Embrapa

48 anos

# Development in gene editing in Brazil and at EMBRAPA

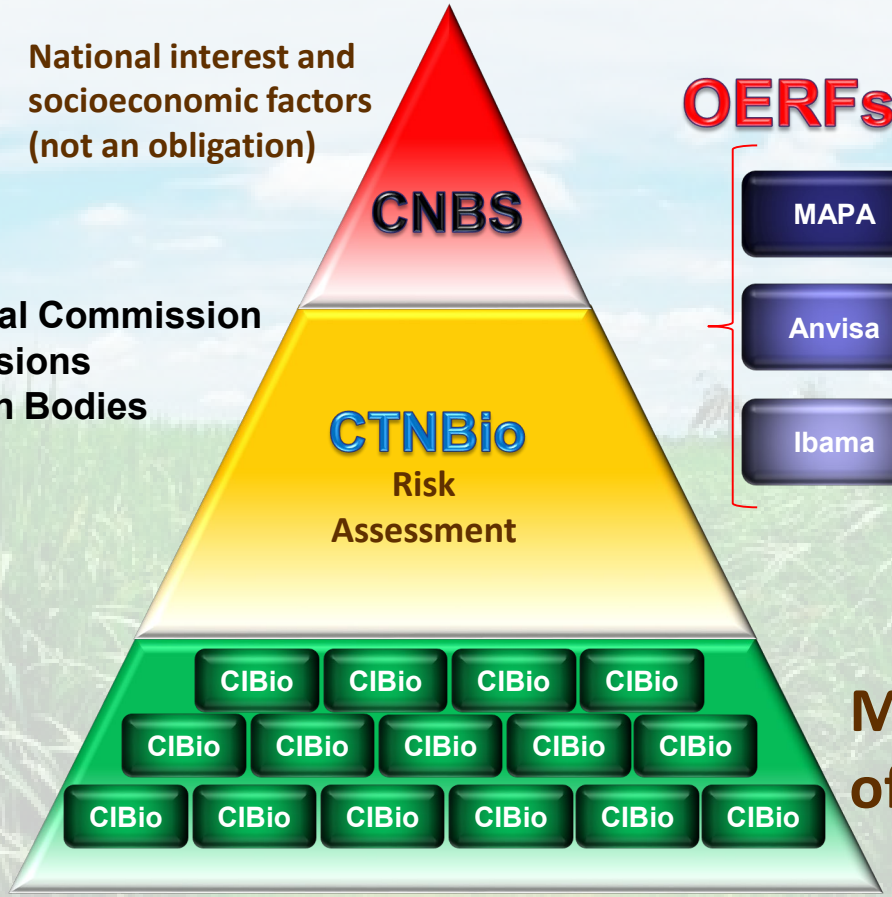
*Alexandre Nepomuceno, Ph.D.*
*General Head*
*Brazilian Soybean Research Center – Embrapa Soja*

# NEW Biosafety Law - 11.105/05

## *Brazilian Biosafety Technical Commission - CTNBio*

comissão técnica nacional CTNBio de biossegurança

- National Biosafety Council
- National Biosafety Technical Commission
- Internal Biosafety Commissions
- Registration and Inspection Bodies




National interest and socioeconomic factors (not an obligation)

**OERFs** — Registration and Inspections agencies

- MAPA — Agriculture
- Anvisa — Human Health
- Ibama — Environmental

Maintenance of biosafety

Embrapa — MINISTÉRIO DA AGRICULTURA, PECUÁRIA E ABASTECIMENTO

# Do you know where biotech crops are grown?

*More than 30 countries have planted biotech crops since 1996. See where they were grown in 2019.*

**17 MILLION**
small, resource-poor farmers and their families totaling >65 million people benefited from biotech crops in 2019

**USA**
is the largest producer of biotech crops in the world, planting 37.6% of global biotech crop area

Biotech canola's adoption rate in Canada reached **95%**

**SPAIN**
is the leading country planting biotech maize in Europe

More than **6 MILLION**
farmers in India planted 11.9 million hectares of Bt cotton in 2019


**10 countries**
in Latin America planted biotech crops in 2019

**BRAZIL**
is the top developing country in the world planting biotech crops

**MALAWI, ETHIOPIA, & NIGERIA**
planted Bt cotton for the first time in 2019

**2.7 Billion**
hectares of biotech crops planted since 1996

Soybeans, Maize, Cotton, Canola, Sugar beets, Alfalfa, Papaya, Squash, Potato, Apples, Eggplant, Sugarcane, Pineapple, Safflower

For more information on biotech crops, visit
**www.isaaa.org**

ISAAA

Source: ISAAA. 2019. Global Status of Commercialized Biotech/GM Crops in 2019. *ISAAA Brief* No. 55. ISAAA: Ithaca, NY.

Animal Feed


Corn Flour

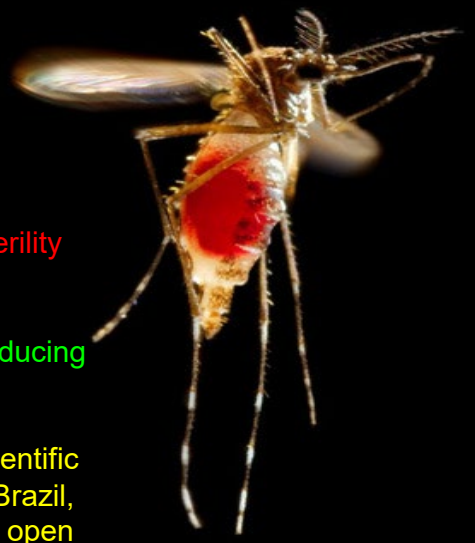

# Today there are many GM products being produced and consumed…

Corn Flour


Gum


Oil

# Mosquito GM



- Insertion of genes to induce male sterility (Tetracycline)
- GM Males compete with Wild Males reducing total population.
- First results were presented to the scientific community recently in Juazeiro, Bahia, Brazil, where the experiment was conducted in open areas with high Mosquito infestations.

**Liberated for Commercial use in Brazil: Abr/2014**

Source: CTNBio, 2011; Oxitec do Brasil e USP, 2012.

Aedes do Bem
Powered by Oxitec

ASSINAR

ENTRAR

HOME | AEDES DO BEM | QUEM SOMOS

# Simples. Seguro. Eficaz.

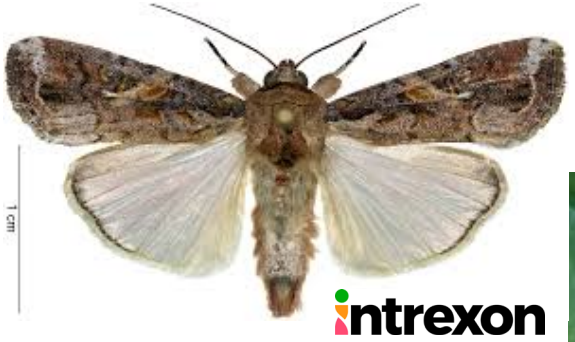
**Aedes do Bem™** é uma solução inovadora no controle biológico do mosquito transmissor da dengue, zika, chikungunya e febre amarela.

## Chegou a hora de viver uma nova vida fora da caixa.

Assine agora por toda a temporada de mosquitos **COM PREÇO ESPECIAL ESQUENTA BLACK FRIDAY** por apenas **R$ 145/mês** e receba seu Kit Caixas + Refis em até 8 dias com **frete grátis**!

