CRISPR Crops for the future

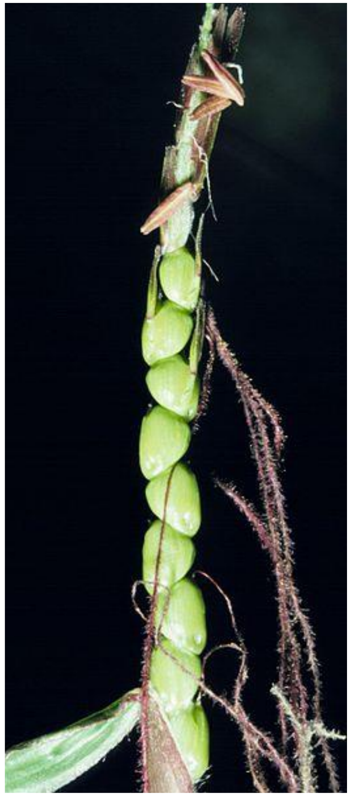
TSN Amgen – 12 July 2023 – Wendy Harwood

John Innes Centre

The importance of access to genetic variation

Wild Cabbage

- Teosinte (corn/maize ancestor)
- First domesticated 9000 years ago



- Very similar at the DNA level
- Teosinte / maize hybrids can be produced
- Few genes or regions of the genome are responsible for the most significant differences.
- Changes in single genes can have dramatic effects

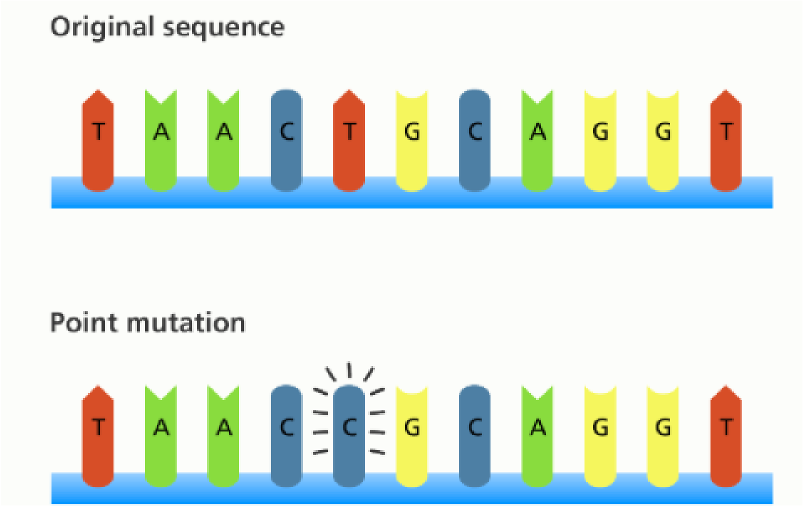
Sources of genetic variation

Conventional plant breeding
10-15 years

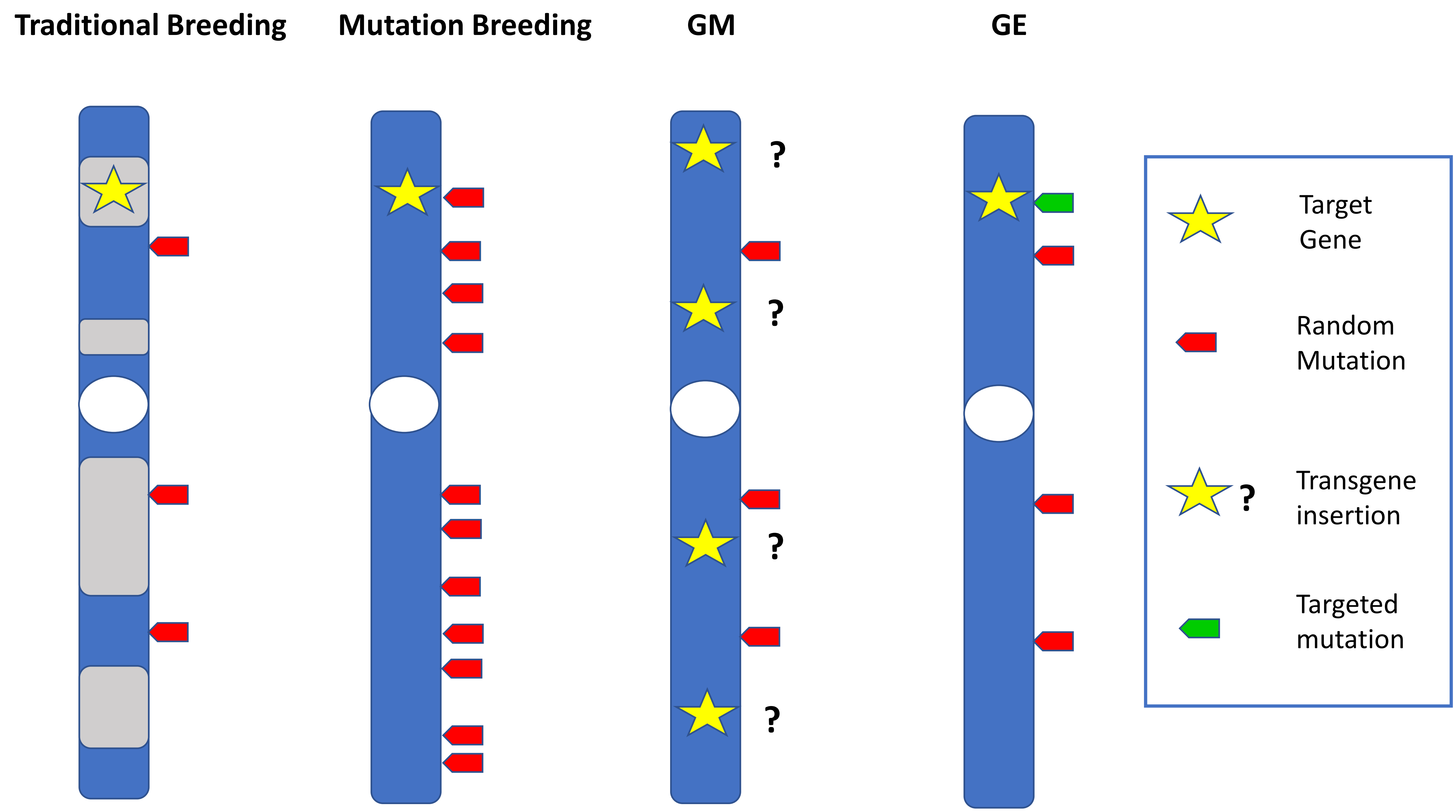
Mutation Breeding

Radiation

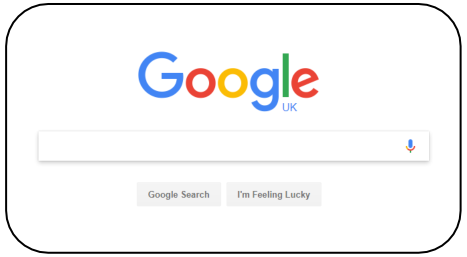
Chemicals (EMS)

