The CRISPR craze

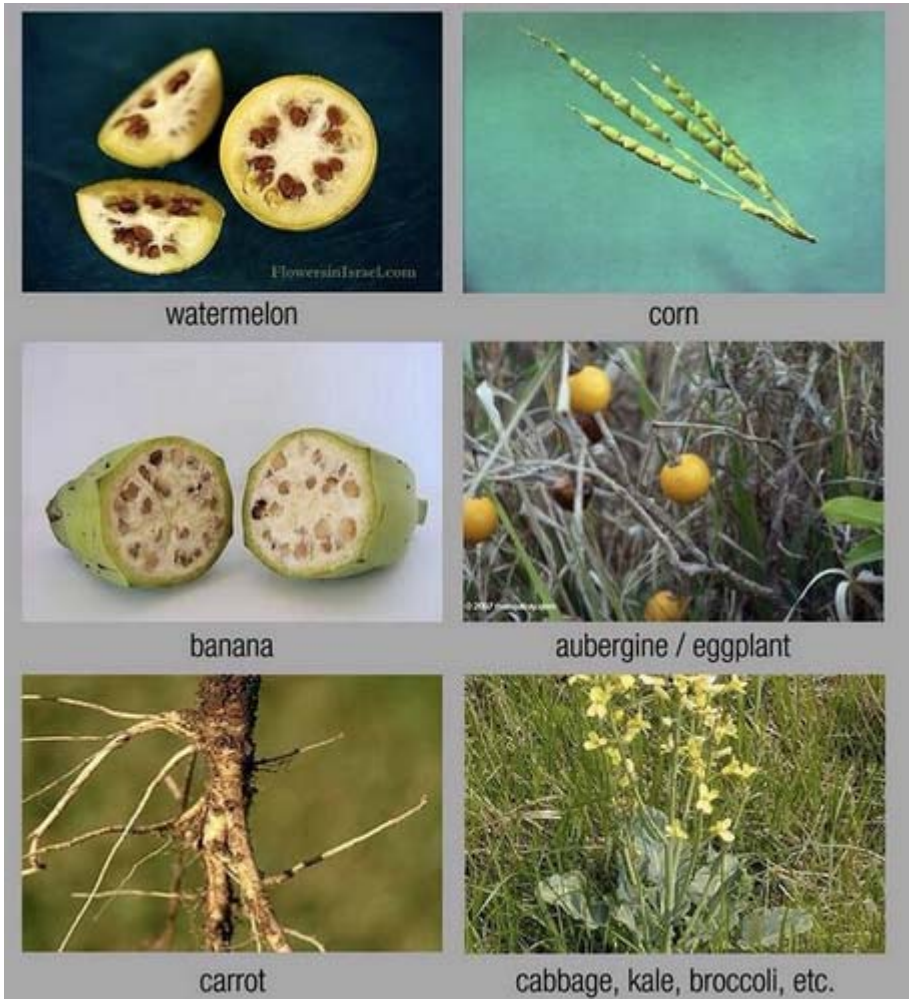
Wendy Harwood

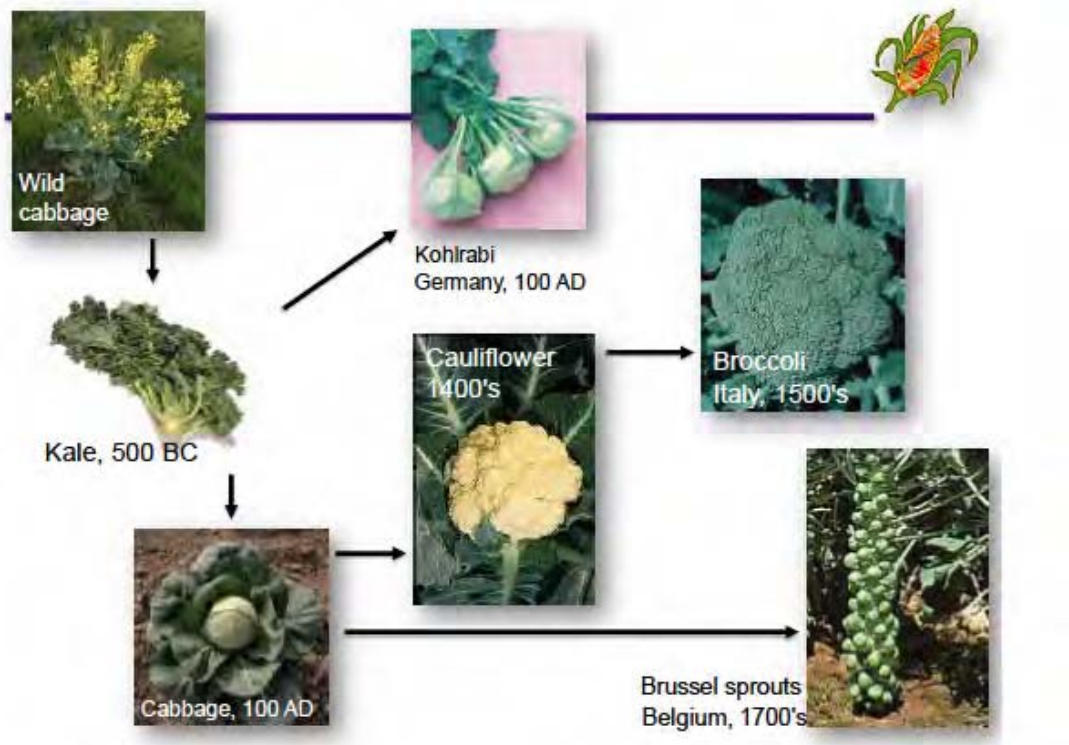
Institute of Food Science & Technology 16 November 2016

