The CRISPR craze

Wendy Harwood
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

Traditional plant breeding

“elite” variety

Related variety

Genetic modification

any gene source
Genetic modification compared to genome editing

### Genetic modification

- Any gene source
- New gene inserted at random location

### Gene editing

#### Type 1
- DNA break in specific gene
- Small change in DNA at precise location

#### Type 2
- Precise replacement of existing gene

#### Type 3
- New gene inserted at precise location
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.
In 1907, Smith and Townsend demonstrated that the Gram-negative soil bacterium, *Agrobacterium tumefaciens*, was responsible for crown gall disease in plants. ‘Nature’s own genetic engineer’
An example of gene editing in a crop

**Section through pod**

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
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
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.