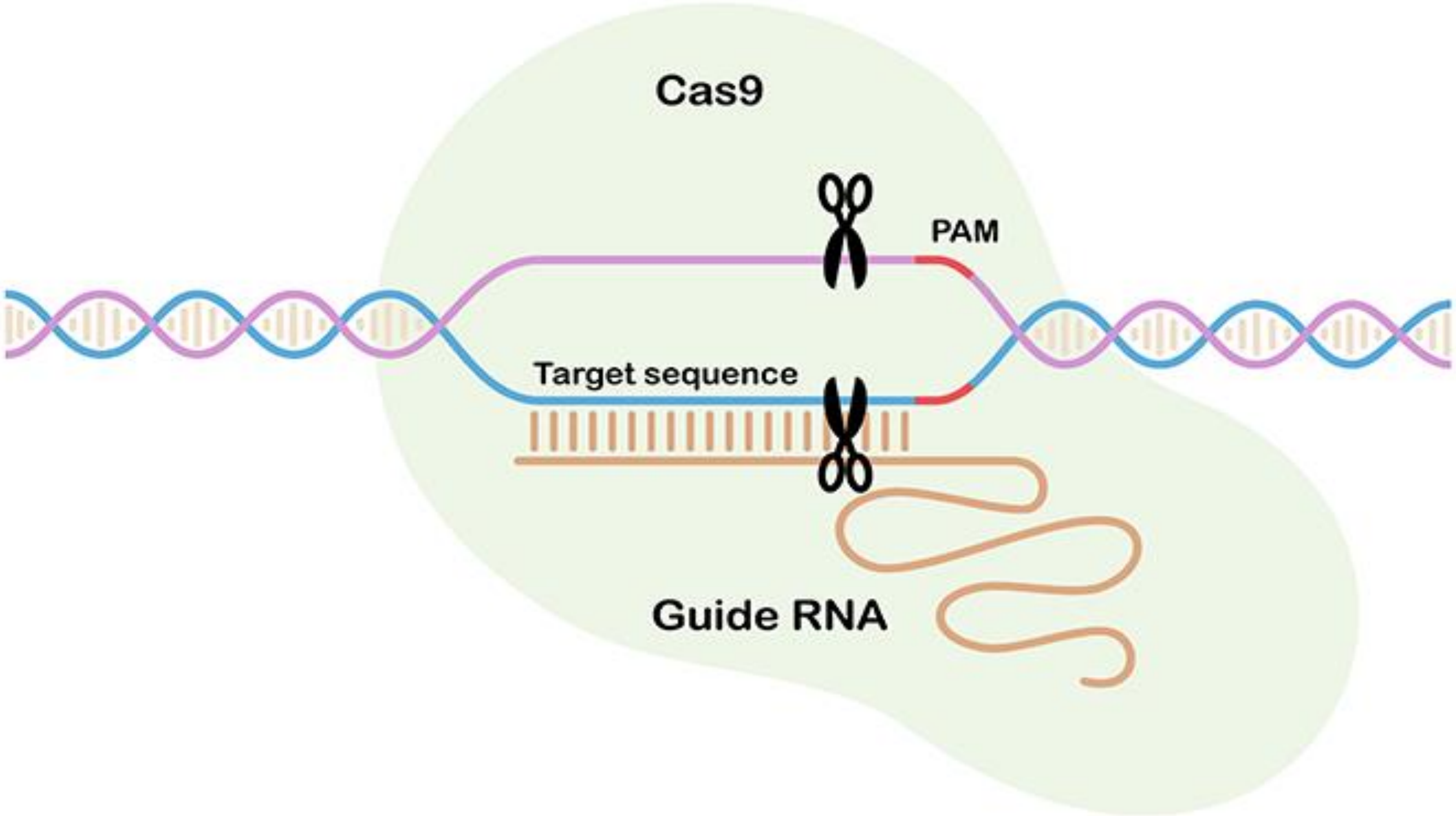


3,200+ varieties are listed on the FAO/IAEA Mutant Variety Database


Traditional Breeding
Mutation Breeding
GM
GE
?
?
?
?
Target Gene
Random Mutation
Transgene insertion
Targeted mutation

Genome editing – CRISPR/Cas9

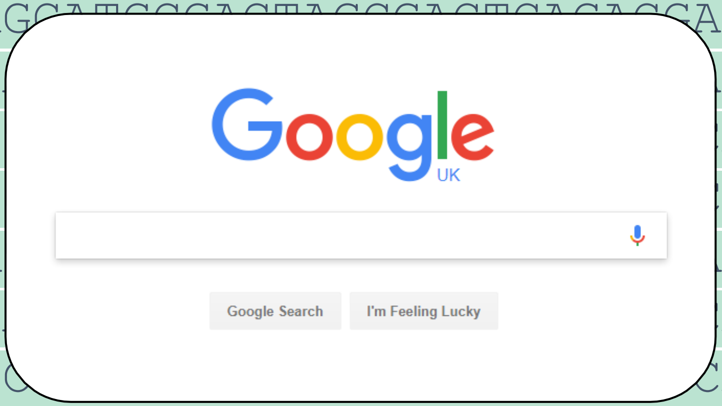




2-part system:

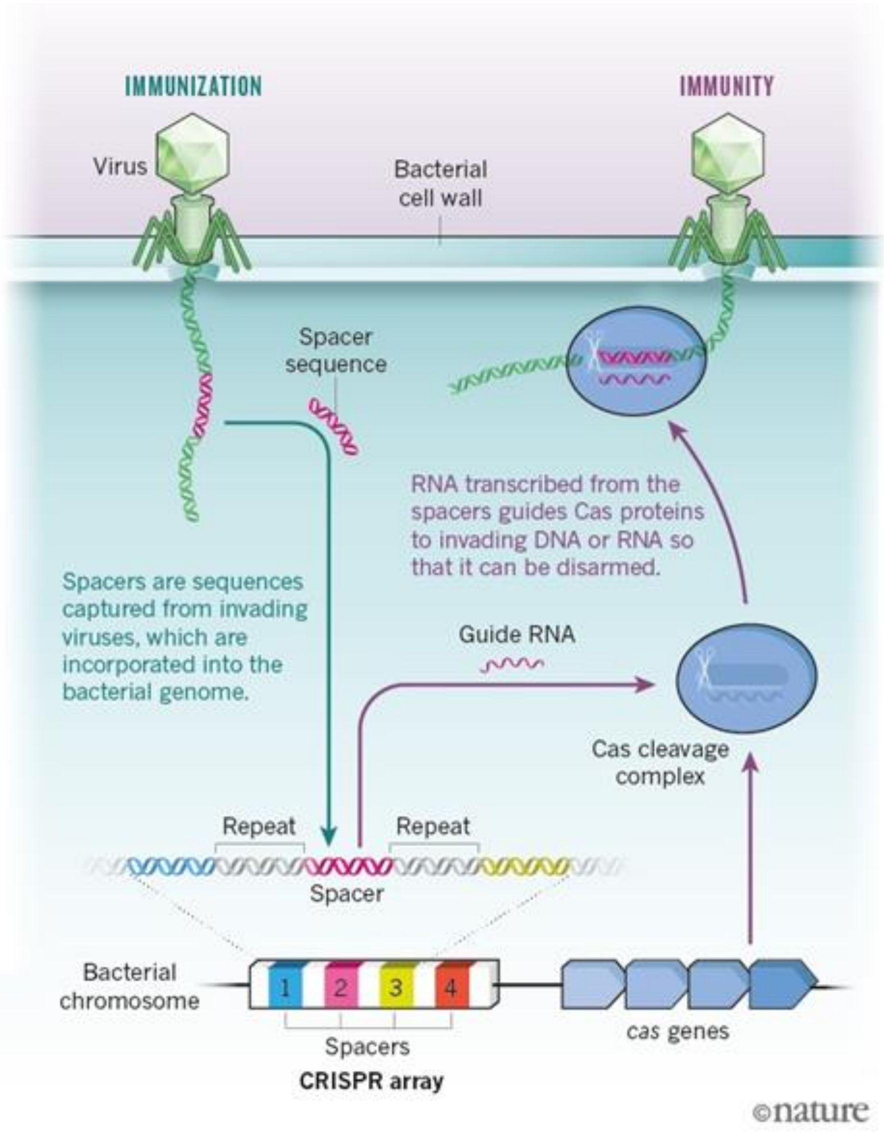
- **1. The guide (guide RNA)** to identify the target.
- **2. The Nuclease** (molecular scissors) to generate a double strand break **(Cas9)**

ACGATACGCATCATCAGCATGCGACTAGCGACTCAGACGACGCAGCAGCATCATCATCTACTATACTCTATTACTACT
ACGCAGCATCATCATCAGATCATCATCATCATCTACACGATACGCATCATCAG ACG
CAGCAGCATCATCATCTACTATACTCTATTACTACTACGCAGCATCATCATC TA
CGCATCATCAGCATGCGACTAGCGACTCAGACGACGCAGCAGCATCATCATC AG
CATCATCATCAGATCATCATCATCATCTACACGATACGCATCATCAGCATGC AG
CATCATCATCTACTATACTCTATTACTACTACGCAGCATCATCATCAGATCA TC
ATCAGCATGCGACTAGCGACTCAGACGACGCAGCAGCATCATCATCTACTAT AT
CATCAGATCATCATCATCATCTACACGATACGCATCATCAGCATGCGACTAG CAT
CATCTAGGATACTCTATTACTACTACGCAGCATCATCATCAGATCATCATCATCATCTACACGATACGCATCATCAGC
ATGCGACTAGCGACTCAGACGACGCAGCAGCATCATCATCTACTATACTCTATTACT CTACGCAGCATCATCATCAG
ATCATCATCATCATCTACACGATACGCATCATCAGCATGCGACTAGCGACTCAGAC ACG AGCAGCATCATCATCTA
CTATACTCTATTACTACTACGCAGCATCATCATCAGATCATCATCATCATCTA AC AC GCATCATCAGCATGCGA
CTAACGACTCAGACGACGCAGCAGCATCATCATCTACTATACTCTATTACTAC ACGC TCATCATCAGATCATC
ATCATCATCTACACGATACGCATCATCAGCATGCGACTAGCGACTCAGACGACGCAG ATCATCTACTATAC
TCTATTACTACTACGCAGCATCATCATCAGATCATCATCATCATCTACACGATACGCATCATC ATGCGACTAGCG
ACTCAGACGACGCAGCAGCATCATCATCTACTATACTCTATTACTACTACGCAGCATCATCATCAC CATCATCATC
ATCTACACGATACGCATCATCAGCATGCGACTAGCGACTCAGACGACGCAGCAGCATCATCATCTACTA ACTCTATT
ACTACTACGCAGCATCATCATCAGATCATCATCATCATCTACACGATACGCATCATCAGCATGCGACTAGCGACTCAG
ACGACGCAGCAGCATCATCATCTACTATACTCTATTACTACTACGCAGCATCATCATCAGATCATCATCATCATCTAC



