xam*-resistant rice declared conventional varieties/lines
- Banana and Brassica on pipeline

Crop	Trait	Edited genes	Stage
Banana	Disease resistance (BXW, Fusarium wilt, BSV)	*DMR6*, BSV sequences	3,1
Cassava	Disease resistance (BB)	*SWEET* gene promoters	3
	Food safety (cyanide-free)	Linamarin synthase	3
	Quality (waxy starch)	*GBSS1*	3
Maize	Disease resistance (MLN)	*C6 QTL*	1
	Weed resistance (*Striga*)	Strigolactone	3
Potato	Disease resistance (PVY[a], late blight)	*eIF-4E, StDMR6-1, StCHL1*	2
Rice	Disease resistance (BLB, RHB)	*SWEET* gene promoters, *AGO4, STV11*	4,3
	Food safety (low arsenic and cadmium)	*OsNRAMP5, OsPT8, LS1, LS2*	3
	Nitrogen remobilization, and methane emission reduction	Unpublished	3
	Insect resistance[a] (BPH)	BPH resistance alleles	2
Wheat	Disease resistance (rusts, mildew)[a]	*Lr67* and others	3

Gene Editing Efforts in the CGIAR

(Pixley at al 2022)

"[a]SDN2 editing required. All other current projects are SDN1"

"Stage of current development: (1) discovery; (2) proof of concept; (3) early development; (4) advanced development; (5) commercialization"

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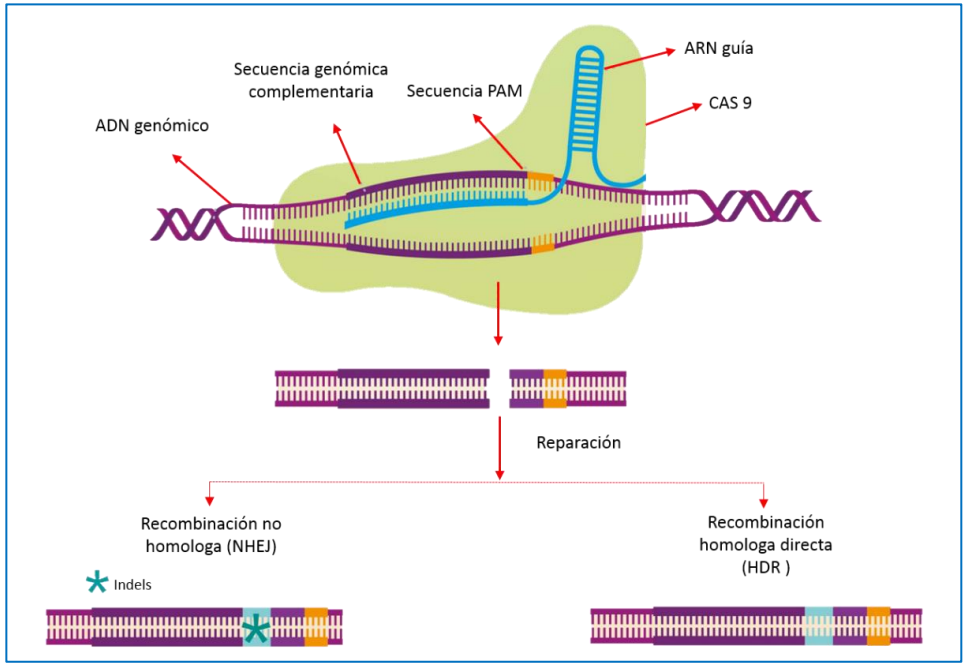
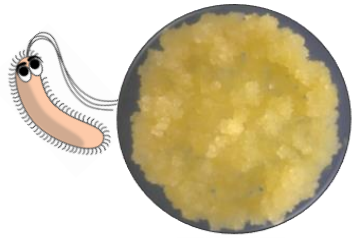
CRISPR-Cas9



Current Cassava's Pipeline



Cells

In vitro GMO+GEd plant

Segregate GMO

In vitro GEd plant

No Foreign DNA in Final Product:

SDN1: DNA repair induces INDELS

SDN2: DNA repair with template

SDN3: Insertion of longer strands of external (transgenes) or autochtonous sequences (cisgenes).

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New Nutations in GBSSI for Waxy Cassava

