ADVISORY COMMITTEE ON ANIMAL FEEDINGSTUFFS

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Presentation Paper
Wendy Harwood – ACAF Member

New Plant Breeding Techniques

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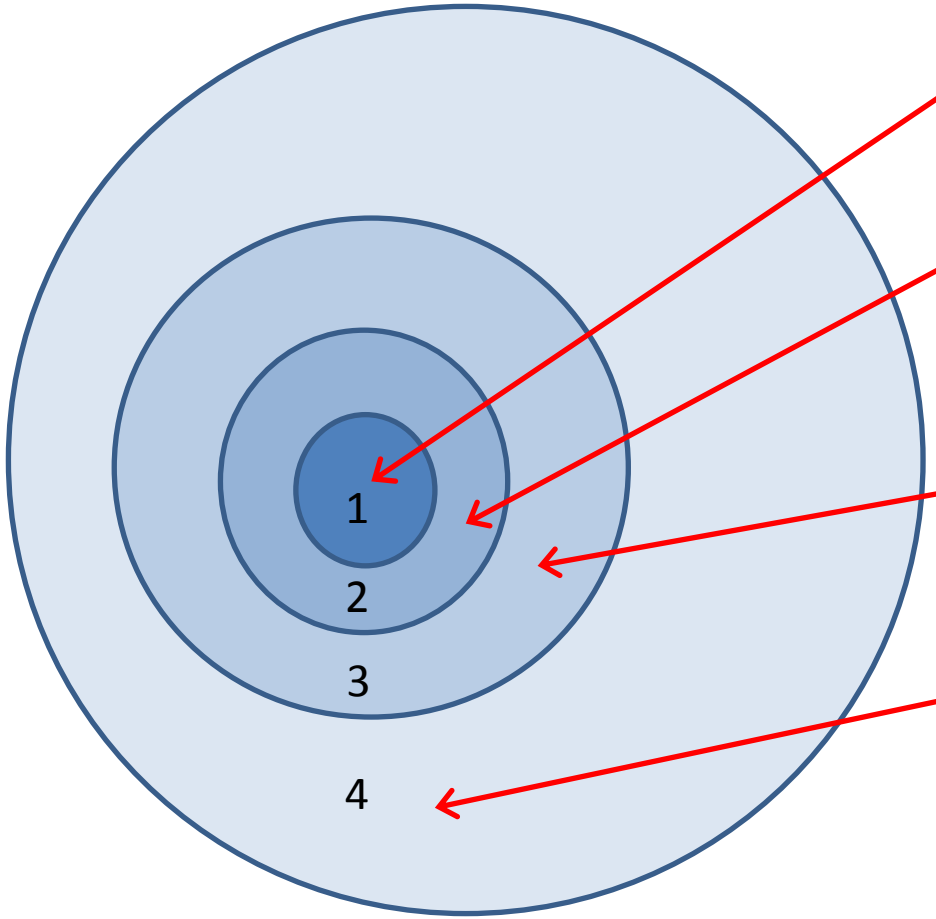
9 May 2014

John Innes Centre

‘Traditional’ Plant Breeding

- Humans have been selecting the best plants to produce future generations for thousands of years.
- Traditional plant breeding has resulted in crops that are very different from their wild ancestors.
- The most important factor for plant breeding is the availability of genetic variation for the characteristic of interest.
- Various methods have been introduced over the years to increase the pool of variation available to plant breeders.
- New technologies also make it easier to recover plants with the required characteristics (for example marker-assisted selection).

Sources of genetic variation for crop improvement



Primary Gene Pool – same and closely related species.

Secondary Gene Pool – more distant species, crosses difficult, may require embryo culture.

Tertiary Gene Pool – Marginally sexually compatible species, crosses usually not successful.

