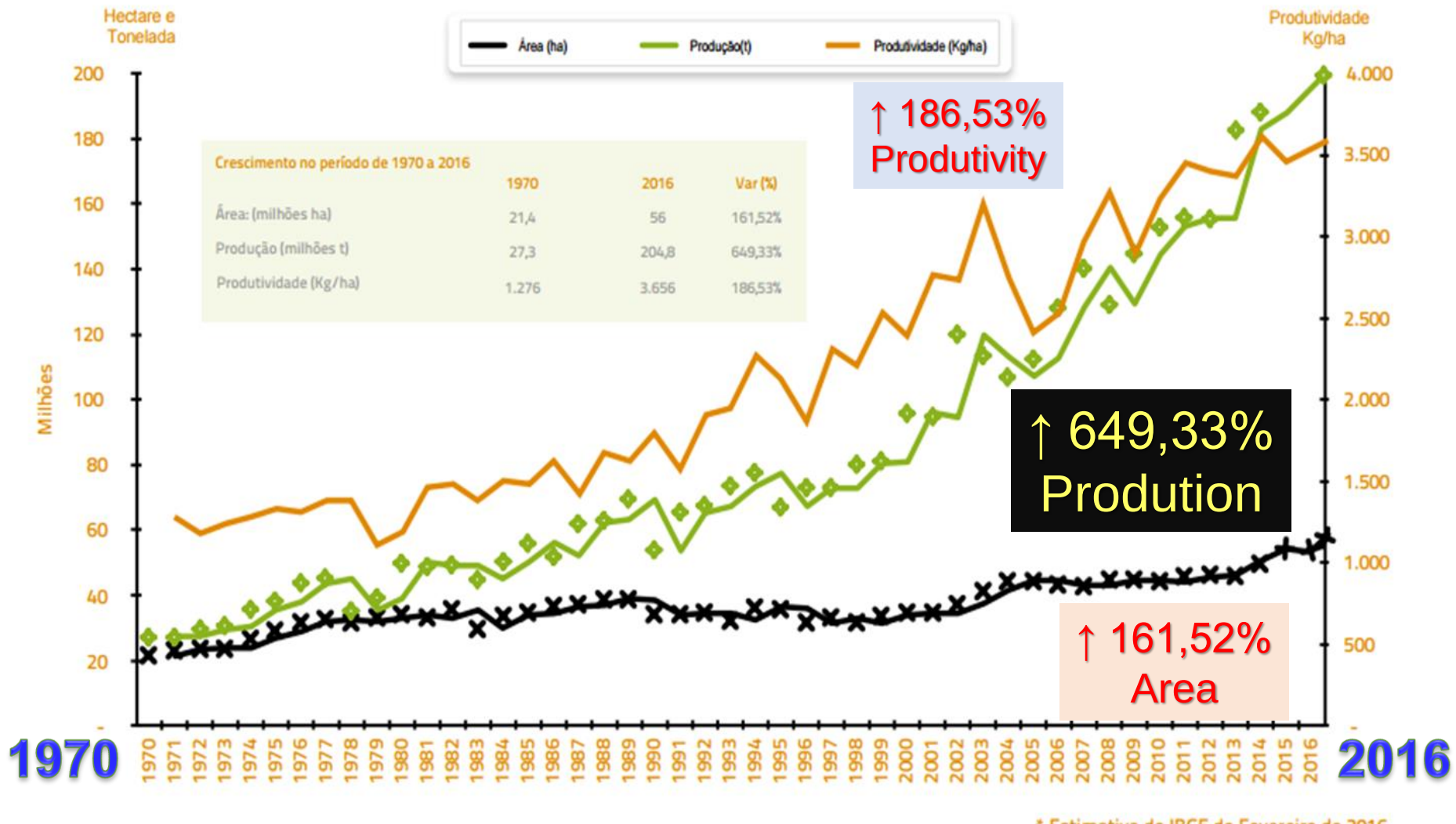


Research & development: Public institutes investing in Plant Breeding Innovation (PBI)