Ingredion

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ATGGCAACTGTAATAGCTGCACATTTAGTTTCCAGGAGCTCACACTTGAGCATCCATGCATTAGAGACTAAGGCTAATAATTTGTCT
CACACTGGACCCTGGACCCAAACTATCACTCCCAATGGTTTAAGGTCCCTCAACACTATGGATAAACTCCAAATGAAGACACAATCA
AAAGCTGTGAAAAAGGTCTCTGCCACCGGCAATGGTAGGCCTGCTGCCAAAATTATTTGTGGTCATGGAATGAATTTAATCTTTGTT
GGAGCTGAAGTTGGTCCCTGGAGCAAAACTGGTGGACTTGGTGATGTTCTTGGAGGACTCCCCCCTGCCATGGCCGCAAGAGGGC
ACCGCGTCATGACAGTGTCTCCCCGCTATGACCAGTACAAGGATGCTTGGGATACCTCTGTATCGGTGGAGATTAAAATTGGAGAT
AGAATTGAAACTGTCCGCTTCTTCCACTCCTACAAAAGAGGAGTTGATCGGGTCTTCGTGGATCATCCAATGTTCCTTGAGAAGGTA
TGGGGCAAAACTGGATCTAAAATATATGGCCCAAGAGCAGGTTTGGATTACCAGGACAACCAACTGCGATTTAGCTTGTTATGCCT
TGCTGCTCTGGAGGCACCGAGAGTTCTGAACTTGAACAGCAGCAAAAATTTCTCAGGACCCTACGGAGAAGAAGTTGCCTTCATTG
CCAACGACTGGCACACTGCTCTGCTTCCATGTTATCTAAAAGCCATTTACCAACCTATGGGGATTTACAAACACGCCAAGGTTGCCTT
TTGCATCCACAACATTGCATATCAGGGAAGATTTGCCTTCTCAGACTTCCCACGACTTAATCTGCCAGATAAATTCAAAAGCTCTTTT
GACTTTATCGATGGGTATGAGAAGCCCGTGAAGGGAAGGAAAATCAATTGGATGAAGGCCGGGATATTGGAATCAGACAGGGTT
TTGACTGTGAGCCCATACTATGCCCAAGAAGTCATCTCTGGAGTTGAAAGAGGCGTCGAGCTGGATAACTTCATTCGTAAAACTGG
CATTGCTGGTATTATAAATGGCATGGACGTCCAGGAGTGGAATCCTGTTACAGATAAATACATTGACATCCACTACGATGCCACAAC
TGTTATGGACGCAAAACCTTTGTTGAAGGAAGCCCTTCAAGCAGAAGTCGGATTGCCTGTTGATAGGAATGTTCCTTTGATAGGCTT
CATTGGTAGATTAGAAGAGCAGAAGGGTTCAGATATTTTTGTTGCAGCTATTTCCCAATTGGTTGAACACAATGTGCAGATAGTAAT
CCTTGGAACTGGCAAAAAGAAATTTGAGAAGCAGATTGAGCATCTGGAGGTTTTGTACCCTGACAAGGCAAGAGGAGTTGCAAAA
TTCAATGTGCCGTTGGCGCACATGATCACAGCTGGTGCTGACTTTATGCTGGTTCCAAGTAGATTTGAGCCCTGTGGTCTCATTCAG
TTGCATGCTATGCGATATGGAACAGTTCCCATTGTTGCTTCTACTGGTGGTCTTGTTGATACTGTTAAAGAAGGTTACACAGGATTC
CAAATGGGGGCCTTGCGCGTTGAATGTGACAAAATTGATTCAGCAGATGTAGCTGCGATAGTTAAAACTGTGGCAAGAGCTCTTG
GCACTTATGCTACCGCTGCATTAAGAGAAATGATCCTGAATTGCATGGCCCAAGACTTGTCATGGAAGGGACCAGCCAGAATGTGG
GAGAAAATGCTCCTGGACCTGGAAGTTACTGGCAGCGAACCTGGCACTGAAGGGGAGGAGATCGCTCCTCTTGCTAAGGAGAACG
TTCCCACGCCTTGA

gRNA name	Sequence 5’-3’ + PAM	Location
sgRNA-GBSSI-5	TTGGGATACCTCTGTATCGG**TGG**	Exon 2
sgRNA-GBBI-1	GGTAGATTAGAAGAGCAGAA**GGG**	Exon 9
sgRNA-GBSSI-4	**CCT**ATGGGGATTTACAAACACGC	Exon 6

Waxy Cassava Harvest June-July 2020

Germination (sprouting) of waxy lines 62 dap

Lines	# of germinated stakes (3 Reps. 10 plants each)	# of plants with shoots >20cm
TMS60444 (control)	9, 8, 9	7, 5, 5
MPER183	6, 5, 2	5, 5, 2
LINE 32	7, 5, 6	5, 2, 2
LINE 8	2, 2, 0	1, 0, 0
LINE 54	0, 0, 0	0, 0, 0

LINE 8

TMS60444 (control)

MPER183

LINE 32

LINE 54

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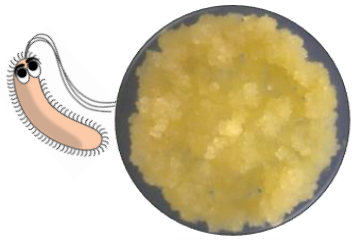
Cassava's Pipeline



