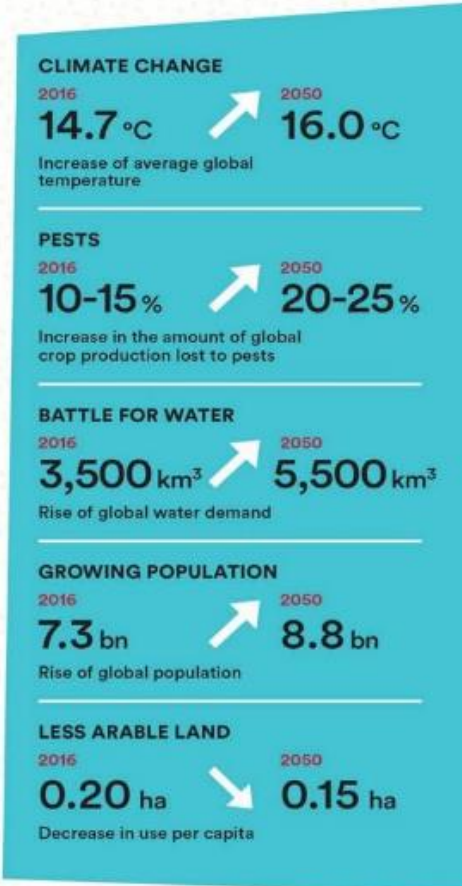
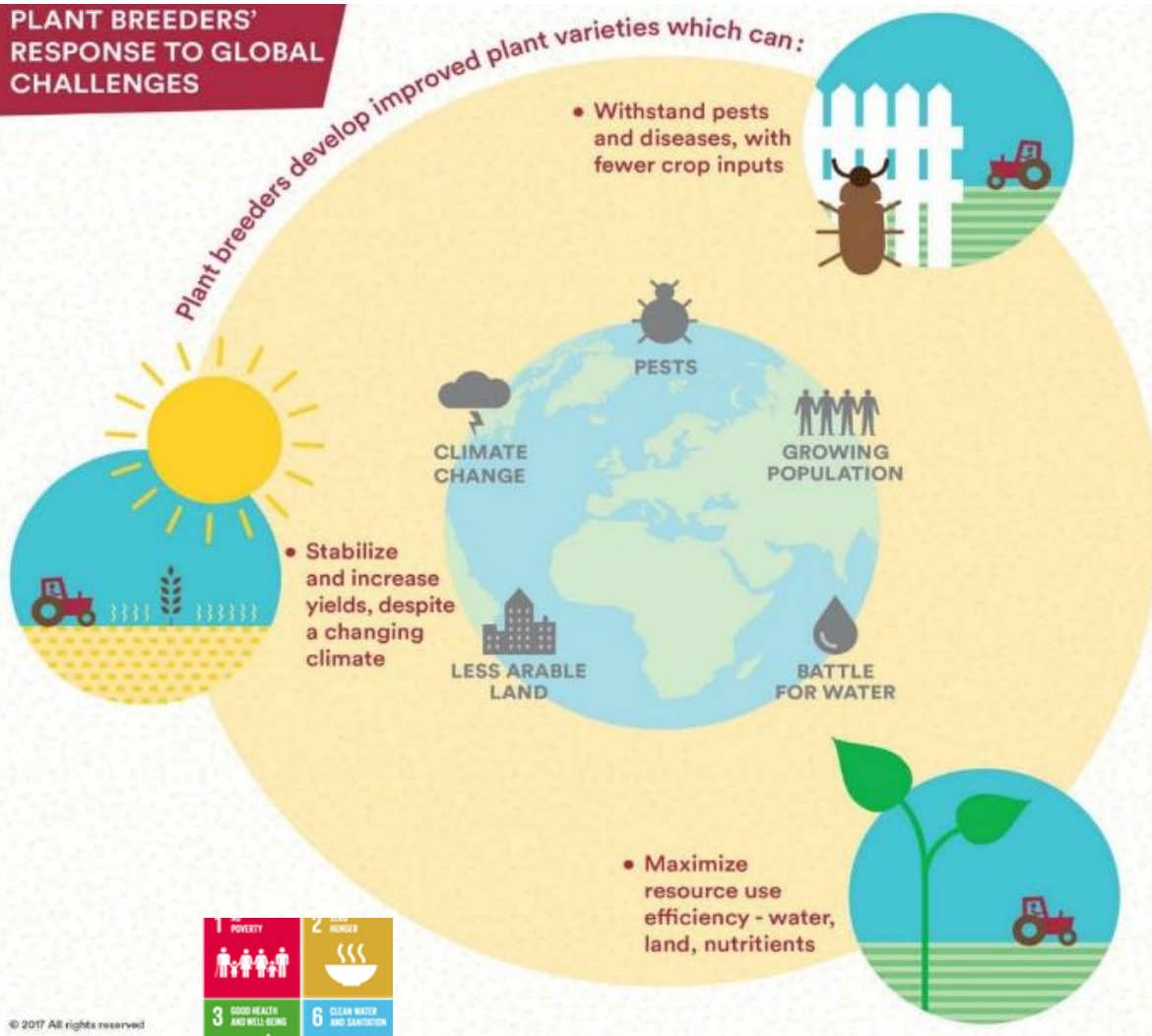


# Genome Editing in Crop Plants: Global Prospects

**B.M. Prasanna**

**Director, Global Maize Program, CIMMYT,  
OneCGIAR Maize Breeding Lead &  
OneCGIAR Plant Health Initiative Lead, Nairobi, Kenya  
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**PLANT BREEDERS' RESPONSE TO GLOBAL CHALLENGES**



**New breeding technologies can, and must, contribute to several of these objectives.**



**ISF** International Seed Federation  
Seed is Life



Source: ISF Breeder's committee meeting 2021 with modifications



# Genome Editing using CRISPR-Cas Technology

2020 Nobel Prize in Chemistry



**Emmanuelle Charpentier** (Max Planck Unit for the Science of Pathogens, Berlin, Germany & **Jennifer Doudna** (University of California, Berkeley, USA)

*Cell* (2016)