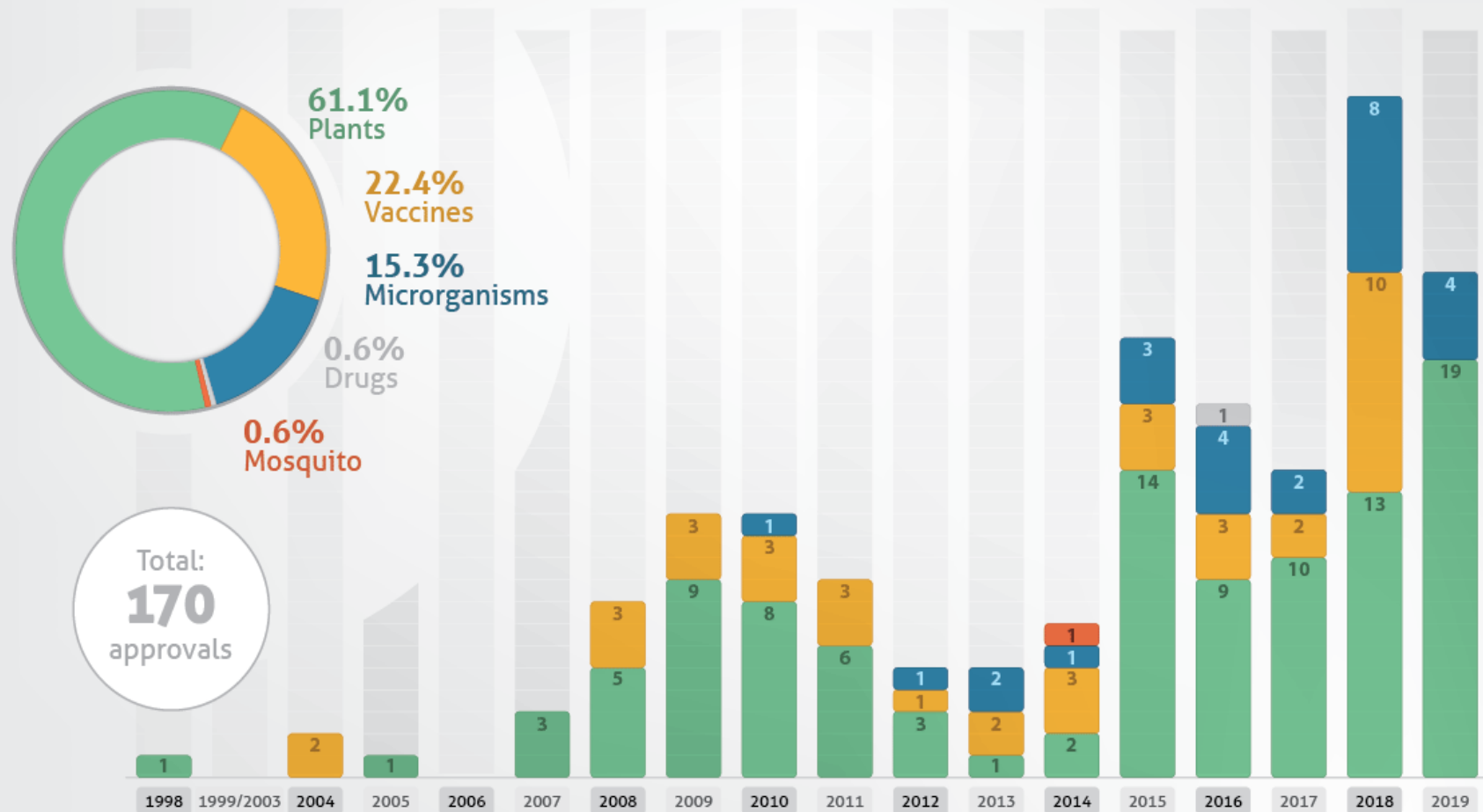
Alexandre Nepomuceno, Ph.D.

Brazil's Grain (Rice, Beans, Corn, Soybean and Wheat) Production Area, Total Production and Productivity



World's largest exporter of **Beef, Coffee, Sugar, Orange juice, Ethanol, Chicken and Soybean** in 2017.

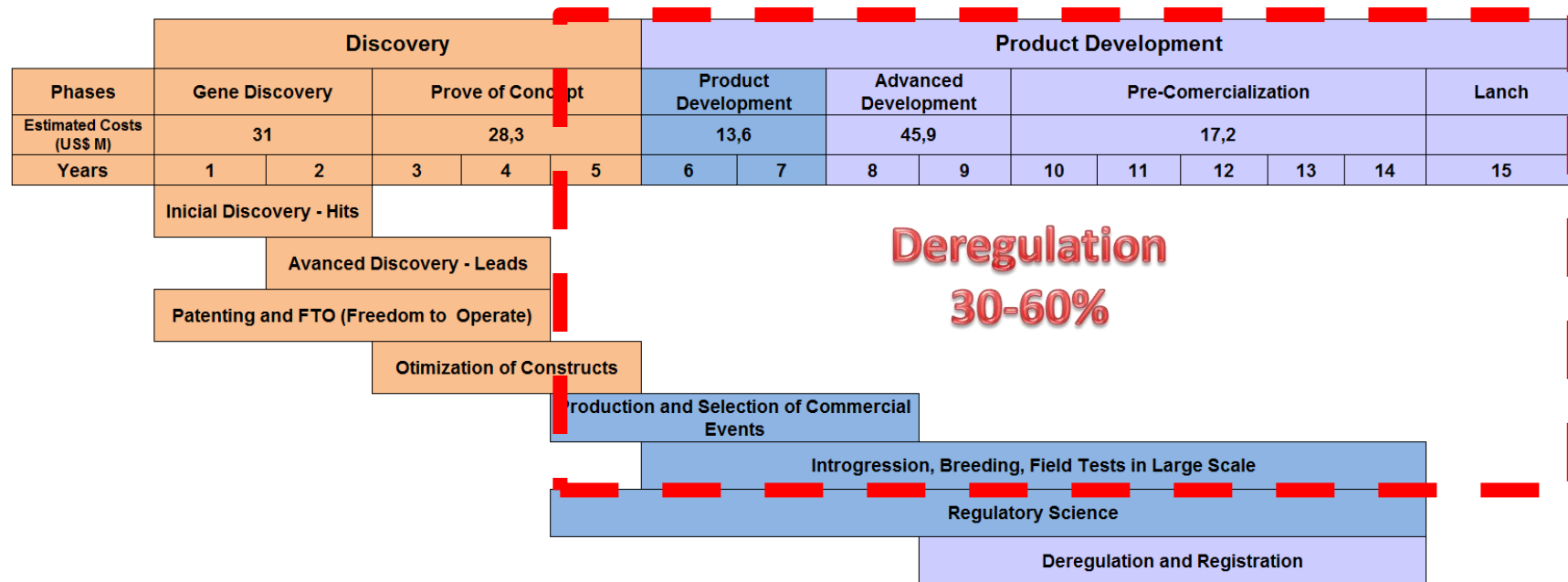
GMO approvals in Brazil



Source: CTNBio 2019.