John Innes Centre

Unlocking Nature's Diversity

How your food would look without the advances made by plant breeders



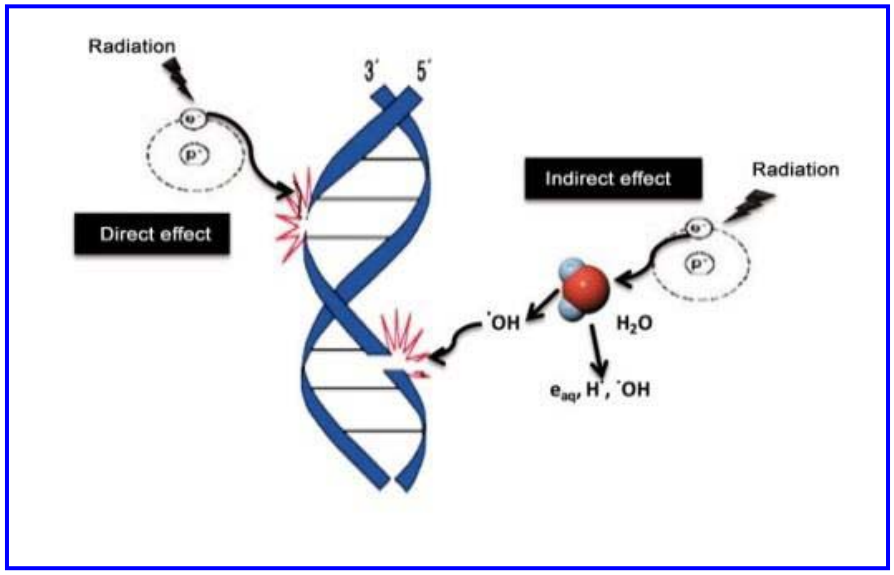


Brassica crops

Plant breeders need variation (mutations) in DNA sequence to improve crops

Plants with mutations in specific genes are also valuable in allowing researchers to understand gene function.

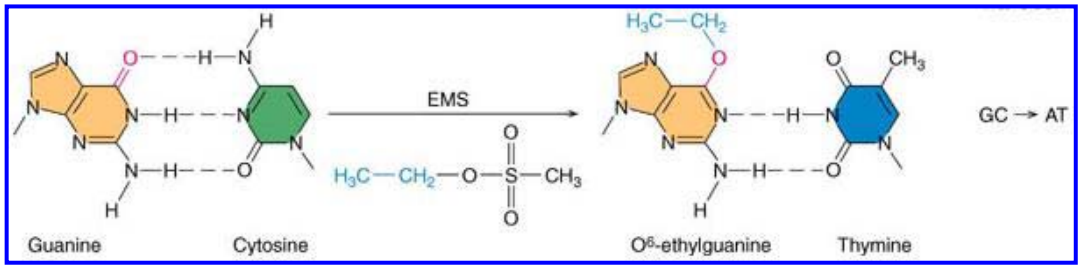
Different methods have been used to increase the variation available to breeders



