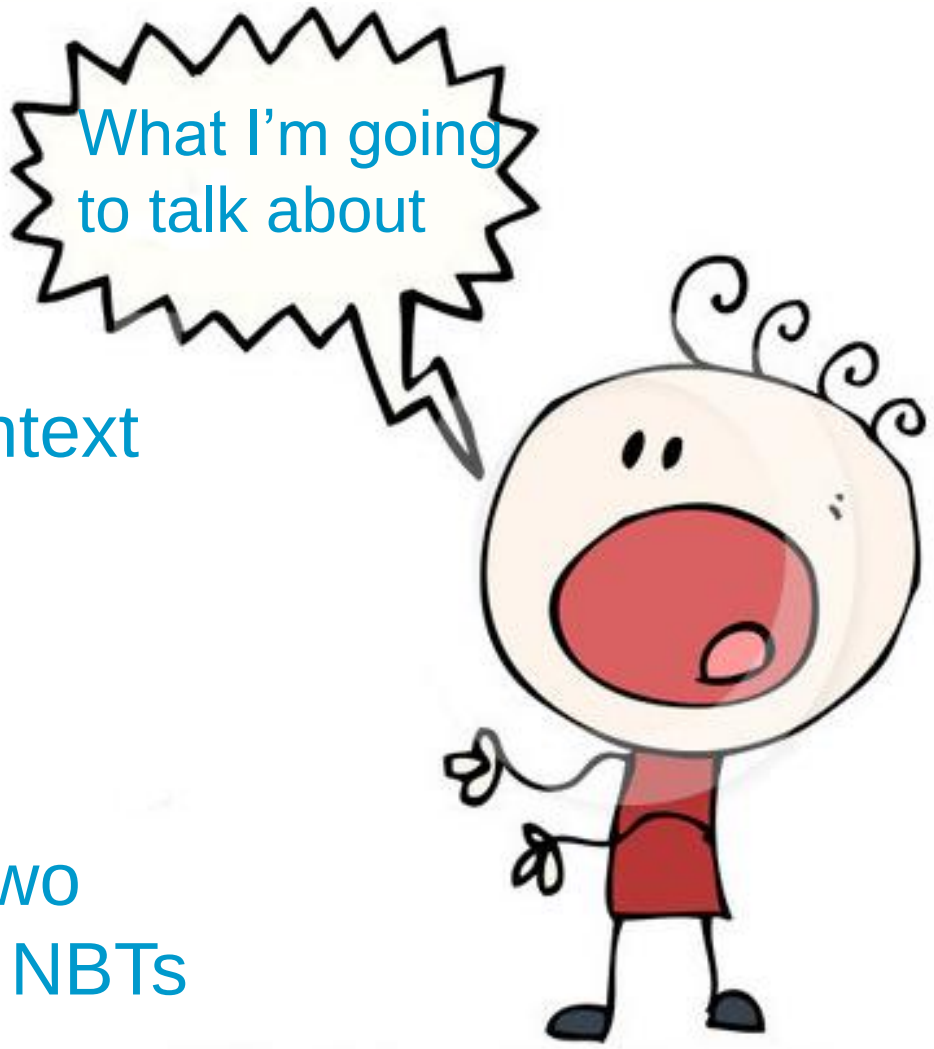
FOOD STANDARDS Australia New Zealand
Te Mana Kounga Kai – Ahitereiria me Aotearoa