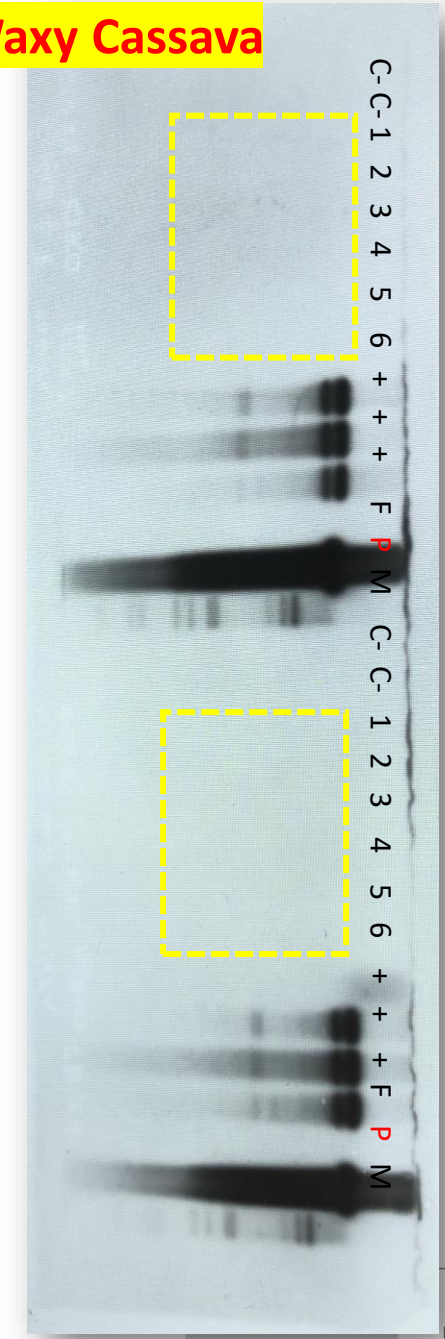
Cells

In vitro GMO+GEd plant

Segregate GMO

In vitro GEd plant

T-DNA-Free, Gene Edited Waxy Cassava



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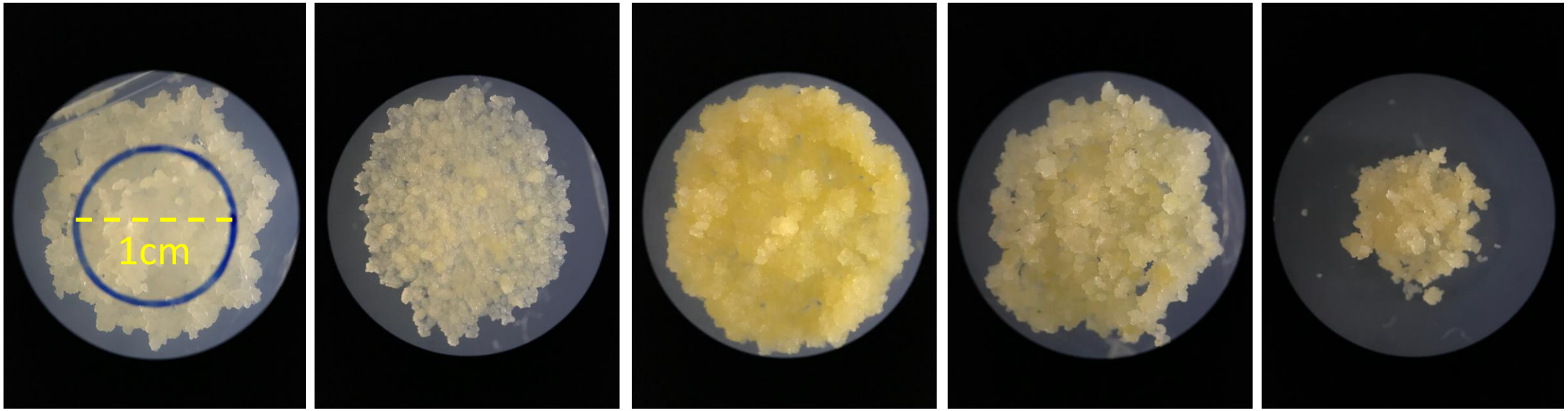
New FEC Lines for Waxy?

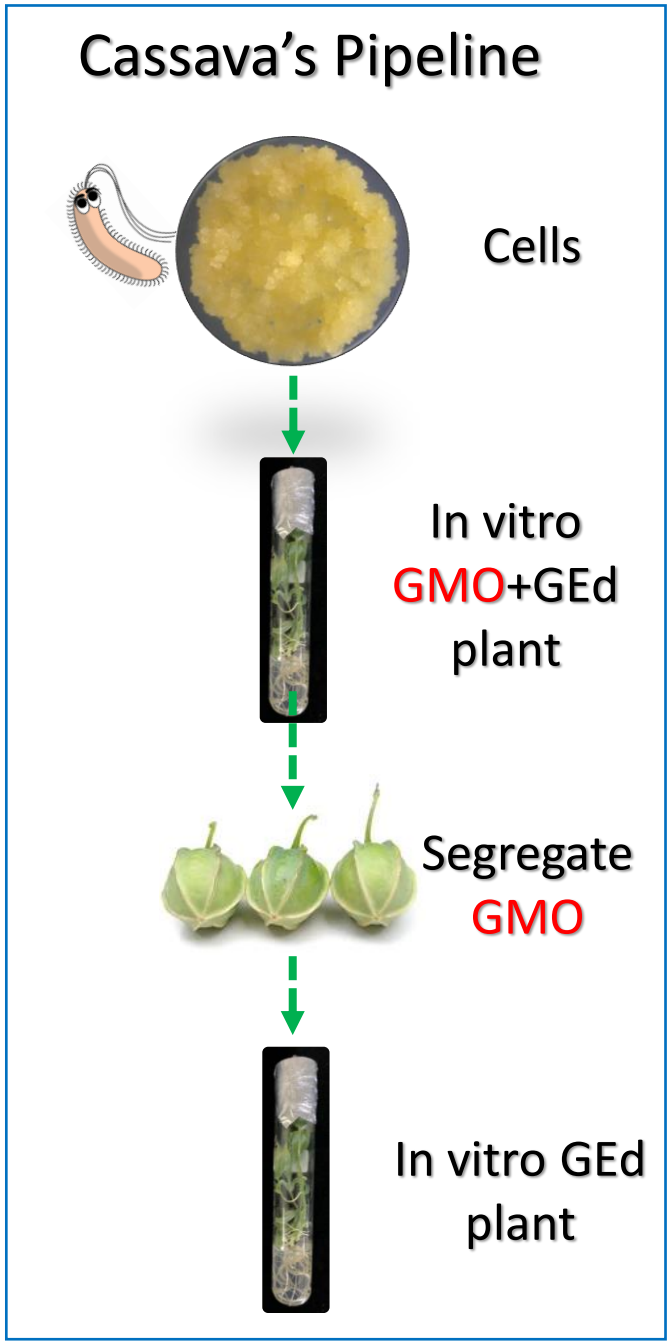


CM4574-7
Caucana
(9 do)
Industrial

MCol2215
(23 do)
Fresh
consump.