Genome Edition – More Institutions and more Crops using Biotechnology solutions for Agriculture

Phases and Costs to Development of a GM Crop



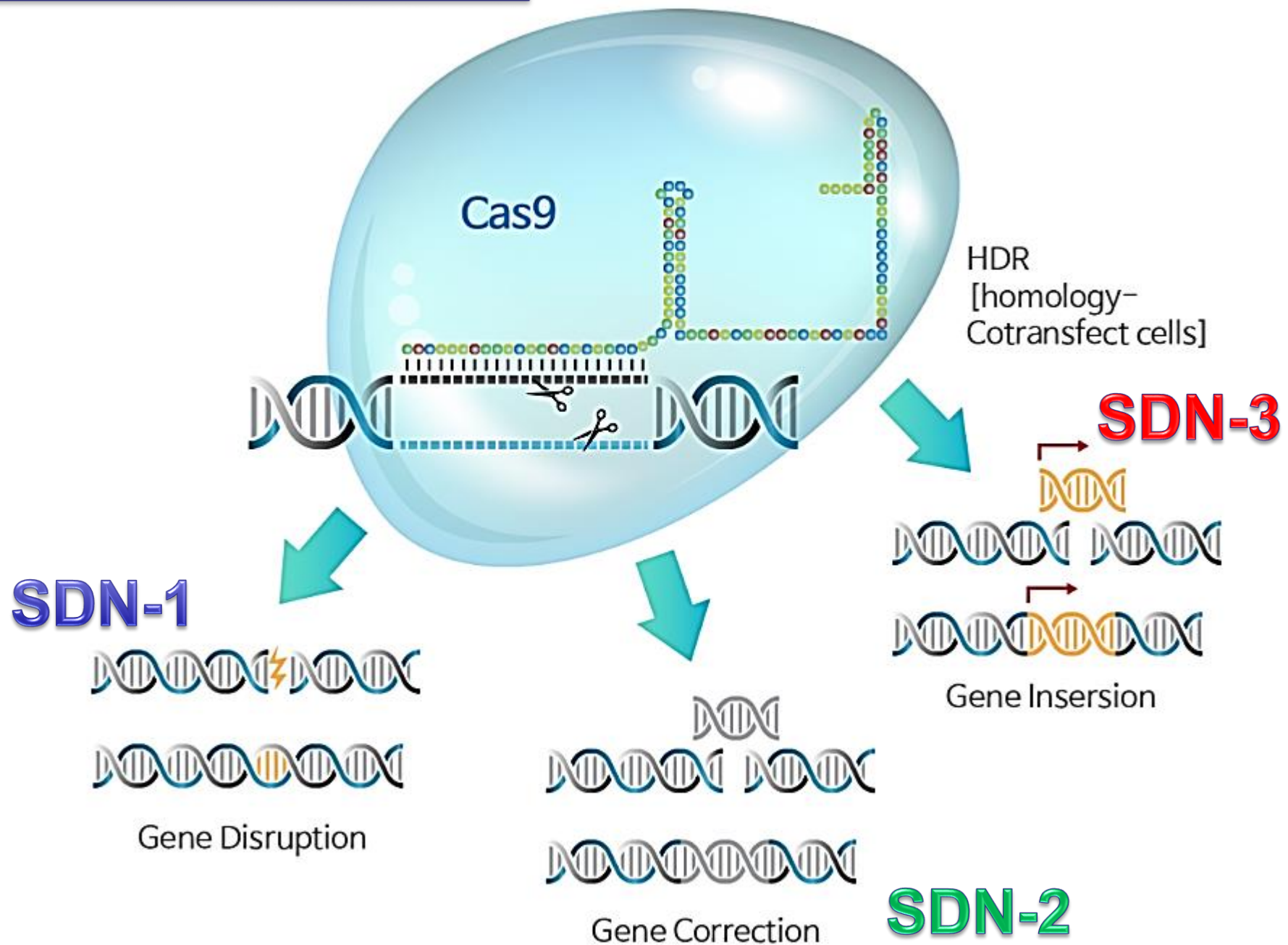
Estimated Costs: ~U\$136 million

Source: McDougall, 2011; Prado et al, 2014

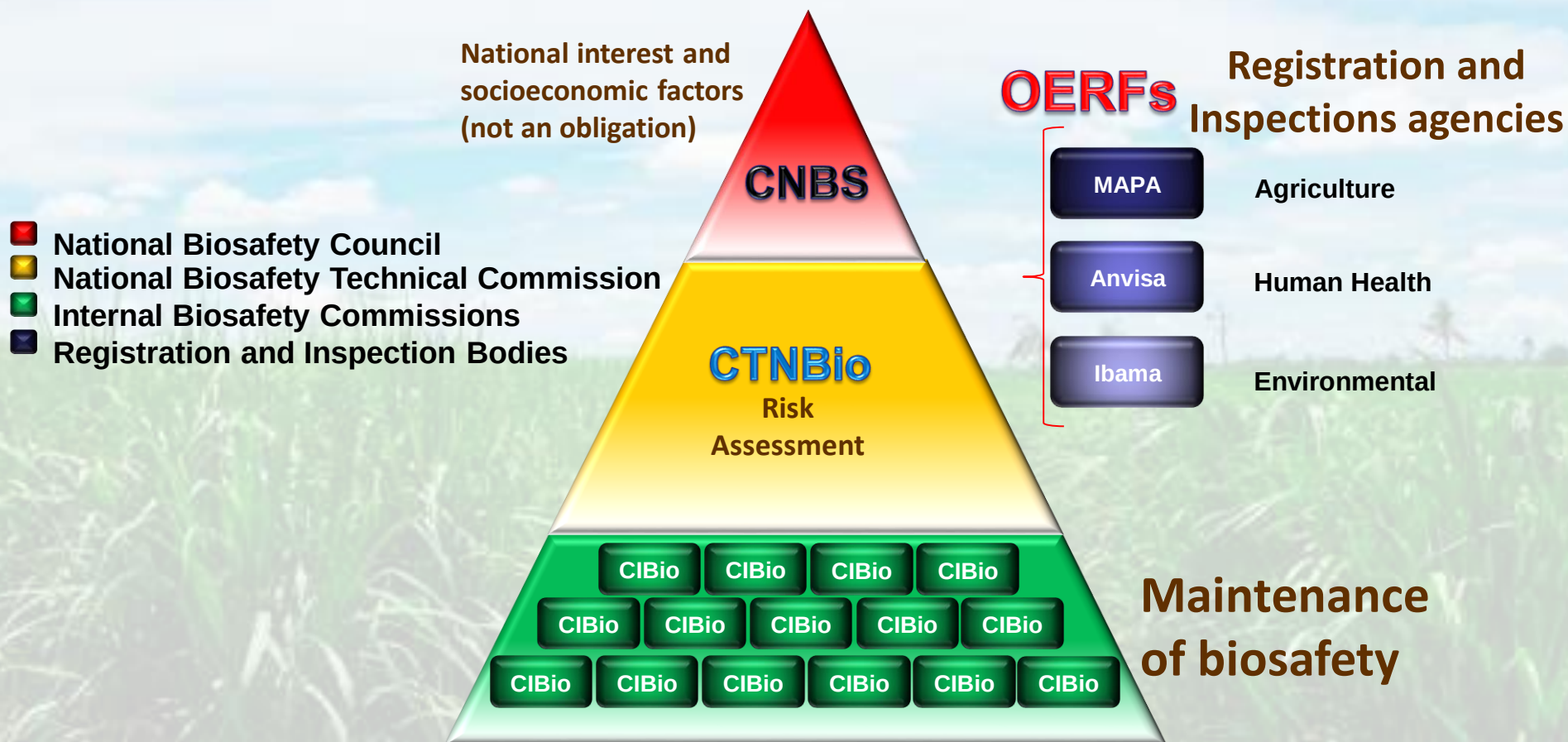
Estimated Costs of Deregulation Phase: ~U\$75 million

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

GENOME EDITING



Biosafety Law - 11.105/05 Structure





DIÁRIO OFICIAL DA UNIÃO



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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

Americas: >60% of world Grains

Canada: Not regulated unless product identified as novel

United States: For plants – USDA 'Am I Regulated?' letters, FDA & EPA under review

Argentina, Brazil, Colombia, Paraguay, Chile: Case by case approaches; foreign DNA insertions generally regulated as GMO