A molecular guide (gRNA) and a nuclease (Cas protein)

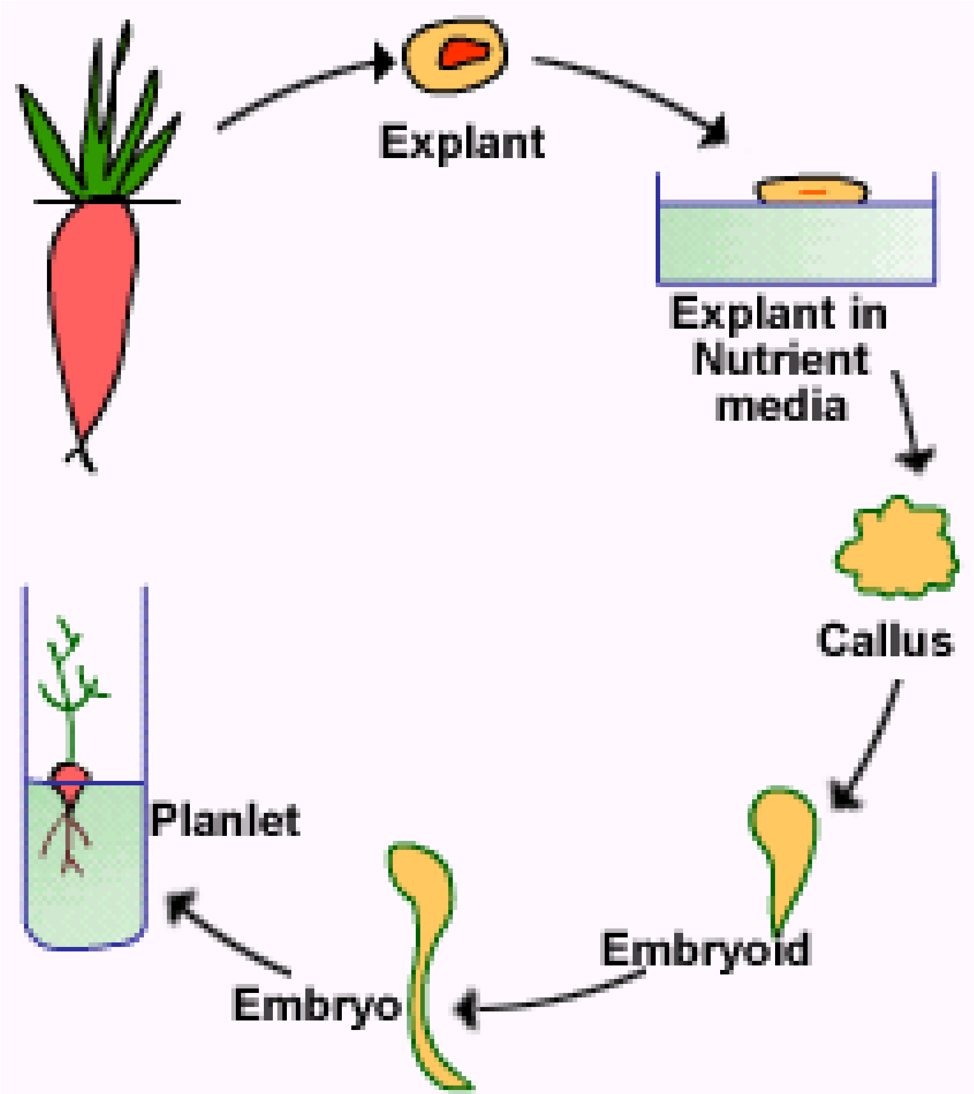
CRISPR - An adaptive bacterial defence strategy



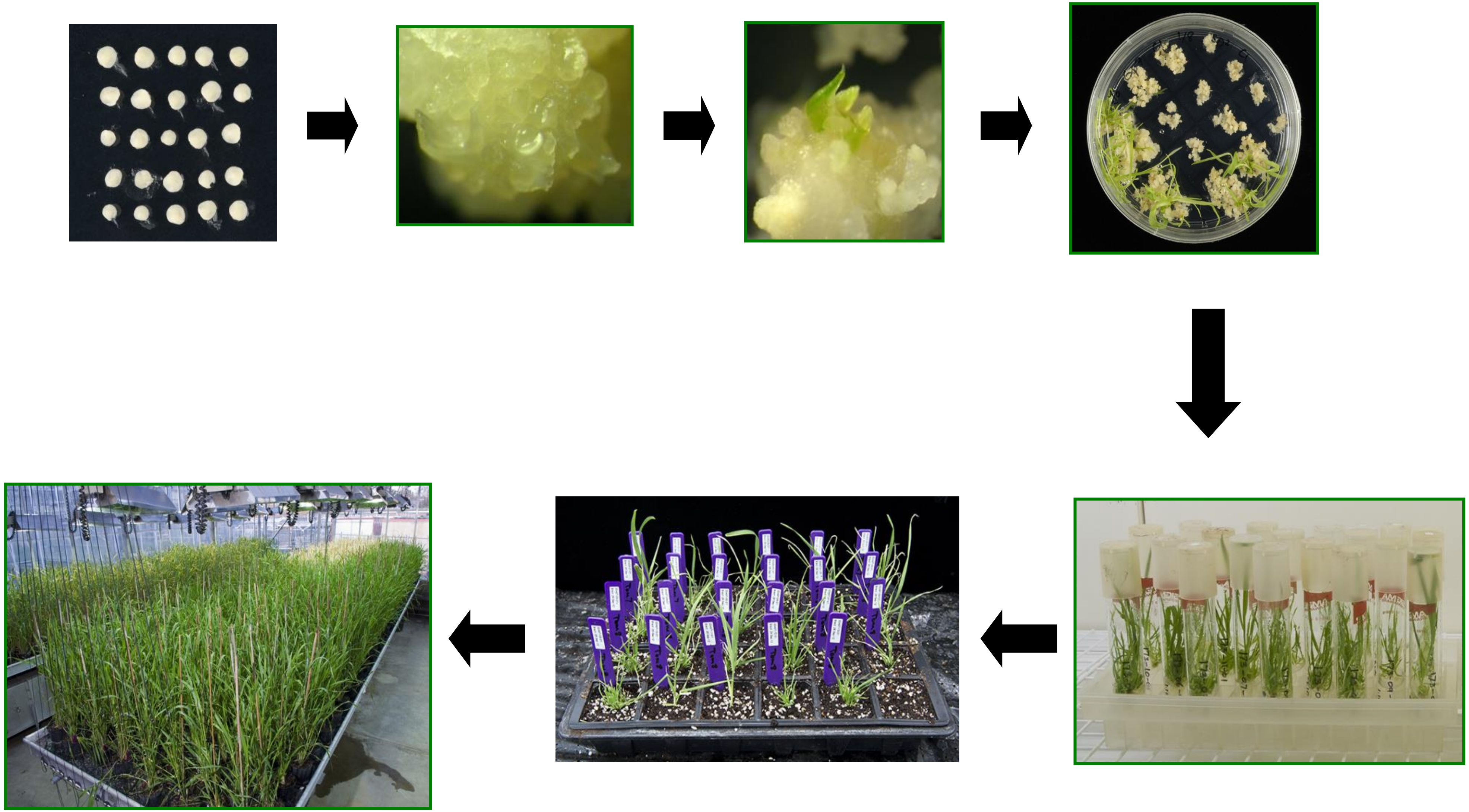
CRISPR History

- 1993 – Discovery of CRISPR and its function (Mojica)
- 2005 – Discovery of Cas9 and PAM (protospacer adjacent motif) (Bolotin)
- 2010 – Cas9 cleaves target DNA causing a double strand break 3 nucleotides from the PAM (Moineau)
- 2012 – Cas9 can be reprogrammed to target a chosen site (Charpentier and Doudna)
- 2013 – CRISPR-Cas9 harnessed for genome editing in eukaryotic cells (Zhang)