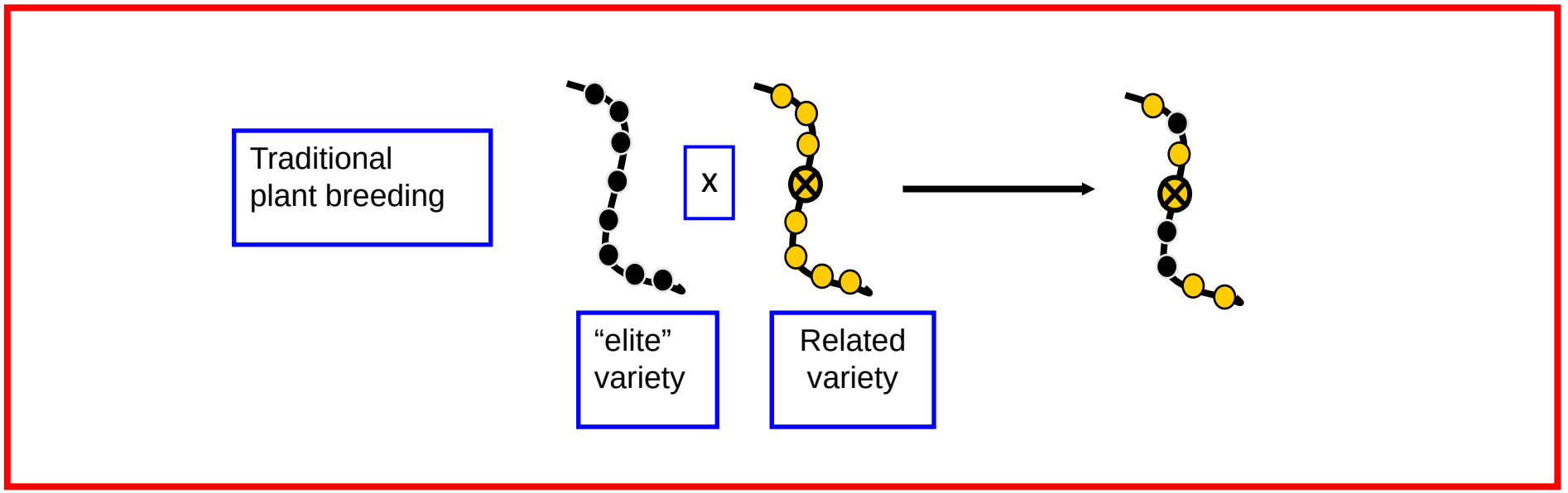
Mutation breeding uses radiation or chemicals to induce mutations

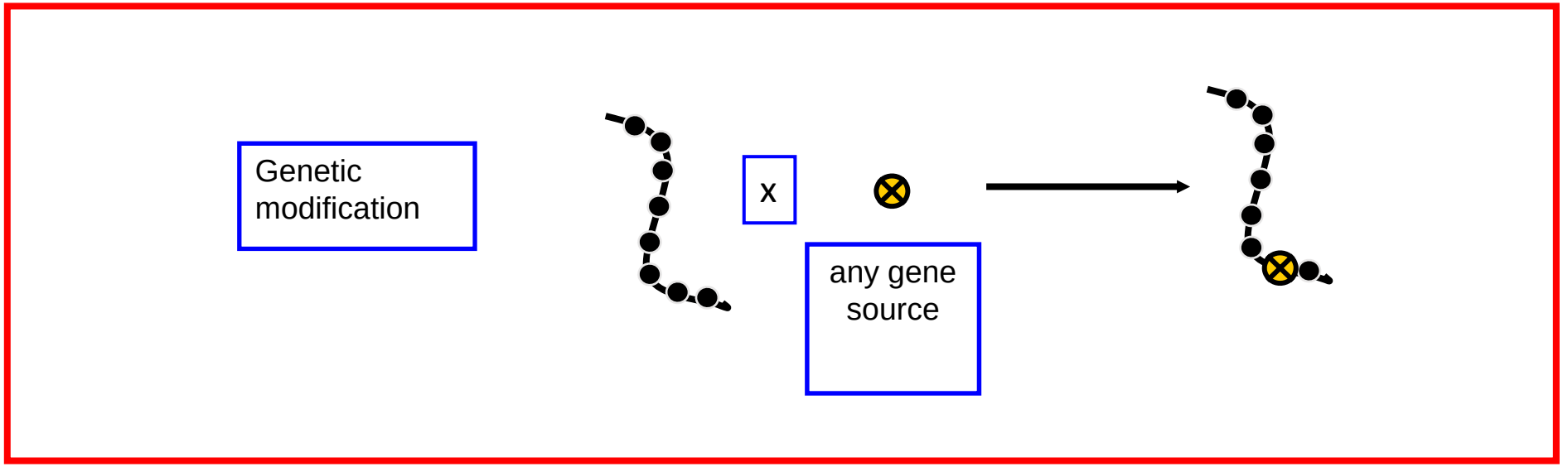
Over 3,000 mutants have been released for commercial cultivation