## The Heroes of CRISPR

Eric S. Lander<sup>1,2,3,\*</sup>

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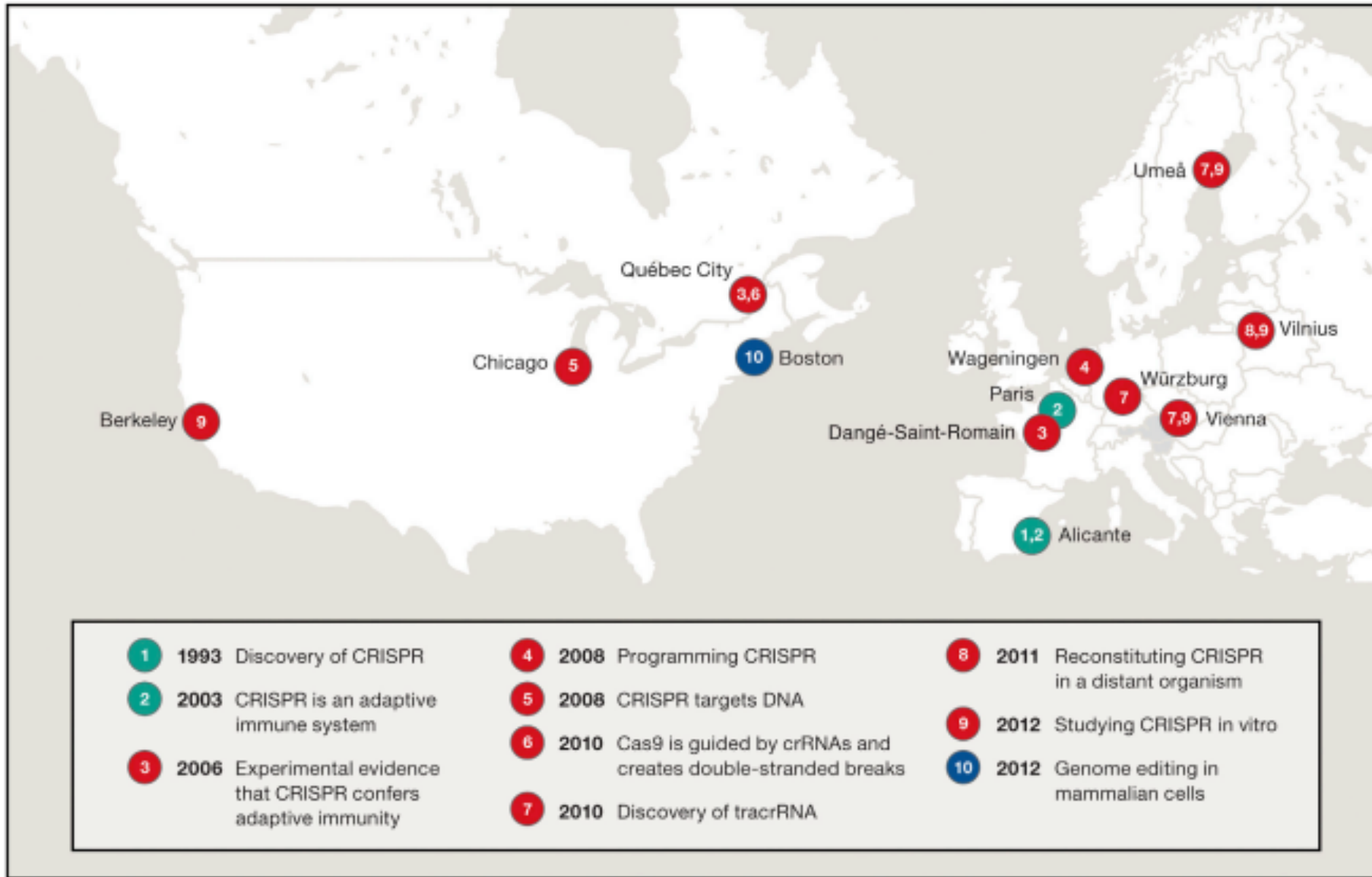
<http://dx.doi.org/10.1016/j.cell.2015.12.041>

*Nature* (2016)

## *THE UNSUNG HEROES OF CRISPR*

The soaring popularity of gene editing has made celebrities of the principal investigators who pioneered the field – but their graduate students and postdocs are often overlooked.

BY HEIDI LEDFORD



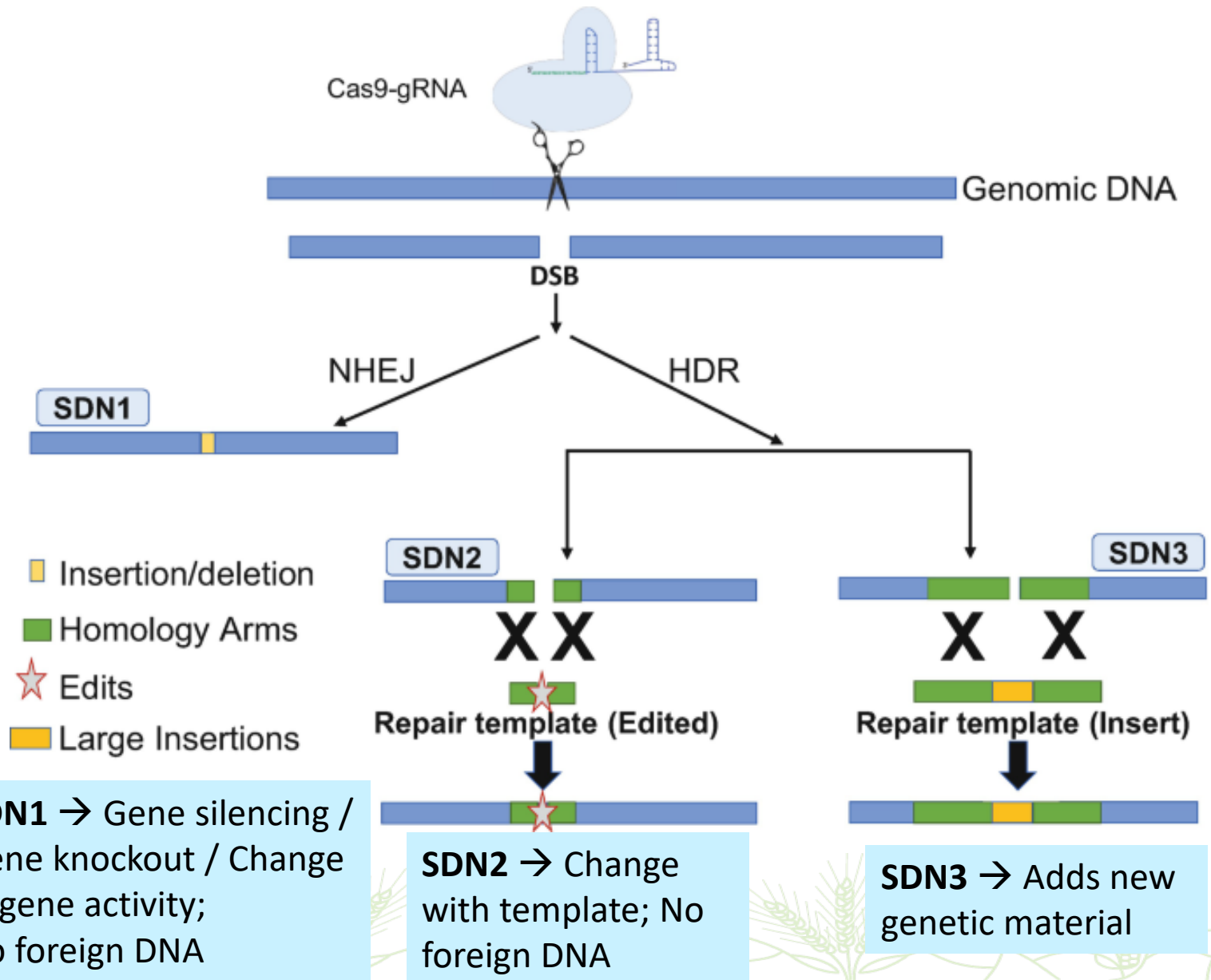
## The 20-year story of CRISPR unfolded across 12 cities in 9 countries!!

**Green circles** refer to the early discovery of the CRISPR system and its function; **Red circles** refer to the genetic, molecular biological, and biochemical characterization; and **Blue circles** refer to the final step of biological engineering to enable genome editing.

