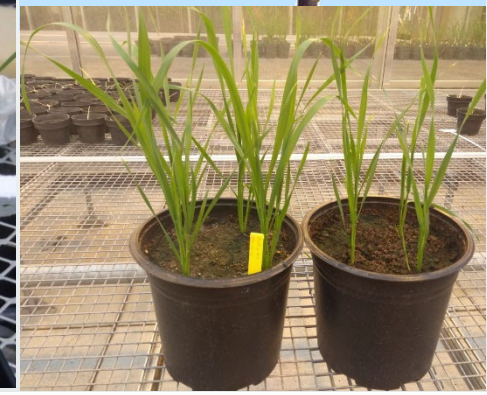




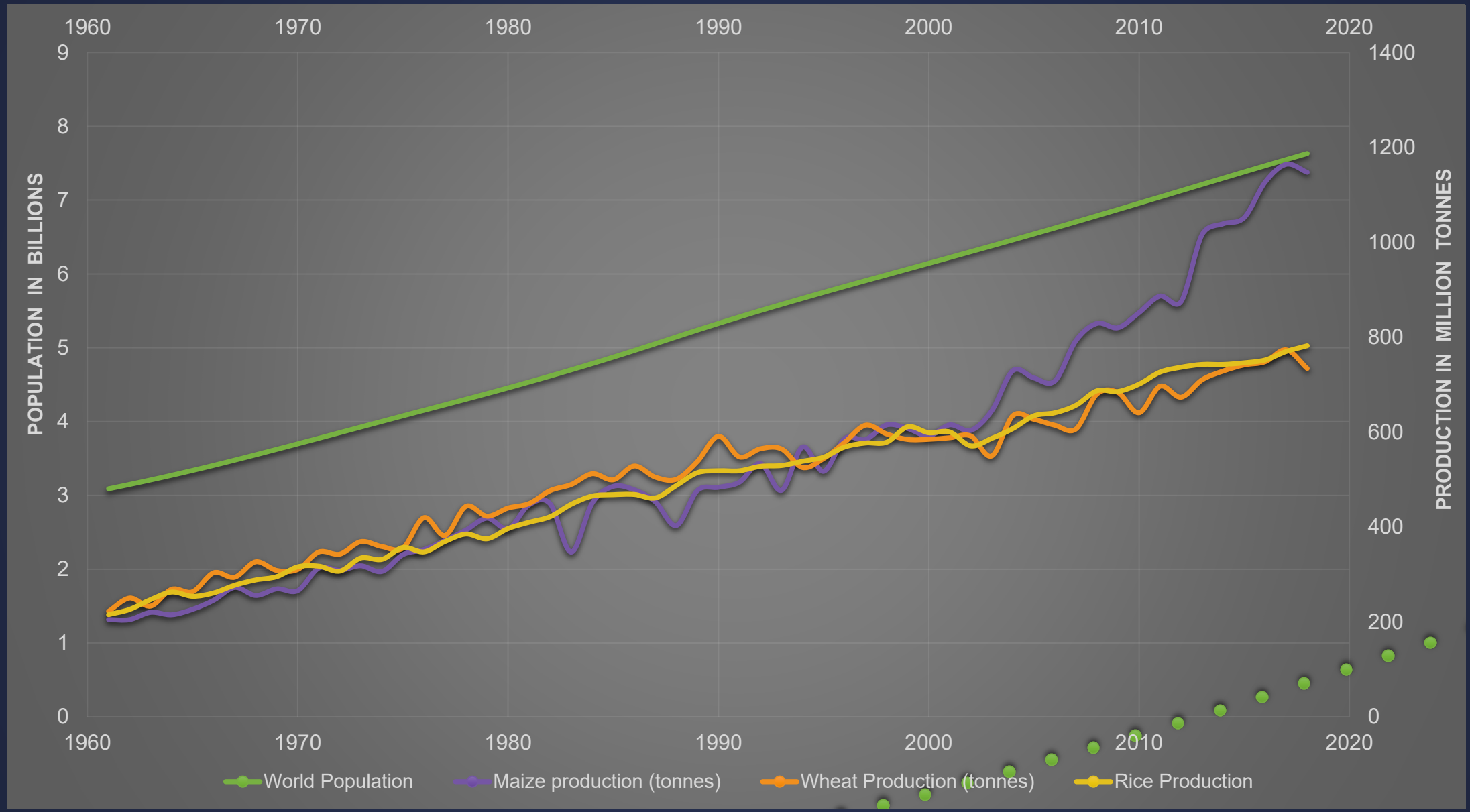


Accelerating cereal breeding through genome editing

Akshaya K Biswal
Scientist, Plant Transformation and Tissue Culture
International Maize and Wheat Improvement Center (CIMMYT)

World Population growth Vs Crop Productivity



Source: http://www.fao.org/faostat/en/#data/QC

Past performance is no guarantee of the future!

The productivity can be challenged by :

- Resistance development among current insect pests
- Outbreak of new pathogens / diseases
- Effects of climate change

Genetic engineering for crop improvement

- Genetic engineering
  - Commercial Glyphosate-resistant corn – 1998
  - 70% of corn in USA
    - Challenges
      - Consumer acceptance of transgenic crops
      - Regulatory stringency
      - Random transgene integration
        - Marker-free transgenics

How to overcome today's agricultural hurdles?

… we should adopt a model that combines the best features of transgenic technology with those of organic and conventional farming…

- Site Specific Nucleases(SSN) for Gene editing
  - Is it a viable alternative for cereal crop improvement?

Outline

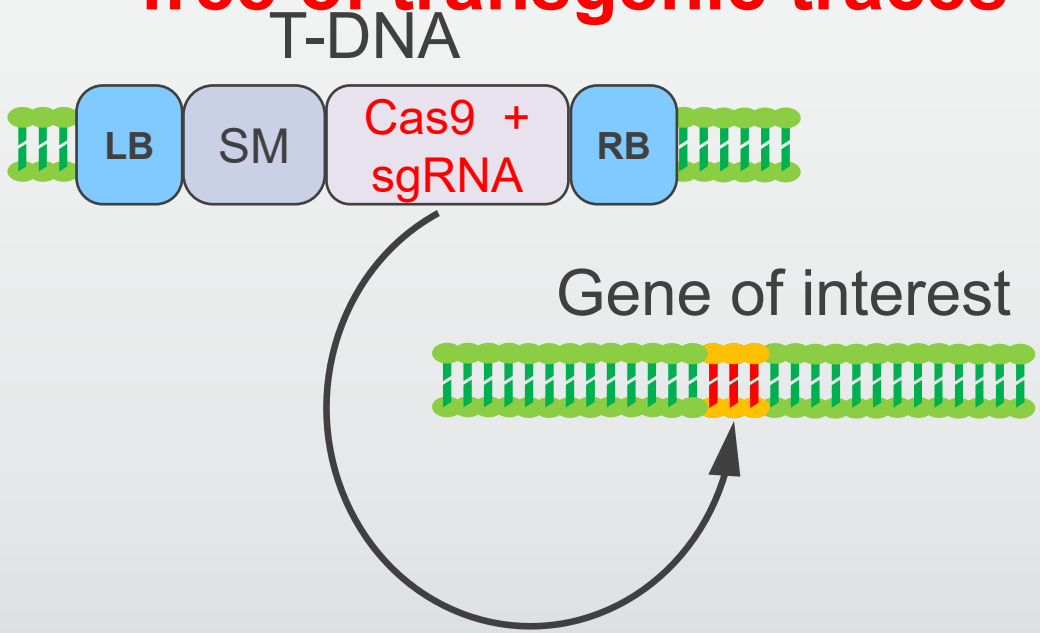
- Genome editing
  - A brief technical overview
  - Promises for crop improvement
    - Herbicide tolerance
    - Disease resistance
    - Abiotic stress tolerance
    - Biofortification and human health
    - Boosting breeding efforts with CRISPR
  - Challenges
  - Regulatory status