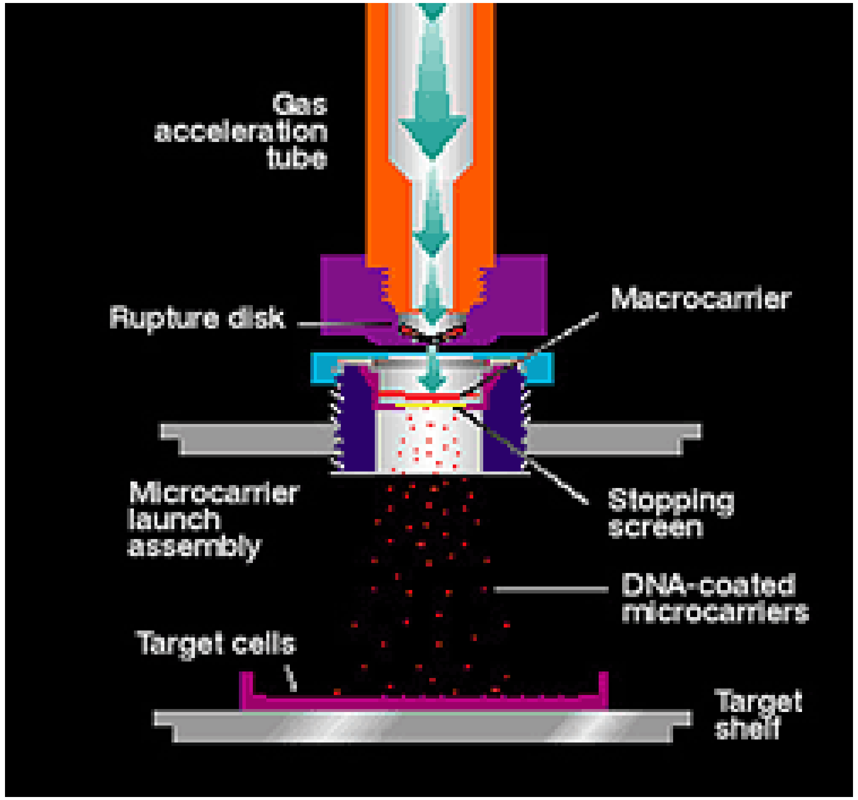
Plant regeneration

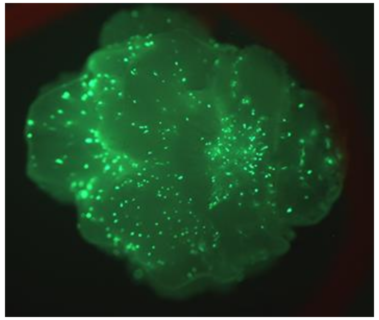


Delivering CRISPR components to plants
Example barley



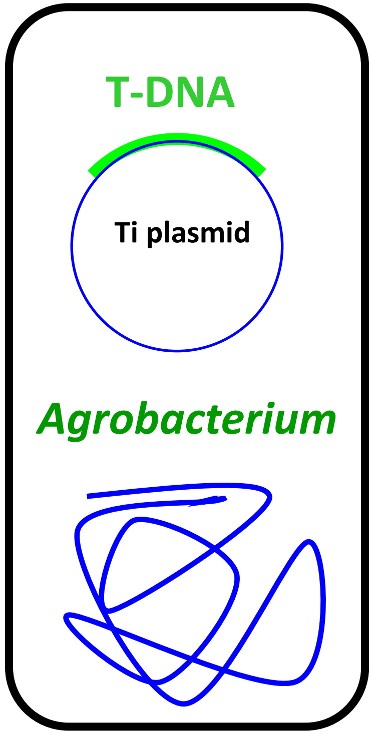
Methods for the introduction of DNA – 1. The Gene Gun

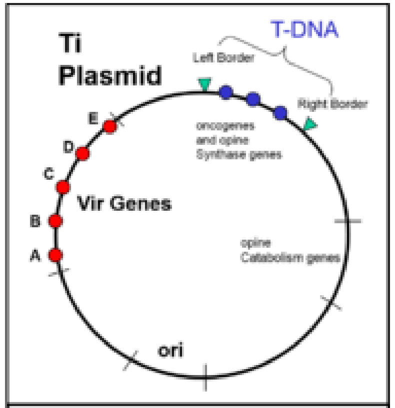




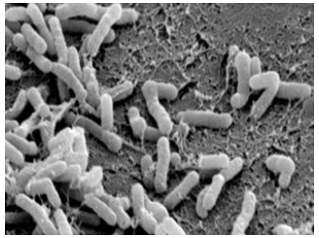
GFP expression following bombardment

2. Agrobacterium

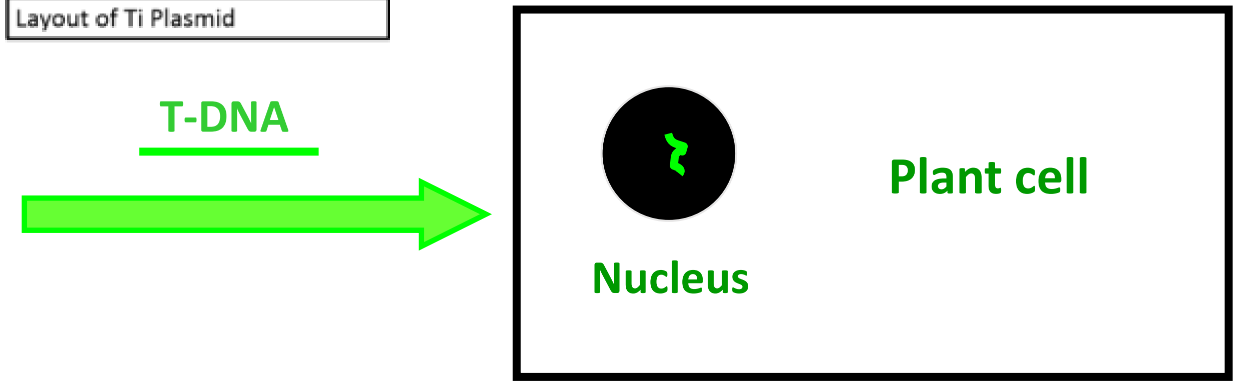




Layout of Ti Plasmid

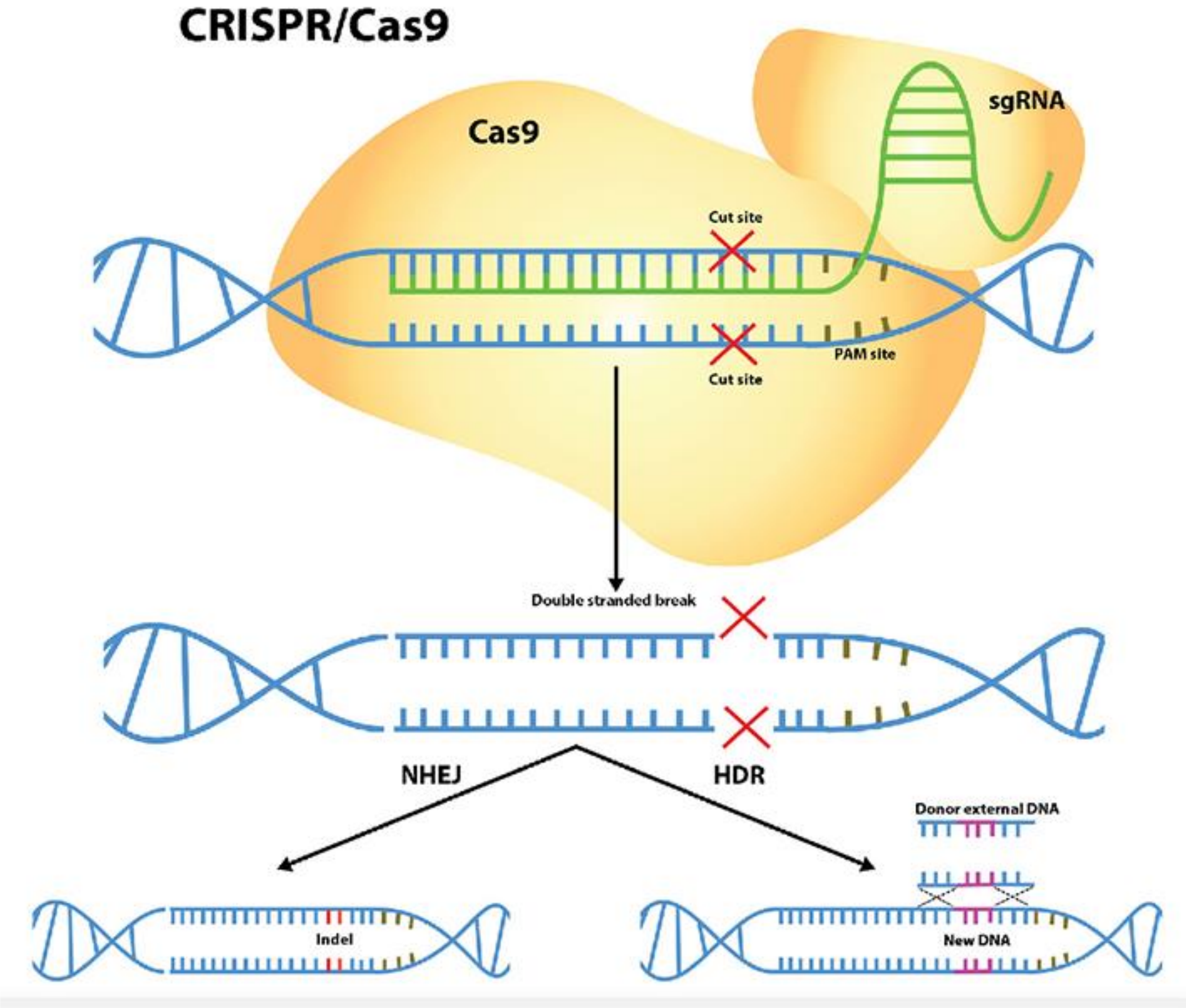


Crown
gall
disease



In 1907, Smith and Townsend demonstrated that the Gram-negative soil bacterium, *Agrobacterium tumefaciens*, was responsible for crown gall disease in plants.