Source: Lander (2016)



# CRISPR-Cas9: A Powerful Tool for Site-directed Mutagenesis

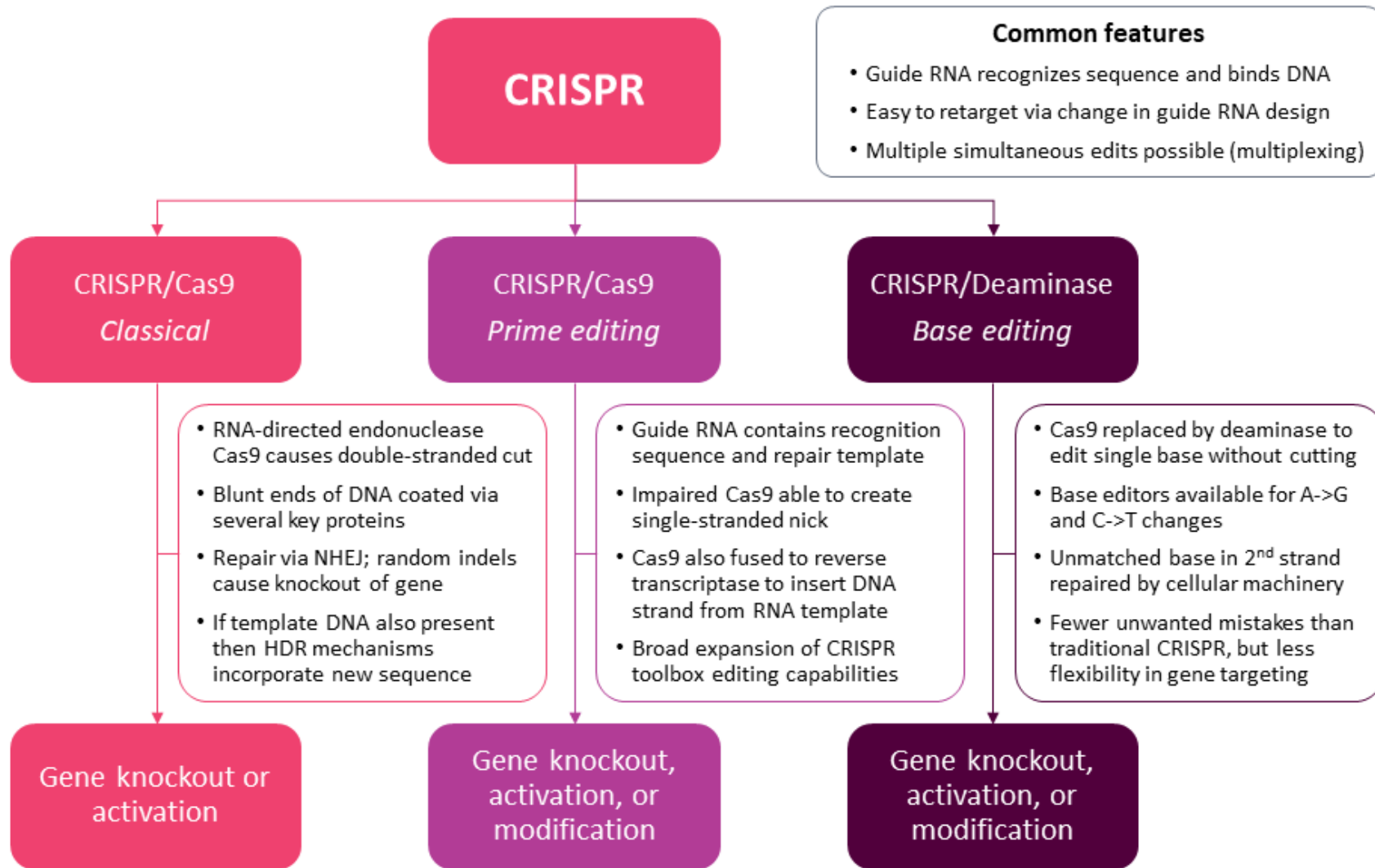


## Major Drivers

1. Inexpensive sequencing
2. Robust genome modification methods to introduce genetic variation
3. Novel plant transformation methods

The goal of **Site-Directed Nuclease (SDN) technology** is to take advantage of the targeted DNA break and the host's natural repair mechanisms to introduce specific small changes at the site of the DNA break. The change can either be a small deletion, a substitution or the addition of a number of nucleotides.

# Technology offers an array of genetic changes with different outcomes...



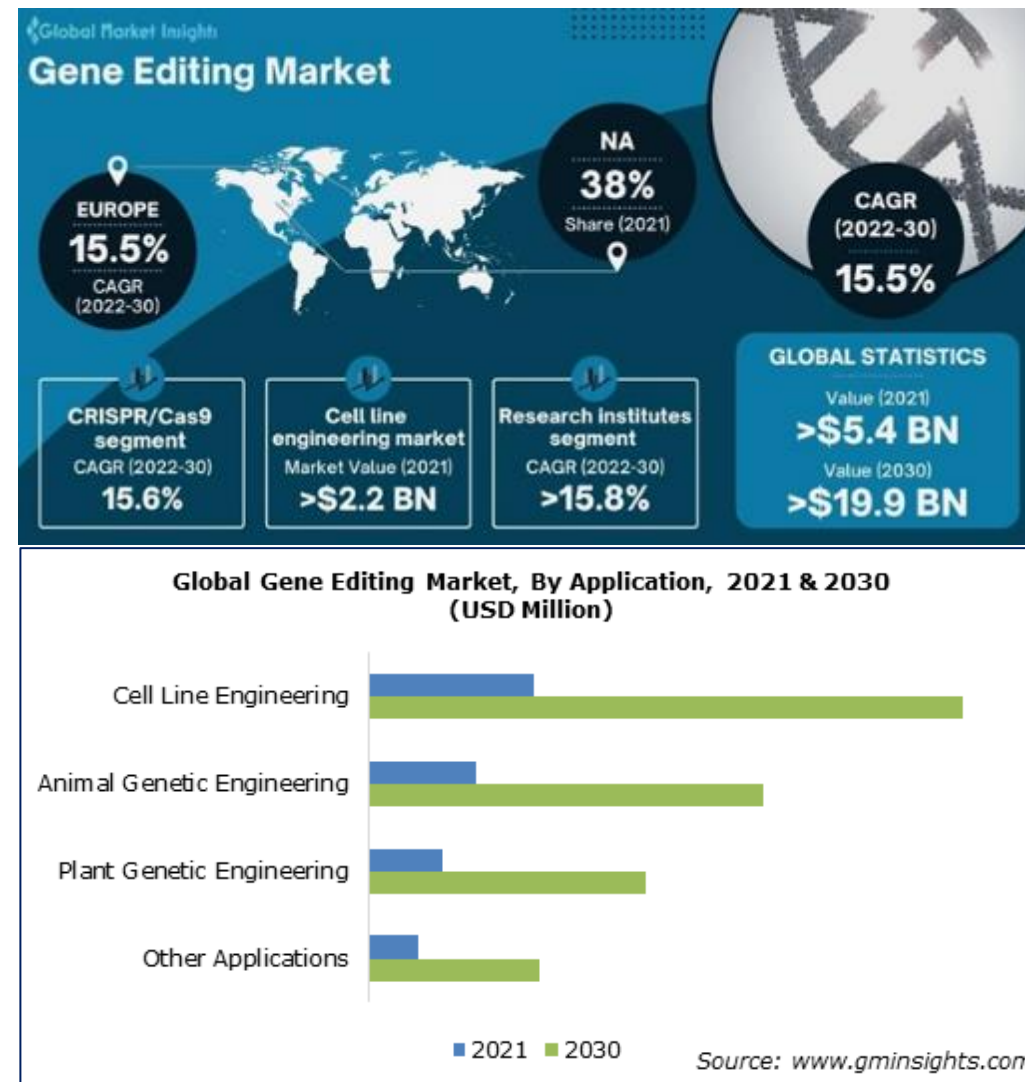
## Powerful alternatives to Cas9 are emerging:

- **Novel Cas nucleases** – e.g., Cpf1 (Cas12), C2c2 (Cas13a), C2c1 (Cas13b) etc.
- **CasMINI** (Cas12f)
- **Cas-CLOVER** (Clo51-Cas9)