“Cut & paste” for Genetic Modification

Genome Editing: Precise modifications at a specific locus

The T-DNA insertion only **delivers** the tool that makes the product

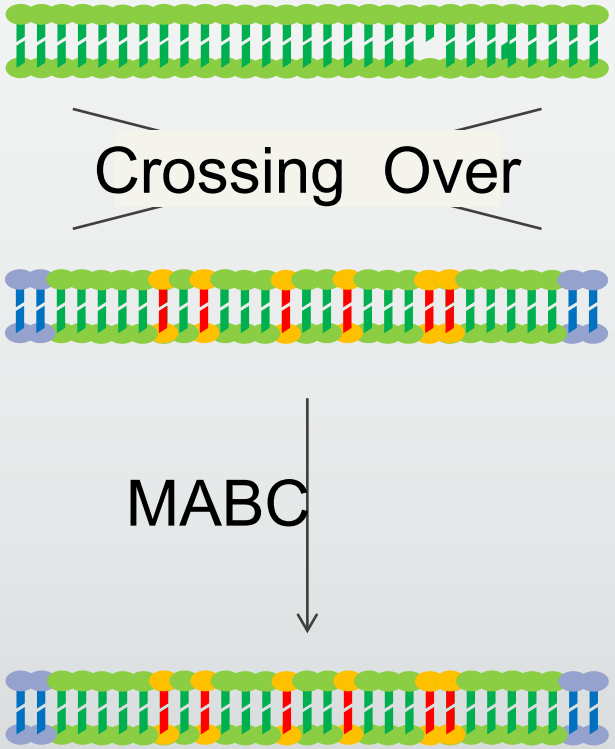
free of transgenic traces



Introgression without linkage drag

7 - 12 Years

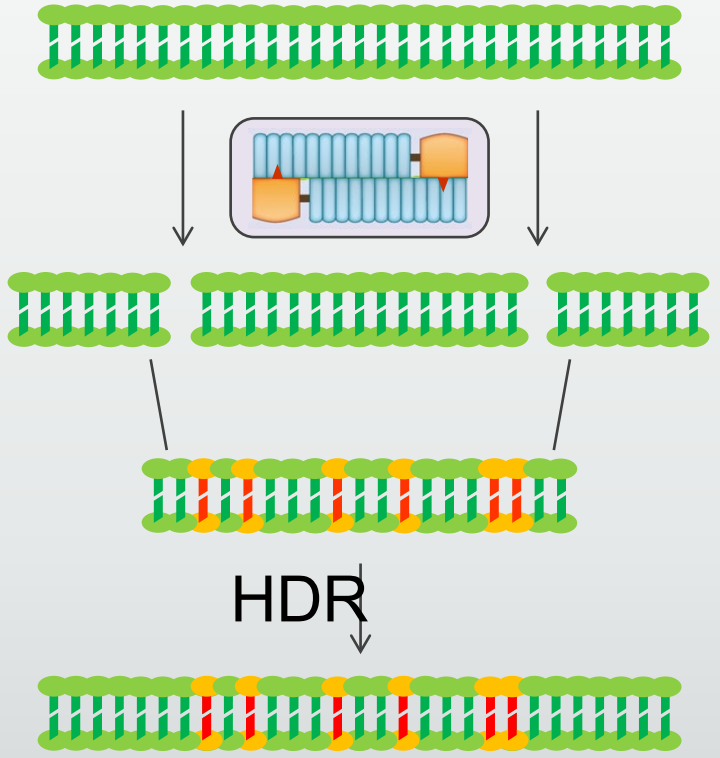
Breeding Equivalent:
QTL Introgression;
Wide Hybridization



=> Near Isogenic Lines (NILs)

Vs

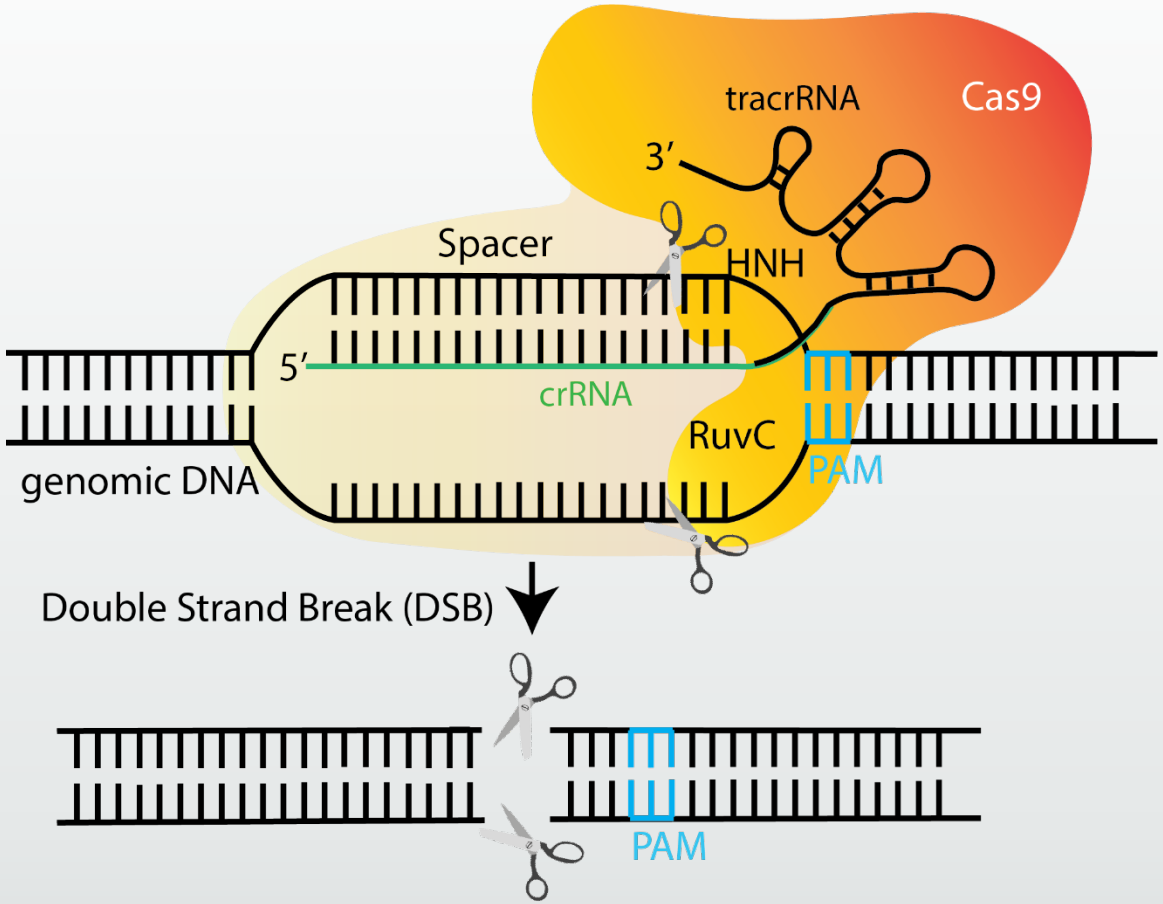
Genome Editing:
Insertion via homologous recombination (HR)

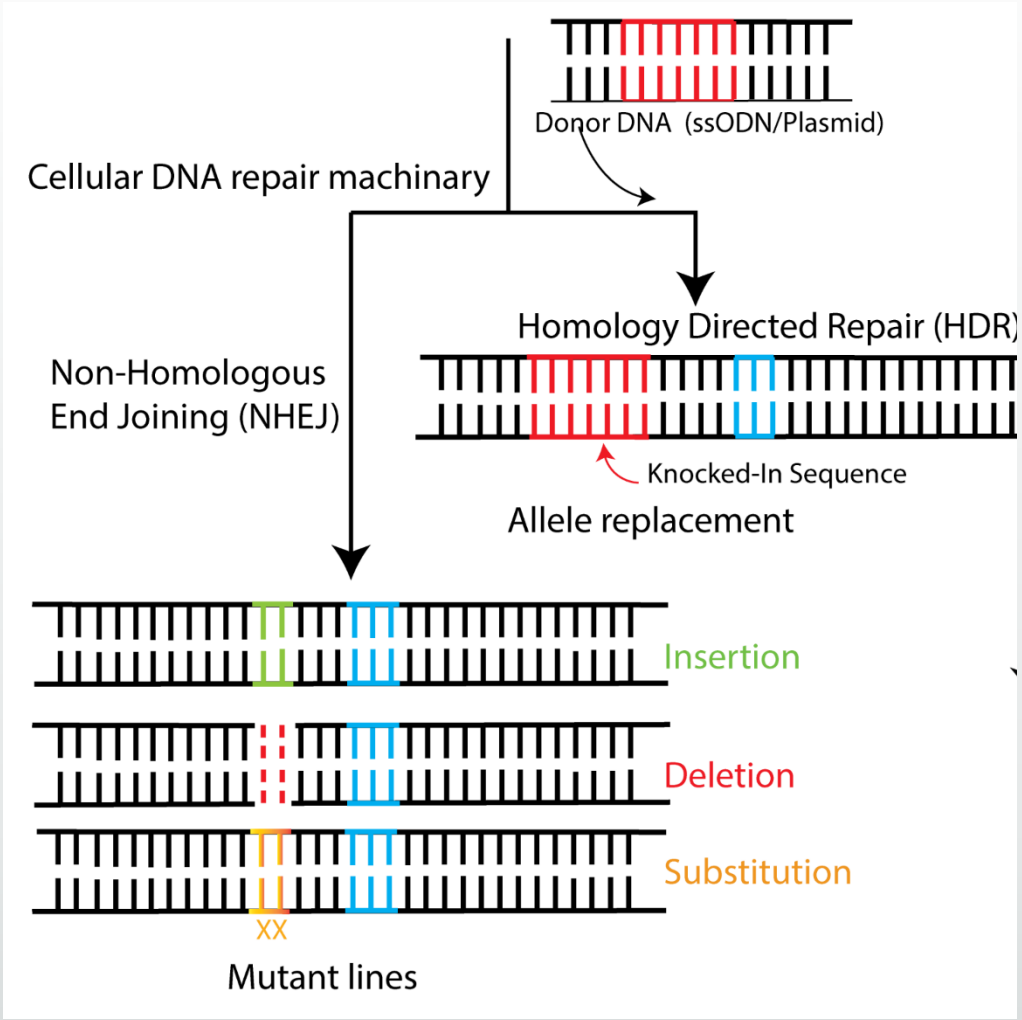


=> Allele replacement

1 - 2 Years

Scheme of gene editing





Biswal et al. 2019

The prospective of Gene Editing

- Herbicide tolerance
- Disease resistance
- Abiotic stress tolerance
- Biofortification and human health
- Boosting breeding efforts with CRISPR

CRISPR can convert existing genes to herbicide tolerance

- Agrobacterium EPSPS gene has been used for glyphosate resistance
- Only two point mutations can convert existing genes resistant to glyphosate

```
Maize QLFLGNAGTAMRPLTAAVTAAGGNA
Wheat KLFLGNAGTAMRPLTAAVVAAGGNA
                  ↑     ↑
                  I     S
```

- Plants can be non-transgenic

Yu et al., Plant Physiol. 2015, Svitashev et al., Plant Physiol. 2015

Disease resistance

- Direct grain yield losses for rice, wheat and maize due to biotic stresses ranges between 20-40%
- Toxic secondary metabolites
- Unavailability of resistant germplasm for a variety of pathogens
- Poor combining ability among resistance donors and cultivars
- Two mechanisms of disease resistance :
  - Single R-gene mediated resistance that is race-specific and
  - broad-spectrum resistance mediated by mutant alleles of the host susceptibility factors or genes.