Overview of techniques & NBT Technical Workshop outcomes

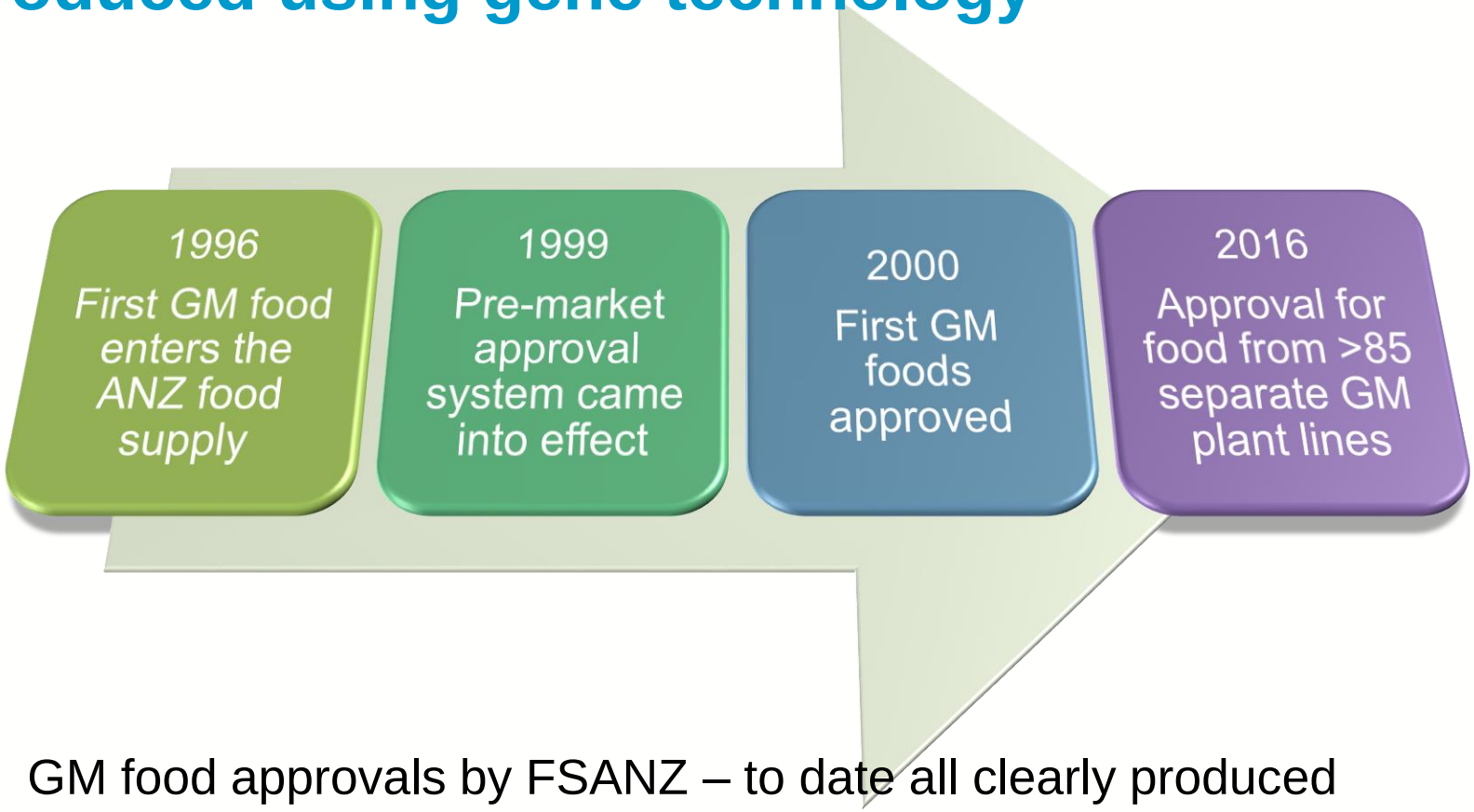
Janet Gorst

Workshop on the Regulatory Status of New Breeding Techniques
31 August 2016



- A bit of history and context
- A layman’s look at the techniques involved
- The outcomes of the two FSANZ workshops on NBTs