# RESEARCH – Authorized for Commercial use in Brazil (Março/2021)

GM Male Sterile Carterpillar (Lagarta do cartucho)
*Spodoptera frugiperda*




Primeira LPMA para pesquisa autorizada em Maio 2019 pela CTNBio

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# GM Salmon

nature International weekly journal of science



NATURE | NEWS

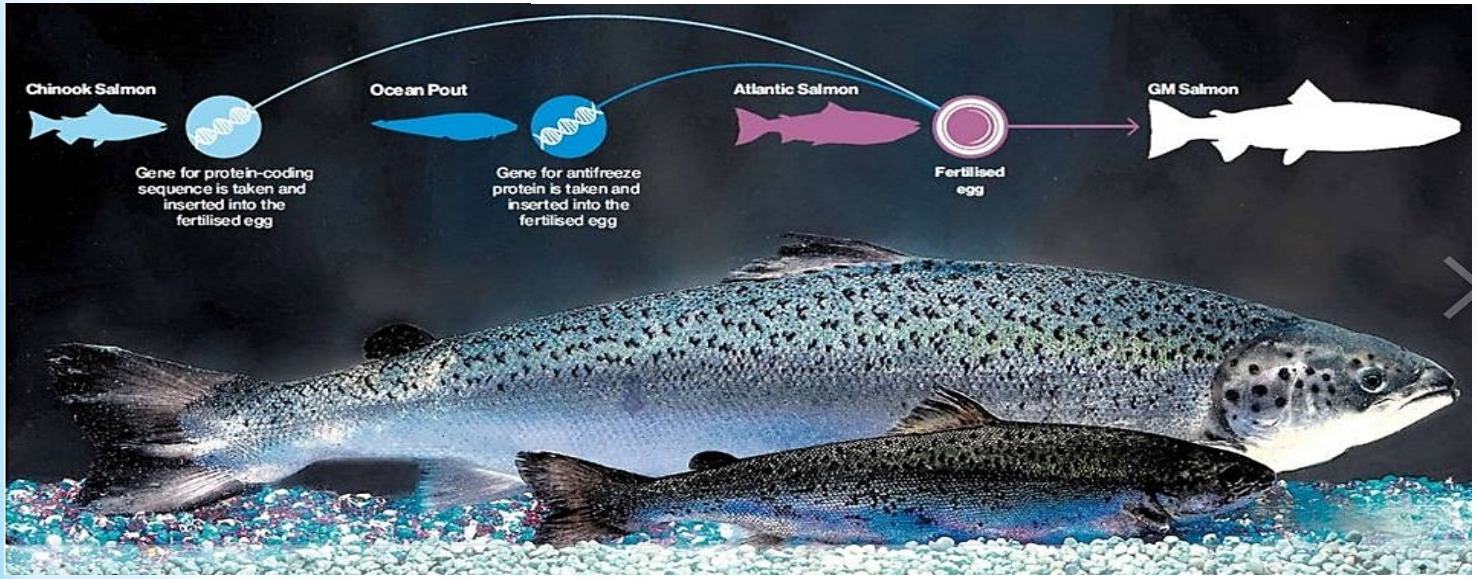
## First genetically engineered salmon sold in Canada

US firm AquaBounty Technologies says that its transgenic fish has hit the market after a 25-year wait.

Emily Waltz

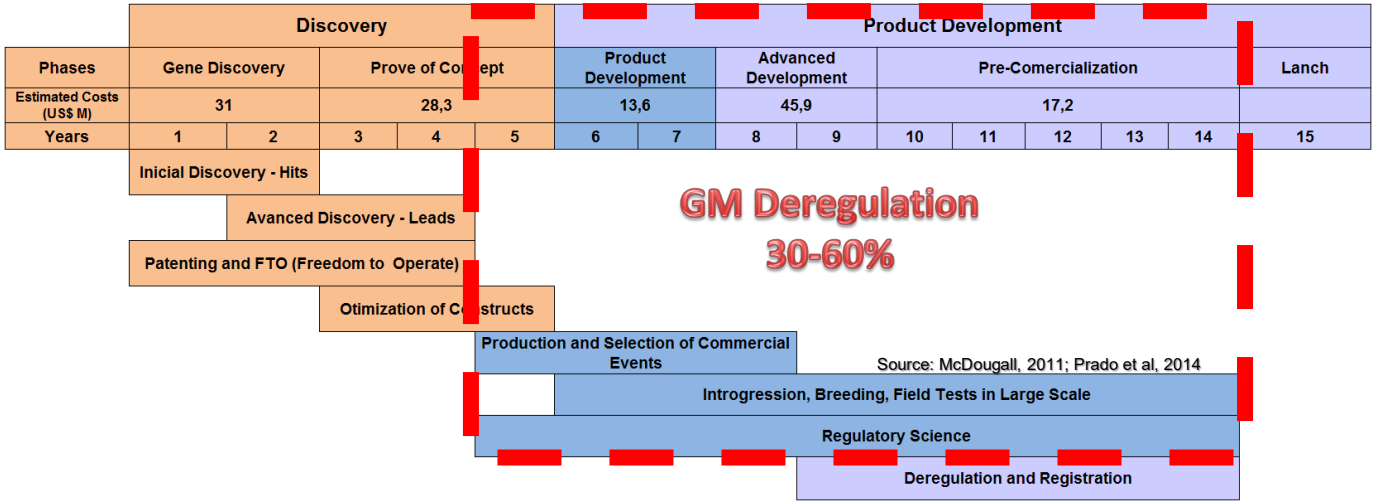
04 August 2017

***Liberado para consumo no BR em Maio/2021***



# OGM: Each country created its own rule

## Phases and Costs to Development of a GM Crop




Today, basically, only four companies can place GM Crop Varieties in the Market

Bayer (+Monsanto)

BASF

Corteva (Dow+DuPont+Pioneer)

Syngenta (+ChemChina)

Estimated Costs: ~U$136 million

Estimated Costs of Deregulation Phase: ~U$75 million

It can take ~12-20 years from discovering a gene(s) and placing a GM Commercial Variety in the Market.

Also, limited the use of Biotech in Agriculture to major crops (Soybean, Cotton, Corn, Eucalyptus, Sugarcane, etc…)

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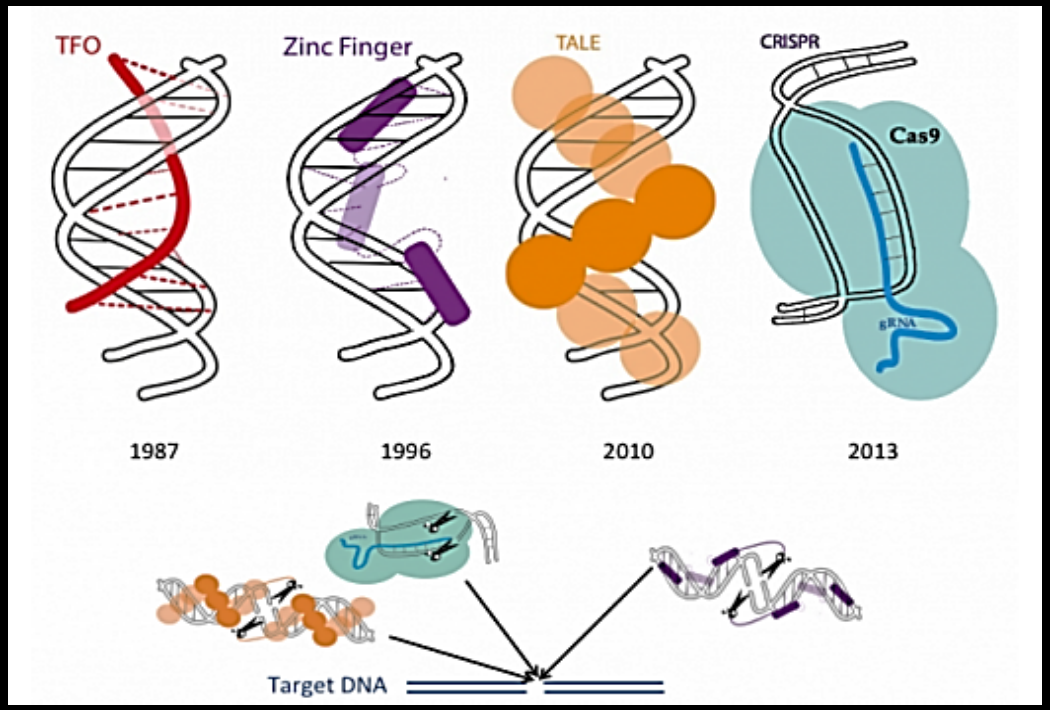
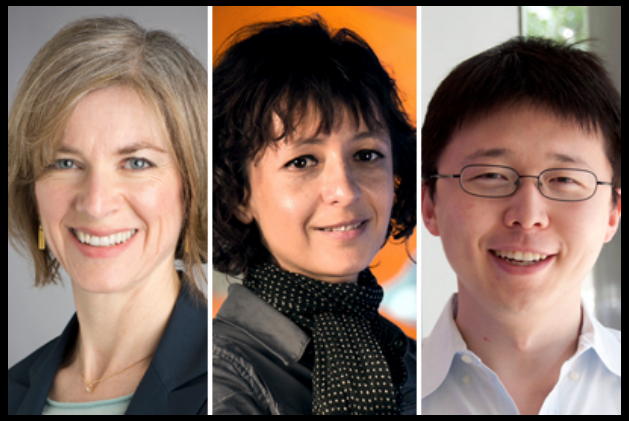
# ... but evolution on genetics keeps moving fast...