Some of the major economically important diseases include:

Wheat:

- Powdery mildew, Wheat RUST, Wheat blast and Fusarium Head Blight (FHB).

Maize:

- Maize lethal necrosis (MLN) and Fusarium ear infection, Tar Spot Complex

Rice:

- rice blast and sheath blight

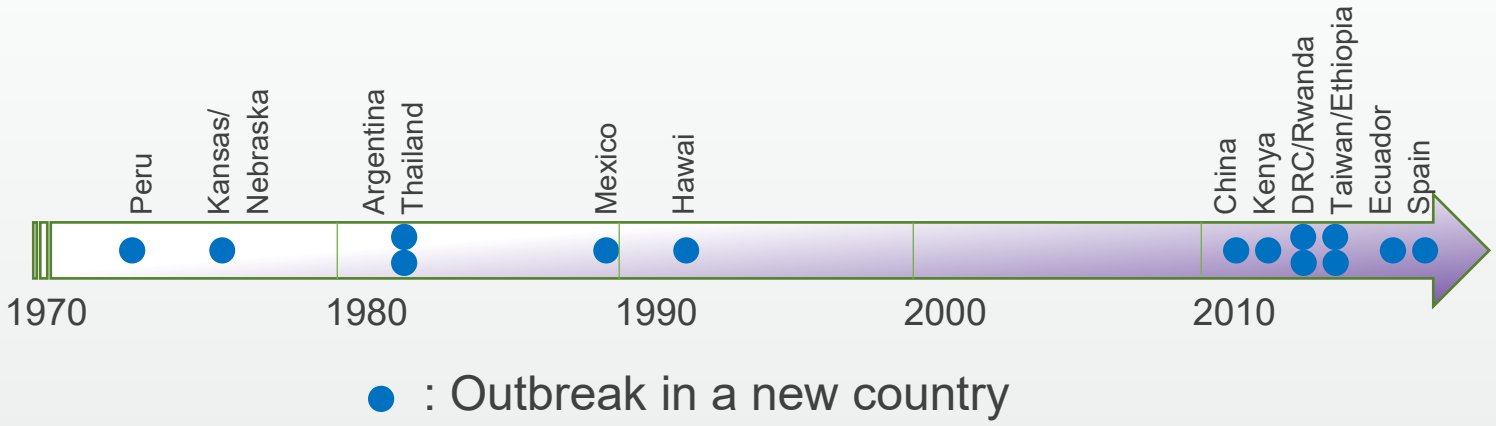
Maize lethal necrosis (MLN): A rapidly emerging viral disease

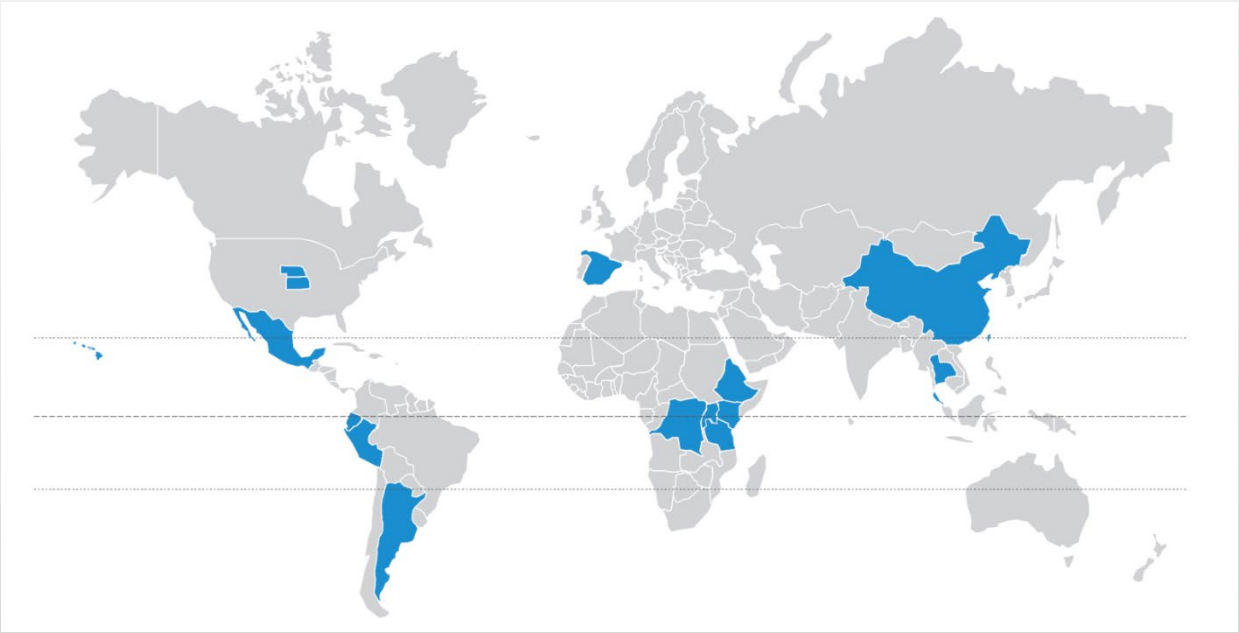
Coinfection of

Maize chlorotic mottle virus (MCMV)

+

Potyvirus (sugarcane mosaic virus / maize dwarf mosaic virus / wheat streak mosaic virus)