# Genome Editing in Crop Plants

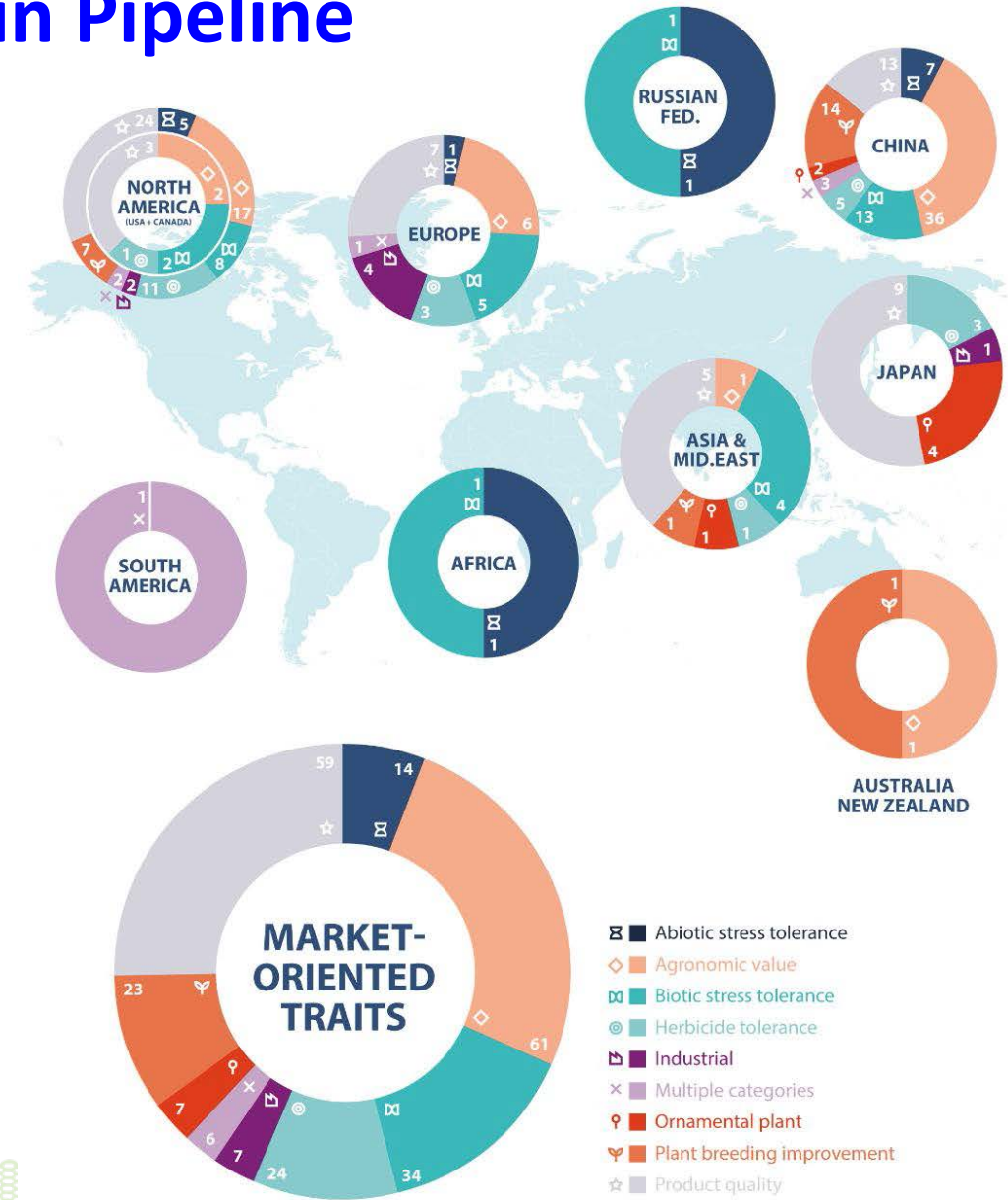
- **Window of Opportunity:** Genome editing is “hot” now; the ship will sail with or without us/CGIAR, early adopters will reap the benefits
- Growing availability of **genomic sequence data** even from orphan crops.
- **Improved landscapes for intellectual property and social acceptance** as compared to transgenics
- **Safety:** Genome editing does not involve substantially greater or different risks than conventional or mutation breeding
- Many partners are eager to avail the technology and are adopting **facilitating regulatory policies**.



The global genome editing market is projected at **USD 11.7 billion by 2026** from **USD 5.1 billion in 2021**; CAGR of 18.2% between 2021 and 2026; could further rise to **USD 36.06 billion** (CAGR of 22.3%) by 2030.

# Market-oriented Genome-edited Crops in Pipeline

- **Genome edits in 63 types of plant species** have been published in scientific literature (Dima et al., 2022).
- **Genome editing is being applied in a much broader variety of crops compared to transgenesis** and the scope of introduced characteristics is much broader as well.
- Many of these crops are globally traded, such as soybean, rice, maize, wheat, banana and oilseed rape → implies that **genome-edited crops may also be traded and move from one part of the world to the other.**





# Genome-edited Crops on the Market

## Gene-edited High Oleic Soybean Oil Now Available in the US

March 27, 2019

Premium quality high-oleic soybean oil developed through gene editing is now available in the US market. The soybean oil known as Calyno™ was developed by experts from Calyxt, Inc. This is the first gene-edited food released for consumers in the US.



Calyxt scientists turned off two genes involved in fatty-acid synthesis. Unlike traditional GMOs, this particular soybean had genes turned off instead of having another organism's gene inserted to it. This resulted to the Calyno oil being 80% higher in oleic acid, 20% less in saturated fatty acids, has 0 grams trans fat per serving, has three times the fry-life and has a longer shelf-life compared to the current soybean oil being sold in the market. Although the same process can be achieved through conventional crossbreeding, gene editing allows scientists to produce the crop with the desired trait more precisely and in less time.

## Japan Starts Sale of Genome-Edited High-GABA Tomato

September 22, 2021



Photo source: Santech Seed

Developed in collaboration with the University of Tsukuba, the genome-edited high-GABA tomato was launched in seedling gardening kits in May 2021 and was received positively by their home gardener consumer panel. The overwhelming response and strong interest from this group prompted commercial sales in September. A puree product made of the same tomato will also be available at a later date.

Sanatech Seed's Sicilian Rouge High GABA tomato was developed using CRISPR-Cas9 gene editing technology. The tomato contains high levels of gamma-aminobutyric acid (GABA), an amino acid believed to aid relaxation and help lower blood pressure. According to Shimpei Takeshita, President of Sanatech Seed and Chief Innovation Officer of Pioneer EcoScience, the exclusive distributor of the tomato, it contains four to five times more GABA than a regular tomato. Sanatech Seed received a parental line of the Sicilian Rouge variety from Pioneer EcoScience, and developed the F1 variety "Sicilian Rouge High GABA" with enhanced GABA content through gene editing.



Emergence of a series of start-ups specifically offering genome editing services in a diverse array of crops....