... CRISPRs Technology brought a revolution in Genome Editing and is democratizing the use of biotechnology in agriculture







comissão técnica nacional
CTNBio
de biossegurança

# Brazilian Biosafety Commission CTNBio NR 16 Genome Edition Normative

DIÁRIO OFICIAL DA UNIÃO

Publicado em: 22/01/2018 | Edição: 15 | Seção: 1 | Página: 2-8
**Órgão: Ministério da Ciência, Tecnologia, Inovações e Comunicações / Comissão Técnica Nacional de Biossegurança**

## RESOLUÇÃO NORMATIVA Nº 16,.DE 15 DE JANEIRO DE 2018

ANEXO I

Estabelece os requisitos técnicos para apresentação de consulta à CTNBio sobre as Técnicas Inovadoras de Melhoramento de Precisão

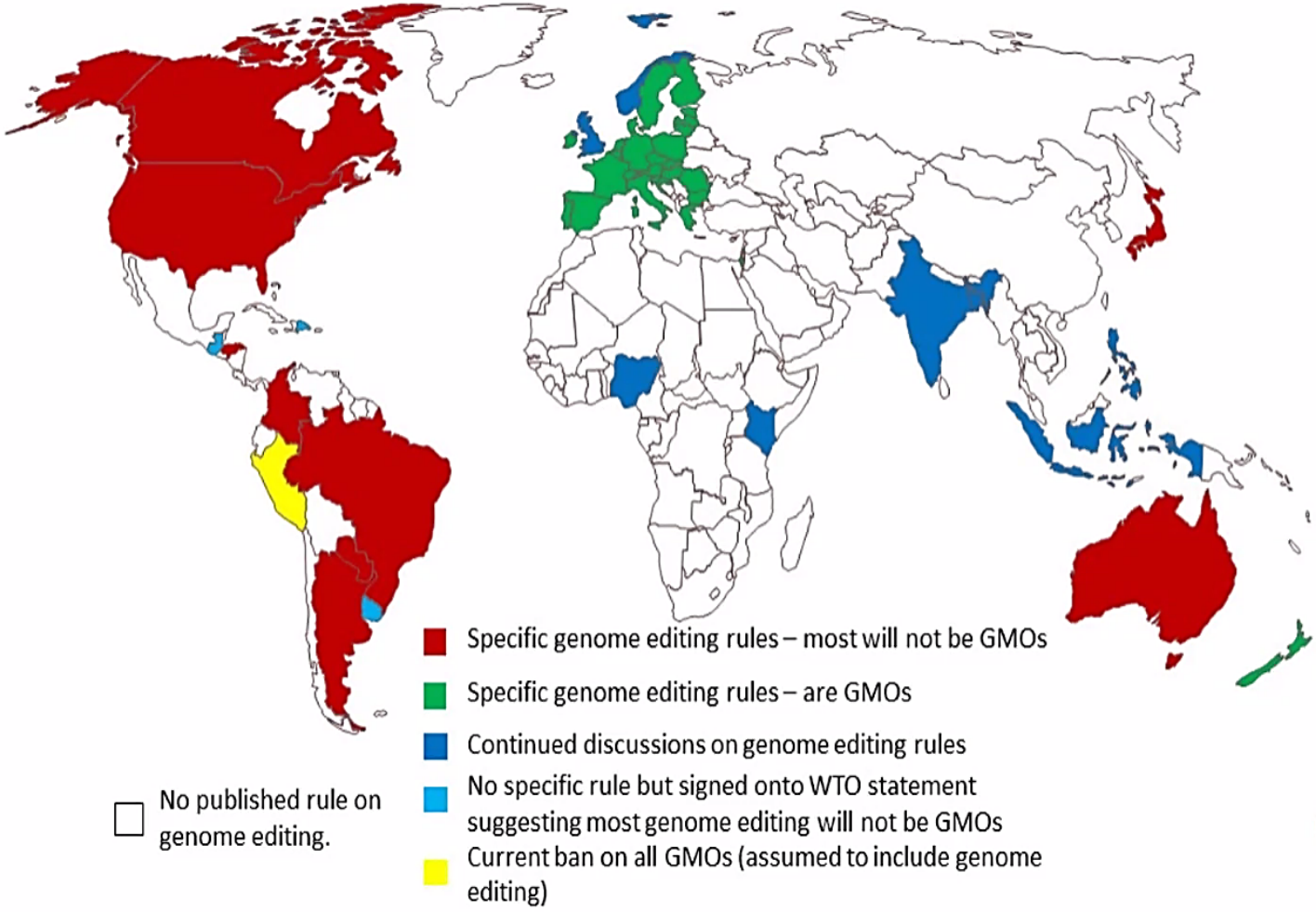
A COMISSÃO TÉCNICA NACIONAL DE BIOSSEGURANÇA - CTNBio, no uso de suas atribuições legais e regulamentares e em observância às disposições contidas nos incisos XV e XVI do art. 14 da Lei nº 11.105, de 24 de março de 2005;

CONSIDERANDO a necessidade de avaliar as Técnicas Inovadoras de Melhoramento de Precisão (TIMP), do inglês Precision Breeding Innovation (PBI) e que também englobam as denominadas Novas Tecnologias de Melhoramento, do inglês New Breeding Technologies -NBTs, à luz dos preceitos previstos na Lei nº 11.105, de 24 de março de 2005;

Considerando que a Lei nº 11.105, de 2005, define moléculas de ADN/ARN recombinante, engenharia genética e organismo geneticamente modificado - OGM nos incisos III, IV e V de seu art. 3º, respectivamente;

Considerando que as TIMP abrangem um conjunto de novas metodologias e abordagens que diferem da estratégia de engenharia genética por transgenia, por resultar na ausência de ADN/ARN

# Global Regulatory Status




IDB
Inter-American Development Bank

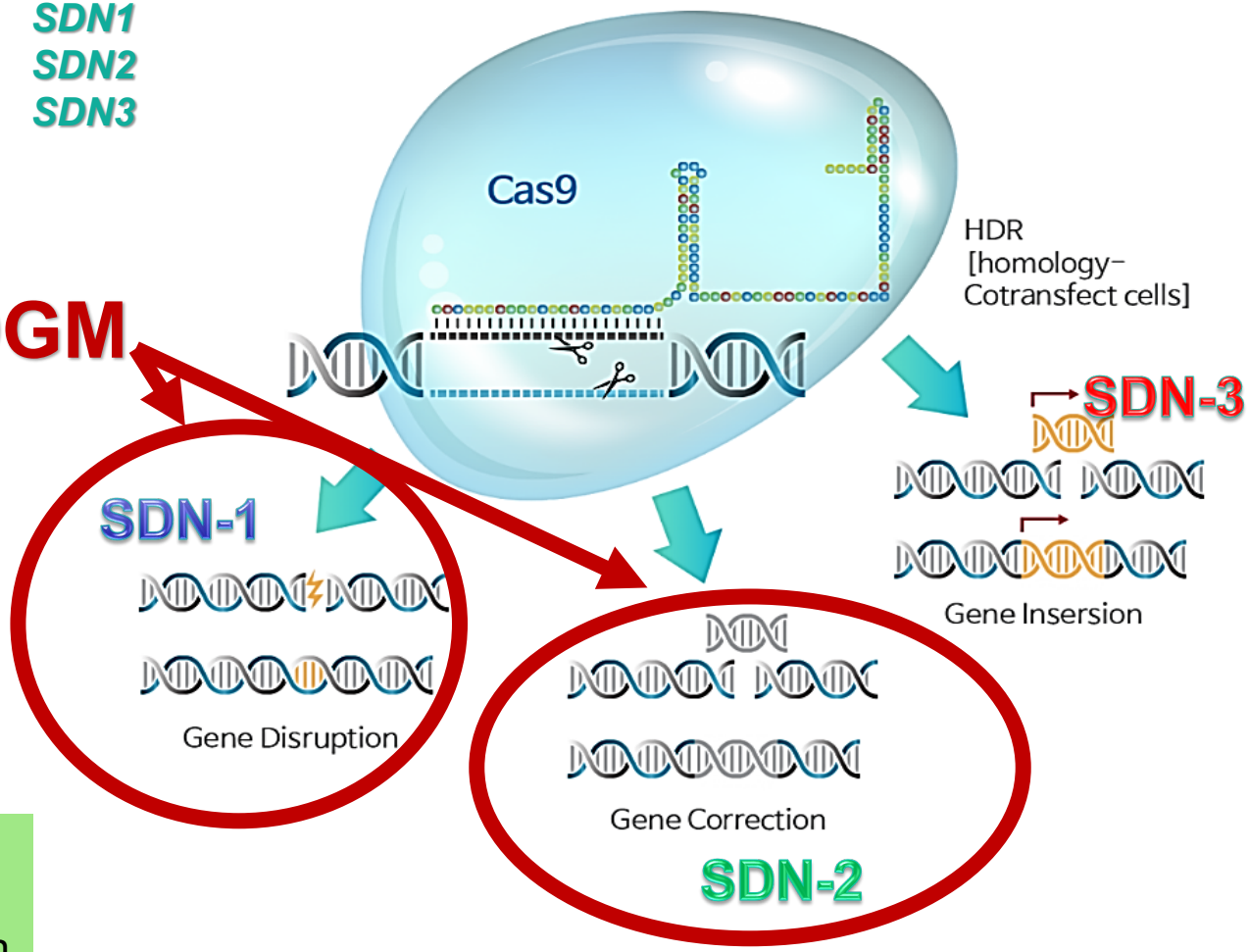
# Genome Edition with CRISPR

**Site Directed Mutagenesis type:**

***SDN1***
***SDN2***
***SDN3***

**Clustered**
**Regularly**
**Interspaced**
**Short**
**Palindromic**
**Repeats**

**Não OGM**




*DNA cutting is done in regions (sequences) chosen with precision*

Similar to mutations that occur in nature and are responsible for evolution on planet earth