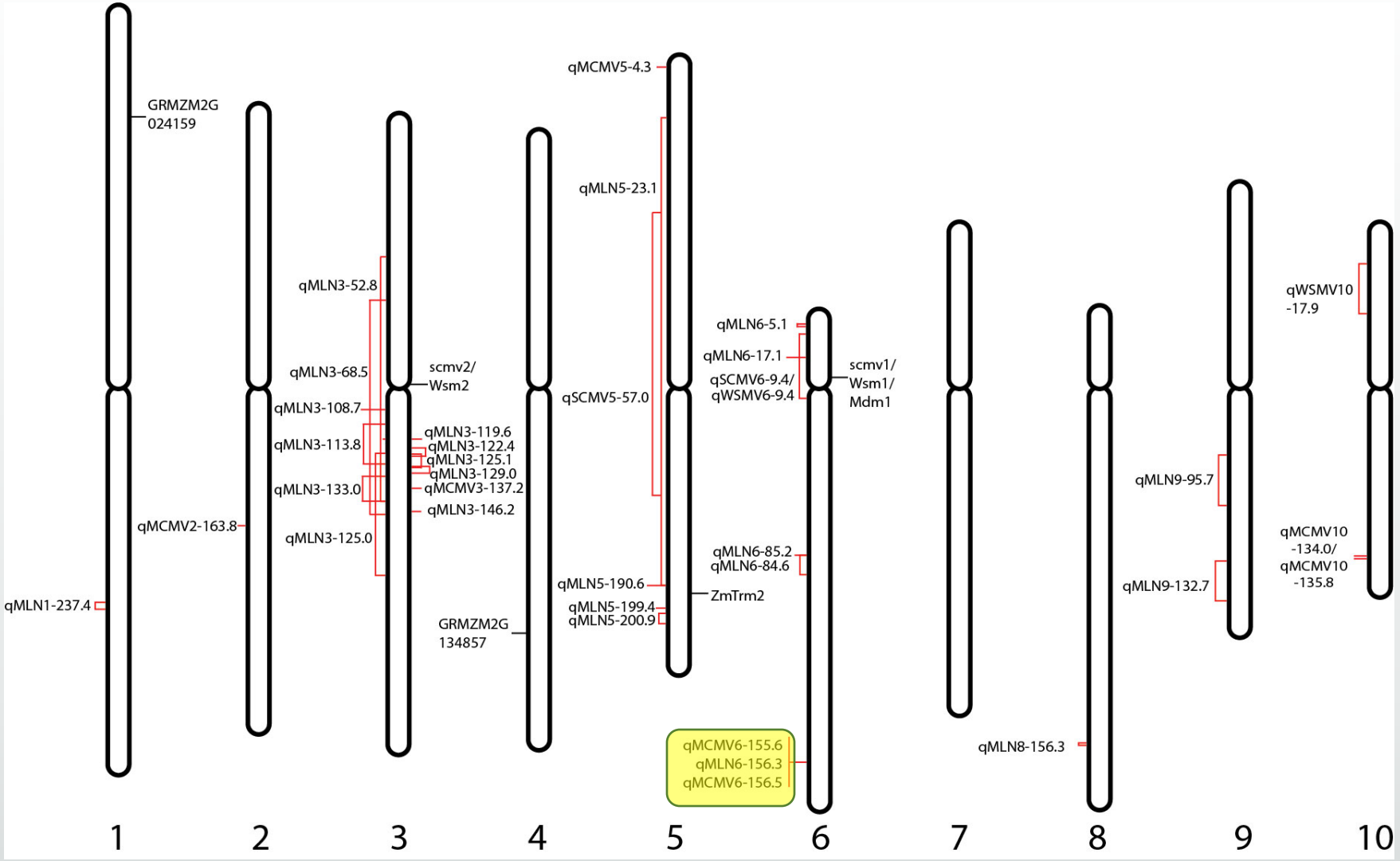


Using CRISPR to develop MLN resistance: 2 ways

1. Targeting viral genome
   - A highly efficient GM approach for viruses

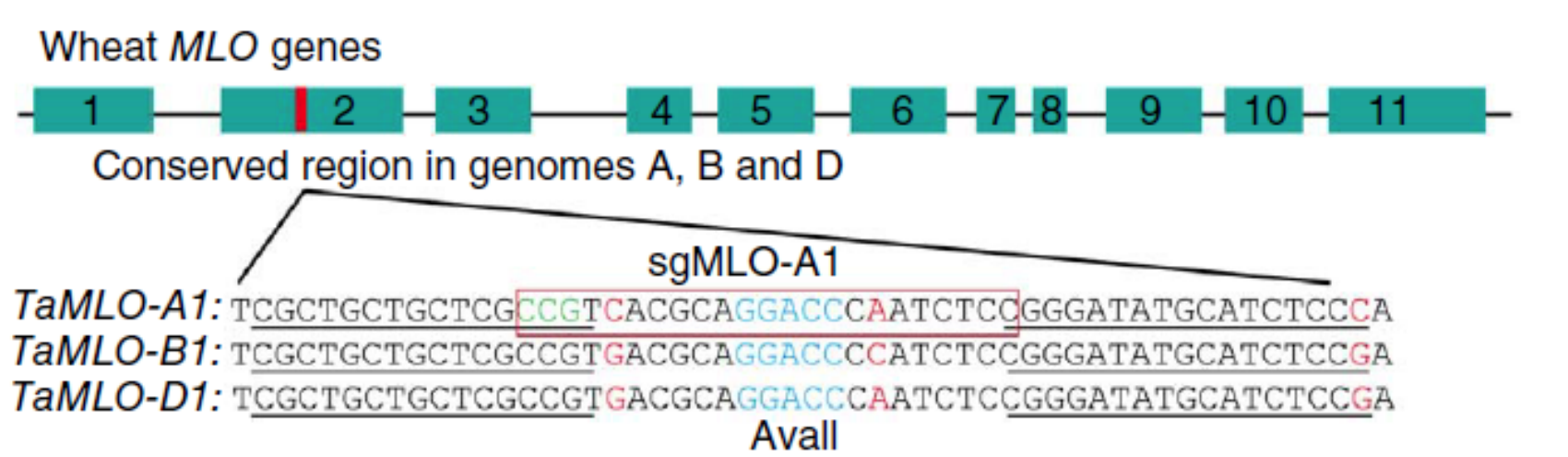
2. Targeting susceptibility factors
   - Transgene free approach for RNA viruses
   - Example: Initiation factors (eIF4E) for developing virus resistant cucumber plants

Targeting susceptibility factors for maize MLN resistance



Powdery mildew in wheat

- Yield losses may be as high as 40%
- Three redundant TaMLO susceptibility genes



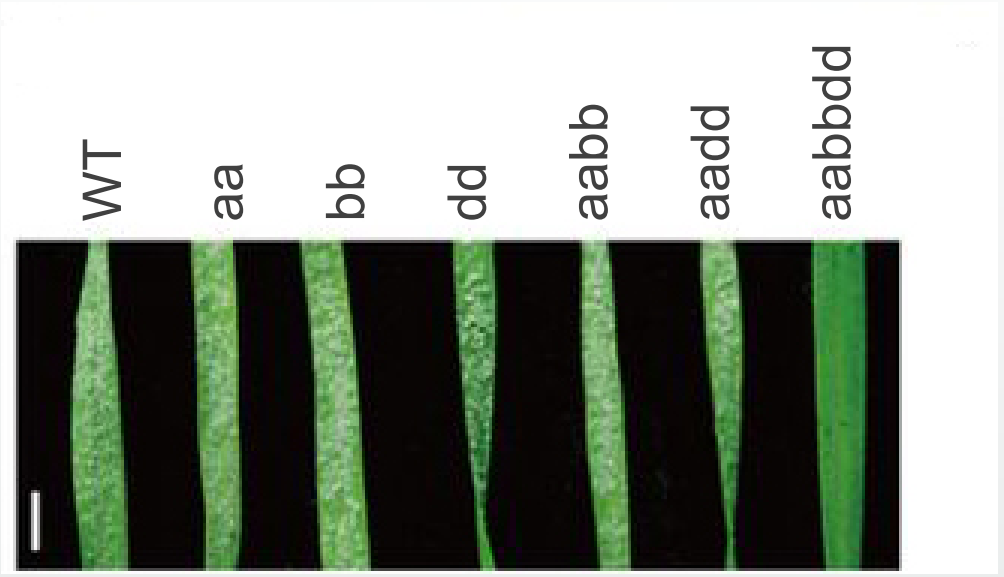
- CRISPR is the technique that can be applied to knock out all three genes
- This is an easy fix - Single gRNA can knock out all three genes

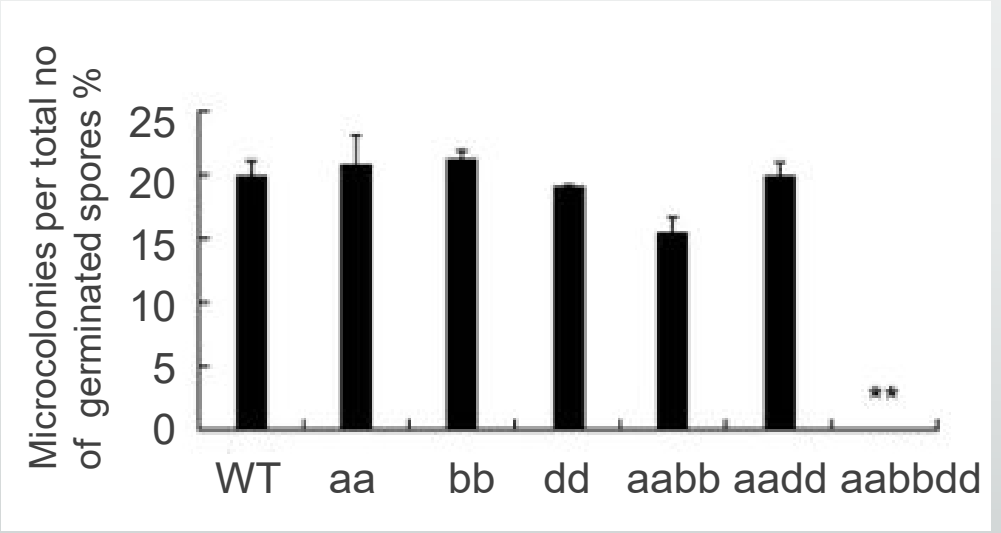
Wang et al., Nat Biotech 2014

Gene editing solved gene redundancy issue for Powdery mildew

TALEN and CRISPR mediated gene knock out of all three genes –

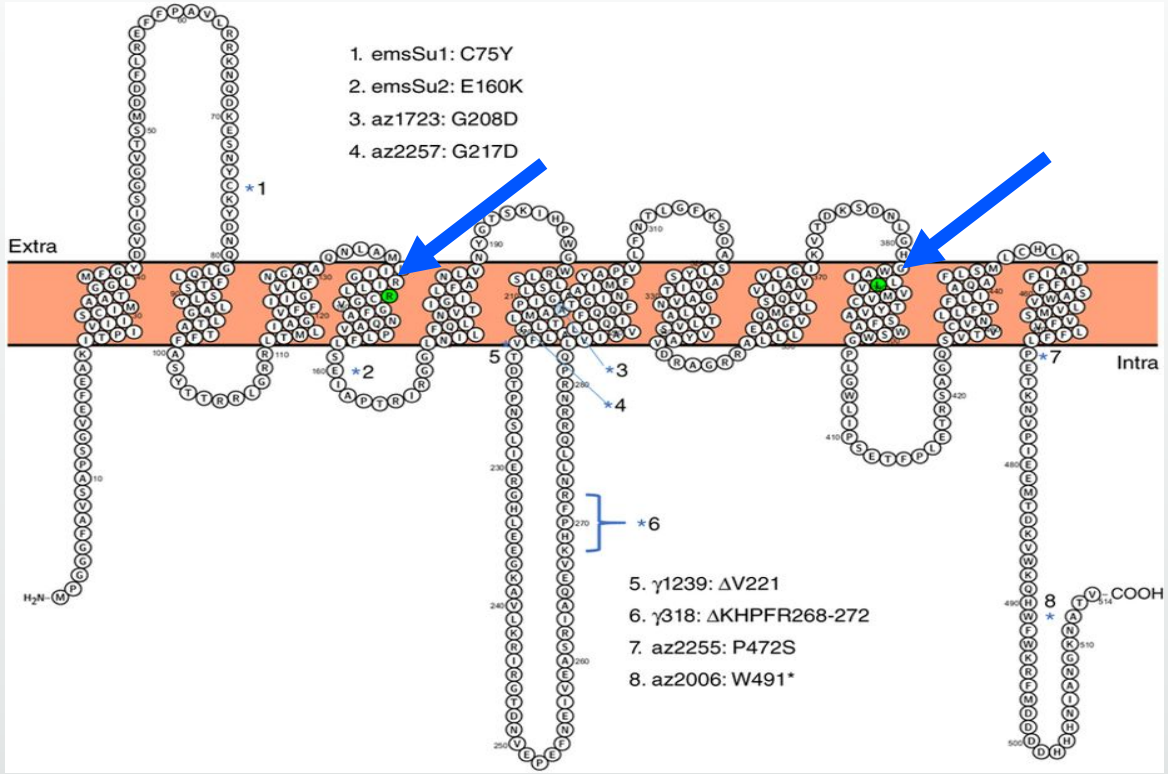
- No macroscopic infection phenotypes
- Heritable nature of resistance