Outcomes from genome editing



Gene knock-out

An example of genome editing in barley

- In barley the inflorescence has 3 single florets.
- In 2-row barley only the middle floret is fertile.
- A single gene, *Vrs1*, an inhibitor of lateral spike development, is responsible for this phenotype.
- RNAi knock-down of expression of *Vrs1* led to partial fertility of lateral florets. (Sakuma et al. 2013)

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An example: Gene editing in a Brassica crop

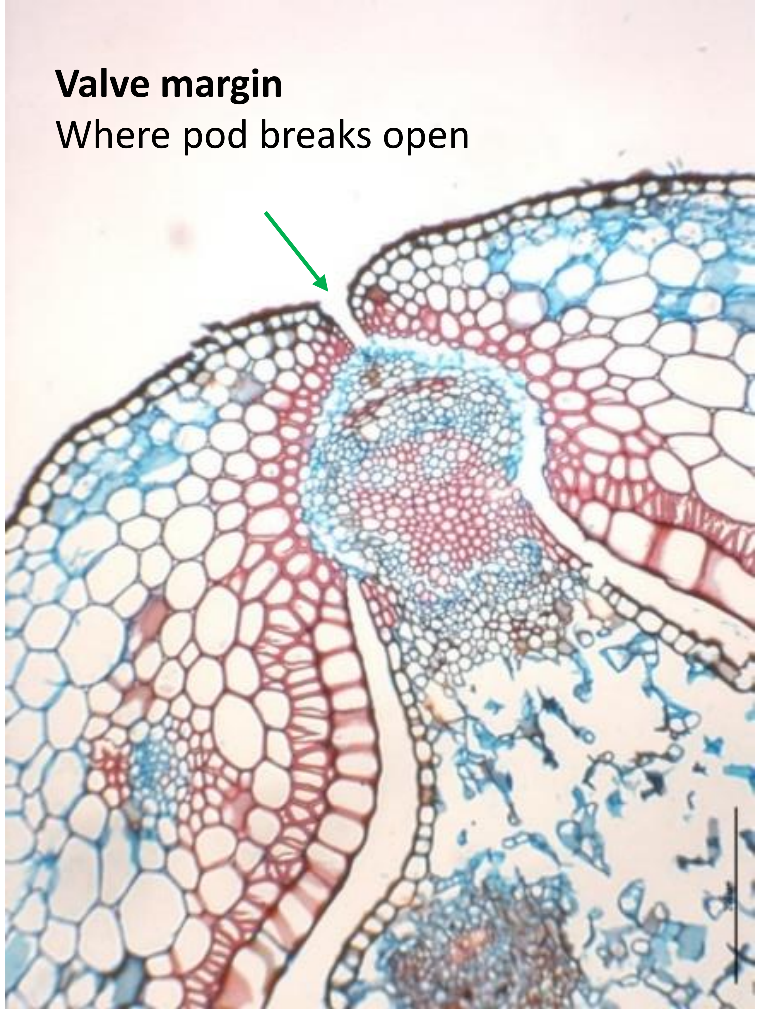
The problem: Pod shatter

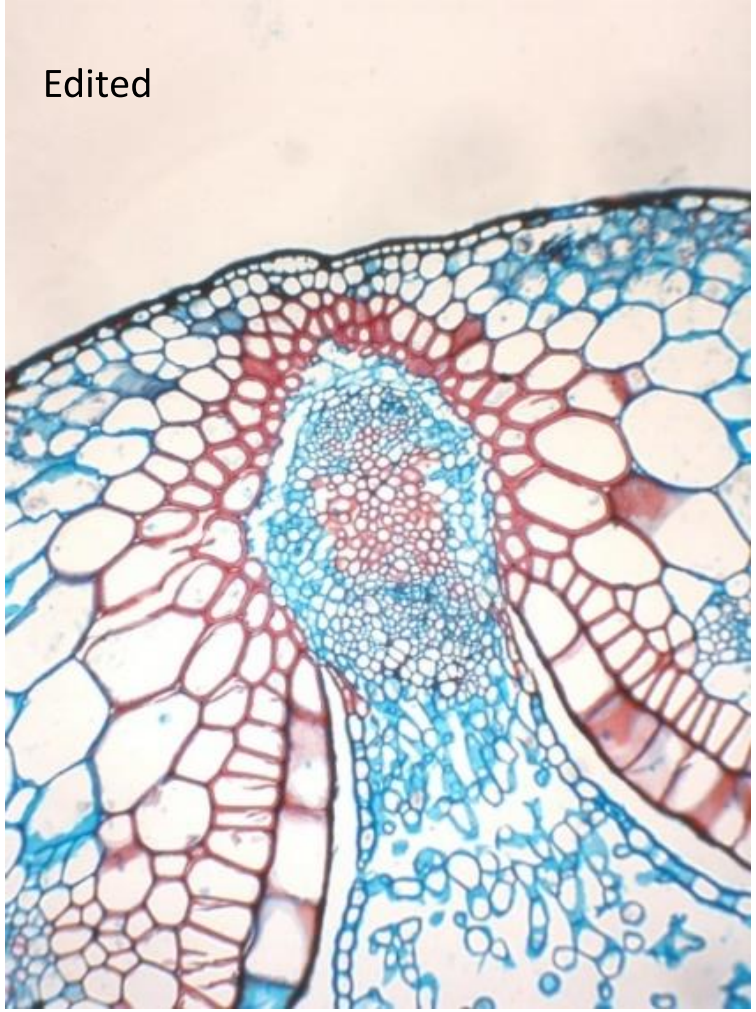
Target gene – GA4 involved in the gibberellin biosynthesis pathway. GA4 mutants in the model plant Arabidopsis are dwarf and have reduced pod shatter.

Lars Ostergaard JIC

Editing a gene involved in valve margin formation

Original ACGATCCCCTCTTGGACGCCGCTCCTTCCCCTCCGGCCGCAAGTGAAAACATCCCTCTCATTGACCTGAA
Edited ACGATCCCCTCTTGGACGCCGCTCCTTCCCCTCT_ GCCGCAAGTGAAAACATCCCTCTCATTGACCTGAA