# Genome Edited Organisms in Brazil

Embrapa

*Considered Non GM by CTNBio (2018)*

# GE Yeast for Alcohol Production from Sugarcane

## First Genome-Edited Organism in Brazil for comercial use

*Saccharomyces cerevisiae*

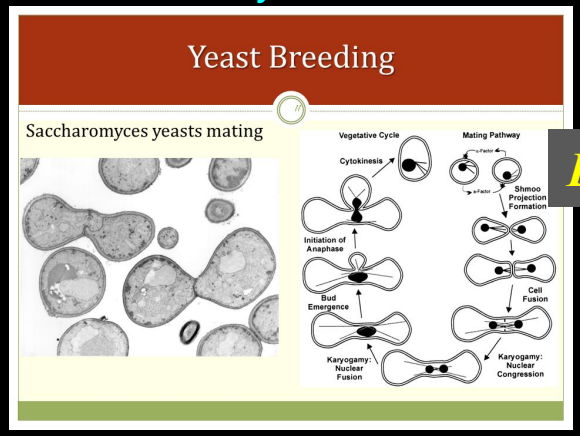
globalyeast
Selected By Excellence.

*Belgian-Brazilian biotechnology startup*

### Development Procedures

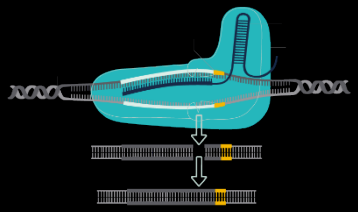
Yeast Germplasm Bank: 80 Strains tested for high alcohol and low glycerol production

*Three S. cerevisiae strains chosen and crossed by classical breeding*

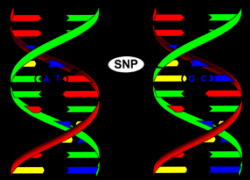



***Excellomol***

***Excellomol 4.0 Next***



All four mutations were introduced by CRISPR/Cas9 into the Excellomol strain




A Forth strain with very high alcohol production was identified. Mutation in 4 genes are responsible for this high efficiency.

Embrapa

# Genome Edition on Diazotrophs organisms

**Considered Non GM by CTNBio (2021)**

Plant Physiology and Biochemistry 167 (2021) 912–920

Contents lists available at ScienceDirect

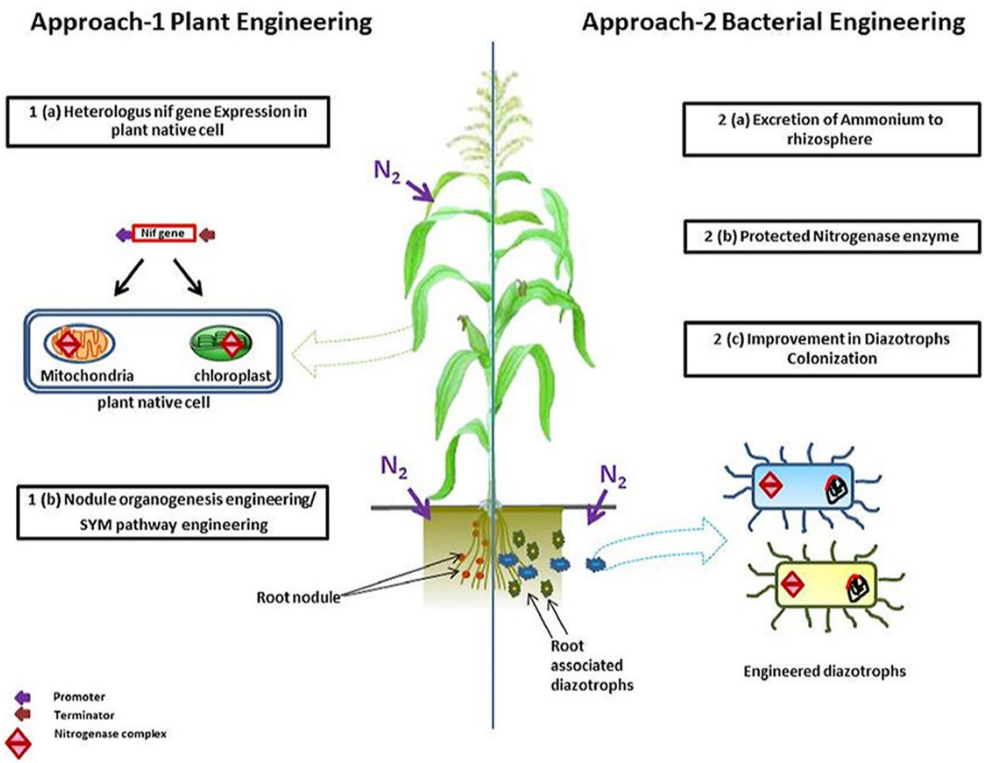
Plant Physiology and Biochemistry

journal homepage: www.elsevier.com/locate/plaphy

## Nitrogen fixing cereal: A rising hero towards meeting food security

Parichita Priyadarshini [a], Sharani Choudhury [b], Jyotsana Tilgam [c,*], Alka Bharati [d], N. Sreeshma [b]

[a] ICAR-Crop Improvement Division, Indian Grassland and Fodder Research Institute, Jhansi, U.P., 284003, India
[b] ICAR – National Institute for Plant Biotechnology, Indian Agricultural Research Institute, New Delhi, 110012, India
[c] ICAR- National Bureau of Agriculturally Important Microorganisms, Maunath Bhanjan, U.P., 274103, India
[d] ICAR-Central Agroforestry Research Institute, Jhansi, U.P., 284003, India




Source: CTNBio, 2021

## Nitrogen Fixation Bacteria *Klebisiela varicola*

**Table 1**
Selected examples of Associative and/or endophytic diazotrophs and their associated plant hosts.

| Associative and/or endophytic diazotrophs | Plant hosts | References |
|---|---|---|
| *Azospirillum* spp. | wheat, maize, rice, *Digitaria decumbens*, kallar grass, rice | Reinhold et al., (1987); Khammas et al., (1989) |
| *Gluconacetobacter diazotrophicus* | sugarcane, sugar beet, coffee, maize, sweet potato, sorghum, tomato, pineapple, wetland rice, wheat, oilseed rape, grass, finger-millet, radish, beetroot, tea, cameroan grass | Eskin et al. (2014) |
| *Herbaspirillum seropedicae* | maize, sorghum, wheat, rice, sugarcane, forage grass | Baldani et al., (1986); James et al., (2002) |
| *Moraxella* sp., *Brevibacillus* sp. and non-cultivated cyanobacterium | peach palm | de Almeida et al. (2009) |
| *Burkholderia* spp. | maize, coffee, rice, sugarcane, tomato, wheat, grapes, peas, *Quercus* sp., *Betula* sp., cotton | Compant et al., (2008); McInroy et al., (1995) |
| *Klebsiella* spp. | maize, rice, sugarcane | McInroy et al., (1995); Palus et al., (1996); Reyna-Flores et al., (2018) |
| *Pseudomonas* spp. | maize, rice | Fisher et al., (1992); James et al., (2000) |
| *Enterobacter* spp. | rice, maize | Fisher et al., (1992); James et al., (2000) |
| *Nostoc* | cycads, hornworts, and liverworts | Rai et al. (2000) |
| *Anabaena* | azolla | Vaishampayan et al. (2001) |
| *Azoarcus* spp. | kallar grass, wild rice | Engelhard et al. (2000) |
| *Bradyrhizobium* spp. | sweet potato | Hill et al. (1983) |

# Considered Non GM by CTNBio (2018)

Food DIVE TOPICS FEATURES

## Inside CRISPR's gene editing technology — and how it could revolutionize the food industry

A new generation of genetically edited organisms has arrived and if early results are any indication, it has the potential to revolutionize the food manufacturing industry, as well as numerous industries.

On Monday, DuPont Pioneer announced the USDA will not subject a waxy corn hybrid created with CRISPR Cas9 gene-editing technology to regulations applied to traditional GMOs. The gene changes the functionality of starch. DuPont's officials believe the corn can be planted in fields in five years. The company is also growing CRISPR-edited wheat.

**Also authorized to be sowed as conventional variety in the US, Canada and Argentina**

Usually Corn Starch has 75% Amylopectin and 25% Amylose


**100% Amilopectina**
**0% Amilose**

comissão técnica nacional CTNBio de biossegurança

Embrapa

*Considered Non GM by CTNBio (2019)*

# Genome Edited Tilapia

**While AquaBounty's GMO salmon remains blocked in US, Argentina exempts the company's sustainable gene-edited tilapia from regulation**

Fish Farming Expert | December 19, 2018

Image Credit: Medium

The Fish Site

Find species, diseases, articles... Breeding & genetics Farm management Health & welfare

## Gene edited tilapia secure GMO exemption

REGULATIONS · POLITICS · GENETICS & BREEDING · BIOTECHNOLOGY

by The Fish Site
18 December 2018, at 2:06p.m.