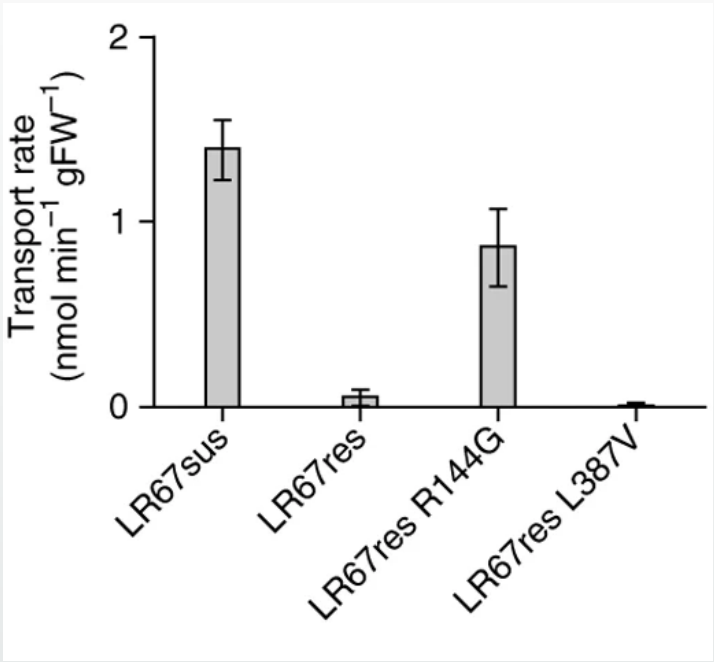




Wang et al., Nat Biotech 2014

Single mutation in Lr67 by CRISPR can provide resistance to Wheat rust

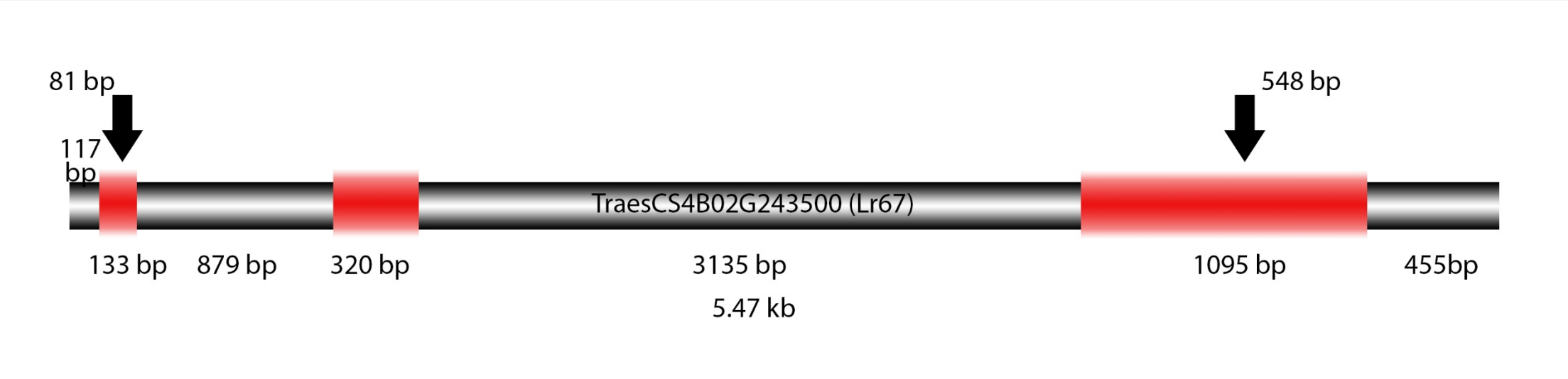




- Alterations to wheat R gene Lr67 gene can provide durable resistance from broad group of fungal pathogens

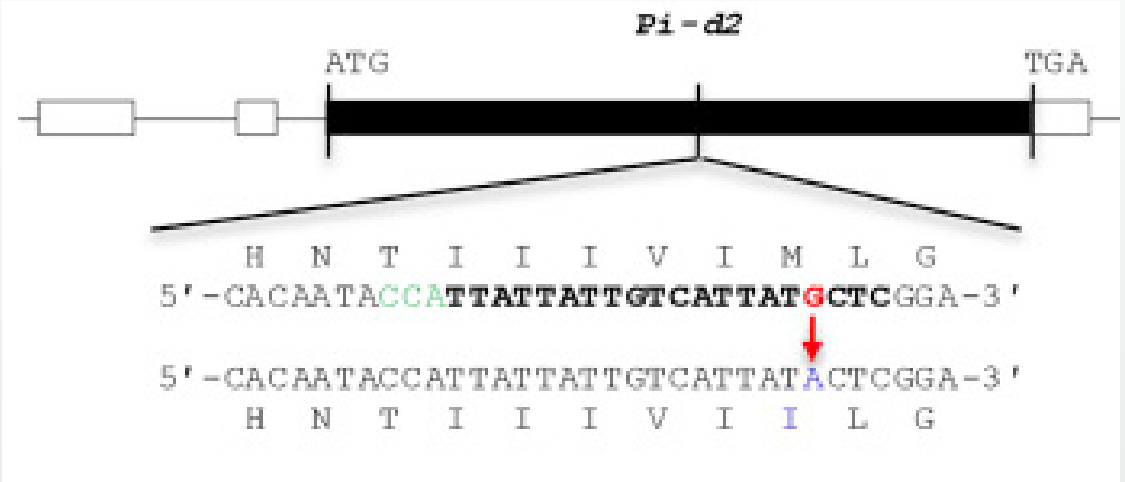
Moore et al Nat Genet 2015, White & Frommer. Nat. Gen. 2015

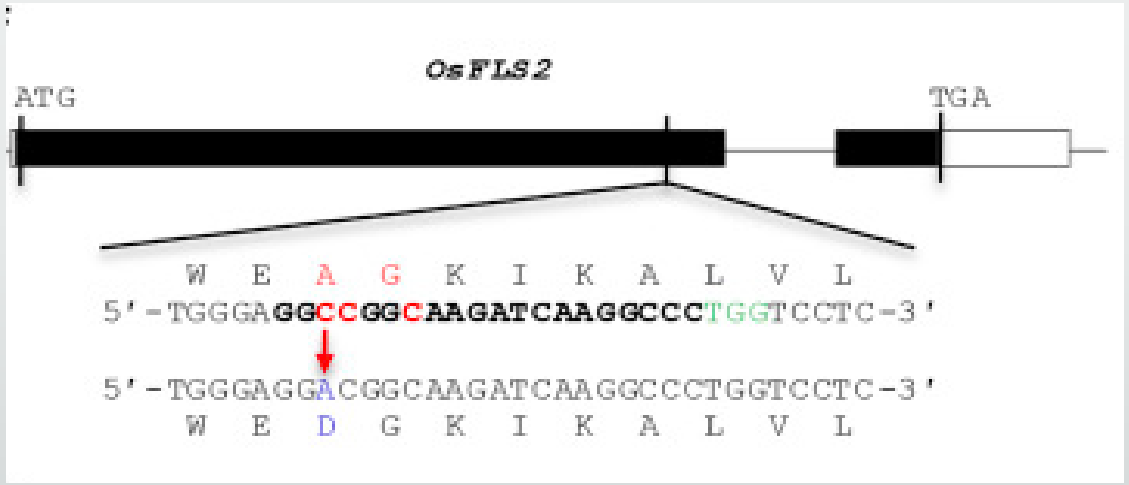
Scheme of editing Lr67 gene for wheat rust resistance