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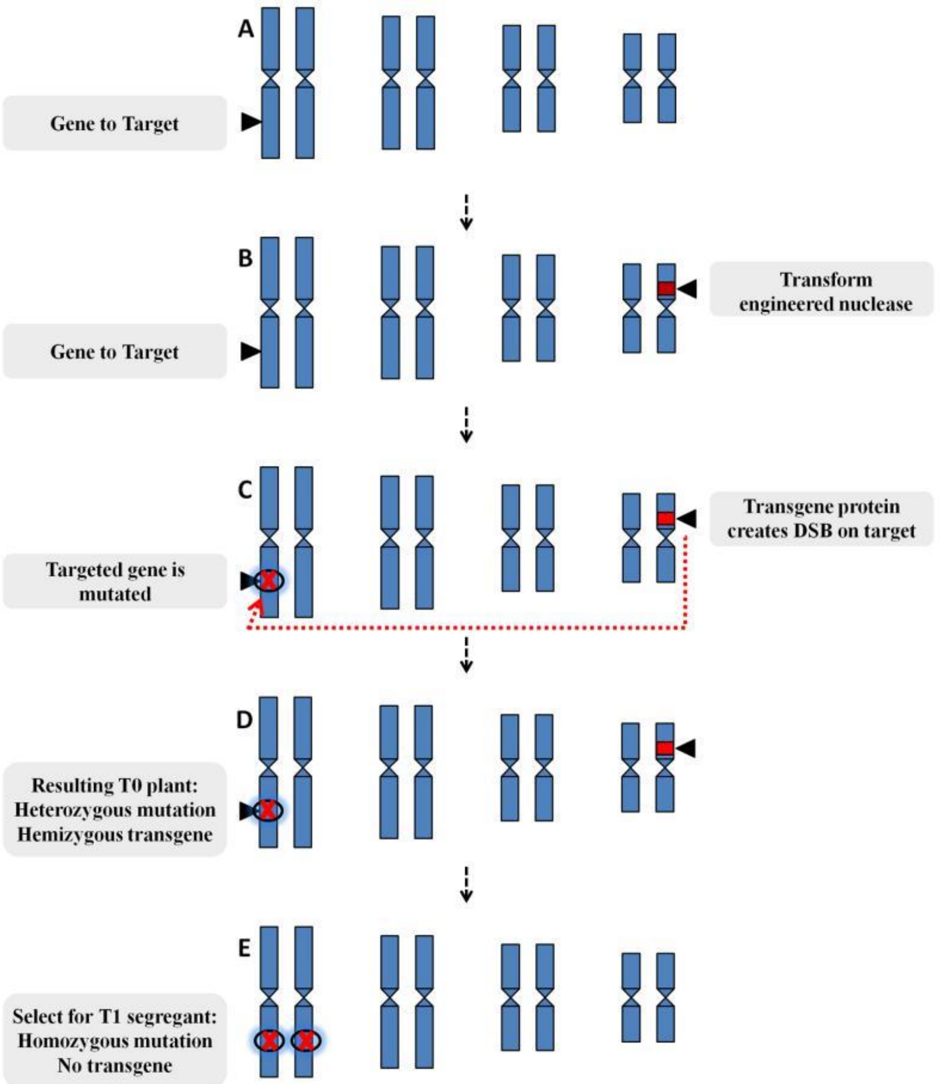
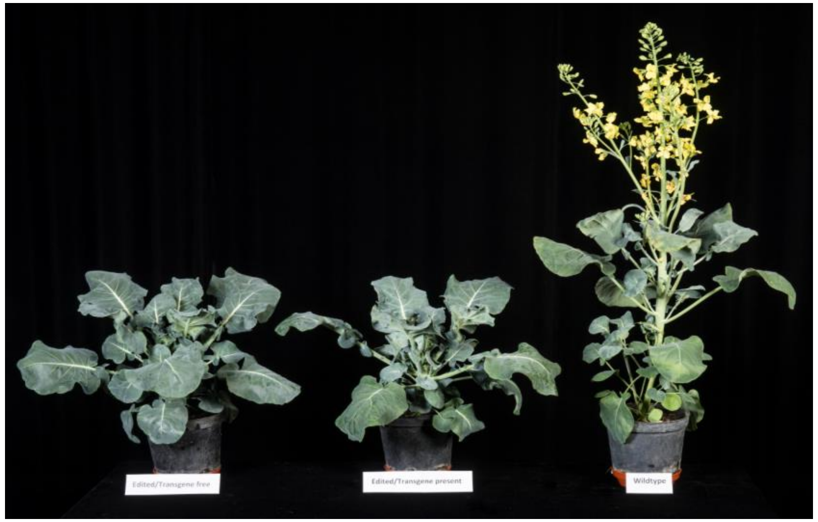
Ostergaard Group

Lawrenson et al 2015

Recovering plants containing only the target mutation and no additional DNA

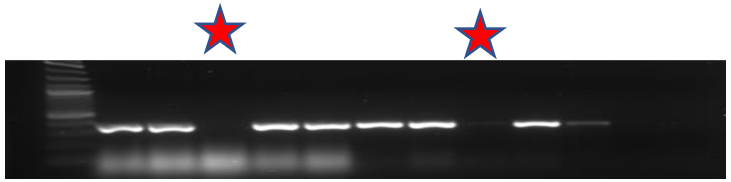




Edited, transgene free

Edited, transgenes present

Wildtype



Plants with the mutation but without any transgenes

Curtin et al. (2012) The Plant Genome 5: 2

CRISPR / Cas applications

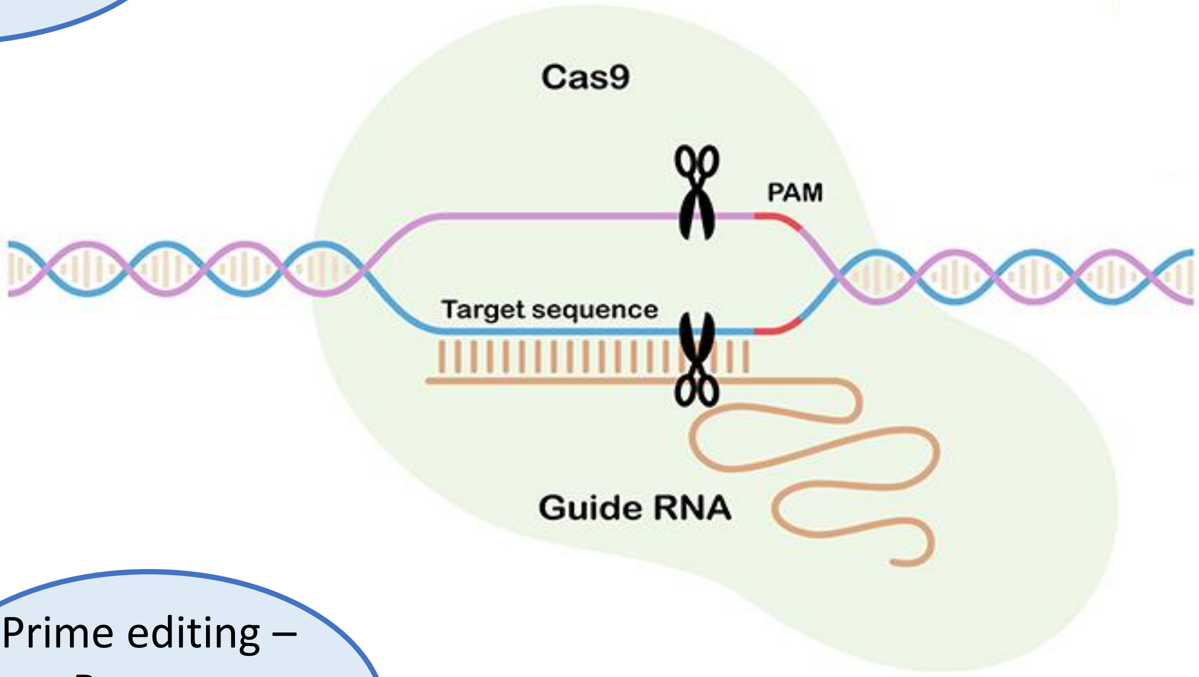
Repression

Base editing - cytosine base editor (C-T, G-A)

Transcriptional activation

Base editing - adenine base editor (A-G, T-C)