A line of tilapia that has been gene edited will not be classified as a genetically modified organisms (GMOs) in Argentina, according to a government advisory commission.

The line, known as FLT 01, has been developed by Intrexon and its subsidiary AquaBounty Technologies., the biotechnology company best known for its AquaAdvantage salmon strain. The tilapia were developed using gene editing

Myostatin gene
Natural Mutation

Bovine Race
Belgian blue bull

# Meat quality - Myostatin

Transgenic Res (2015) 24:147–153
DOI 10.1007/s11248-014-9832-x

ORIGINAL PAPER

**Genome edited sheep and cattle**

Chris Proudfoot · Daniel F. Carlson · Rachel Huddart · Charles R. Long · Jane H. Pryor · Tim J. King · Simon G. Lillico · Alan J. Mileham · David G. McLaren · C. Bruce A. Whitelaw · Scott C. Fahrenkrug

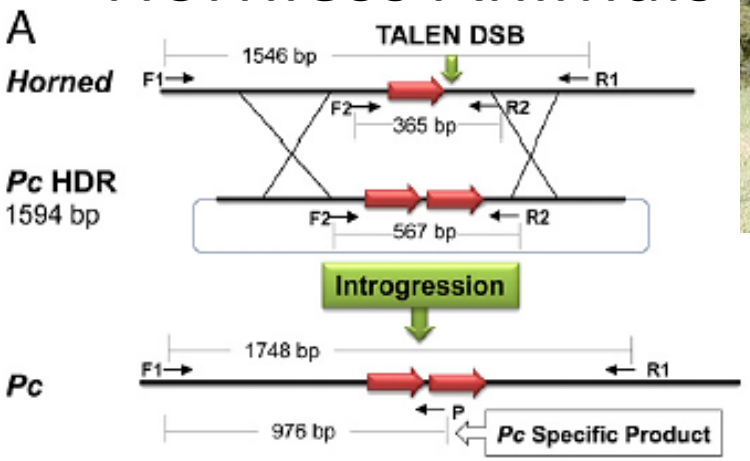

***Considered Non GM by CTNBio (2021)***

*Nelore bull semen with genome edited to Myostatin inactivation*
*Considered Non GM by CTNBio*

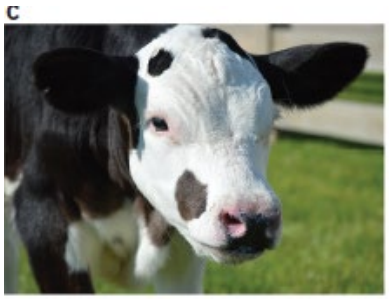
Increase of ~20% in meat production

# *Considered GM by CTNBio (2018)*

## Hornless Animais




- ***Polled allele*** introduced by ***Homologous Recombination*** in fibroblast
  - Calves produced by somatic cell nuclear transfer (animal cloning)





**Production of hornless dairy cattle from genome-edited cell lines**

*Daniel F Carlson[1], Cheryl A Lancto[1], Bin Zang[2], Eui-Soo Kim[1], Mark Walton[1], David Oldeschulte[3], Christopher Seabury[3], Tad S Sonstegard[1] & Scott C Fahrenkrug[1]*

*[1]Recombinetics, St. Paul, Minnesota, USA.*
*[2]Program in Scientific Computation, University of Minnesota, Minneapolis Minnesota, USA.*

# Genome Edited Organisms evaluated by CTNBio until August 2021

**PRODUTOS AVALIADOS - Técnicas Inovadoras de Melhoramento de Precisão**

Data da última atualização: 26/08/2021

| Requerente | Processo | Parecer | produto |
|---|---|---|---|
| Globalyeast JV CO Brasil S.A | 01250.011076/2018- 19 | 5905/2018 | levedura para produção de bioetanol |
| Globalyeast JV CO Brasil S.A | 01250.011076/2018- 19 | 5904/2018 | levedura para produção de bioetanol |
| Ourofino | 01250.017539/2018-56 | 6236/2018 | cepa vacinal de parvovírus canino |
| Agro Partners Consulting | 01250.045811/2018-98 | 6125/2018 | Bovino sem chifres (Parecer Cancelado) |
| Lallemand Brasil Ltda. | 01250.014824/2018-15 | 6167/2018 | linhagem M15980 de Saccharomyces cerevisiae para produção de etanol |
| Du Pont do Brasil | 01250.033737/2018-67 | 6208/2018 | milho ceroso |
| AquaBounty Technologies | 01250.012915/2019-05 | 6527/2019 | tilápia com um fenótipo de maior rendimento de filé |
| Forest | 01200.700832/2016-10 | 6655/2019 | mosquito tratado com RNA de interferência para esterilidade |
| Lallemand Brasil Ltda. | 01250.008028/2019-24 | 6711/2019 | linhagem melhorada M18447 de Saccharomyces cerevisiae |
| Lallemand Brasil Ltda. | 01250.008066/2019-87 | 6710/2019 | linhagem melhorada M20544 de Saccharomyces cerevisiae |
| Lallemand Brasil Ltda. | 01250.006695/2020-14 | 7021/2020 | linhagem melhorada M22993 de Saccharomyces cerevisae |
| Lallemand Brasil Ltda. | 01245.003067/2020-48 | 7130/2020 | linhagem melhorada M25319 de Saccharomyces cerevisiae |
| Tevah Consultoria Regulatória | 01250.020346/2020-05 | 7098/2020 | aditivo seco para ração e água destinado à criação de aves |
| Evolutta Agro Biotecnologia Ltda | 01245.003068/2020-92 | 7247/2020 | dsRNA para o silenciamento de genes de Spodoptera frugiperda e Helicoverpa armigera que atacam as lavouras cultivadas |
| Pivot Bio | 01250.010577/2020-01 | 7271/2020 | Produto Kv137-3933 um inoculante para cultura do milho a base de Klebisiela variicola, visando a otimização do nitrogênio |
| Pivot Bio | 01250.010588/2020-82 | 7248/2020 | Produto Kv137-1034 um inoculante para cultura do milho a base de Klebisiela variicola, visando a otimização do nitrogênio |
| YesSinergy Agroindustrial Ltda | 01245.003594/2021-33 | 7519/2021 | Produto produto *Saccharomyces cerevisiae* CEPA YS2101 - produção de etanol |
| Acceligen do Brasil Biotecnologia e Pesquisa Científica Ltda | 01245.006161/2021-30 | 7520/2021 | Sêmen de touro da raça Nelore (Samson) com aumento da massa muscular por edição gênica do gene da Miostatina |
| Evolutta Agro Biotecnologia Ltda | 01245.003068/2020-92 | 7581/2021 | dsRNA para o silenciamento de genes de Spodoptera frugiperda e Helicoverpa armigera |

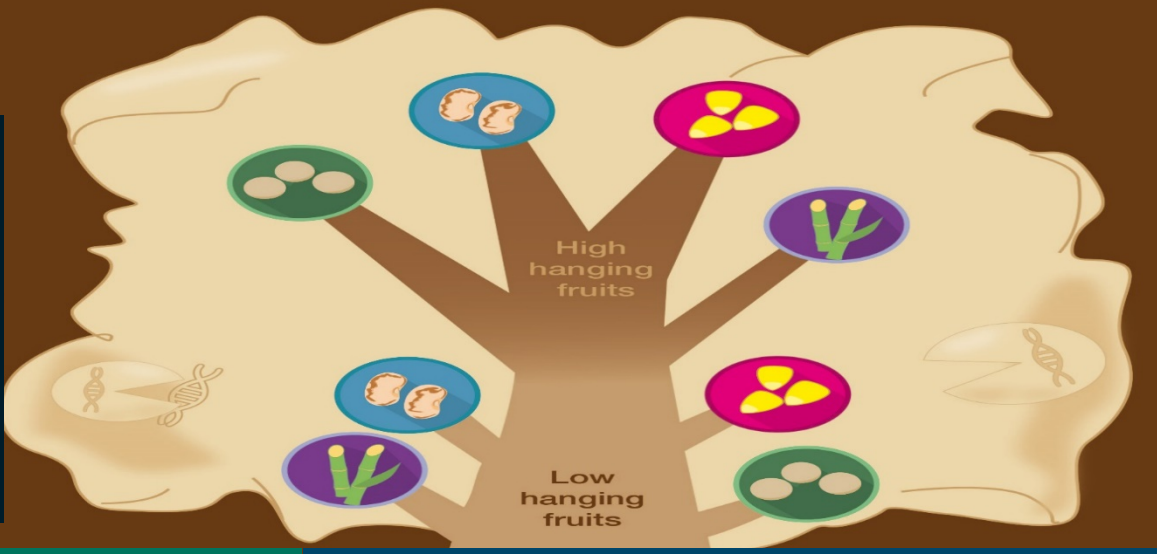
# Research on Genome Edition in Brazil

Embrapa

# CRISPRevolution

## Four Crops and Two Strategies

Leading project on Genome Edition at EMBRAPA




**Knock-out (SDN1)**

Soybean: Anti-nutritional Factors
Sugarcane: Cell wall structure (2G Ethanol)
Corn: Cell wall structure (2G Ethanol)
Common Bean: Tegument Color