Quaternary Gene Pool – All organisms including animals and microbes. Gene transfer has to be by transgenic (**GM**) methods. (GM crops are grown on more than 12% of the world's arable land)

Other sources of variation for crop improvement:

- Tissue culture induced variation
- Mutation breeding
- **New Plant Breeding Technologies (NPBTs)**
  - NPBTs use biotechnology and molecular biology approaches and go beyond ‘traditional ‘ GM techniques.
  - Some allow more precise modification of plant genomes than was previously possible.
  - Some plants developed using NPBTs will be indistinguishable from plants derived by traditional plant breeding. The genetic variation incorporated in these crops could also have originated naturally.
  - There is an on-going debate as to whether crops derived using NPBTs, and their products should be covered by the same regulations as GM crops.
  - The first crops obtained through NPBTs are close to commercialisation.

New Plant Breeding Technologies include:

- **Cisgenesis / intragenesis** - uses the same techniques as GM but the DNA introduced comes from the same or cross-compatible species only.
- **Genome editing** - Zinc finger nucleases, TALENS & CRISPRs – allows specific changes at specific locations in the plant DNA.
- **GM rootstock grafting** – A graft is made between a GM and a non-GM plant.
- **Reverse breeding** – Allows homozygous lines to be produced by silencing the normal recombination process followed by production of double haploids.
- **Oligonucleotide-directed mutagenesis (ODM)** – uses chemically synthesised oligonucleotides to induce mutations at specific target sites in the plant DNA.
- **RNA-dependent DNA methylation** – A way of silencing genes by methylation of promoter sequences.
- **Agro-infiltration techniques** – A range of methods for infiltrating plant tissues with a suspension of Agrobacterium carrying a gene of interest.

Uptake of NPBTs by industry

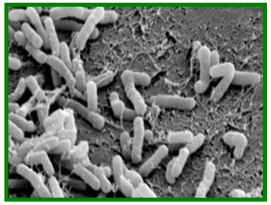
Maria Lusser et al. 2012 conducted a survey to determine the extend to which plant breeders were adopting NPBTs and to examine the development of commercial products.

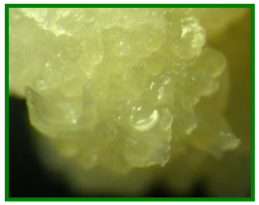
- The survey showed that all of the NPBTs listed above were being used by between 2 and 4 out of 17 plant breeding companies who responded.
- Some crops developed using these techniques have reached an advanced commercial development stage (phase 3).
- Herbicide tolerant oilseed rape and maize has been developed using ODM.
- Zinc-finger nucleases are being used in breeding maize, oilseed rape and tomato.
- Cisgenesis / intragenesis based products also include maize, oilseed rape and potato.

Uncertainly regarding the regulatory status and possible high regulatory costs were reasons given limiting the use of the technologies.

‘Traditional’ GM

- Introduced genes can come from any source
- Insertion of the genes into the plant genome is at a random location
- A tissue culture step is often needed
- A selectable marker is often needed but can be removed later