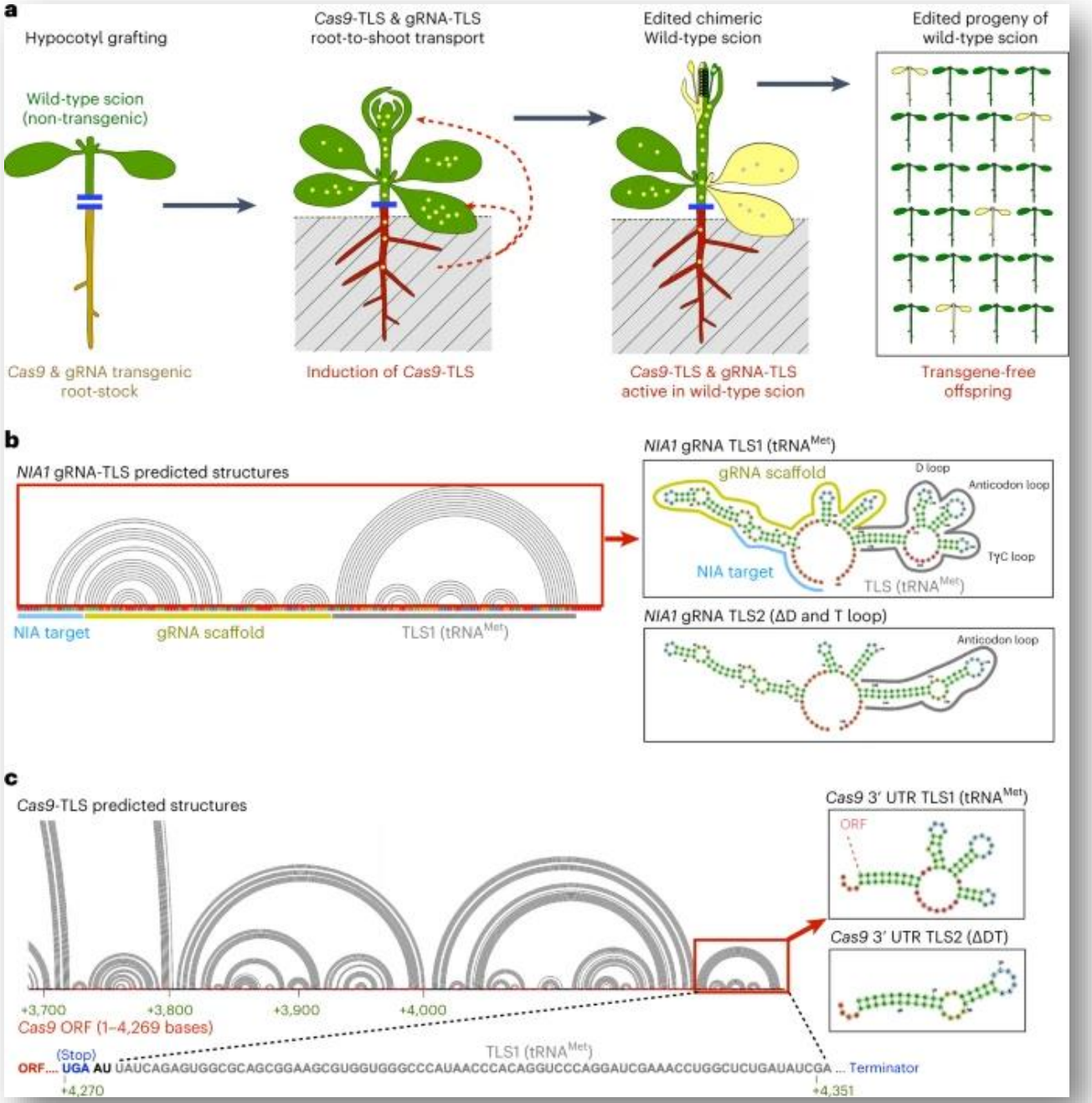
TMS60444
(23 do)

KU50
(16 do)
Industrial



Remember

Segregation still needed since, for non-GMO, Gene-Edited cassava there is no DNA-free gene editing approach, efficient and reliable, available yet.



Heritable transgene-free genome editing in plants by grafting of wild-type shoots to transgenic donor rootstocks

Lei Yang et al Nat. Biotechnol. (2023)
https://www.nature.com/articles/s41587-022-01585-8

- Drawback: cleaning out chimerism in next true-seed generation. Feasible for cacao, not cassava.