# Genome Editing in Crop Plants: Key Requirements

- Selection of right traits and right genes to edit
- Skilled scientists with the necessary technical know-how (molecular biology, bioinformatics, transformation & regeneration)
- Intellectual property (IP) rights/FTO
- Partnerships
- Financial resources for sustained efforts
- An enabling regulatory environment for faster and smoother deployment of products in the target market
- Communications and outreach (at various levels)



# Disease Resistance

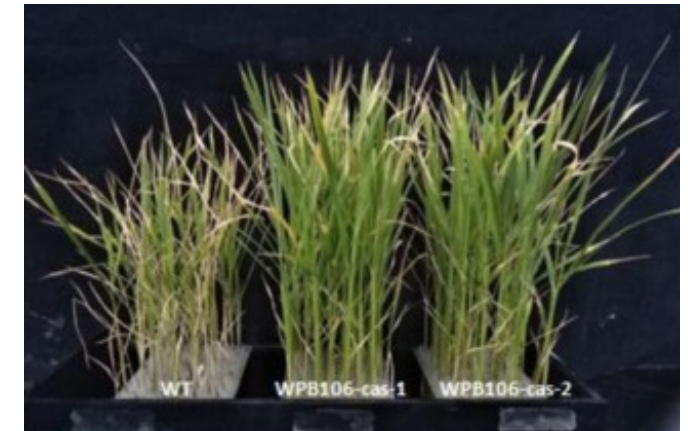
Crop	Traits	Gene(s) targeted	References
Rice	Bacterial leaf blight; Leaf blast; Powdery mildew; Rice Tungro	OsSWEET 14 promoter; OsSWEET11 promoter; OsSWEET13; Os8N3; OsERF922; TaEDR1; eIF4G	Jiang et al., 2013; Zhou et al., 2015; Xu et al., 2019; Wang et al., 2016; Zhang et al., 2017; Macovei et al., 2018
Wheat	Powdery mildew	TAMLOs	Wang et al., 2014
Banana	Banana streak virus	eBSV	Tripathi et al., 2019
Cassava	Cassava brown streak disease	ncbp1/2	Gomez et al., 2019
Grape	Powdery mildew	VvMLO3	Wan et al., 2020
Potato	Potato virus Y	Colin C-terminal	Makhotenko et al., 2019
Tomato	Powdery mildew; TLCV; Bacterial speck	SIMLO1; Coat and replicase protein of TYCV; SIJAZ2;	Nekrasov et al., 2017; Tashkandi et al., 2018; Ortigosa et al., 2019

Improving disease resistance in crop plants will be one of the most powerful applications of genome editing.



# Abiotic Stress Tolerance

Crop Species	Traits	Gene(s) targeted	References
Rice	Drought tolerance	EPFL9	Yin et al. (2017)
	Salinity tolerance	OsRR22	Zhang et al. (2019)
	Early flowering	Hd2, Hd4, Hd5	Li et al. (2017)
Maize	Drought tolerance	ARGOS8	Shi et al. (2017)
Banana	Semi-dwarfing	Ma04g15900 Ma06g27710 Ma08g32850 Ma11g10500 Ma11g17210	Shao et al. (2020)



Salinity tolerance (Zhang et al. 2019)

Except in a few cases where a few genomic regions/genes have a major effect on abiotic stress tolerance (e.g., in crops like rice), the power of genome editing could be limited.



# Nutritional Quality

Crop	Traits	Gene(s) targeted	References
Rice	Increased amylose	OsNramp5	Tang et al., 2017
	Increased carotenoids	GR-1 & GR-2	Dong et al., 2020
Maize	Reduced phytate levels	IPK1; ZmPDS, ZmIPK1, ZmIPK, ZmMRP4	Shukla et al., 2009; Liang et al., 2014
Wheat	Low gluten for reduced allergenicity	Alpha-gliadin array, Gli-2 locus	Sánchez-León et al., 2018
Sorghum	Reduced kafirins	K1C genes	Li et al., 2018
Soybean	Altered oil levels	FAD2-1A, FAD2-1B, FAD3A	Demorest et al., 2016
Brassica	Increased oleic acid	FAD2	Okuzaki et al., 2018
Banana	Increased beta-carotene	LCYe	Kaur et al., 2020
Cassava	Reduced starch	PTST1, GBSS	Bull et al., 2018
Groundnut	Increased oleic acid	FAD2	Wen et al., 2018
Potato	Reduced starch	GBSS	Andersson et al., 2017
Tomato	Increased anthocyanin		Cermák et al., 2015; Filler Hayut et al., 2017; Deng et al., 2018)

Excellent opportunities to enhance the nutritional quality as many genes influencing the nutritional quality pathways have been well-characterized.

# Yield Improvement and Specialty Traits

Crop	Traits	Gene(s) targeted	References
Rice	Grain number and yield	DEP1, Gn1A	Huang et al., 2018
	Growth and yield	PYL1, 4, 6	Miao et al., 2018
	Grain traits and yield	OsGs3, OsGW2, OsGn1A	Zhou et al., 2019
Maize	Waxy maize	Waxy	Gao et al., 2020
Wheat	Grain weight and yield	TaGW2, TaGW7	Wang et al., 2018, 2019

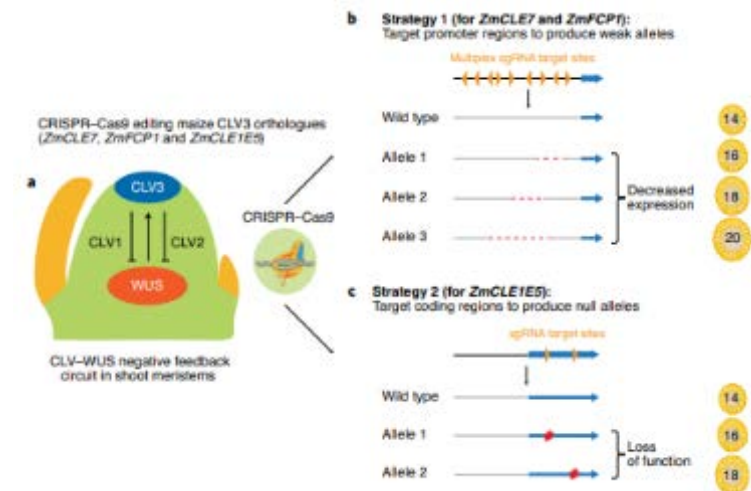


Gao et al. (2020)

## Towards knowledge-driven breeding







Manipulating three important genes in the CLAVATA-WUSCHEL signalling pathway quantitatively enhances grain-yield-related traits in maize.

Qiuyue Chen and Feng Tian



# Genome Editing at CGIAR

- CGIAR is not-for-profit; trusted by partners as “honest brokers” – respects every country’s right to decide if, when, and how to use genome editing.
- Competent scientists in CGIAR, and in partner ARIs plus NARS institutions in the Global South
- Together with partners, CGIAR has a unique network of breeding and phenotyping platforms, useful for proof-of-concept under diverse agro-climatic conditions.
- CGIAR is in a leadership position to provide beneficial technologies to the resource-poor farmers in Africa, Asia and Latin America.
- We all have a moral obligation to enable the option / choice to use the best that science can offer.

	<b>Banana</b>	Disease resistance (BXW, Fusarium wilt, BSV)	3 & 1 <sup>b</sup>
	<b>Cassava</b>	Bacterial Blight Resistance	3
		Safety trait (cyanide-free)	3
		Quality trait (waxy starch)	3
	<b>Rice</b>	Disease resistance (BLB, RHB)	4, 3
		Low arsenic & cadmium & yield	3
		Nitrogen use efficiency, and methane reduction	3
	<b>Maize</b>	Insect resistance (BPH) <sup>a</sup>	2
		Disease and weed resistance (MLN)	4, 1
	<b>Wheat</b>	Durable disease resistance (rusts, mildew)	3
	<b>Potato</b>	Disease resistance (late blight)	2

## Potentially Coming Soon

Cassava: Bacterial blight, Brown Streak virus, Haploid inducers

Bean: Nutritional quality, digestibility

Sorghum: Striga resistance

Maize: Nutrition (low phytic acid, provitamin A), Striga resistance

Wheat: Bread quality (low polyphenol oxidase), nutrition (low phytate), less acrylamide (low asparagine)

Rice: Reduced glycemic index, reduced postharvest loss, hybrid-facilitating traits.