**HDR (SDN2)**

Soybean: Drought
Sugarcane: Drought
Corn: Drought
Common Bean: Drought

Projeto em parceria com as Unidades Descentralizadas: Embrapa Agroenergia, Embrapa Arroz e Feijão, Embrapa Milho e Sorgo, Embrapa Recursos Genéticos e Biotecnologia, Embrapa Soja e Unidade Mista de Pesquisa GenClima

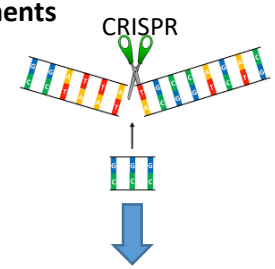
Embrapa

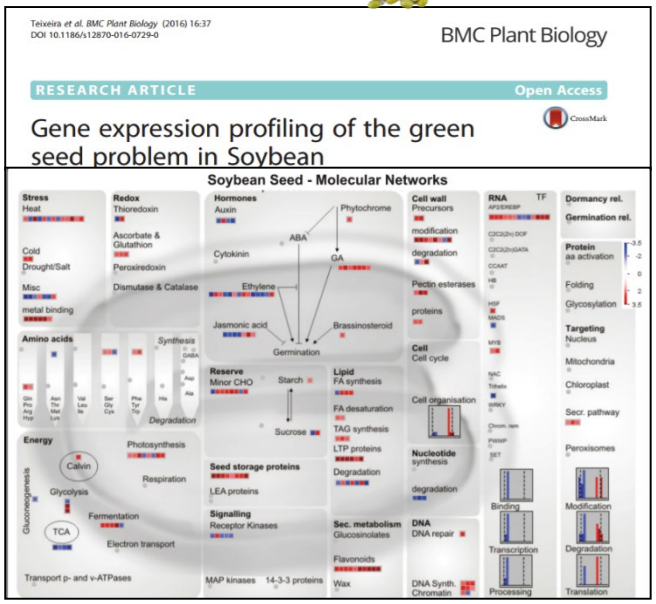
## Embrapa Soybean and Embrapa Cenargen

# CRISPRs Systems in Soybean

# Soybean: Seed Quality, Anti-Nutricional factors and Drought Tolerance

**Knock Out and HR at promoter elements**



Teixeira et al. BMC Plant Biology (2016) 16:37
DOI 10.1186/s12870-016-0729-0

BMC Plant Biology

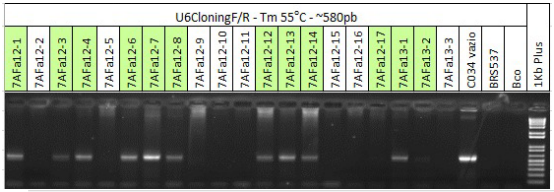
RESEARCH ARTICLE Open Access

Gene expression profiling of the green seed problem in Soybean

Soybean Seed - Molecular Networks

Candidate genes for Knock Out and HR:
Stay green1 (D1); Stay green2 (D2); Pheophorbidase (PH2) , DREB, AREB, DRIP, etc

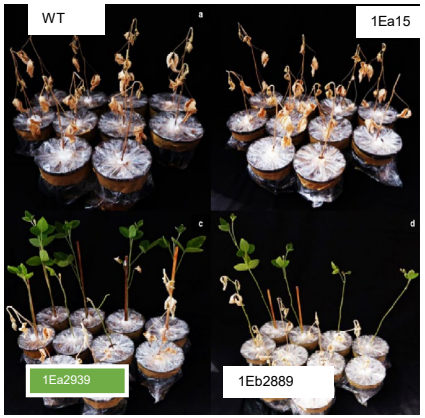
# Embrapa Soybean: Anti-Nutricional factors and Drought Tolerance

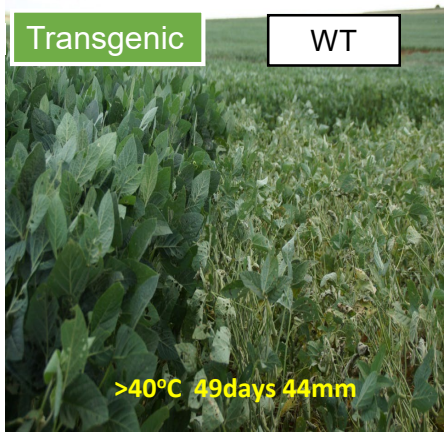



## SDN1

It will be submitted soon to CTNBio under RN16


## SDN1 e SDN2 for drought




(Marinho *et al*., 2016; Fuganti-Pagliarini *et al*., 2017)

Probably the first Genome Edited Sugarcane in the world to be evaluated by a biosafety commission

Submitted to CTNBio under RN16

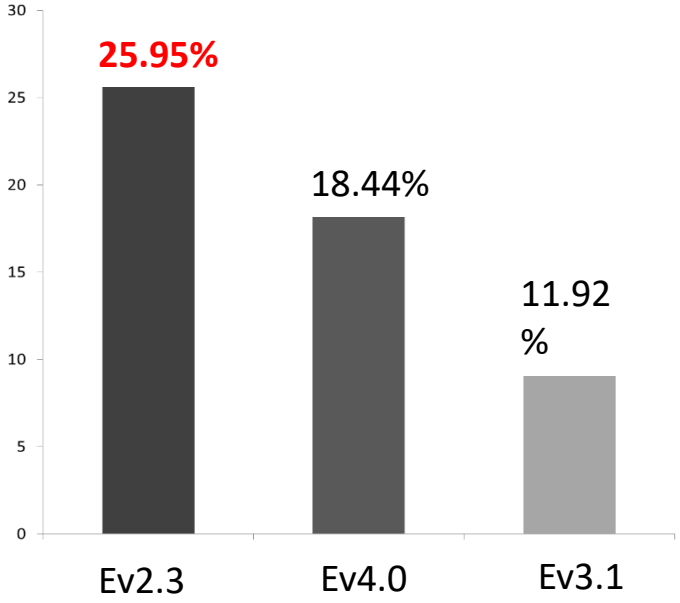
# Sugarcane BAHD01 – Increase in glucose convertion

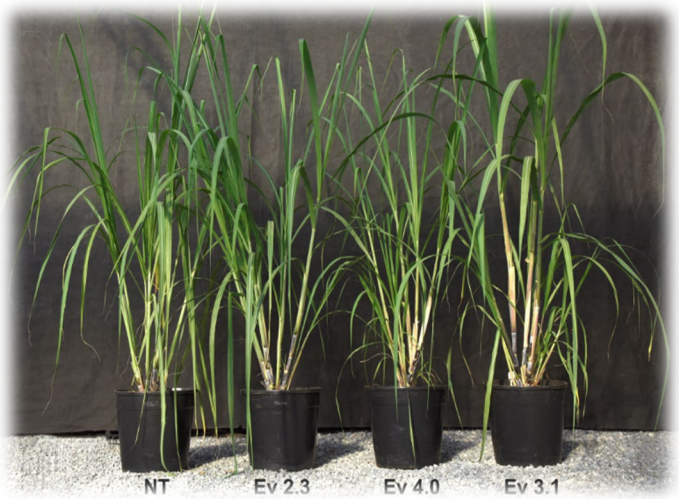
***Embrapa Agroenergia***

- More than 70% of the biomass cellulose was converted to glucose;
- No change in percentage of cellulose, hemicellulose and lignin;
- ART content of the biomasses Flex was equal to or greater than WT