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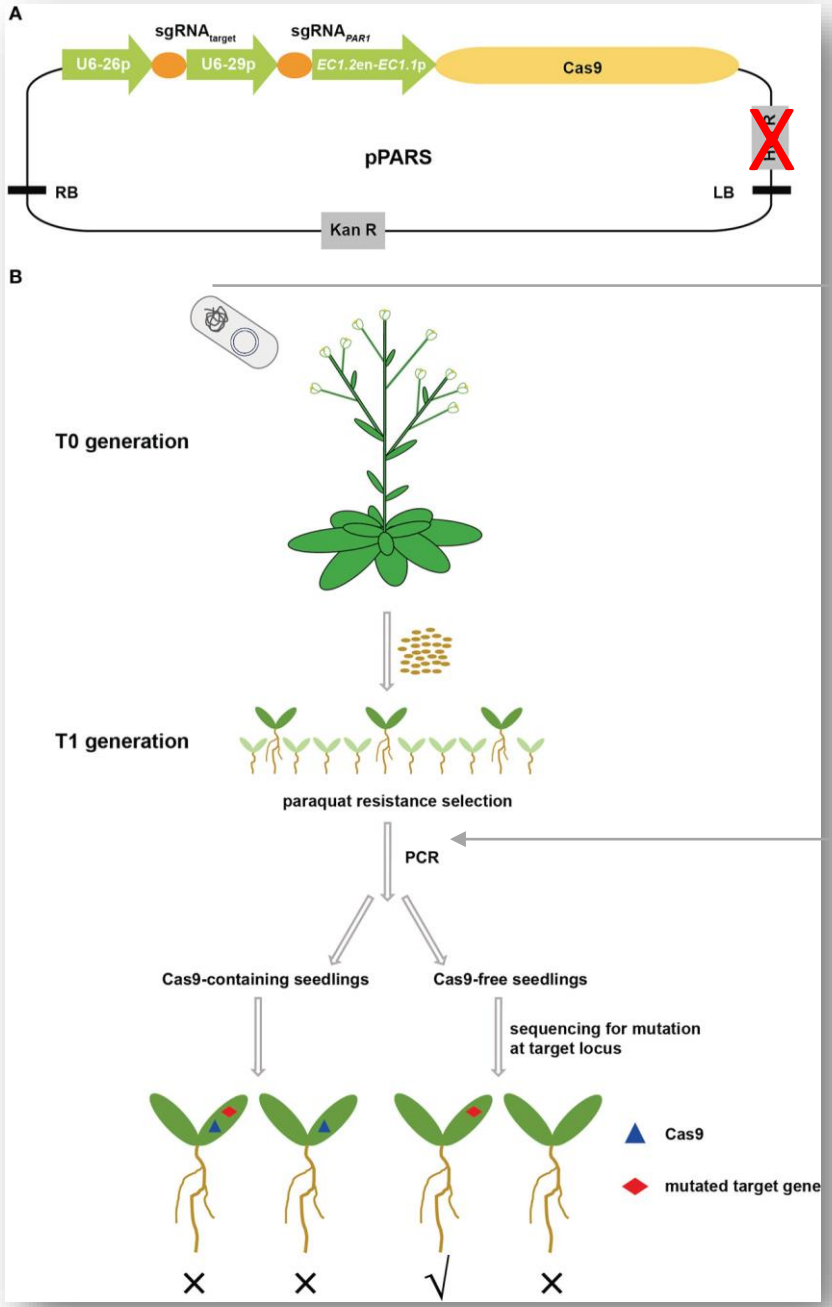
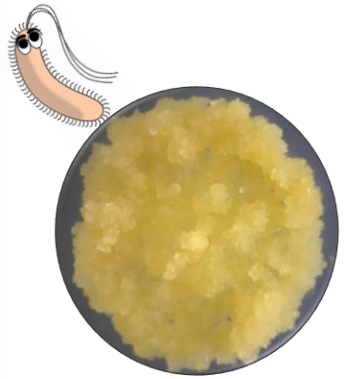




FEC

T0 generation

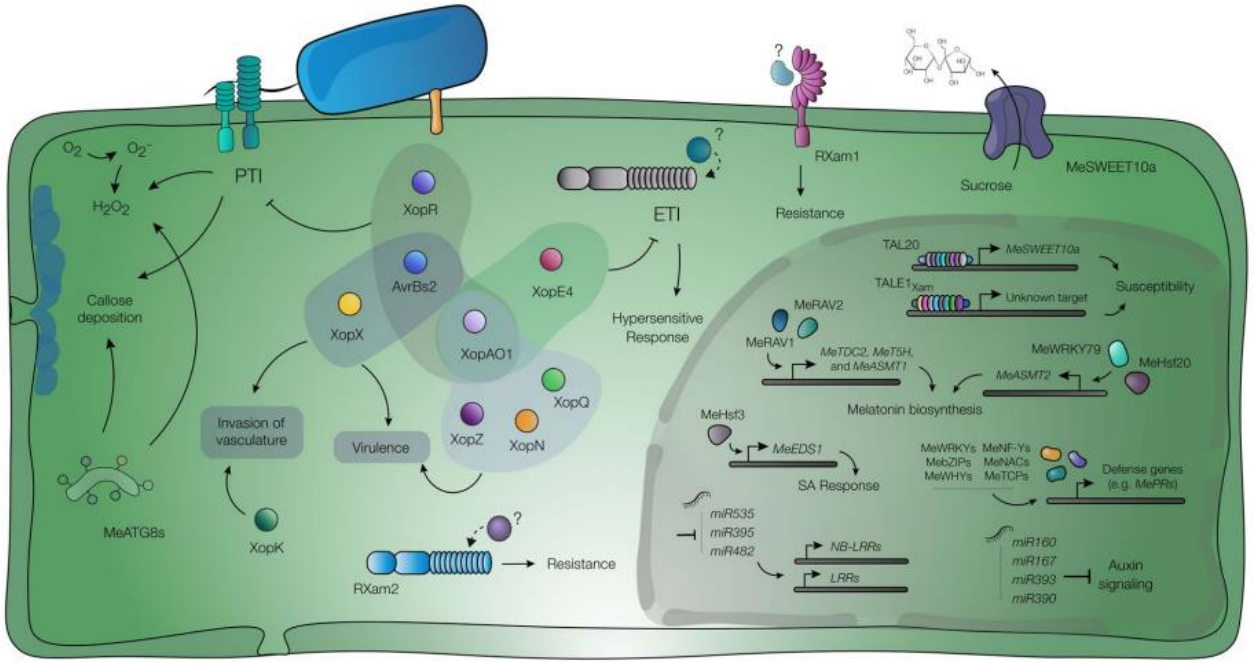
A simple and efficient strategy to produce transgene-free gene edited plants in one generation using paraquat resistant 1 as a selection marker

Xiangkiu Kong et al (2022)
https://www.frontiersin.org/articles/10.3389/fpls.2022.1051991/full

- "PAR1 can be used as a coediting marker to enrich mutants of target loci."
- Being implemented for cassava using FEC, selection with paraquat (not Hygromycin)

Gene Editing SWEET genes to Confer resistance to Cassava Bacterial Blight produced by *Xanthomonas phaseoli pv. manihotis (Xpm)*

Boris Szurek & Paula Díaz & The Alliance



(Zarate et al. 2021)

Institut de Recherche pour le Développement FRANCE

UAN UNIVERSIDAD ANTONIO NARIÑO

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Design of SgRNA's in the MeSweet 10a gene.