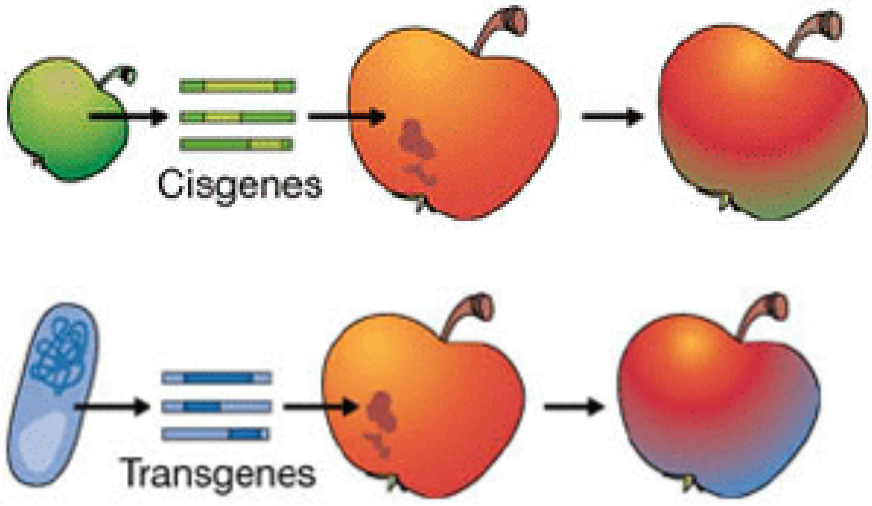




Isolate target tissue and introduce new gene(s) using *Agrobacterium*

Select and then regenerate GM plants

Cisgenesis / Intragenesis



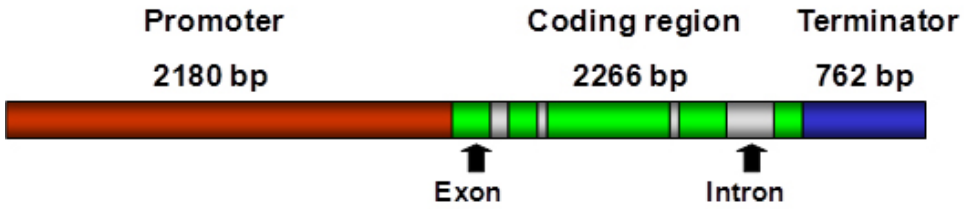
- Uses the same gene pool as traditional plant breeding but is quicker and does not transfer unwanted genetic material along with the desired gene (linkage drag).
- The same techniques used for GM are used to produce cisgenic plants.
- Intragenesis is like cisgenesis but allows different combinations of genes and promoters to be used rather than ruling that a gene must have its own promoter and terminator.

Example of the possible use of cisgenesis in animal feed

Barley with improved grain phytase activity.

- Phytases release phosphate groups from phytic acid.
- They are important for making phosphate available to monogastric animals like pigs and poultry.
- The phytase activity in barley grain is not sufficient therefore phytase from a microbial source or extra phosphate is commonly added to feed.
- If there is insufficient phytase activity then phosphorus not utilised by the animal will be excreted and can lead to problems in the environment.

- A barley phytase gene was used and expressed during grain filling.



- Marker genes were removed and no additional sequences were present. Plants had a 2-3 fold increase in phytase activity.

- Currently in field trials in Denmark, next step is to apply for permission to study this barley in animal feeding trials.

- There are also currently trials of a similar, but transgenic, barley in the Czech Republic.

- Other crops modified using cisgenic approaches include potato, apple, strawberry and grapevine.

- EFSA has evaluated crops generated by cisgenesis and concluded that existing guidance for GM food and feed is applicable but that the amount of risk assessment data required could be reduced on a case by case basis.

Genome editing (targeted gene modification technologies)

- Overcomes one of the main arguments against the use of GM crops - that the random integration of the gene of interest might cause problems in the host genome.

- The effects produced are very similar to those produced by natural variation or by mutation breeding although they are much more specific.

- Genome editing has been used to generate herbicide tolerant plants and this may be one of the first commercial applications of relevance to animal feeds.

- The method relies on two components:
  - a sequence specific DNA binding system
  - linked to a nuclease to cause breaks in the targeted DNA sequence.

- These technologies can lead to:
  - targeted inactivation of a specific gene (targeted mutagenesis)
  - targeted gene insertion
  - gene replacement

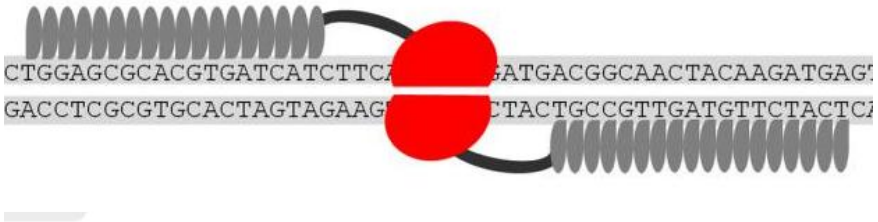
Genome editing

Nature Methods named targeted gene modification technologies, or genome editing, as the **method of the year 2012**. There were originally three proteins that could be used:

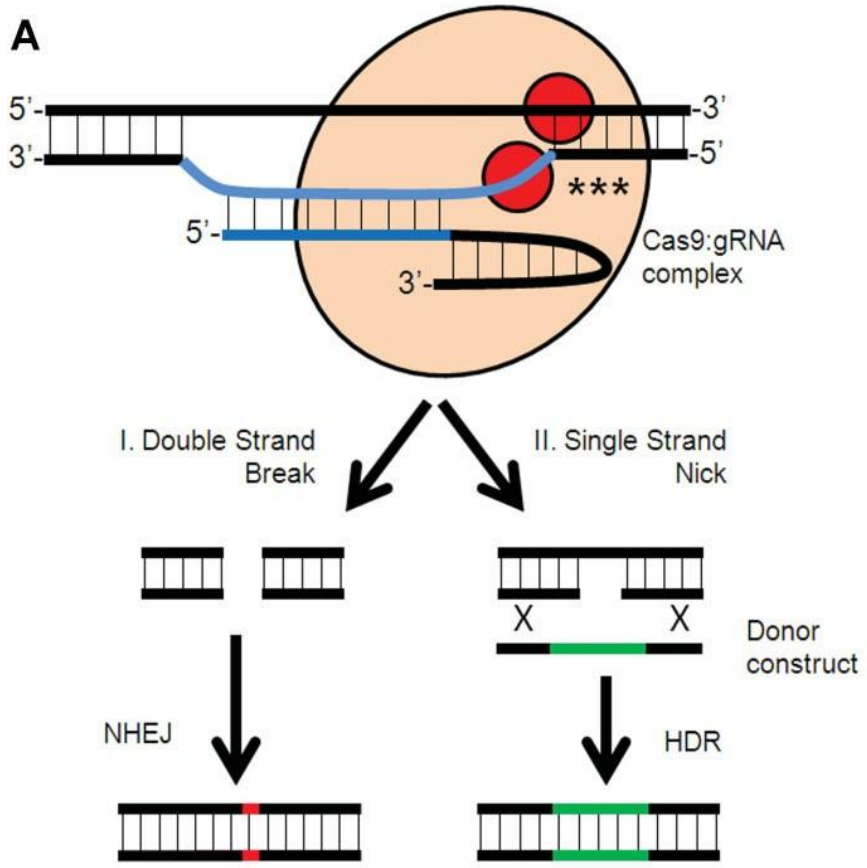
Zinc Finger Nuclease

LAGLIDADG Homing Endonuclease (LHE)“meganuclease”

Transcription Activator-Like Effector Nuclease (TALEN)