RNP
Transient Expression

**Increased glucose in 48 hours of enzymatic hydrolysis**



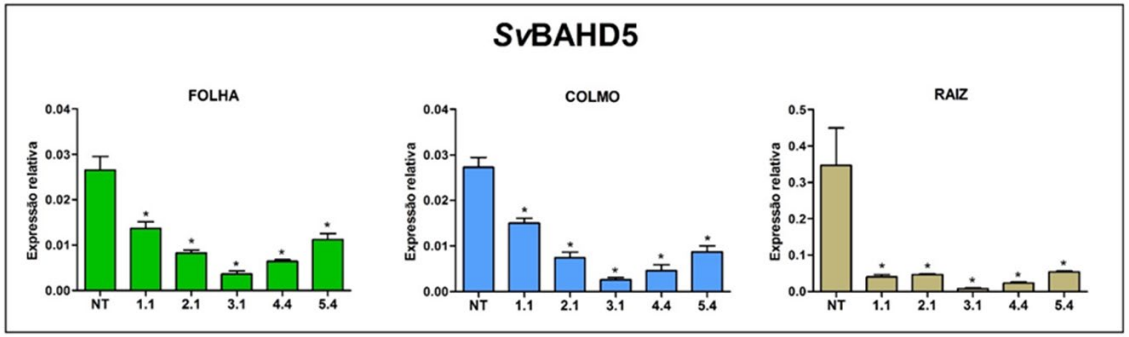

De Souza et al., 2019. **Biotechnology for Biofuels**, v. 12, n. 111, p. 1-14

Fonte: Hugo Molinari, 2021

# Embrapa Maize and Sorghun

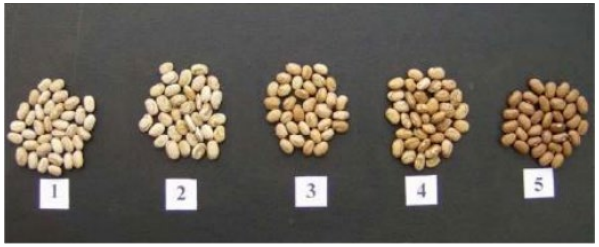
## BAHD genes – New gene for sucrose improvement in Corn and Sugarcane

- Metabolic profile (HPLC-HRMS): greatest difference observed for the BAHD5 events;
- Desreplication: sucrose main discriminant metabolite in the BAHD5 events;
- We observed an increase in the sucrose content in leaf (Ev. 1.1 = 52.94%; Ev. 3.1 = 85.19%) and culm (Ev. 1.1 = 95.80%; Ev. 2.1 = 96.22%; Ev. 3.1 = 94.05%) in comparison with NT plants.
- No significant diffences in biomass and structural carbohydrate content.




# EMBRAPA Rice and Beans Center


## CRISPR in Common Beans




Dr. Josias Correa de Faria

Dr. Rosana Vianello

# EMBRAPA Dairy Cattle

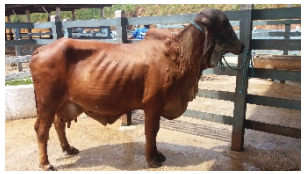
## Gene Editing in Animals

**Increasing thermotolerance in European dairy (Holstein) and beef (Angus) cattle**

- SNPs found in criollo breeds in Central and South American cattle are associated to high tolerance to heat
  - ✓ Contribute to improve reproductive and productive performace of Angus and Holstein in the tropics

**Increasing milk yield in Indian (Zebu) dairy cattle**

- SNPs found in Holstein and Jersey breeds are associated to milk and protein production
  - ✓ Contribute to improve productive perfomance of zebu cows (Gir)



Holstein, Angus and Jersey images: CRV Lagoa
Caracu and Gir images: Camargo, LS

# Improving drought tolerance in maize via targeting of candidate genes with CRISPR

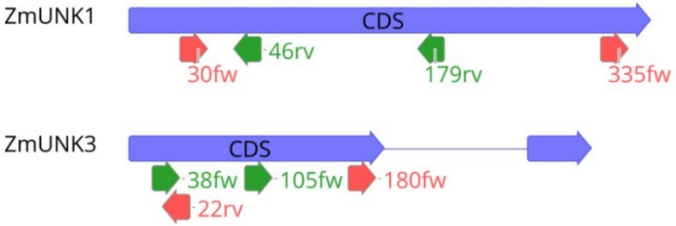
Team: José Hernandes Lopes, Viviane C.H. da Silva, Ricardo Dante, Isabel Gerhardt, Juliana Yassitepe, Paulo Arruda

1) **Overall problem:** Drought stress is an important concern for maize production, often resulting in large yield losses during extreme events. Discovery of new genes efficacious at improving drought tolerance via biotechnology approaches is challenging due to the trait genetic complexity and IP protection. We thus have focused discovery efforts at thus far uncharacterized genes associated with large-effect stress tolerance genes and pathways.

2) **Objective:** Development of non-GM (SND1) maize lines with improved drought tolerance via knock-out of thus far largely uncharacterized genes.

3) **Results:**

| Candidate gene | Modulation under drought | Score | Differential expression under drought | | |
|---|---|---|---|---|---|
| | | | Treatments | Stages | Tissues |
| *ZmUNK1* | Up/Down | 20 | 35.7% | 36.4% | 38.5% |
| *ZmUNK3* | Up/Down | 29 | 57.1% | 54.5% | 61.5% |
| *ZmUNK5a* | Up | 16 | 28.6% | 27.3% | 30.8% |
| *ZmUNK8* | Up | 42 | 100.0% | 100.0% | 92.3% |
| *ZmUNK10a* | Up/Down | 34 | 71.4% | 81.8% | 69.2% |
| *ZmUNK11* | Up | 16 | 21.4% | 36.4% | 30.8% |




1) Identification of genes of unknown function co-expressed with drought-response drivers;

2) Selection of six candidate genes for knock-out by expression profiling under drought conditions;

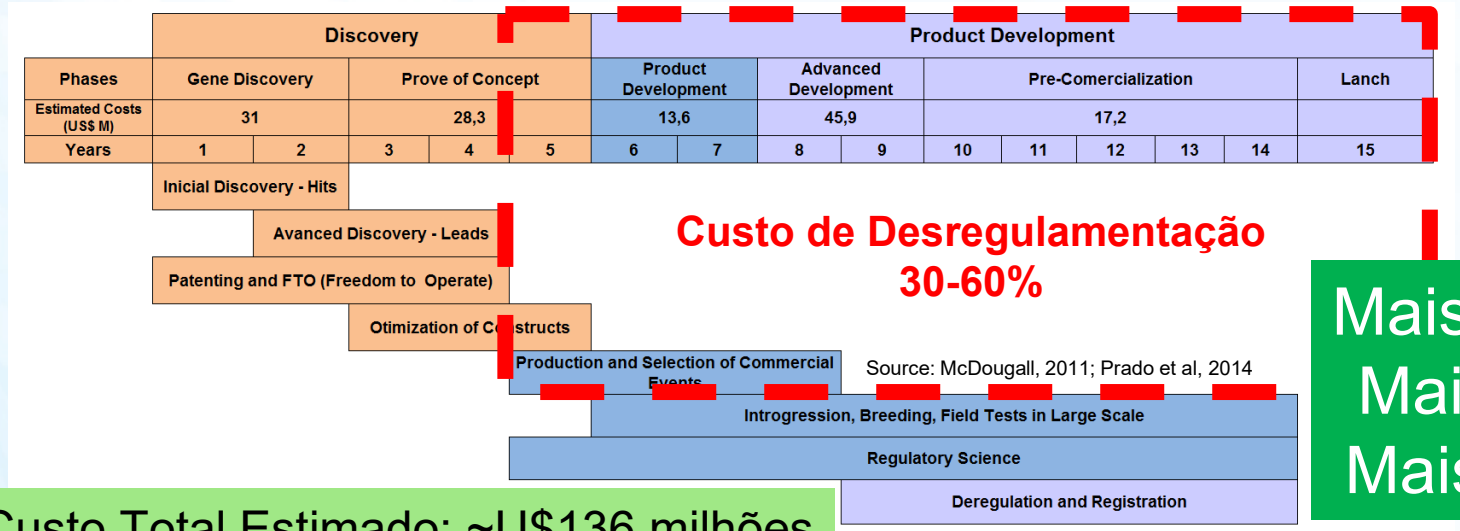
3) Two sets of sgRNAs were designed to knock-out (SDN1) each selected gene; Such sgRNAs were cloned into expression vectors containing the CRISPR machinery.

4) In progress: transformation (Agrobacterium), tissue culture and selection of the edited plants via Sanger Sequencing.