SgRNA information for the EBE site of the MeSweet10a gene:

gRNA name	Sequence 5'-3'+ PAM	Location
XamEBE-1	GGGCGAGAAGCGTTTATATAGGG	EBE (TAL20 Xam668)
XamEBE-2	CTATGTTGTGCAATGATGGATGG	EBE (TAL20 Xam668)

XamEBE-1 site for TAL20
XamEBE-2 site for TAL20
CCA = PAM region
5'UTR region
First exon

TTCTTCAAAAAAAATAATAAAAGAAACAAGGCCACTGTTACATTGACATATTTTATTCACTTTAATCATGCATGCAACTTGACTTCATT
CCGTTCCCTGGATTCCTCCCCTATATAAACGCTTCTCGCCCATCCATCATTGCACAACATAGCTAGAGTTTCCTCTTGAGAAAGAGAGT
TTCCTCTGCACAAGGGAAAGAGAGTCTCTACTATAGCCGGAGAATGGCCTTGCACTTGTCATTGGACTTCGTTTTTGGCGTTTTAGGTA

SWEET10a_EBE1

gRNA

```
Control          TCCGTTCCCTGGATTCCTCCCCTATATAAACGCTTCTCGCCCATCCATCATTGCACAACATAGCTAGAGTTTCCTCT  WT
A-1(1)_all1      TCCGTTCCCTGGATTCCTCCCCTAT----------CTCGCCCATCCATCATTGCACAACATAGCTAGAGTTTCCTCT  -10
A-1(1)_all2      TCCGTTCCCTGGATTCCTCCCCTATATAAACGCTTCTCGCCCATCCATCATTGCACAACATAGCTAGAGTTTCCTCT  WT
B-2(4)_all1&2    TCCGTTCCCTGGATTCCTCCCCTAATATAAACGCTTCTCGCCCATCCATCATTGCACAACATAGCTAGAGTTTCCTCT +1
(5)_all1&2       TCCGTTCCCTGGATTCCTCCCCTAATATAAACGCTTCTCGCCCATCCATCATTGCACAACATAGCTAGAGTTTCCTCT +1
D-1(6)_all1      TCCGTTCCCTGGATTCCTCCCCTAT----------CTCGCCCATCCATCATTGCACAACATAGCTAGAGTTTCCTCT  -10
D-1(6)_all2      TCCGTTCCCTGGATTCCTCCCCTATATAAACGCTTCTCGCCCATCCATCATTGCACAACATAGCTAGAGTTTCCTCT  WT
D-2(7)_all1      TCCGTTCCCTGGATTCCTCCCCTAT----------CTCGCCCATCCATCATTGCACAACATAGCTAGAGTTTCCTCT  -10
D-2(7)_all2      TCCGTTCCCTGGATTCCTCCCCTATATAAACGCTTCTCGCCCATCCATCATTGCACAACATAGCTAGAGTTTCCTCT  WT
G-1(12)_all1&2   TCCGTTCCCTGGATTCCTCCCCTATATAAACGCTTCTCGCCCATCCATCATTGCACAACATAGCTAGAGTTTCCTCT  WT
E-2(15)_all1&2   TCCGTTCCCTGGATTCCTCCCCTATATAAACGCTTCTCGCCCATCCATCATTGCACAACATAGCTAGAGTTTCCTCT  WT
K-2(17)_all1&2   TCCGTTCCCTGGATTCCTCCCCTATATAAACGCTTCTCGCCCATCCATCATTGCACAACATAGCTAGAGTTTCCTCT  WT
(20)_all1        TCCGTTCCCTGGATTCCTCCCCTAATATAAACGCTTCTCGCCCATCCATCATTGCACAACATAGCTAGAGTTTCCTCT +1
(20)_all2        TCCGTTCCCTGGATTCCTCCCCTATATAAACGCTTCTCGCCCATCCATCATTGCACAACATAGCTAGAGTTTCCTCT  WT
P-1(21)_all1     TCCGTTCCCTGGATTCCTCCCCTATAATAAACGCTTCTCGCCCATCCATCATTGCACAACATAGCTAGAGTTTCCTCT +1
P-1(21)_all2     TCCGTTCCCTGGATTCCTCCCCTATATAAACGCTTCTCGCCCATCCATCATTGCACAACATAGCTAGAGTTTCCTCT  WT
```

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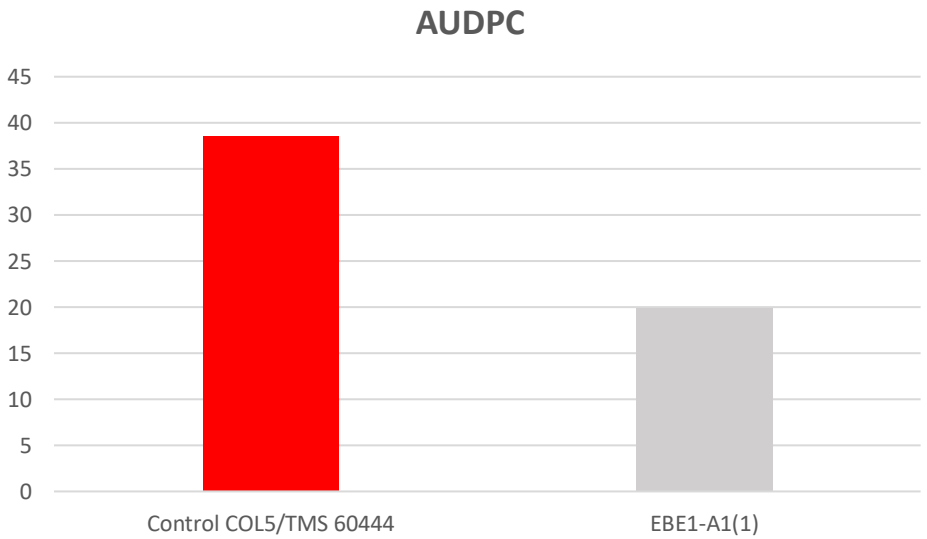
CONTROL TMS 60444–R4.