Many crops grown today are the result of mutation breeding but the process relies on random changes in the DNA sequence

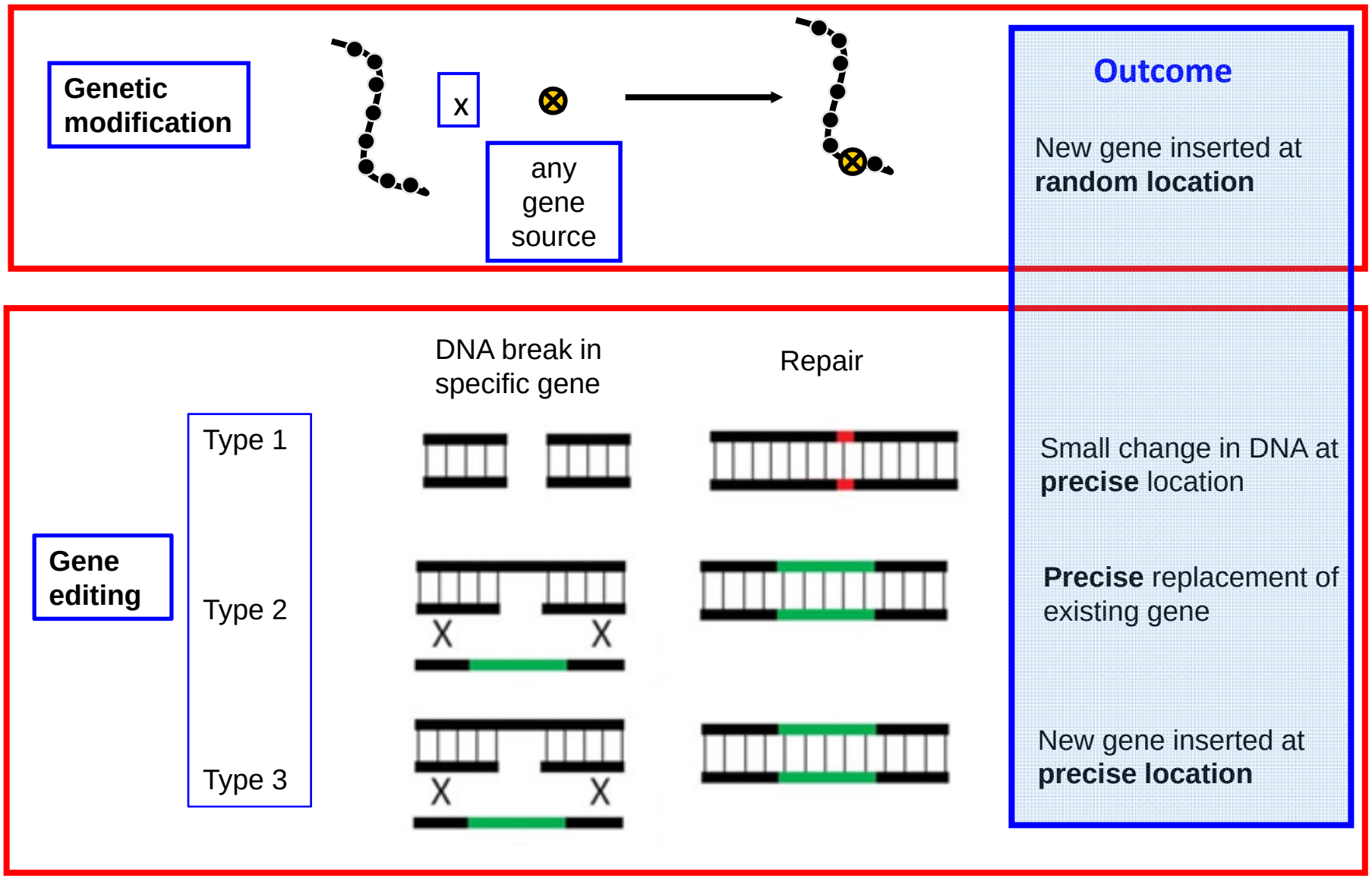


Traditional plant breeding compared to genetic modification

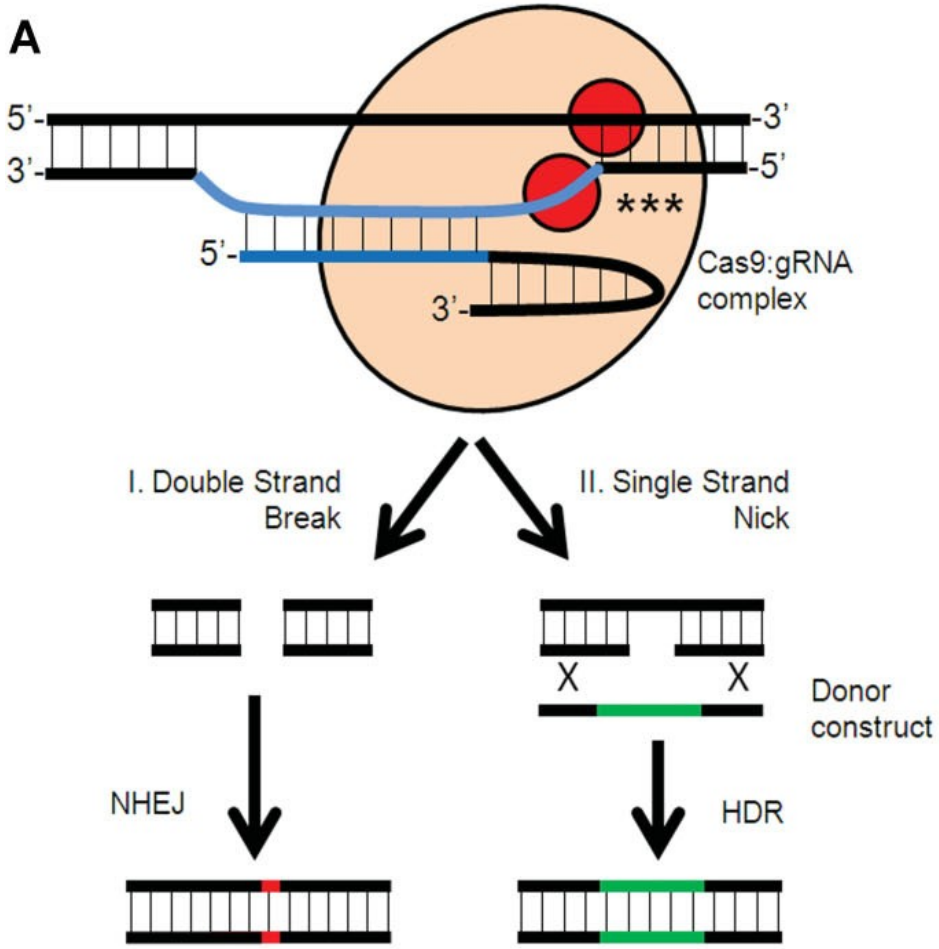




Genetic modification compared to genome editing



CRISPR / Cas 9 genome editing



Insertions / deletions caused by error prone DNA repair **(Knock out)**

Gene replacement / insertion **(Knock in)**

Two components are required to achieve genome editing.

- **The Cas9 nuclease**
- **The 'guide RNA' (gRNA).**

These components can be introduced by genetic modification or simply introduced as a protein / RNA complex.