Improving disease resistance through base editing

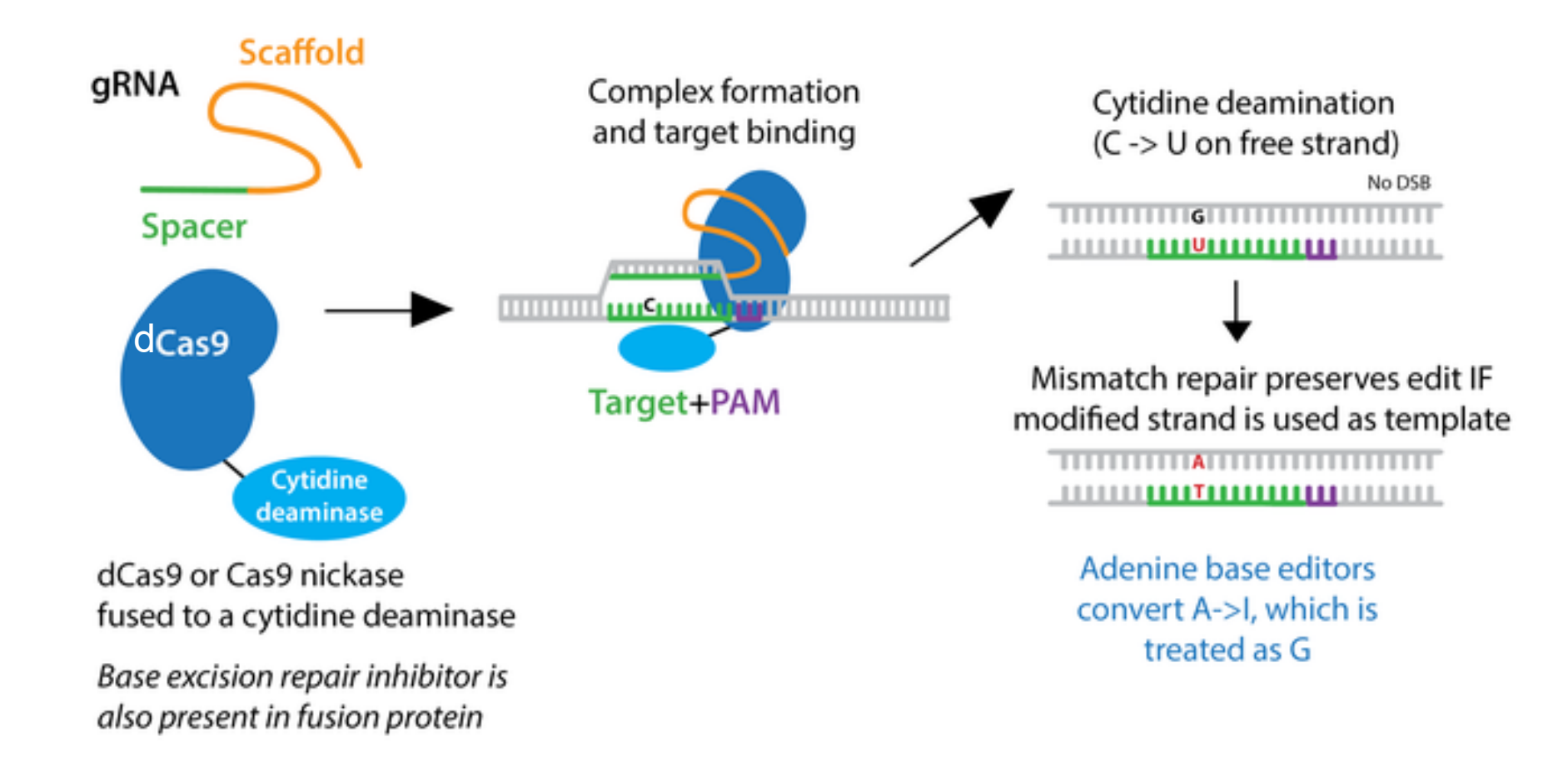
- Most of plant diseases occur due to change in single base in the coding sequence
- A hyperactive human activation induced deaminase (hAID) Mutant was employed to edit rice blast R gene Pi-d2 and also FLS2 for resistance to bacterial pathogens





Ren et al 2018

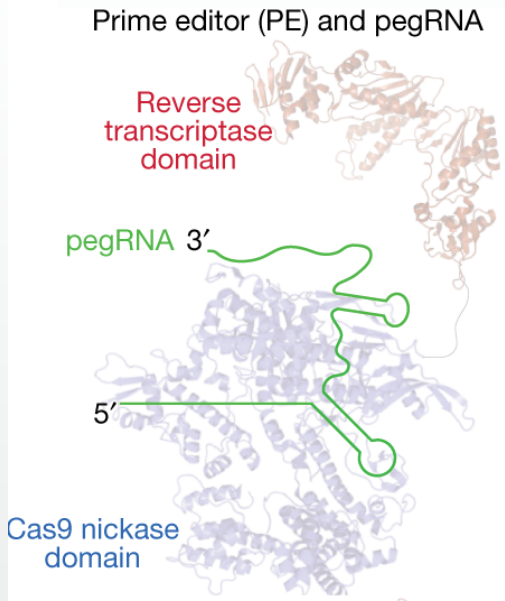
What is Base editing?

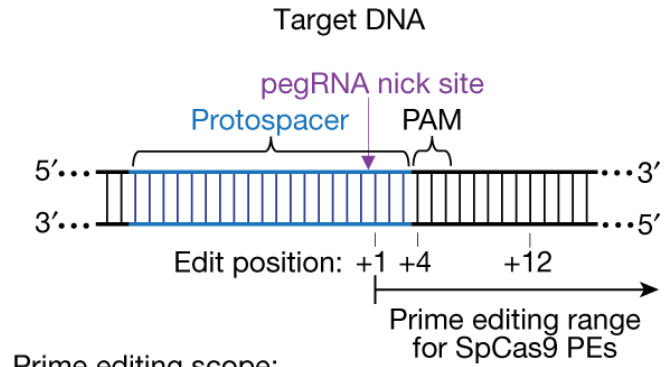


- Generate artificial diversity
- Generate high frequency random alleles of any gene

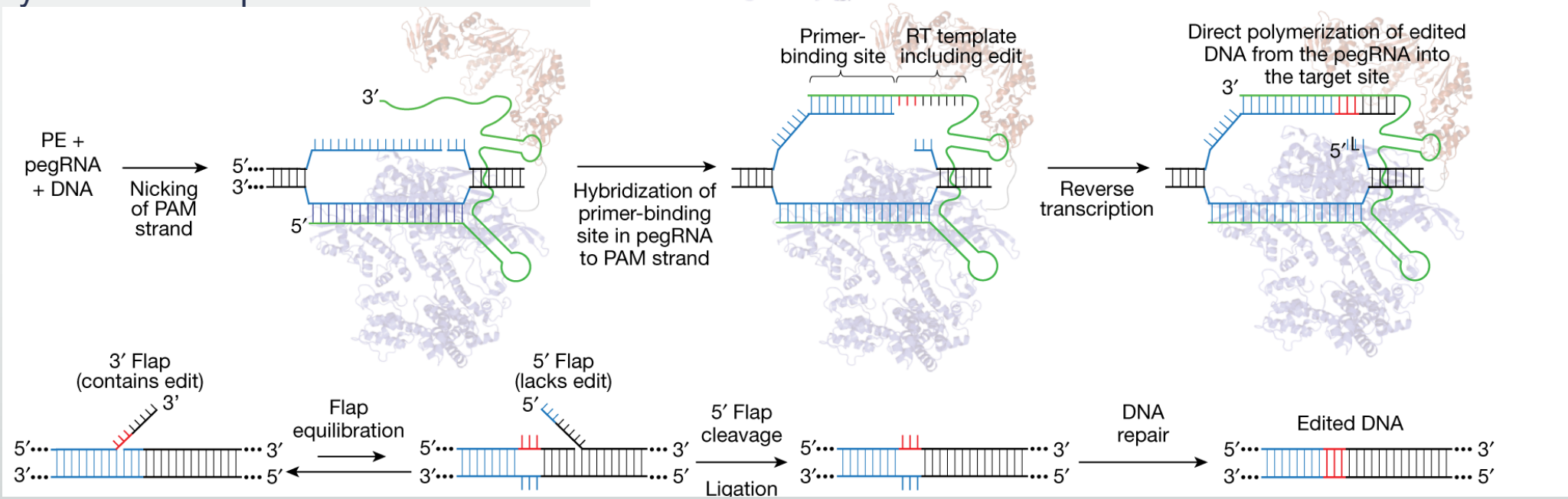
Prime editing

- Precise DNA edits including
  - generation of “indels”
  - replacement of individual bases in all possible combinations
- No need of
  - double-strand breaks (DSBs) or
  - co-delivery of donor templates



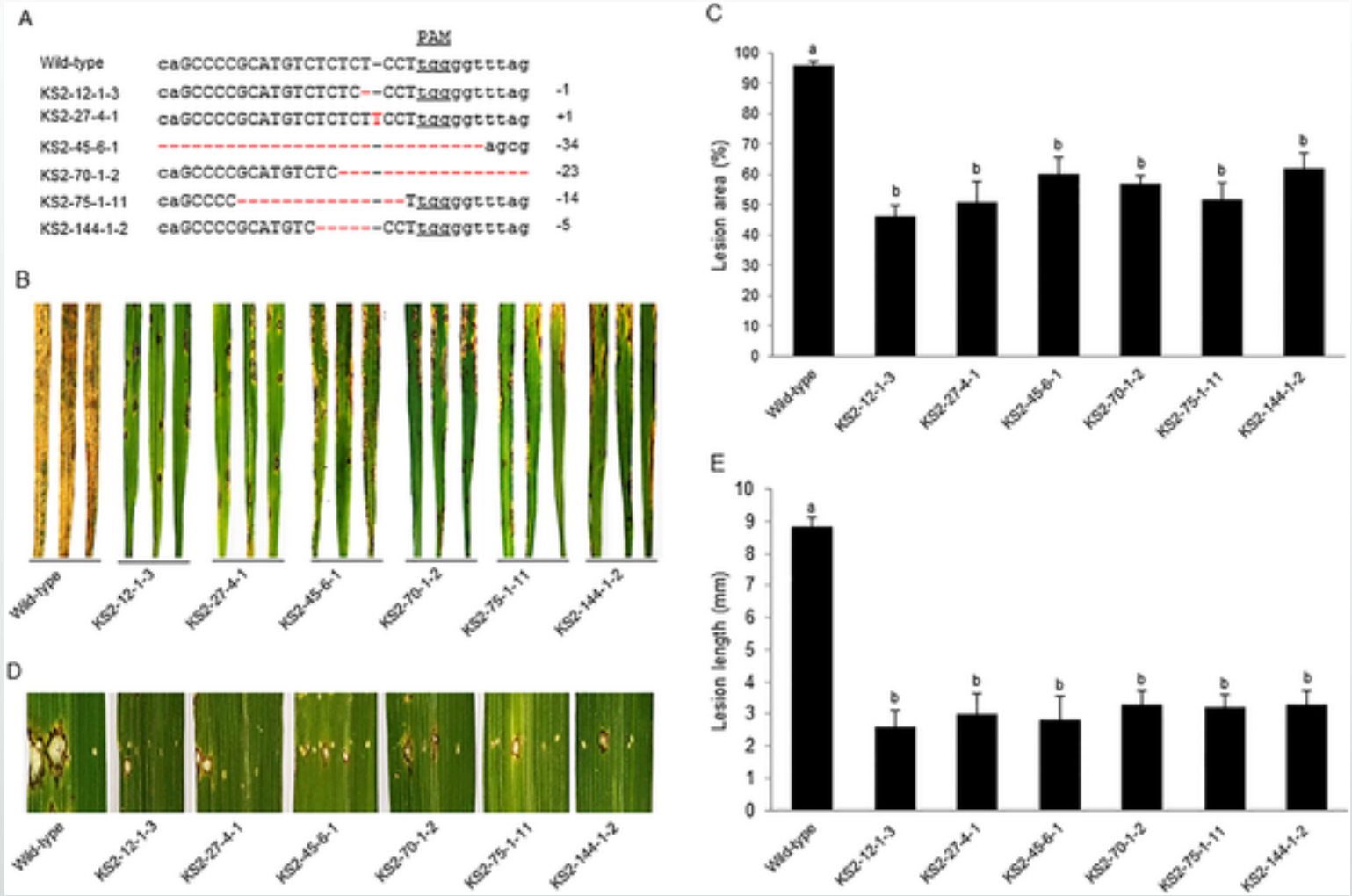


Prime editing scope:
All 4 transition point mutations
All 8 transversion point mutations
Insertions (1 bp to ≥ 44 bp)
Deletions (1 bp to ≥ 80 bp)
Combinations of the above