MeSWEET 10A -EBE1-A1(1)- R1



Haploid Induction

EMBO J. 2017 Mar 15;36(6):707-717. doi: 10.15252/embj.201796603. Epub 2017 Feb 22.

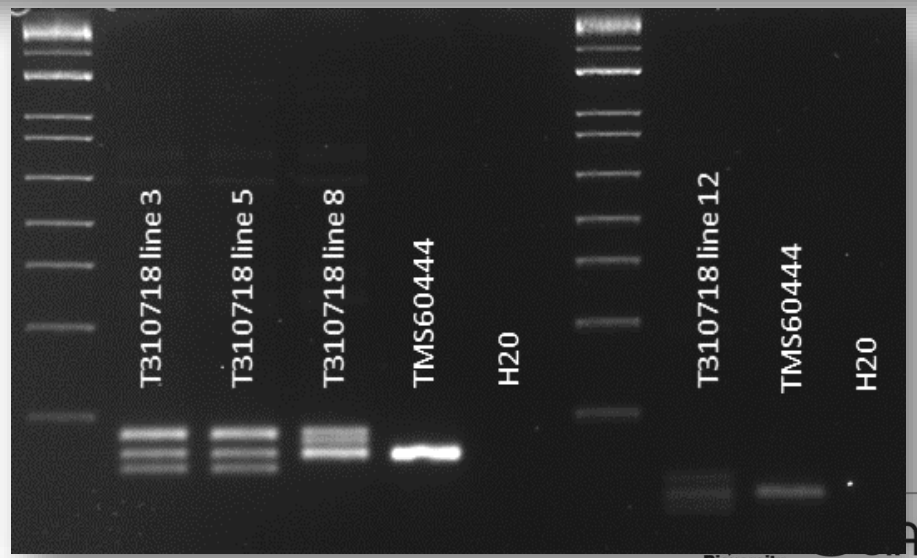
Loss of pollen-specific phospholipase NOT LIKE DAD triggers gynogenesis in maize

Laurine M Gilles [1 2], Abdelsabour Khaled [1 3], Jean-Baptiste Laffaire [2], Sandrine Chaignon [1], Ghislaine Gendrot [1], Jérôme Laplaige [1], Hélène Bergès [4], Gensériс Beydon [4], Vincent Bayle [1], Pierre Barret [5], Jordi Comadran [2], Jean-Pierre Martinant [2], Peter M Rogowsky [1], Thomas Widiez [6]

Nature. 2017 Feb 2;542(7639):105-109. doi: 10.1038/nature20827. Epub 2017 Jan 23.

MATRILINEAL, a sperm-specific phospholipase, triggers maize haploid induction

Timothy Kelliher [1], Dakota Starr [1], Lee Richbourg [1], Satya Chintamanani [2], Brent Delzer [3], Michael L Nuccio [1], Julie Green [1], Zhongying Chen [1], Jamie McCuiston [1], Wenling Wang [1], Tara Liebler [1], Paul Bullock [2], Barry Martin [1]



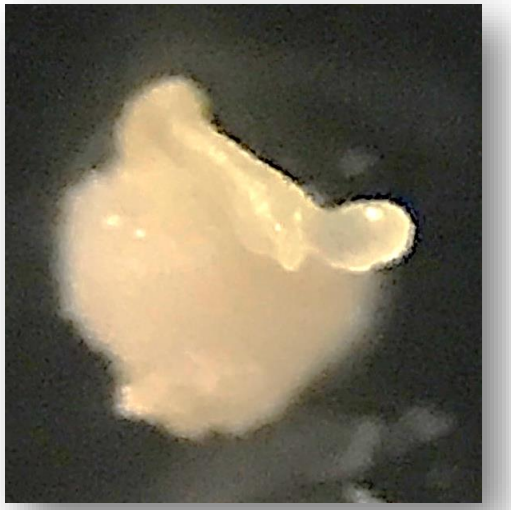
(Haploid Induction) Crosses & Embryo Rescue

Crosses:
SM 3359-11 x Hapl mol 17 - A
SM 3359-11 x Hapl mol 17 - 1A
SM 3359-11 x Hapl mol 17 - 2A
SM 3359-11 x Hapl mol 17 - 3A
SM 3359-11 x Hapl mol 9 - A
SM 3359-11 x Hapl mol 9 - B

WT

Haploid Inducer

Somatic embryos from 2 months old seed from cross SM3359 **X** Hapl-mol17-2A



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Leveraging Landrace Genomics to Rapidly Engineer Thermotolerant Cassava

Grey Monroe , Kehan Zhao & Evan Long

Gene Editing Cassava Team at CIAT

- Genomics data from Grey Monroe et al (online)

Genome Sequencing Landraces

- Total *in vitro* landraces in TC: 307
- DNA extracted: 328 varieties and Landraces.
- Missing: 72 plants to complete the goal of 400

Pan-Genome

- Total of *in vitro* plants: 8
- DNA extracted: 8 Landraces for pan-genome
- Missing: 2 plants to complete the goal of 10