Targeted Mutation



Precise deletions

Multiple gene knockouts

Gene targeting

Prime editing – Reverse transcriptase fusion

Epi-editing - DNA methyl transferase fusion

Nuclease variants

Gene replacement

Lawrenson et al. (2021) Frontiers in Genome Editing

ProVitamin D_3 enriched tomatoes

Use for waste

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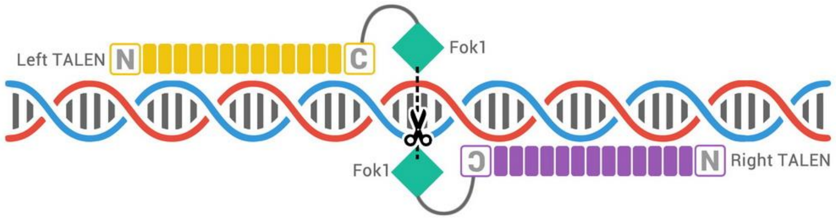
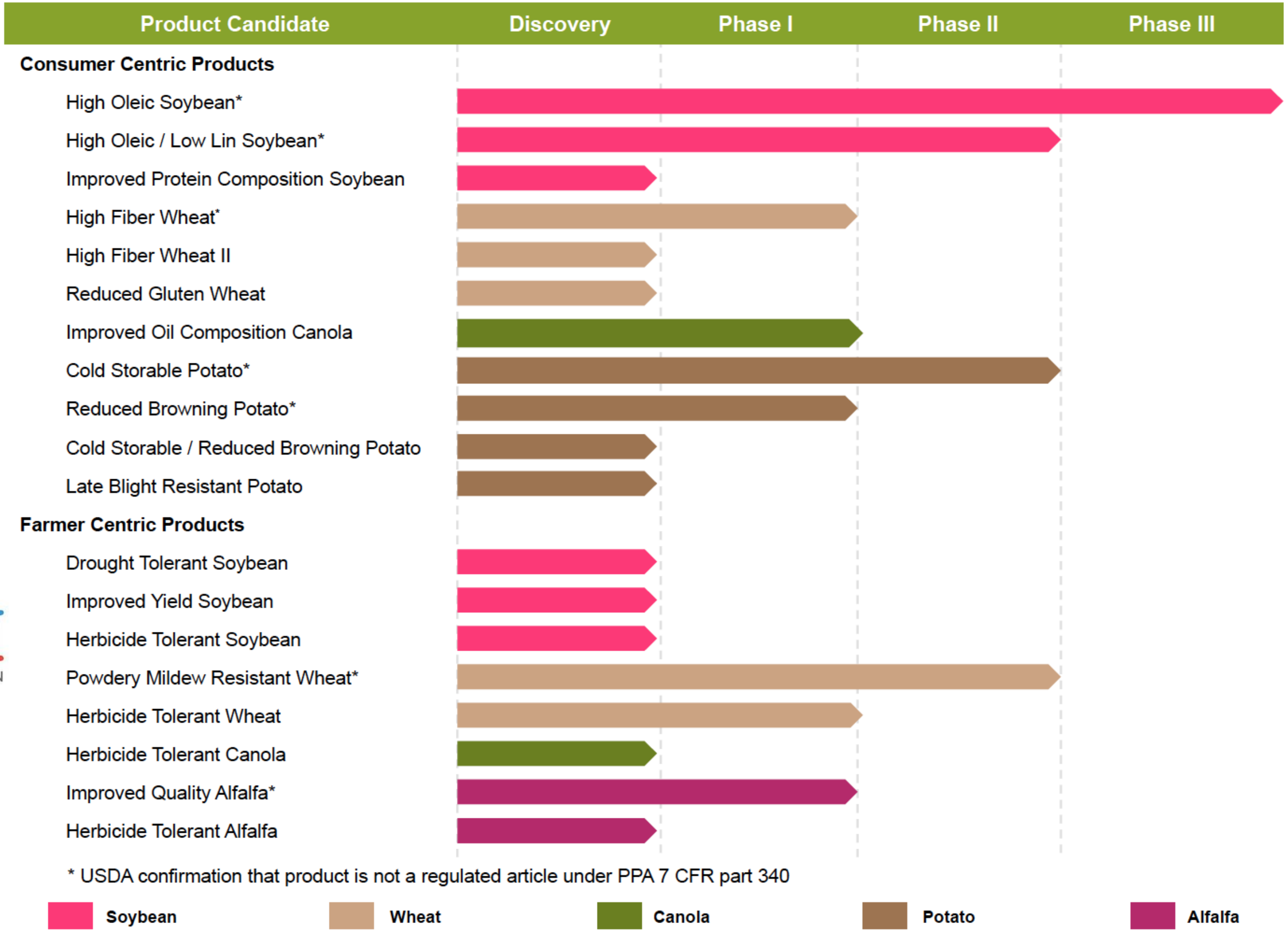
Genome edited crops in the pipeline

TALEN technology

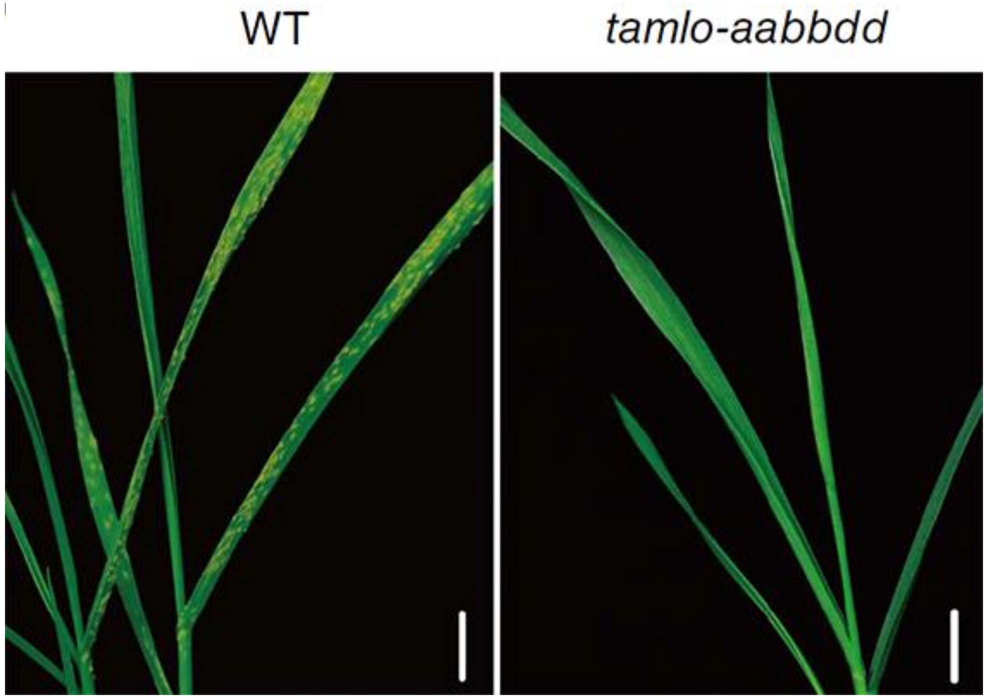




http://www.calyxt.com/products/products-in-our-development-pipeline/

Powdery mildew resistance in wheat

- Genome editing used to target all three copies of the Mildew Locus O gene (MLO) in wheat.
- This reduced susceptibility to mildew infection.
- The same natural mutation in barley has protected spring barley in the field for 40 years – a reliable weapon to protect plants.

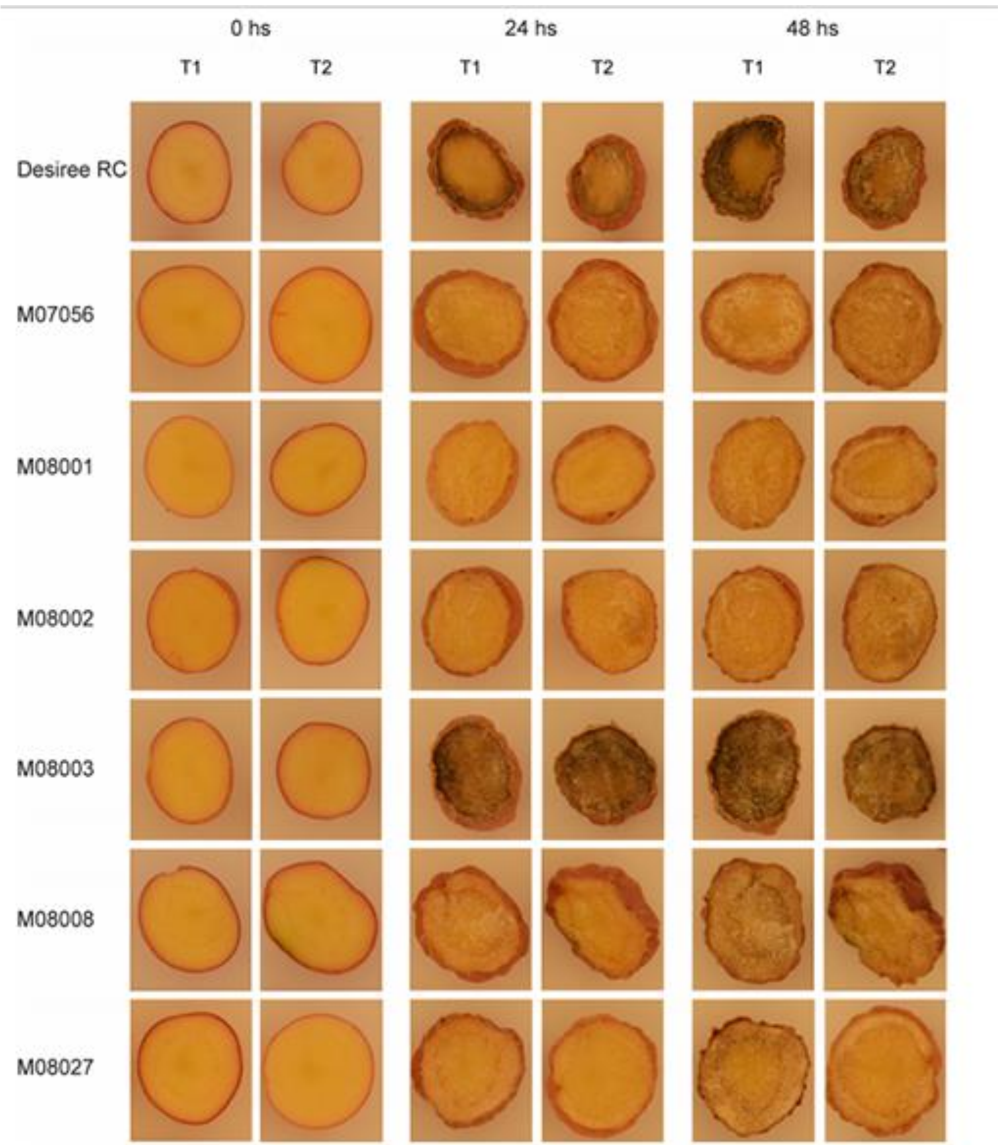


Wang et al. 2014

Reducing food loss and waste

Reduced browning in potato

- An average of 33% of all fruit and vegetables produced globally are never eaten.
- In potato - Gene editing was used to induce mutations in a polyphenol oxidase (PPO) gene.
- A 73% reduction in browning was achieved
- The potato is in field trials in Argentina



Gonzalez et al. (2020) Front. Plant Sci.