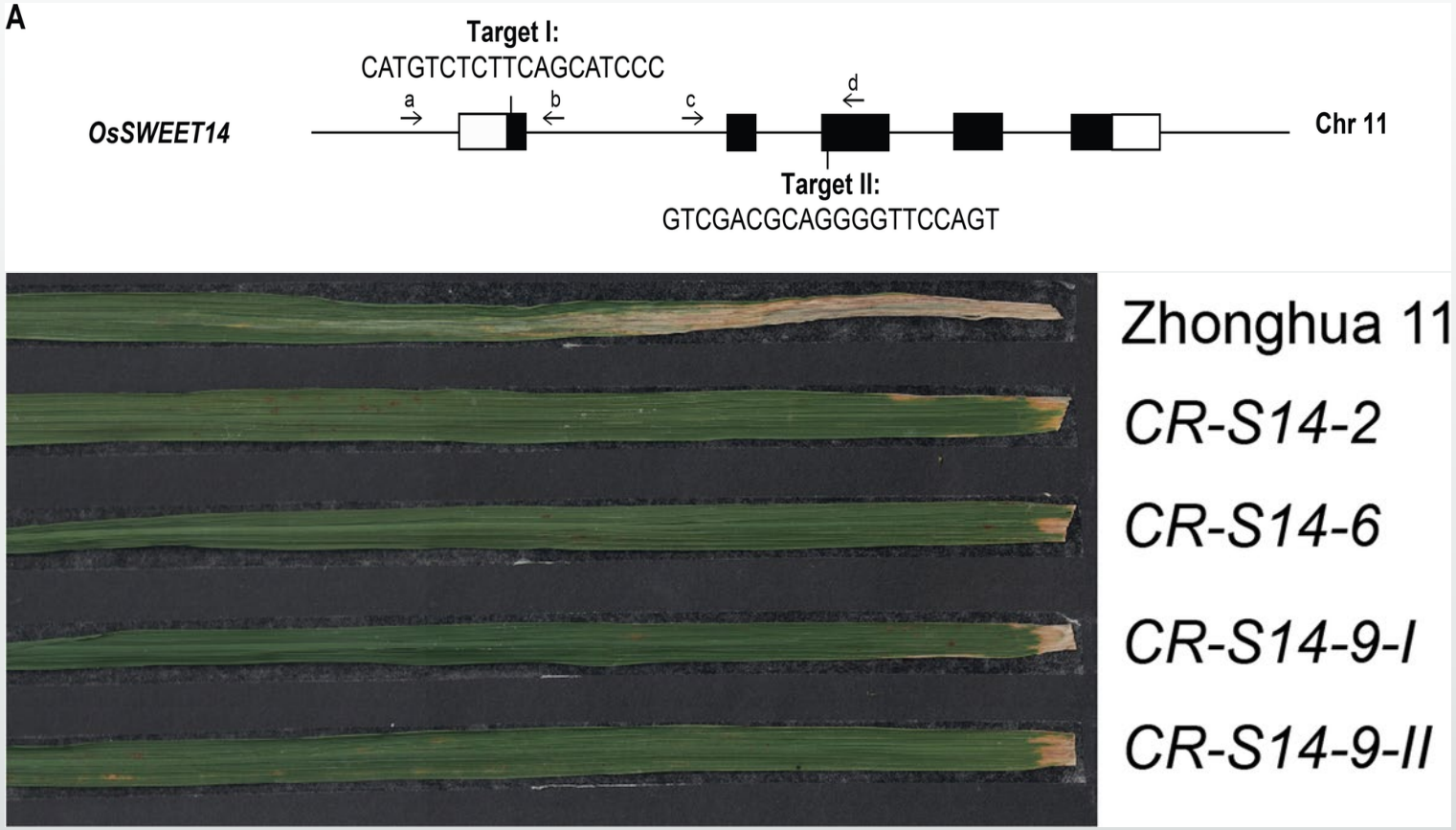
Enhanced Rice Blast Resistance by CRISPR/Cas9-mediated Mutagenesis

Knocking out of an ethylene responsive factor *OsERF922* enhanced the resistance against rice blast disease

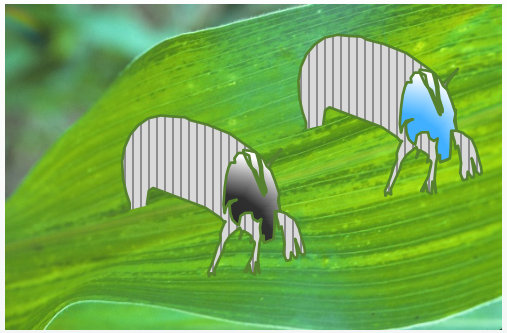


CRISPR/Cas9-mediated mutation of *OsSWEET14* confers resistance to BLB

- Six-week-old rice plants were inoculated with AXO1947.
- Genome edited lines confer strong resistance to AXO1947 after 14 days of inoculation with AXO1947.



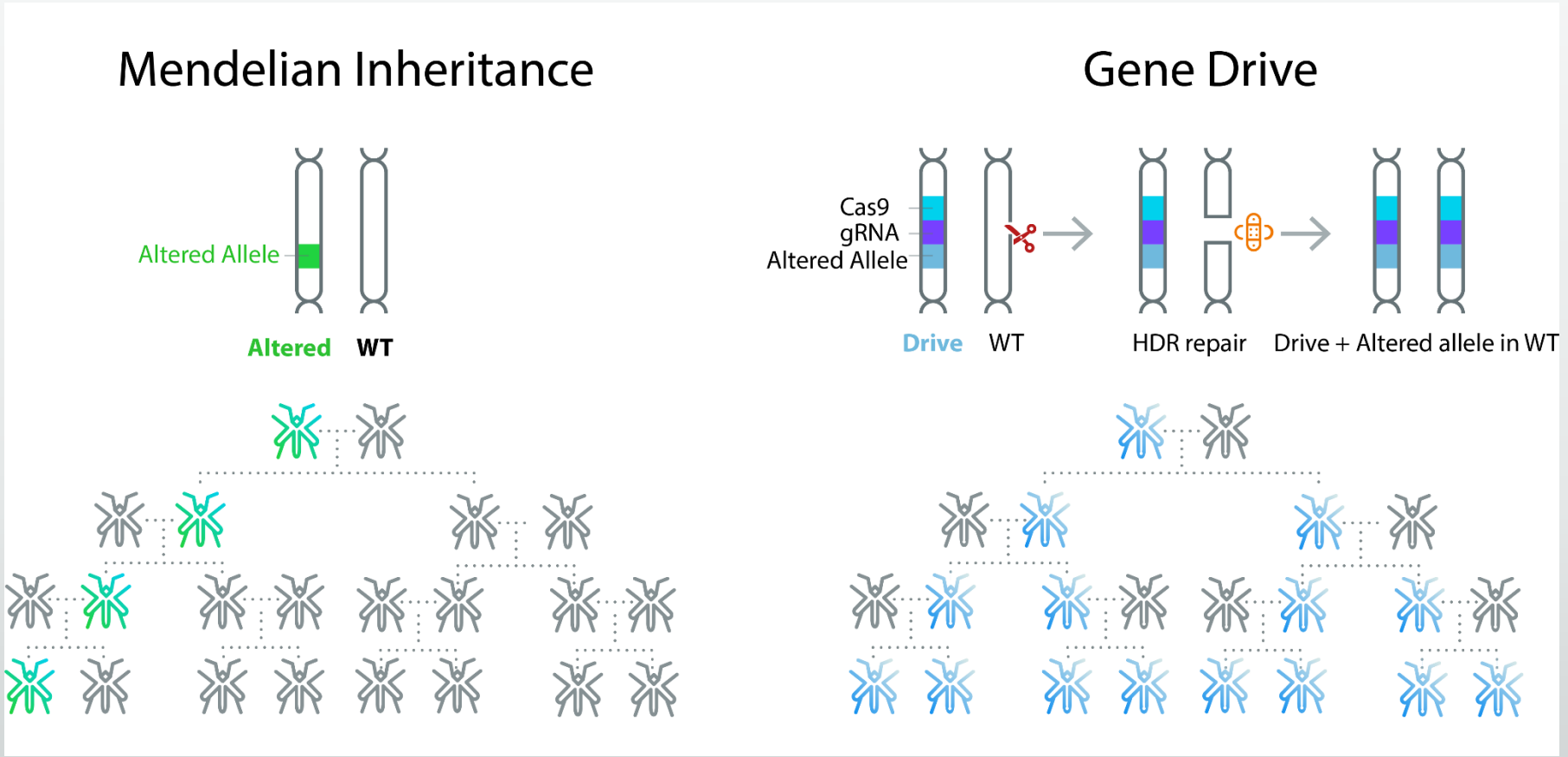
CRISPR Gene Drive



STOP the spread of the disease:

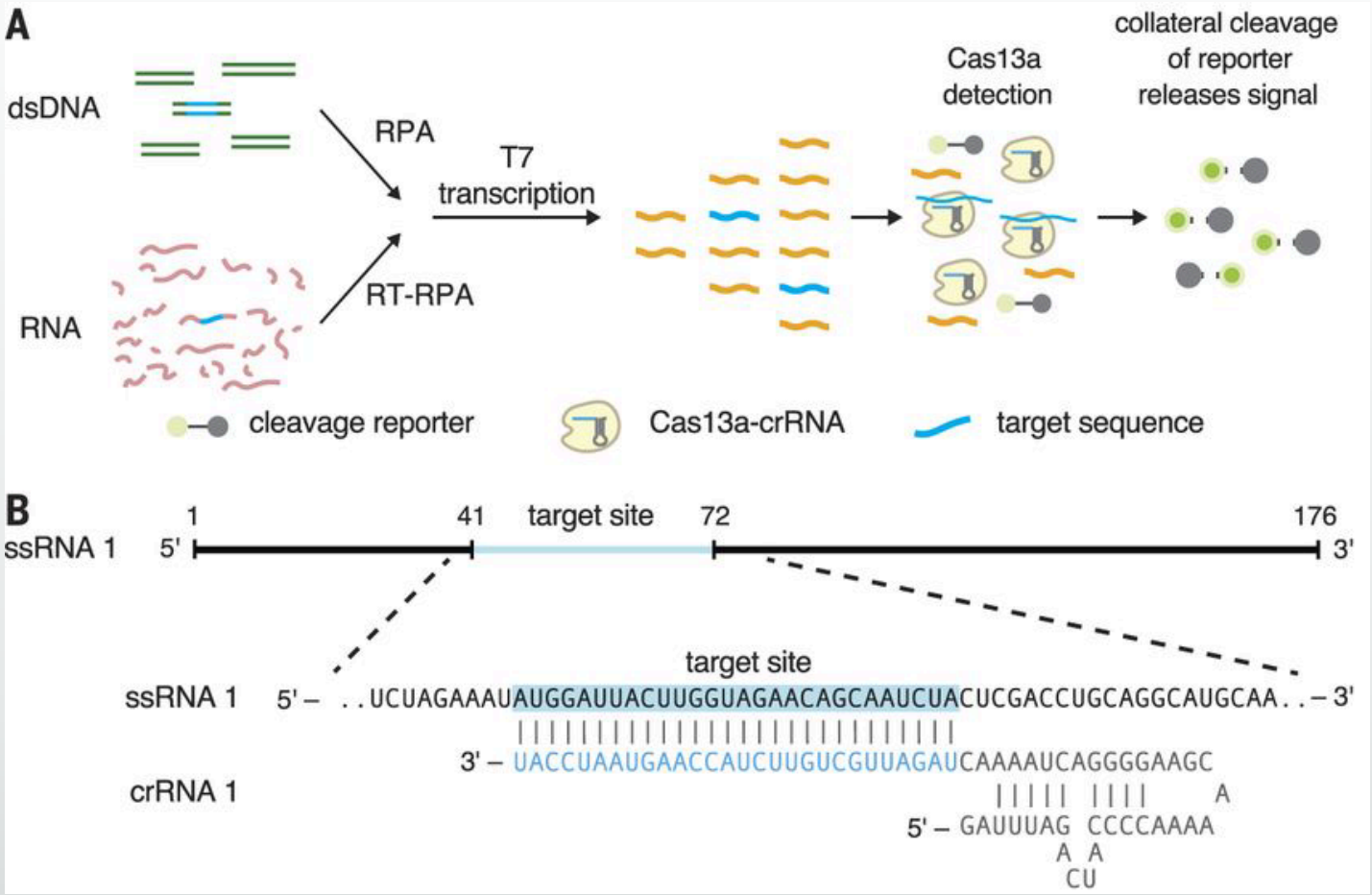
Gene Drive can create a resistance in the insect vector

- End the insect menace
  - Ex.: Fall Army Worm



Power of CRISPR/Cas system in diagnostics

- SHERLOCK is capable of single-molecule nucleic acid detection



Abiotic stress tolerance: How CRISPR can do the rescue

- HDR can be used to introduce genes such as DREB
- ARGOS8 (negative regulator of ethylene responses) promoter modification
  - improved maize grain yield under field drought stress conditions

Shi et al. Plant Biotech J. 2017

Biofortification and human health

- Lysine content
  - Knockout lysine deg
- Iron and Zinc
  - Knockout phytate-re

Plant Biotechnology Journal

Plant Biotechnology Journal (2018) **16**, pp. 902–910 doi: 10.1111/pbi.12837

Low-gluten, nontransgenic wheat engineered with CRISPR/Cas9

Susana Sánchez-León[1,#], Javier Gil-Humanes[2,*,#], Carmen V. Ozuna[1], María J. Giménez[1], Carolina Sousa[3], Daniel F. Voytas[2] and Francisco Barro[1,*]

- Grain Quality & human health
  - Gluten free wheat
  - Polyploidy is the main problem
  - Forty five genes regulate the level of Gutein content

Yu et al. Plant Physiol. 2015

Boosting breeding efforts

- Decoding genetic circuits
  - Non-availability of natural mutants due to polyploidy
  - Eg. Hexaploid bread wheat
- Pyramiding of multiple beneficial traits into the elite lines in a single generation
- SHERLOCK can detect heterozygosity and trait stacking
- CRISPR based mutant library

Generation of artificial diversity for breeding using CRISPR

Breeders have difficulty due to:

- Narrow genetic diversity
- Low efficiency of chemical mutagenesis

CRISPR-mediated Base Editing can create targeted polymorphism around a specific locus for:

- developing novel alleles
- reverse genetics
- More than 90,000 T0 rice lines with high rate of targeted mutation have been generated using an sgRNA pool targeting 34,234 genes in rice
- Above 700 maize candidate genes also edited to generated artificial mutation

Summary up to here

Gene editing can

- Generate agronomically important traits
- Develop artificial diversity for breeders
- Generate new alleles of interest

Challenges for editing plant genes !

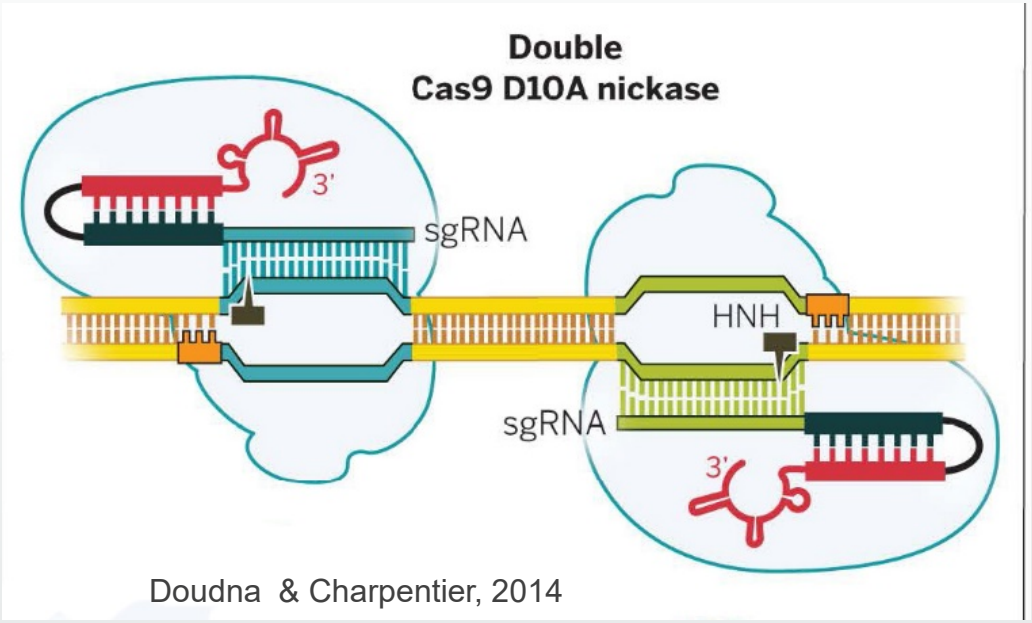
1. Off-target activity
2. Difficulty in delivering the genetic scissors

Off-target activity

- Accidental mutation of non-target genes may
  - Deactivate useful genes
  - Activate some unwanted genes
  - Lead to translocations of chromosomal segments and genome instability.

Off-target activity

- Strategies to reduce the off-target risks of Cas9:
  - CRISPR Off-Finder
  - Use of paired Cas9 nickases
  - Screening of the off-target mutants by NGS
  - Backcrossing to remove the non-targeted mutation



Doudna & Charpentier, 2014

Deployment of CRISPR-edited crops

- USDA/Japanese government
  - No regulation of plants that could otherwise have been developed by conventional breeding techniques
- Israel, Brazil, Argentina, Colombia, Paraguay and Chile
  - Gene-edited crops and foods are regulated as conventional plants unless they contain foreign DNA
- India:
  - Draft proposal indicates case to case consideration

THANK YOU