Bulk genotypes for common garden experiments

Date of field: 14-Dec-2022

09-FEB-2023

- Landraces: 240
- Varieties: 16
- Total plants: 648

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Clonal Macro-Propagation of Cassava using Hydroponic system

Cassava mini cuttings (8-10 cm)

5-6 weeks

Direct transplanting to field

Springer Link

Developmental Biology | Published: 26 January 2017

A simple hydroponic hardening system and the effect of nitrogen source on the acclimation of *in vitro* cassava (*Manihot esculenta* Crantz)

Oscar Castañeda-Méndez, Satoshi Ogawa, Adriana Medina, Paul Chavarriaga & Michael Gomez Selvaraj

3 weeks after direct transplanting to field

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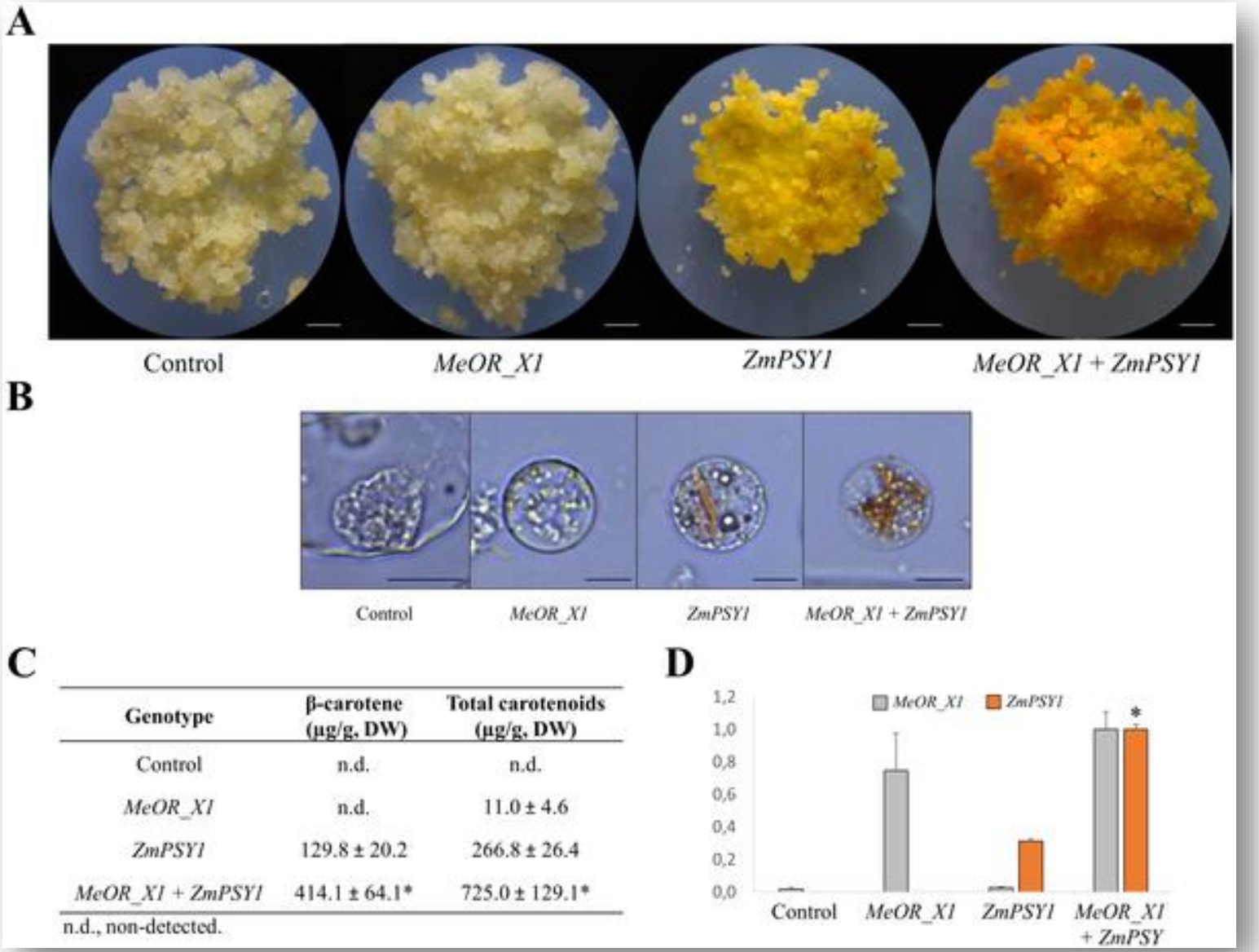

Genotype	β-carotene (µg/g, DW)	Total carotenoids (µg/g, DW)
Control	n.d.	n.d.
MeOR_X1	n.d.	11.0 ± 4.6
ZmPSY1	129.8 ± 20.2	266.8 ± 26.4
MeOR_X1 + ZmPSY1	414.1 ± 64.1*	725.0 ± 129.1*

n.d., non-detected.

Jaramillo AM, Sierra S, Chavarriaga-Aguirre P, Castillo DK, Gkanogiannis A, et al. (2022) Characterization of cassava ORANGE proteins and their capability to increase provitamin A carotenoids accumulation. PLOS ONE 17(1): e0262412. https://doi.org/10.1371/journal.pone.0262412
https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0262412

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Perspectives

- **DNA-Free or Transgene-Free Methods.**
- **For Haploid Induction**: MORE MALE FLOWERS; Histo-Cellular characterization
- **Training partners in Asia/Africa (even for rice)**

Reasons to continue:

- > 40 crops[11]
- > 25 countries.
- Agronomy, food and feed quality, biotic/abiotic stress tolerance.
- Commercially seven crops: soybean, canola, rice, maize, mushroom, camelina and tomato.


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Gracias!