Uruguay: Proposal identical to other south America countries

Norway: Proposed; foreign DNA insertion regulated; case-by-case tiered approach – notification, expedited, standard review

Europe: European Court of Justice ruling genome editing techniques must be regulated as GMOs

China, Korea: Issue still being debated; no formal guidance

Israel: Foreign DNA insertions regulated

Philippines, Japan: Under consideration; Foreign DNA insertions generally regulated as GMO

Australia: Under review; possibly regulated whenever when templates involved; case-by-case review

New Zealand: Initial "non-GMO" ruling for gene editing struck down by courts

 Countries with regulatory policy with exclusions

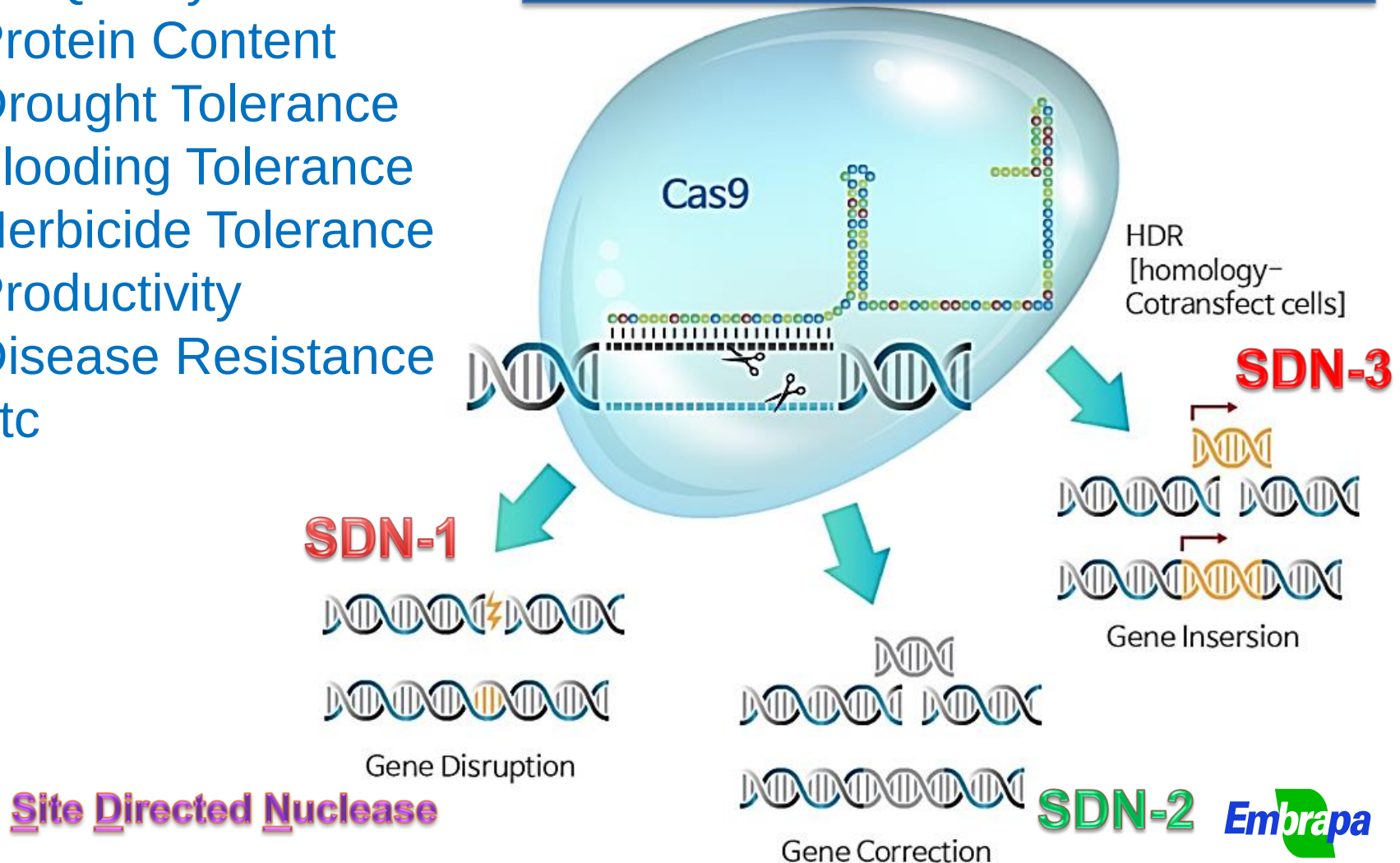
 Countries with **pending** policies, regulations, or legal rulings