# Regulamentação no Mundo para uso da Biotecnologia no AGRO

## Legislação mais assertiva mantendo a Biossegurança

## Fases e Custos para desenvolver uma Planta GM



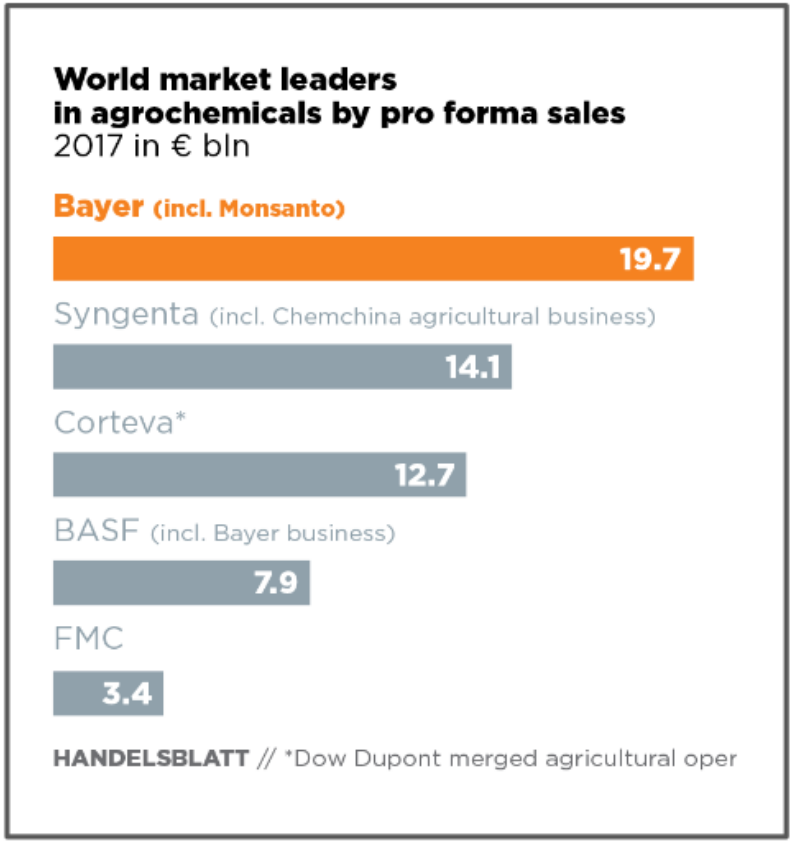

Custo Total Estimado: ~U$136 milhões

Mais Empresas
Mais Culturas
Mais Soluções

**Pode levar de 12 a 20 anos desde a descoberta de um gene até a colocação de uma variedade comercial no mercado**

# Legislação Harmonizada entre vários países tem permitido surgimento de mais "players"

## Regulated Technologies: Consolidation




Handelsblatt 5.6.2018

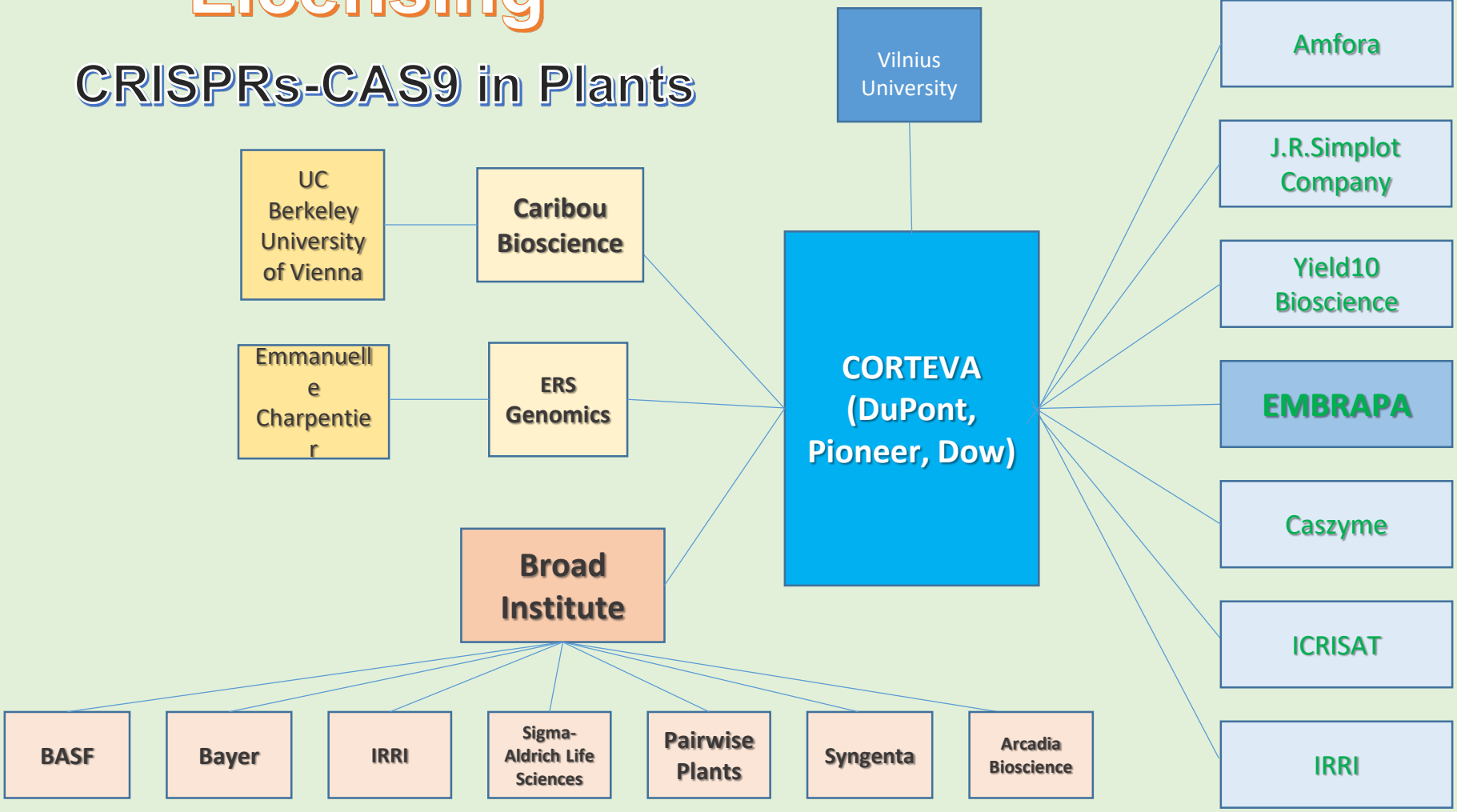
## Non-Regulated Tech.: Diversification

- Caribou Bioscience
- Eden Research
- Inari
- Biothalys
- Green Biologics
- Zymergen
- CiBO
- Indigo
- Invaio
- Pacific Bioscience
- BioAmber
- Arcadia
- EcoVative Design
- Pairwise
- Provivi
- Iden
- BioAtlantis
- Phyllotec
- Lemnatec
- Edison Agroscience
- AgriHouse
- Benson Hill
- Evogene
- Performance Plant
- GreenVenus
- Tropic Bioscience
- Agrosphere
- Agricell
- AgriScience
- Grassroots Biotech
- Eurogentec
- Mendel
- Oxitec
- .....

https://www.ventureradar.com/keyword/Agricultural%20Biotechnology?

# Licensing

## CRISPRs-CAS9 in Plants




Source: Bagley, 2021 - Genome Editing in Latin America: CRISPR Patent and Licensing Policy

# “Open source” new nucleases

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Original research

## Genome editing in plants with MAD7 nuclease

Qiupeng Lin [a, b, 1], Zixu Zhu [a, b, 1], Guanwen Liu [c, d, 1], Chao Sun [a, b], Dexing Lin [a, b], Chenxiao Xue [a, b], Shengnan Li [c, d], Dandan Zhang [c, d], Caixia Gao [a, b], Yanpeng Wang [a, b, *], Jin-Long Qiu [c, d, *]

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Commercial use

### ABSTRACT

MAD7 is an engineered nuclease of the Class 2 type V-A CRISPR-Cas (Cas12a/Cpf1) family with a low level of homology to canonical Cas12a nucleases. It has been publicly released as a royalty-free nuclease for both academic and commercial use. Here, we demonstrate that the CRISPR-MAD7 system can be used for genome editing and recognizes T-rich PAM sequences (YTTN) in plants. Its editing efficiency in rice and wheat is comparable to that of the widely used CRISPR-LbCas12a system. We developed two variants, MAD7-RR and MAD7-RVR, that increase the target range of MAD7, as well as an M-AFID (a MAD7-APOBEC fusion-induced deletion) system that creates predictable deletions from 5′-deaminated Cs to the MAD7-cleavage site. Moreover, we show that MAD7 can be used for multiplex gene editing and that it is effective in generating indels when combined with other CRISPR RNA orthologs. Using the CRISPR-MAD7 system, we have obtained regenerated mutant rice and wheat plants with up to 65.6% efficiency.

European Commission

JRC TECHNICAL REPORTS

**Explanatory Note**

**Challenges for the detection of genetically modified food or feed originating from genome editing**

*EU Reference Laboratory*

*in consultation with the*

SDN1 and SND2 human made mutations indistinguishable from natural mutations

# 8 Conclusions

With respect to the challenges for GMO detection laboratories as framed in the scenario above the following can be concluded:

Most of the mutations induced by genome editing technologies cannot be unequivocally distinguished from natural mutations as they may also occur naturally. Plant genomes have inherently a considerable sequence variability which is at present not only insufficiently documented for any crop, but the genomes keep also changing over time.

Moreover, mutations obtained by new mutagenesis techniques can currently not be differentiated from those induced by conventional mutagenesis techniques, which have been incorporated in traditional breeding programs and are often not thoroughly documented.

# Obrigado

## Thank You

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CTNBio
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