Sampson & Weiss 2013, Bioessays 36:34-38

Targeted gene knockouts in Brassica

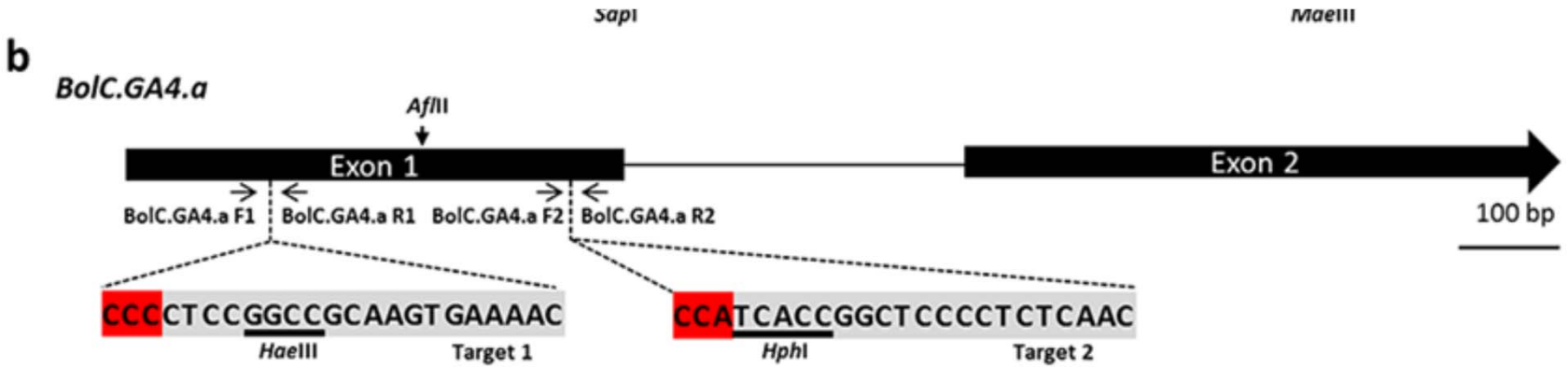
The problem: Pod shatter

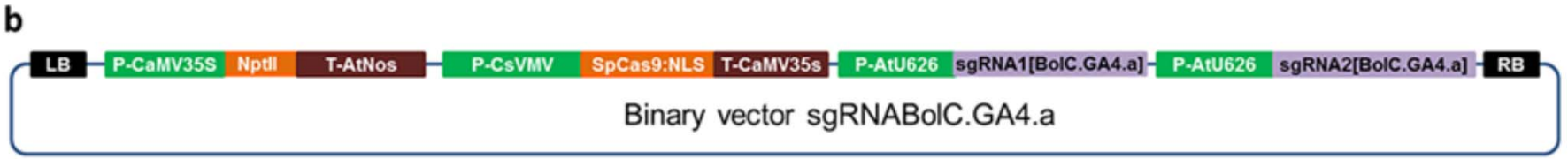
Target gene – GA4 involved in the gibberellin biosynthesis pathway. GA4 loss-of-function mutants in the model plant Arabidopsis have dwarf stature and reduced fruit dehiscence (**pod shatter**).

Lars Ostergaard JIC

Gene editing in Brassica oleracea

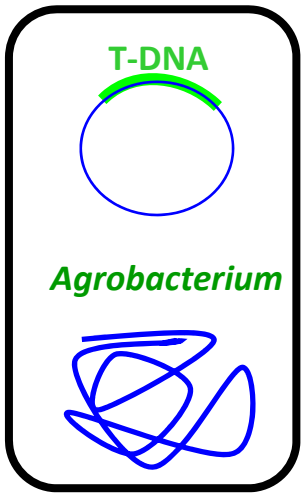
In Brassica there are 2 copies of GA4 – ‘a’ and ‘b’.
Only the ‘a’ copy was targeted.





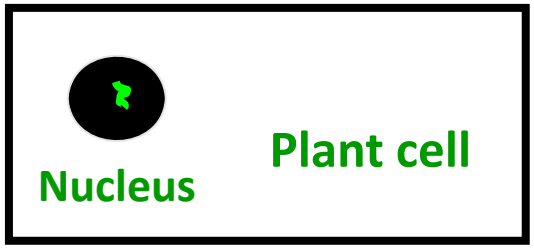
Introduction into Brassica

Agrobacterium- mediated transformation

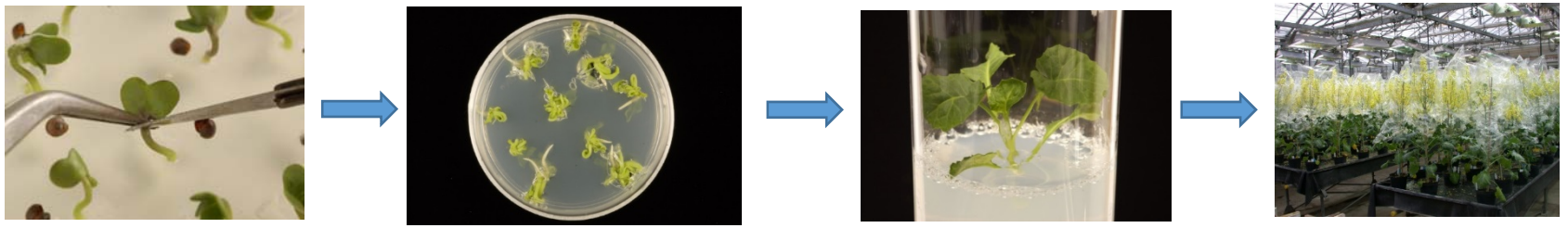


In 1907, Smith and Townsend demonstrated that the Gram-negative soil bacterium, *Agrobacterium tumefaciens*, was responsible for crown gall disease in plants.