 Countries with policy with no exclusions

Genome Editing in Brazilian Agriculture

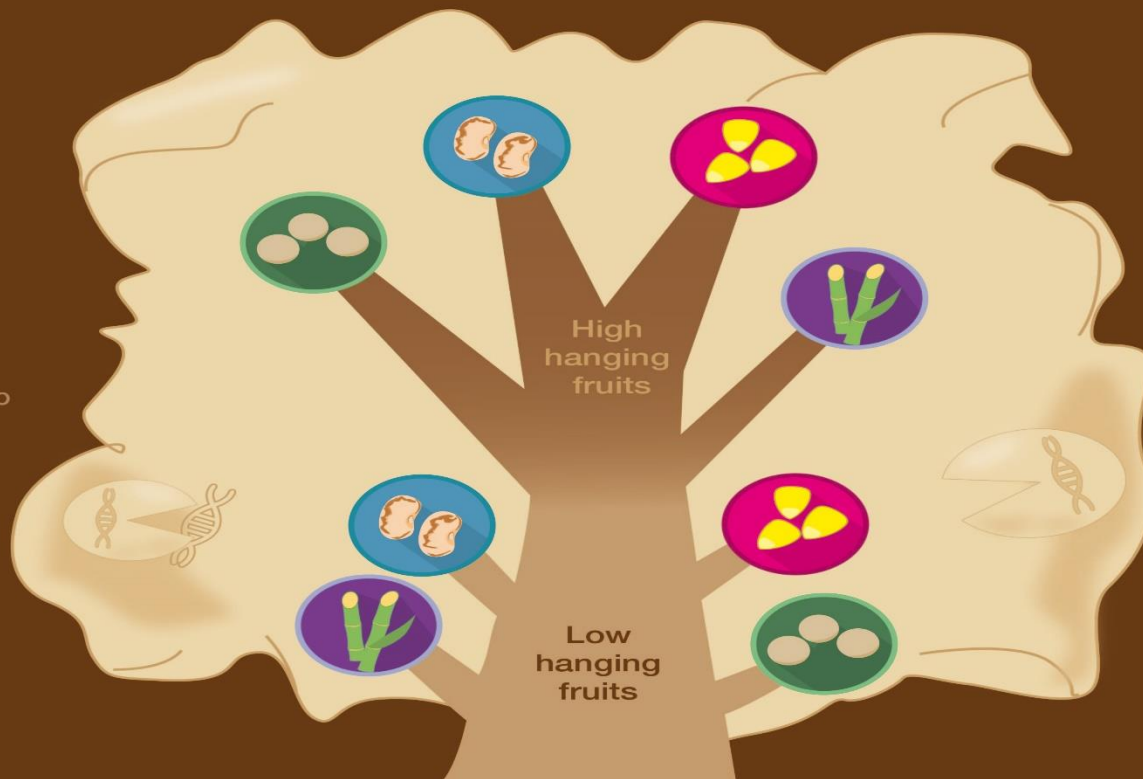
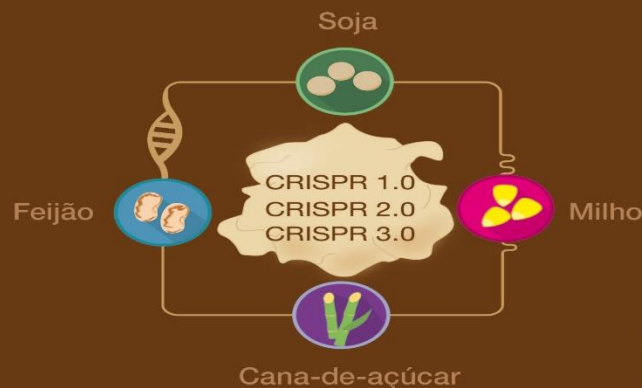
- Grain/Seed Quality
- Oil Quality
- Protein Content
- Drought Tolerance
- Flooding Tolerance
- Herbicide Tolerance
- Productivity
- Disease Resistance
- etc