Nutritional benefits

Tomato that can help lower blood pressure

- Gene editing (CRISPR/Cas9) used to produce a tomato with 5x the normal amount of the amino acid GABA (Gamma-Amino Butyric Acid)

- GABA is effective in lowering blood pressure and relieving stress

- The first gene edited food to be approved by the government in Japan

Tropic Biosciences (Norwich Research Park)

Aim to develop high-performing commercial varieties of tropical crops which promote grower wellbeing, consumer health, and improved sustainable environmental practices, using cutting edge non-GMO gene editing techniques.

The U.S. Department of Agriculture (USDA) and the Honduran Ministry of Agriculture and Livestock (SAG) have issued exemptions from regulation for our gene edited non-browning Cavendish banana. This is the world's first gene edited banana cleared for commercial release by regulatory authorities.

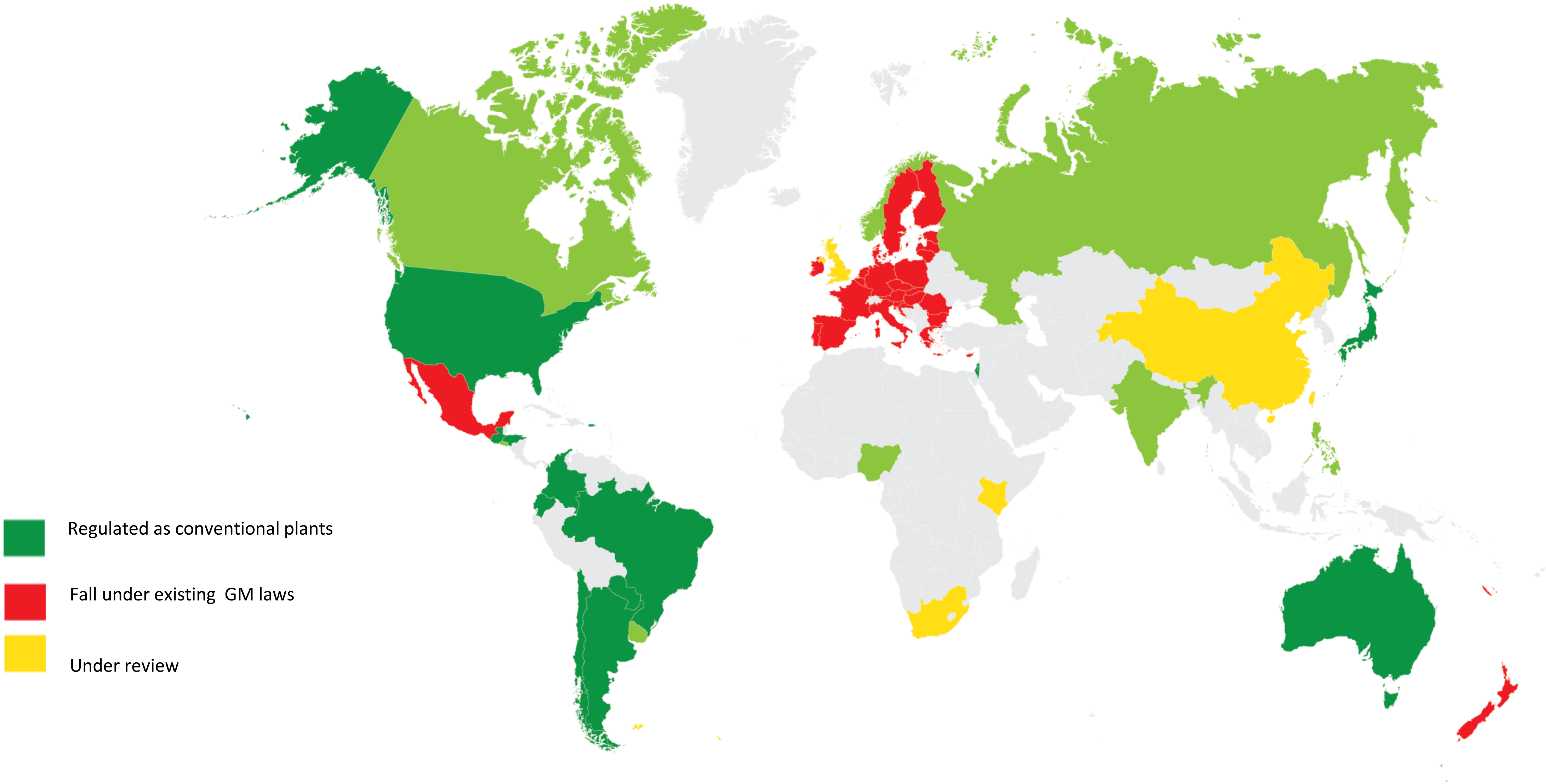
We estimate that our non-browning bananas could reduce food waste and CO_2 equivalent emissions along the supply chain by over 25%.

Gene editing for complex traits

Low gluten bread

Targeting 35 genes

Regulatory status of gene-edited crops (when no foreign DNA is inserted)



New Genetic Technologies (Precision Breeding) Bill

A Bill to make provision about the release and marketing of, and risk assessments relating to, precision bred plants and animals, and the marketing of food and feed produced from such plants and animals; and for connected purposes.

Will apply to Precision Bred Organisms (PBOs) where the end product could have been achieved through traditional breeding approaches.

1. April 2022: Defra introduced changes to make it easier to carry out research field trials. Requiring a notification to Defra and removing the burden of needing a GMO approval.
   https://www.gov.uk/government/collections/genetically-modified-organisms-applications-and-consents

2. Bring forward primary legislation to amend the definitions of a GMO to remove organisms that have genetic changes that could have been achieved through traditional breeding methods.

The Genetic Technology (Precision Breeding) Act passed into law and received Royal Assent on 23 March 2023.


2023: Gene edited wheat – altered grain size
2023: High Iron GM Wheat
Wildtype
single
double
triple
Weight
- -
+ 6 %
+ 11 %
+ 21 %
Bawburgh Field Station

New EU proposal:

A long-awaited draft regulation from the EU Commission to deregulate the authorisation, risk assessment and labelling of a whole range of new genetically modified organisms (GMOs) was published this month.

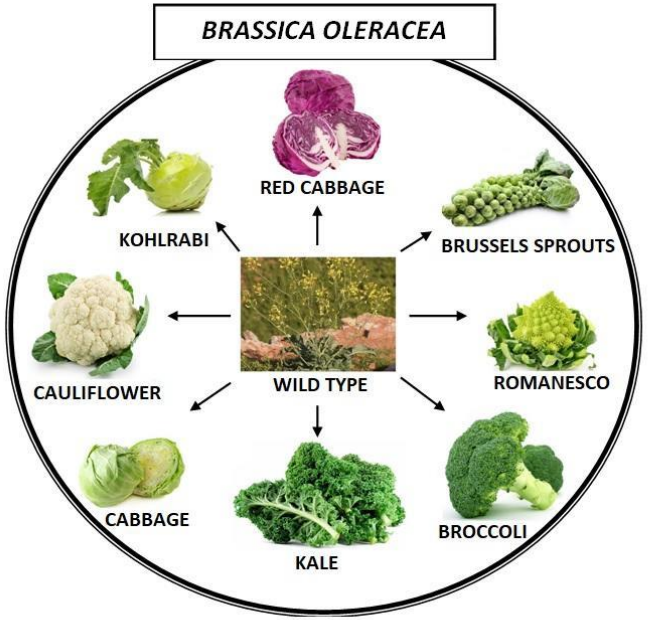
New Genomic Techniques (NGT) are introduced as an umbrella term for certain genetic engineering techniques that aim to modify DNA more precisely than "old" methods by targeting changes to pre-defined loci in the genome.

The proposal creates two distinct pathways for NGT plants to be placed on the market. "Category 1 NGTs" are considered equivalent to conventionally bred plants and no longer require prior case-by-case risk assessment.

But: It may be some time before the new "law on plants derived from new genomic techniques" comes into force.

Plant Breeding is essential and relies on access to genetic variation

This variation can natural, induced, introduced, or achieved by precision breeding



GM

Gene editing

ALL new varieties undergo several years of field evaluation regardless of the method used.

ALL technologies are likely to be required to meet future challenges supplying sufficient nutritious food in a sustainable way.