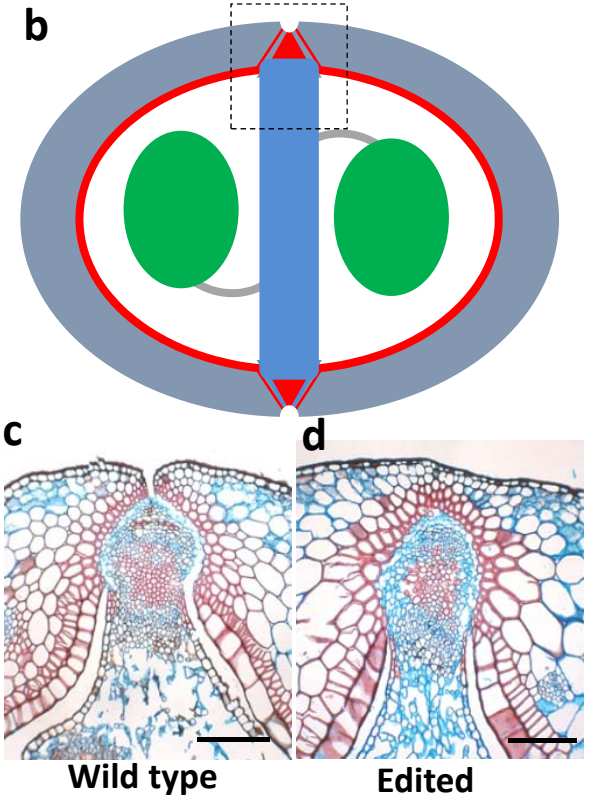
T-DNA



'Nature's own genetic engineer'



An example of gene editing in a crop



Section through pod

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BolC.GA4.a                ACGATCCCCTCTTGGACGCCGCTCCTTCCCCTCCGGCCGCAAGTGAAAACATCCCTCTCATTGACCTGAA
T0-L2E1_17.1 Allele 1     ACGATCCCCTCTTGGACGCCGCTCCTTCCCCTCT-GCCGCAAGTGAAAACATCCCTCTCATTGACCTGAA
T0-L2E1_17.1 Allele 2     ACGATCCCCTCTTGGACGCCGCTCCTTCCCCTCA-GCCGCAAGTGAAAACATCCCTCTCATTGACCTGAA
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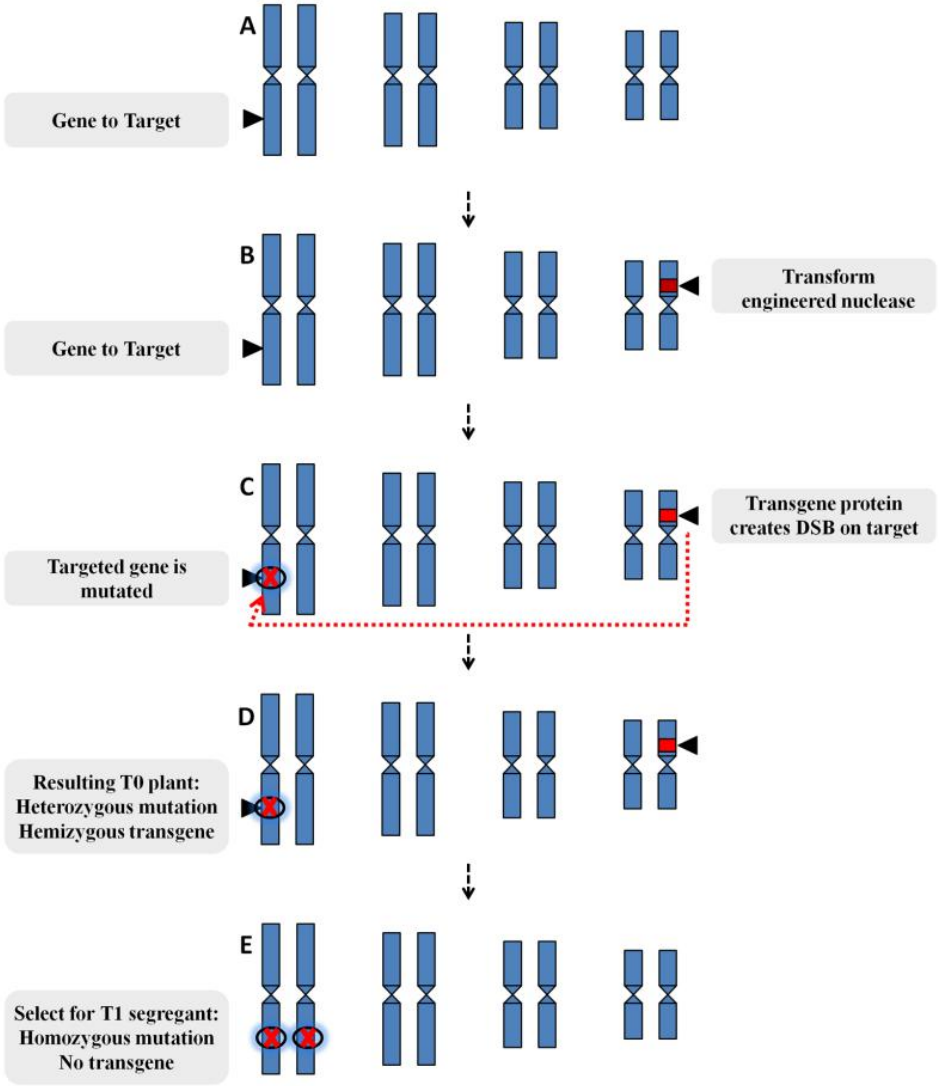
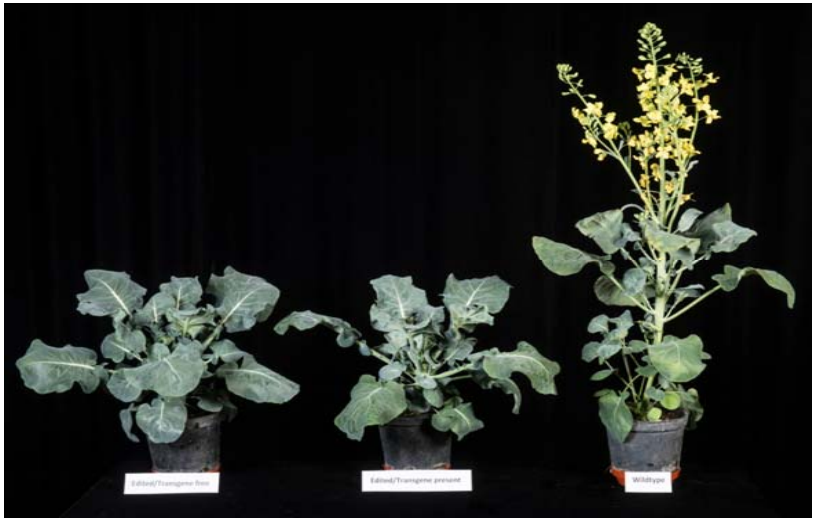
Lawrenson et al. Genome Biology 2015