Soybean, Corn, Cotton, Sugarcane,
Eucalyptus, Grapes, Common Beans,
Pastures, etc



PROJETO CRISPRevolution

Plantas de importância econômica com genoma editado pela tecnologia CRISPR visando melhoria da qualidade nutricional e industrial e tolerância a estresse hídrico



Cultivares / Variedades superiores para aumento da competitividade do agronegócio

- Geneticamente editado para redução do escurecimento da semente
- Geneticamente editada para redução dos fatores antinutricionais
- Geneticamente editada para aumento da digestibilidade da biomassa
- Geneticamente editado para aumento da digestibilidade da biomassa

Geneticamente editados para tolerância à seca

Partnerships

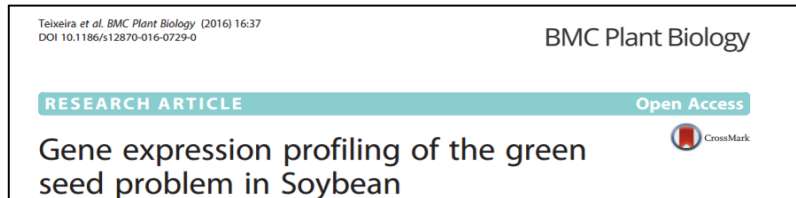
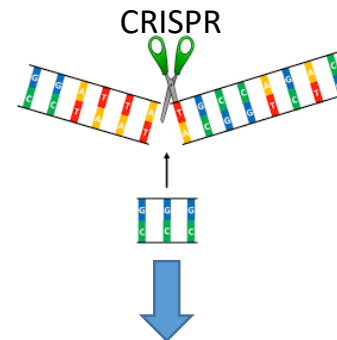


EMBRAPA Soybean CRISPRs Systems

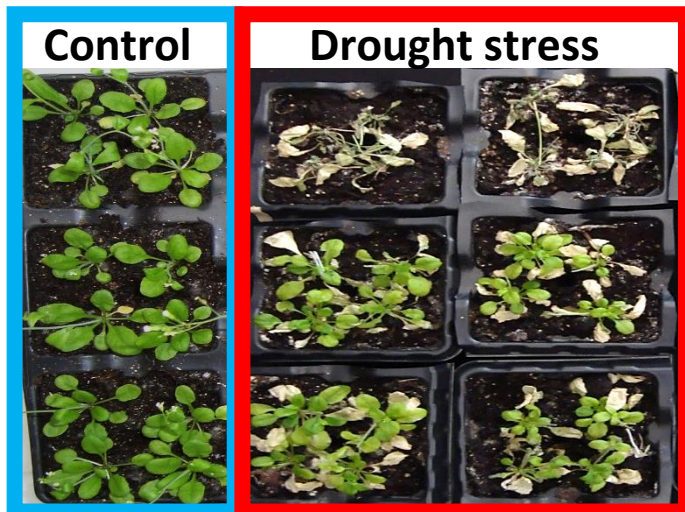
Soybean: Seed Quality, Anti-nutricional factors and Drought Tolerance



Knock Out and HR at promoter elements



REHYDRATION AFTER 2 WEEKS OF DROUGHT STRESS



Wild Type
F5-8
F3-6

Dr. Alexandre Nepomuceno
Dr. Liliane Henning
Dr. Elibio Rech

Enhanced drought tolerance

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

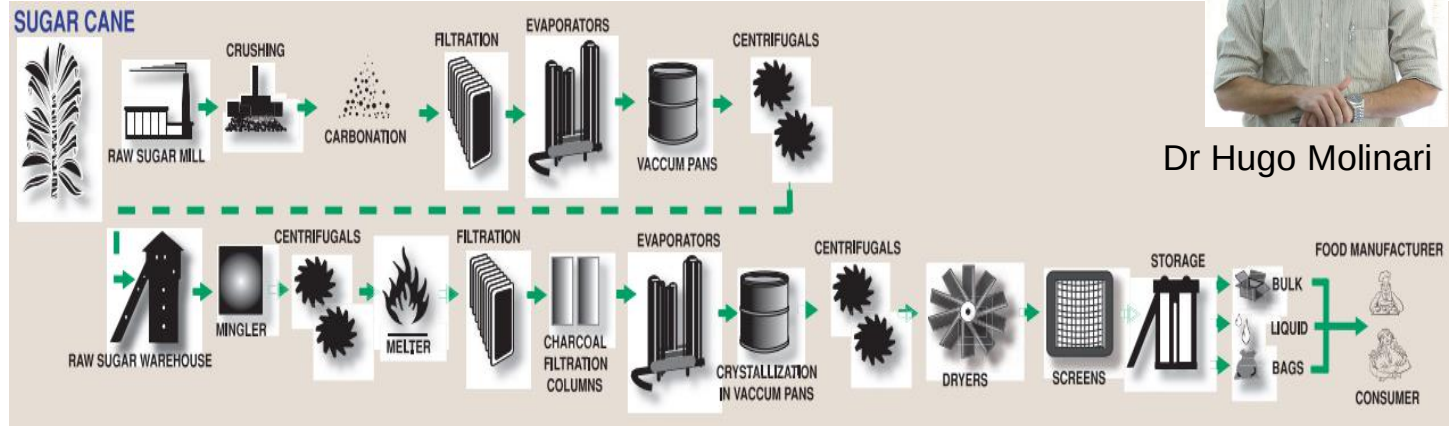


CRISPR-CANE PPO and Drought

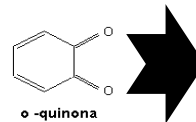
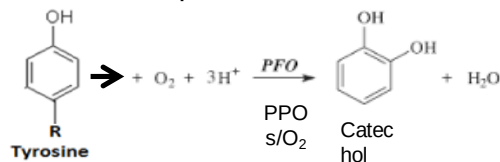