CRISPR / Cas 9



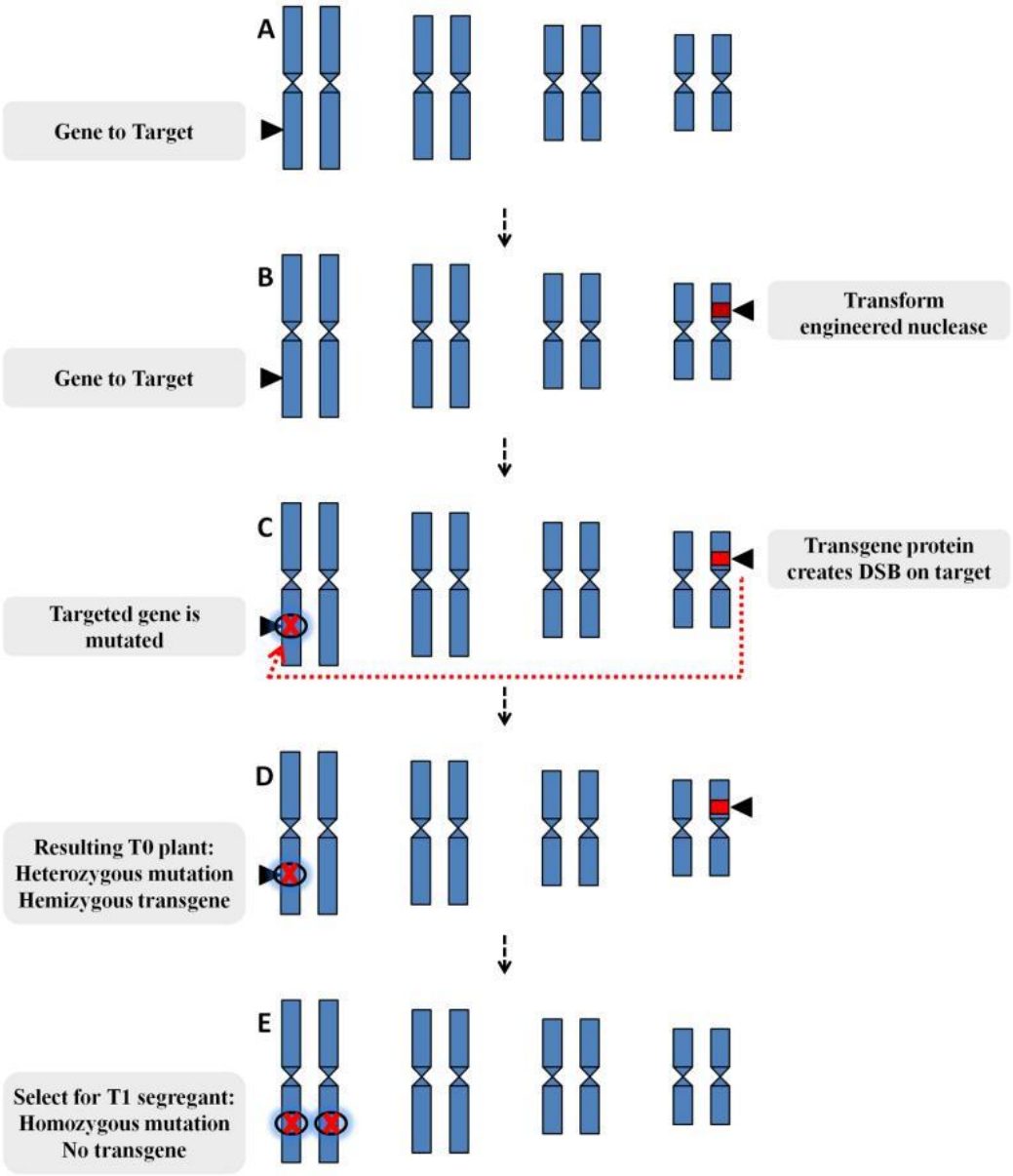
Non-homologous end joining

Homology-directed repair

- The most recent tool for genome editing is the CRISPR/Cas9 system (**c**lustered **r**egularly **i**nterspaced **s**hort **p**alindromic **r**epeats).
- Uses a guide RNA to direct the nuclease.
- Discovered as a bacterial defence system against invading foreign DNA.
- Cas9 targeting systems could be very significant in future gene therapy applications.
- They could also have huge implications for crop improvement. They have been shown to work in rice, wheat, maize and barley.
- Advantages over previous genome editing systems include easy to design, flexible, affordable and efficient.

Sampson & Weiss 2013, Bioessays 36:34-38

Recovering plants containing only the target mutation



Challenges for detecting plants and products derived using the new techniques in animal feeds.

- Some changes made using the new techniques could also be generated by other mutagenesis techniques used in traditional plant breeding or could arise due to natural genetic variation.

- A change made using genome editing could only be detected if information on the target DNA sequence and on its flanking sequences was available.

- For cisgenic plants, similar prior information would be needed to allow the design of appropriate primers for PCR detection.

- Plants derived by the RNA-dependent DNA methylation method would have no changes to the DNA sequence itself as it is just the methylation pattern that is modified. These could not therefore be easily detected.

Conclusions

- New Plant Breeding techniques are evolving very quickly.
- They are being adopted by industry because of the advantages they offer. There have been numerous recent patent applications on the CRISPR/Cas system.
- It is likely that some of the first commercial products using these techniques will be for animal feeds.
- Regulation of these new techniques is currently vague and the debate surrounding them is on-going.
- These techniques allow plant genome modifications which are indistinguishable from those introduced by conventional breeding and chemical or physical mutagenesis. Crops produced in this way may be classified as non-GM.
- ACRE has issued advice on the new plant breeding techniques and highlighted areas where legal clarification is needed. ACRE advises that the changes made using these techniques should be considered in the context of the large amount of variation already present in the same species.