Recovering plants containing only the target mutation



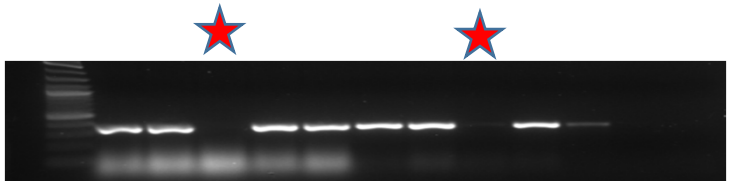
Curtin et al. (2012) The Plant Genome 5: 2



Edited, transgene free

Edited, transgenes present

Wildtype



★ Plants with the mutation but without any transgenes

Applications of gene editing in agriculture

- Resistance to disease and pests
- Enhanced nutritional value (e.g. increased levels of anthocyanins)
- Stress tolerance including drought tolerance
- Improved energy crops
- Improved yield (changes in phenotype, dwarf stature, reduced pod shatter)
- Improved shelf life

John Innes Centre

Unlocking Nature's Diversity

CRISPR crops already available

Gene-edited CRISPR mushroom

A fungus engineered using CRISPR–Cas9 can be cultivated and sold without oversight.

The US Department of Agriculture (USDA) will not regulate a mushroom that has been genetically modified with the gene-editing tool CRISPR–Cas9, the agency has confirmed.

The mushroom resists browning.

- In the EU there is current discussion as to whether these techniques lead to products which are subject to legislation as described in the European Union (EU) Directive 2001/18/EU on Deliberate Release of Genetically Modified Organisms (GMOs).
- Competent authorities in some EU countries have given opinions that gene edited crops that have a small mutation and no transgenes should not be regulated as GM (e.g. Sweden)

Nature 2016

The first CRISPR dinner

Mail Online

Is THIS the meal of the future? Scientists create the world's first gene-edited dinner

'Tagliatelle with green, fried CRISPRy vegetables'

Umeå researcher served a world first (?) CRISPR meal

[2016-09-05] For (probably) the first time ever, plants modified with the "genetic scissors" CRISPR-Cas9 has been cultivated, harvested and cooked. Stefan Jansson, professor in Plant Cell and Molecular Biology at Umeå University, served pasta with "CRISPRy" vegetable fry to a radio reporter.