Dr Hugo Molinari

Sugarcane refinery



- The sugar refinement requires addition of chemicals, demands sophisticated physical structure, requires high cost and results in the removal of >90% of their vitamins and minerals;
- CRISPR-CANE PPO is an promising strategy due to the reduction of costs of refinement and maintenance of the nutritional characteristics of sugar;
- It can be applied to several commercial cultivars;
- Global expansion of the Brazilian sugar market;



It forms (melanin) compounds responsible for the development of dark coloration (brown pigments)



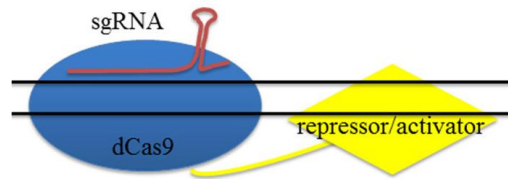
DROUGHT-TOLERANCE AND NEMATOTE RESISTANCE IN SOYBEAN, COTTON AND SUGARCANE



dCas9-
Activator

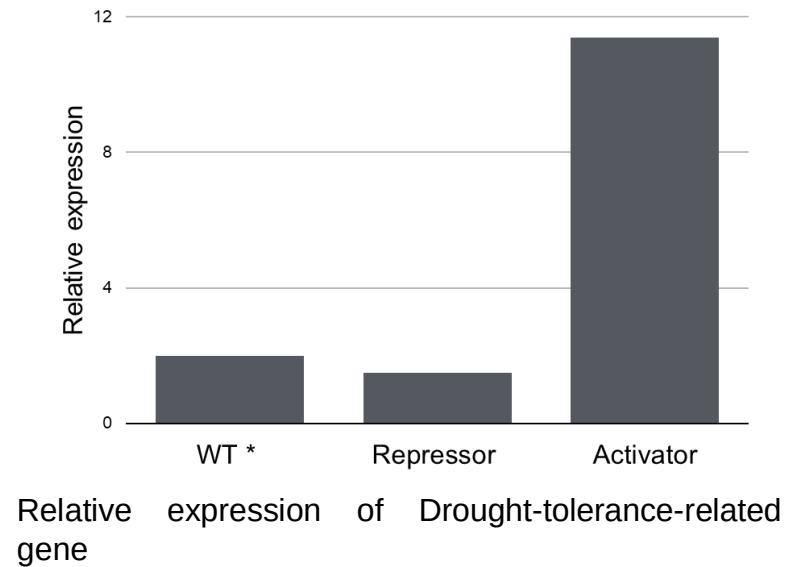
dCas9-
Represso
r

WT



Embrapa

Dr. Maria
Fátima
Grossi-de-Sá



CRISPR in Common Beans (Feijão)

COMMON BEAN **CRISPR** TARGETS:

- DROUGHT
- TEGUMENT DARKENING (POLIFENOL OXIDASES?)
- ANTINUTRITIONAL FACTORS (RAFINOSE, ESTAQUIOSE, VERBASCOSIDE, ETC)
- PROTEIN CONTENT/QUALITY



Dr. Josias Correa de Faria

Dr. Rosana Vianello



Development of a tool kit for genome edition with new enzymes (Nucleases) from Brazilian biodiversity

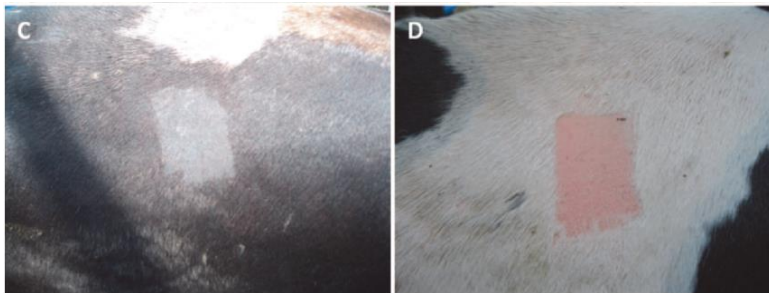


Animal Genome Edition



Increase the thermotolerance

- By the introgression of genes or mutations associated to heat tolerance
 - Ex. Slick hair locus derived from cattle breeds like Senepol and Criollo (Caribbean *Bos taurus*) associated to an increased thermotolerance
 - short hair length
 - Holstein cows with short hair - more tolerant to heat stress



Evidence of a major gene influencing hair length and heat tolerance in *Bos taurus* cattle^{1,2,3}

T. A. Olson^{*4}, C. Lucena[†], C. C. Chase, Jr.[‡], and A. C. Hammond^{§5}

^{*}University of Florida, Gainesville 32611; [‡]USDA, ARS, Subtropical Agricultural Research Station, Brooksville, FL 34601; and [†]Universidad Centrooccidental "Lisandro Alvarado," Barquisimeto, Venezuela