Potato: Heat tolerance

BXW, banana Xanthomonas wilt; BSV, banana streak virus; BLB, bacterial leaf blight; RHB, rice hoja blanca virus; BPH, brown plant hopper; MLN, maize lethal necrosis.

<sup>a</sup>SDN2 editing required; all other current projects are SND1.

<sup>b</sup>**Stage of current development:** 1) Discovery, 2) Proof of concept, 3) Early development, 4) Advanced development, 5) Commercialization.



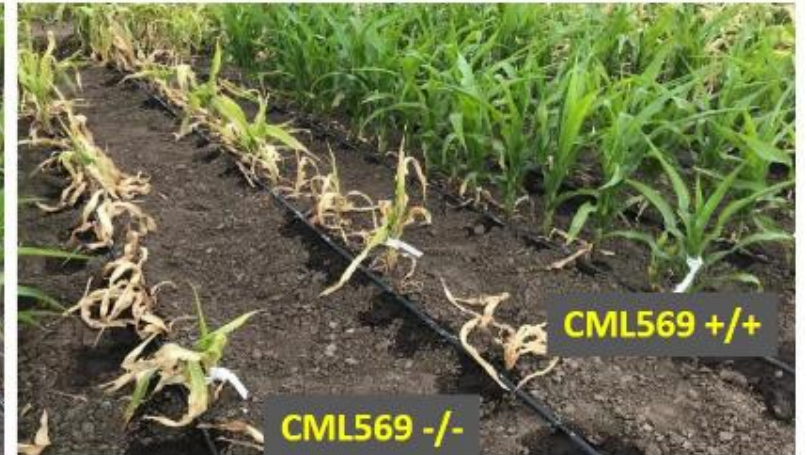
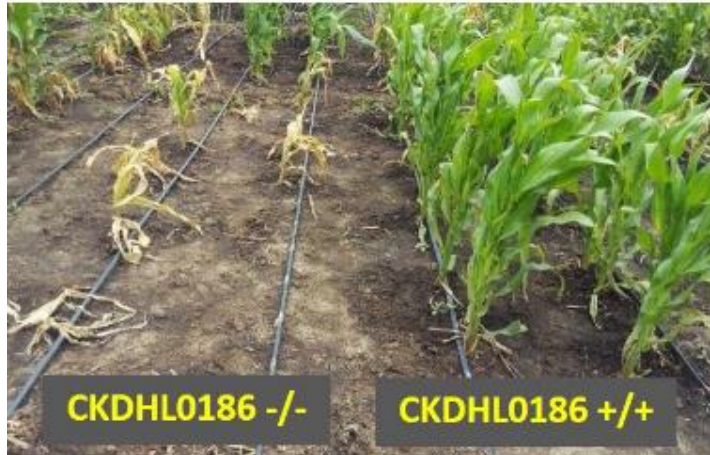
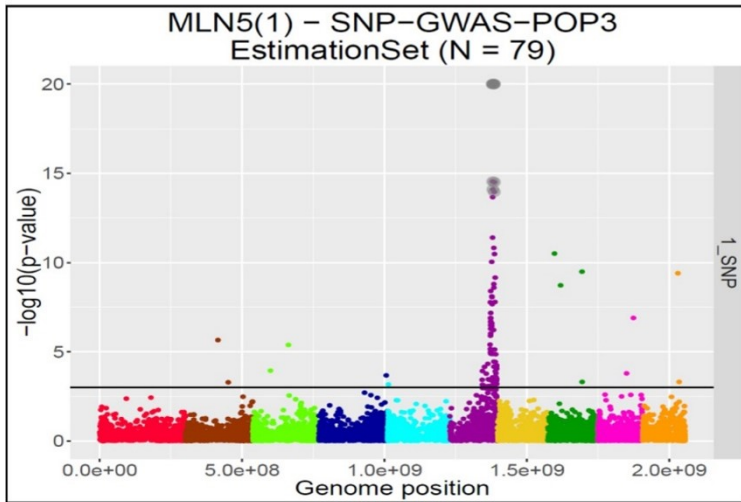
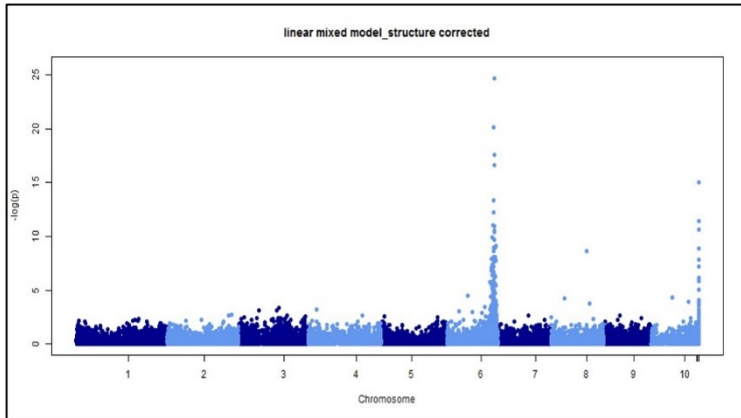
# Maize Lethal Necrosis (MLN) in Eastern Africa



- **MLN first appeared in Kenya in 2011** and then quickly spread to several countries across Eastern Africa, including Uganda, Tanzania, Rwanda, D.R. Congo, and Ethiopia by 2014.
- Losses to maize production in farmers' fields due to MLN in the impacted countries were significant. In Kenya alone, it was estimated to be 0.5 MMT, at a value of **US\$ 180 million** (De Groote et al., 2016).

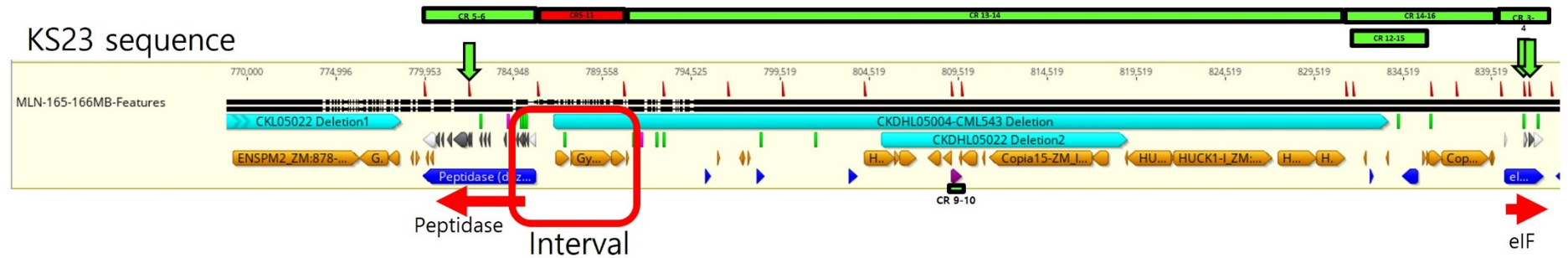


# Effect of KS23-6 allele on MLN Resistance



Marker-assisted backcrossing undertaken by CIMMYT team in Africa to convert **52** elite, drought-tolerant but MLN-susceptible lines into MLN-resistant versions using KS23-6 resistant allele (in 3 years).

# Genome Editing for MLN Resistance



- **15 Editing projects – 14 successful**
  - KS23
  - CML536
- Designs completed in 2019
- Transformations in Jan 2020
- G1 seed ready; planted G2 increase/phenotyping underway
- No foreign DNA in G2 seed

- **Fine-mapping, cloning and editing** the causal genomic region(s) for MLN resistance.
- **Establishing a pipeline to edit for MLN resistance** in MLN-susceptible lines that are parents of CIMMYT-derived commercial maize hybrids in Africa.