J. Dairy Sci. 97:5508–5520
<http://dx.doi.org/10.3168/jds.2014-8087>
© American Dairy Science Association[®], 2014.

The *SLICK* hair locus derived from Senepol cattle confers thermotolerance to intensively managed lactating Holstein cows

S. Dikmen,^{*} F. A. Khan,^{†1} H. J. Huson,^{‡2} T. S. Sonstegard,[‡] J. I. Moss,[†] G. E. Dahl,[†] and P. J. Hansen^{†3}

^{*}University of Uludağ, Faculty of Veterinary Medicine, Department of Animal Science, Bursa 16059, Turkey

[†]University of Florida, Department of Animal Sciences, Gainesville 32611-0910

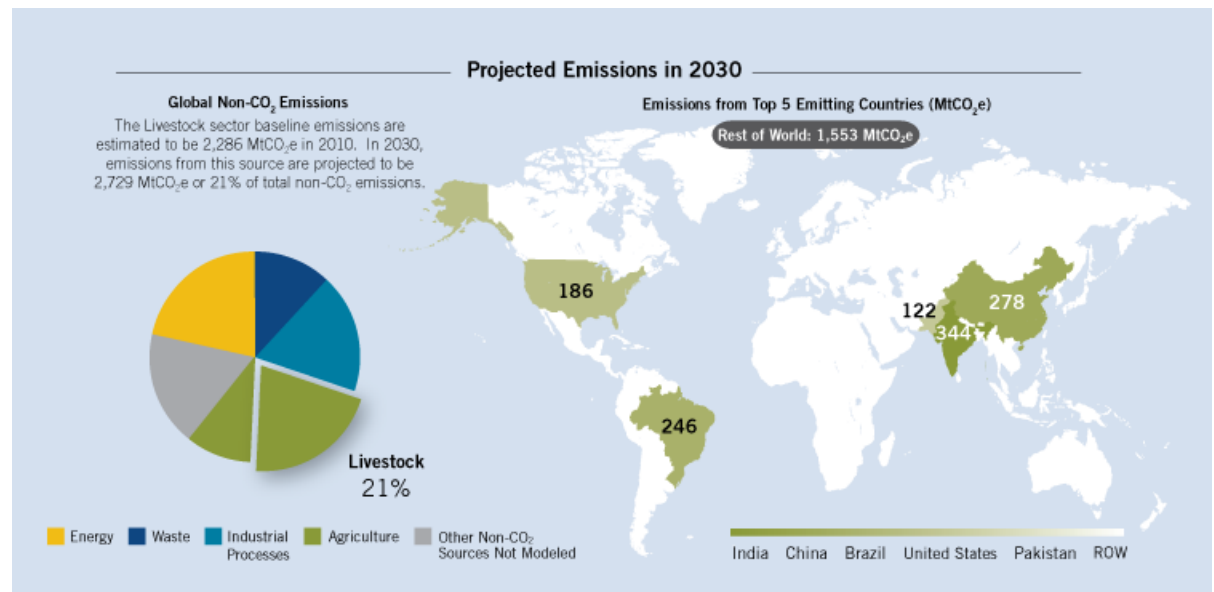
[‡]USDA, Agricultural Research Service, Animal Genomics and Improvement Laboratory, Beltsville, MD 20705-2350

Animal Genome Edition

Reduce methane emission

Increase the rumen digestibility

- Low digestibility of tropical forages – due to high content of hemi-cellulose and/or lignin
 - Can not be digested by rumen microbiome - lack of enzymes
 - Modification of rumen microbiome to express fibrolytics or ligninolytic enzymes:
 - Xylanases (hemi-cellulose), laccases (lignin), etc
- Emission of non-CO₂ greenhouses gases (e.g. methane)
 - 21% of non-CO₂ emission is projected to be from livestock in 2030



Embrapa's Soybean Reference Genome

Embrapa's Variety Sequencing (BR537) as
“*BioChassis*” for a more precise genome
editing



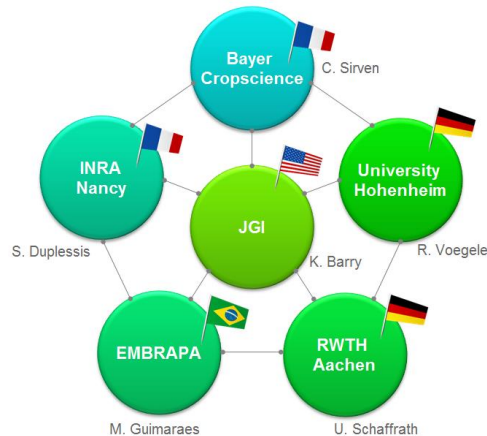
soybean seeds

**≈ 40% protein
≈ 20% oil**

Reference genome of *Phakopsora pachyrhizi* (Soybean Rust)



<http://jgi.doe.gov/collaborate-with-jgi/community-science-program/>



Public project
Community Science
Program
DOE JGI

Project leader: INRA S. Duplessis
Bayer: C. Sirven/ Jochen Kleemann
Embrapa: F. Marcelino-Guimaraes
University Hohenheim: R. Voegelé
University Aachen: U. Schafrath

Whole reference genome
Resequencing
Transcriptomics
Small RNAs

Bilateral project

Project leaders
Bayer: R. Bortolan
EMBRAPA: M. Meyer

- ✓ **Whole reference genome**
- ✓ **Resequencing:** genomic wide variations studies of Avr/Effectors and fungicides targets
44 isolates (29 from Brazil)
- ✓ Monitoring resistance to fungicides and Rpp genes



Obrigado

Alexandre Nepomuceno

alexandre.nepomuceno@embrapa.br