# Genome Editing Facility at CIMMYT-Mexico

Testing for altered function



Molecular biology



**CIMMYT's  
Genome Editing  
Facility at Mexico**

Transformation and  
regeneration

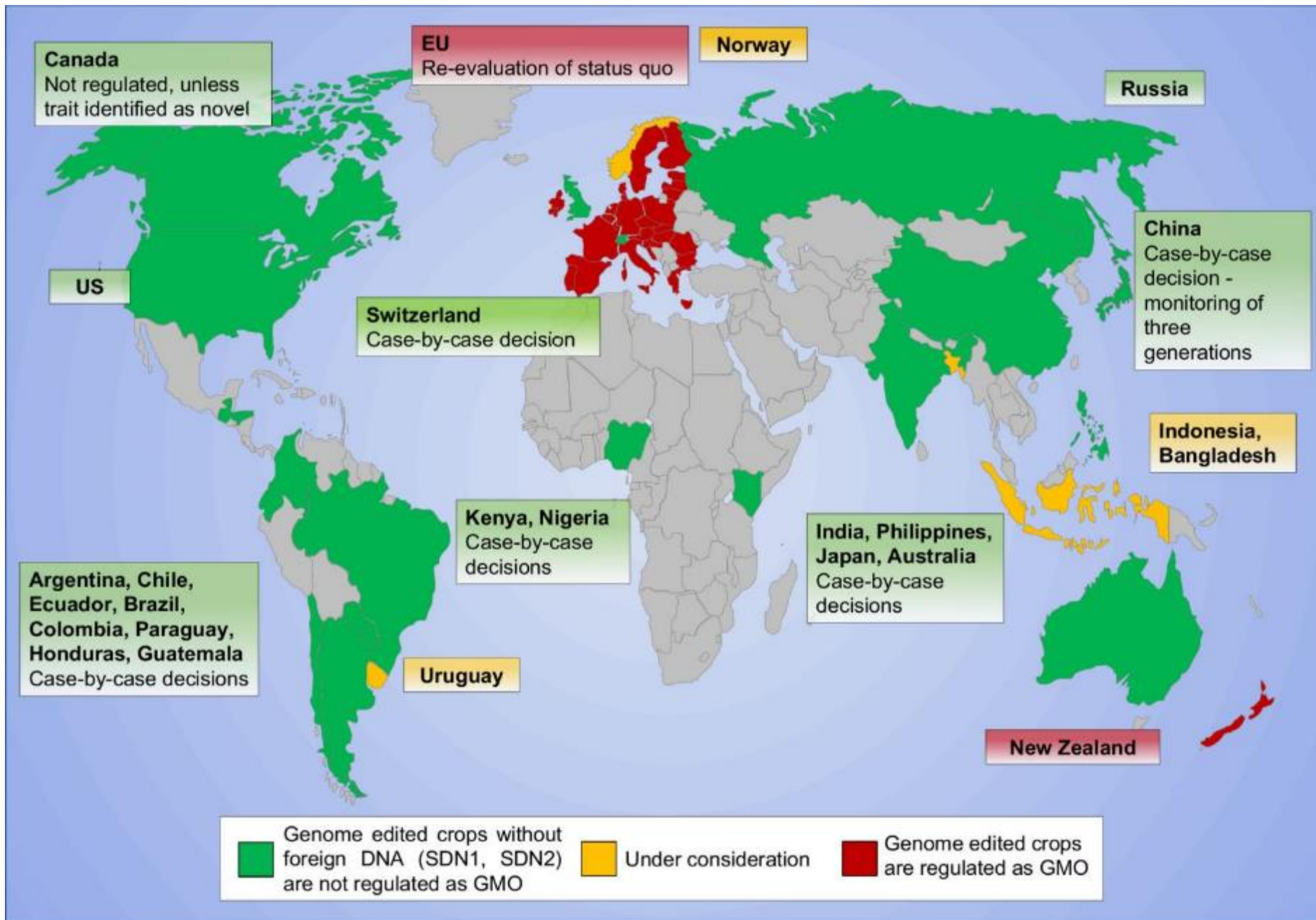


Screening for alterations



# Genome Editing: Regulation Status for Crop Plants

Regulation Status		Countries
Determined: No Unique Regulations	Gene-edited crops that do not incorporate DNA from another species are regulated as conventional plants with no additional restrictions.	USA, Israel, Argentina, Brazil, Ecuador, Paraguay, Chile, Colombia
Lightly Regulated	Some or all types of gene editing are regulated more strictly than conventional agriculture, but not as strictly as transgenic GMOs.	Japan, Canada, Australia
Proposed: No Unique Regulations	Decrees under consideration; Gene-edited crops that do not incorporate DNA from another species would not require unique regulations beyond those imposed on conventional breeding.	India, Norway, Central America, Uruguay
Ongoing Research; Regulations In Development		China, Africa, Russia, Switzerland
Highly Regulated		New Zealand
Mostly Prohibited		UK, EU
Limited Research; No Clear Regulations		Mexico, Ukraine
Prohibited		



Many countries in the Americas and Asia have implemented legislation that regulates genome-edited crops, and now a few African countries are following suit.

# Genome Editing: IPR Landscape

- **At least six parties are fighting for their “piece of the cake” when it comes to the Cas9 foundational patents.**
- **The patent landscape for CRISPR-Cas alone has become highly complex in a period of a few years.** A recent study identified 7427 patent families related to CRISPR filed by 1850 institutes and companies. Out of these, 1232 related specifically to plant modification.
- **Cpf1—an alternative to Cas9**—already gathers 899 patent families in the current landscape.
- Additional relevant patents: (i) delivery of the editing machinery; (ii) efficient generation of plants after editing; and (iii) technologies to enhance HDR-mediated repair (SDN2/3).
- The final landscape will differ from country to country with a strong regional bias. **Depending on the specific use of the Cas9 enzyme four or more licenses may be necessary for a simple edit in a plant.**

The Broad Institute and Corteva Agriscience in 2017 agreed to mutually license interested parties with foundation Intellectual Property for the use of CRISPR-Cas9 in agriculture. They are licensing technology for those developing smallholder farmer uses in developing countries at essentially no cost.

- <https://openinnovation.corteva.com/crispr-cas/>
- <https://www.broadinstitute.org/news/duPont-pioneer-and-broad-institute-join-forcesenable-democratic-crispr-licensing-agriculture>.

# Genome Editing: Some Concerns

Concern	Explanation
<b>Non-target edits and their possible effects</b>	<ul style="list-style-type: none"> <li>▪ Genome editing may cause random, non-target mutations at frequencies similar or less than natural mutations. Non-target mutation frequency is 1000 times greater when using chemical or radiation mutagenesis.</li> <li>▪ As for every new variety, extensive field trials will confirm the competitiveness of genome-edited varieties.</li> </ul>
<b>Genome editing avoids natural barriers to reproduction</b>	<ul style="list-style-type: none"> <li>▪ Same as chemical mutagenesis, genome editing avoids sexual reproduction steps that might eliminate unfavorable mutations.</li> <li>▪ Genome editing does not present substantially different risks than those for well accepted chemical mutagenesis methods</li> </ul>
<b>Inadequate stewardship by researchers</b>	<ul style="list-style-type: none"> <li>▪ Researchers must implement responsible stewardship during genome editing research and assure that the final varieties are free of transgenic elements.</li> <li>▪ Possible that emerging methods will soon enable genome editing without using an intermediate transgenic step.</li> </ul>





# Genome Editing: Some Concerns

Concern	Explanation
<b>Technology may lead to greater inequity between small and large farmers</b>	<ul style="list-style-type: none"> <li>▪ The technology per se is relatively affordable, so it is being applied in many crops and for many traits of interest to resource-poor and wealthy farmers and consumers.</li> <li>▪ The more complex and costly the regulatory framework, the more we will exclude resource-poor farmers and orphan crops. If we demand product isolation or labeling, this adds costs that can only be affordable to commercial/large producers.</li> </ul>
<b>Ambiguity about IP rights</b>	<ul style="list-style-type: none"> <li>▪ Important to resolve the ongoing IP uncertainties, preferably keeping the fundamental processes in the public domain. Needing to pay royalties or lose access to the technology discourages many institutions in the LMICs who would otherwise avail it.</li> </ul>
<b>Insufficient institutional capacity in the public sector to fully avail genome editing technology</b>	<ul style="list-style-type: none"> <li>▪ Translating and delivering products from the laboratory to farmers and consumers requires numerous, complex capabilities.</li> <li>▪ New partnerships and institutional models emerging in Global South that can support the use of genome editing.</li> <li>▪ Support to public institutions must be holistic and sufficient to assure their capacity to deliver the benefits of genome editing to those who wish to avail them.</li> </ul>



# Detection of genome-edited products may not be straightforward..

- Detecting a certain alteration does not automatically mean that this alteration has been introduced with genome editing. It could have also occurred spontaneously, or it could have resulted from conventional breeding.
- A substantial amount of additional information is necessary to enable the determination of the probability that the presence of the alteration is due to the presence of genome-edited plant material.
- Tracing genome-edited products through both internal markets and across external borders would be challenging.

In Vitro Cellular & Developmental Biology - Plant (2021) 57:595–608  
<https://doi.org/10.1007/s11627-021-10214-z>

SPECIAL ISSUE ON GENOME EDITING

## Detection of genome edits in plants—from editing to seed

Raymond D. Shillito<sup>1</sup>  • Sherry Whitt<sup>1</sup> • Margit Ross<sup>2</sup> • Farhad Ghavami<sup>3</sup> • David De Vleeschauwer<sup>4</sup> • Katrijn D'Halluin<sup>4</sup> • Annelies Van Hoecke<sup>4</sup> • Frank Meulewaeter<sup>4</sup>

*Frontiers in Plant Science* (2019)

## Detection and Identification of Genome Editing in Plants: Challenges and Opportunities

Lutz Grohmann<sup>1\*</sup>, Jens Keilwagen<sup>2†</sup>, Nina Duensing<sup>1</sup>, Emilie Dagand<sup>1</sup>, Frank Hartung<sup>2</sup>, Ralf Wilhelm<sup>2</sup>, Joachim Bendiek<sup>1</sup> and Thorben Sprink<sup>2</sup>



# Will Genome Editing become a widely used, disruptive technology?

## Two Possible Scenarios

### Scenario 1: “Democratization”

- Breeding-by-editing becomes a standard and is used on many crops in many countries.
- In 10 years >50% of all varieties comprise at least one genome-edited characteristic.
- In 20 years essentially all commercial varieties will comprise characteristics obtained by genome editing, many will comprise 5 or more of such characteristics.

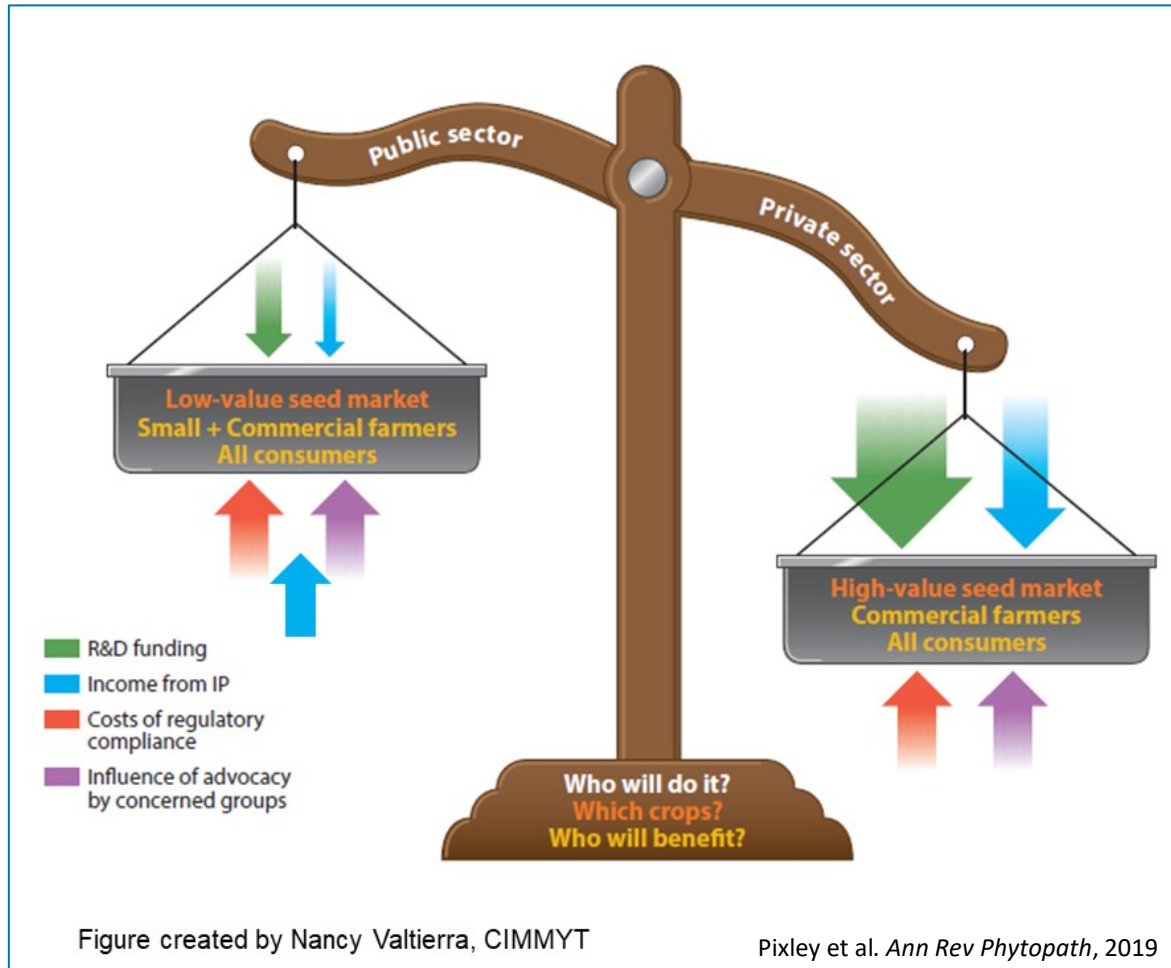
### Scenario 2: “The World As We Know It”

- An unfavorable regulatory framework with high regulatory and stewardship costs.
- An unfavorable “IP framework” which limits the use of NBTs to a few institutions.
- Genome editing remains complex and regulated, and in consequence limited to a few major crops (i.e., maize, rice, soybean) and “controlled by a few multinational companies”.

Need to bridge the gap between the capacity and needs of small-scale producers, national food security strategies, and breeding efforts of public and private sectors.

Source: Kock MA (2021)

# Who should benefit from a technology, and how should this happen?



## Critical Qs

1. Who should have the opportunity to benefit?
2. Who should decide this?
3. How should we proceed forward?