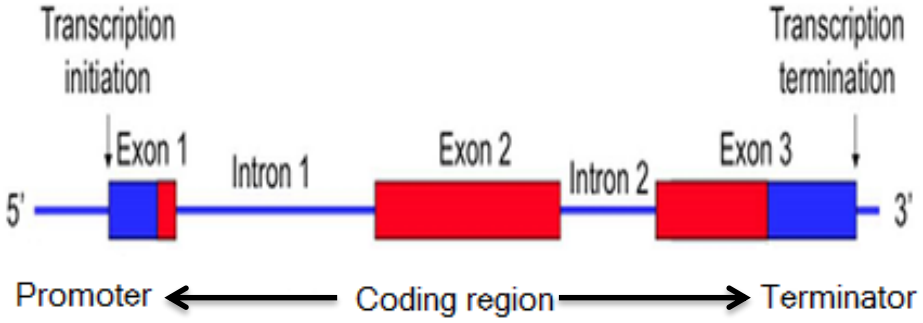
Standard 1.5.2/Schedule 26 - Food produced using gene technology



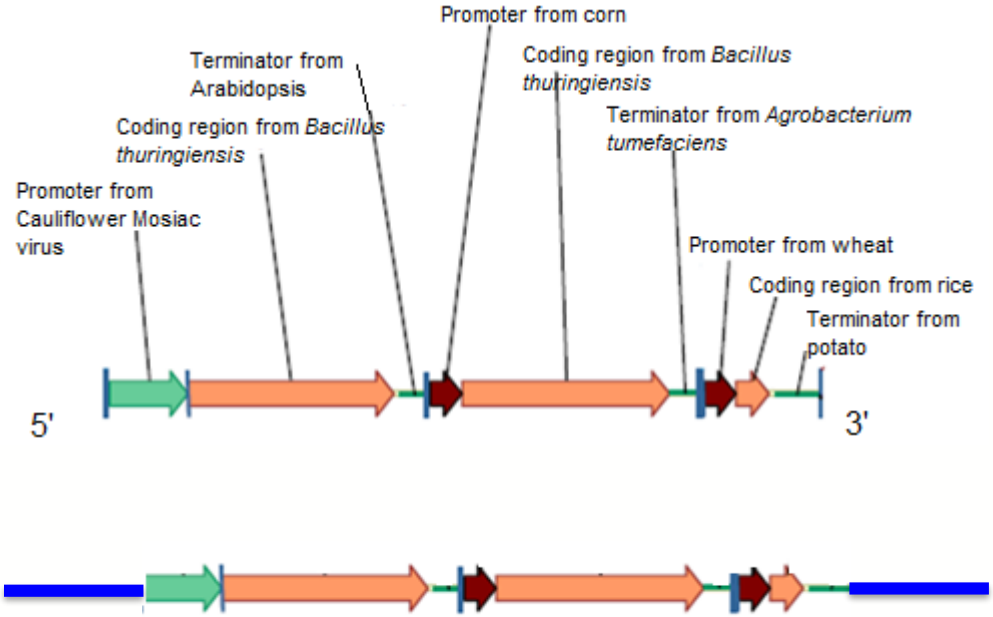
GM food approvals by FSANZ – to date all clearly produced by transgenesis and all plants

What is transgenesis?

Generalised gene structure



A construct = gene(s) artificially assembled together in the lab



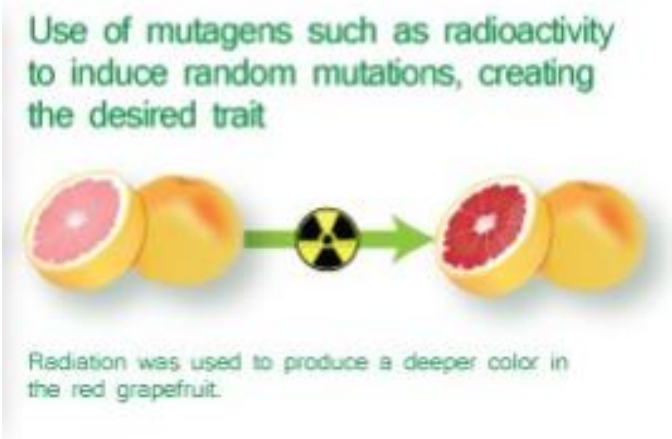
The construct is incorporated somewhere into the genome of the host – via Agrobacterium *or biolistics*

What is not food produced using gene technology?

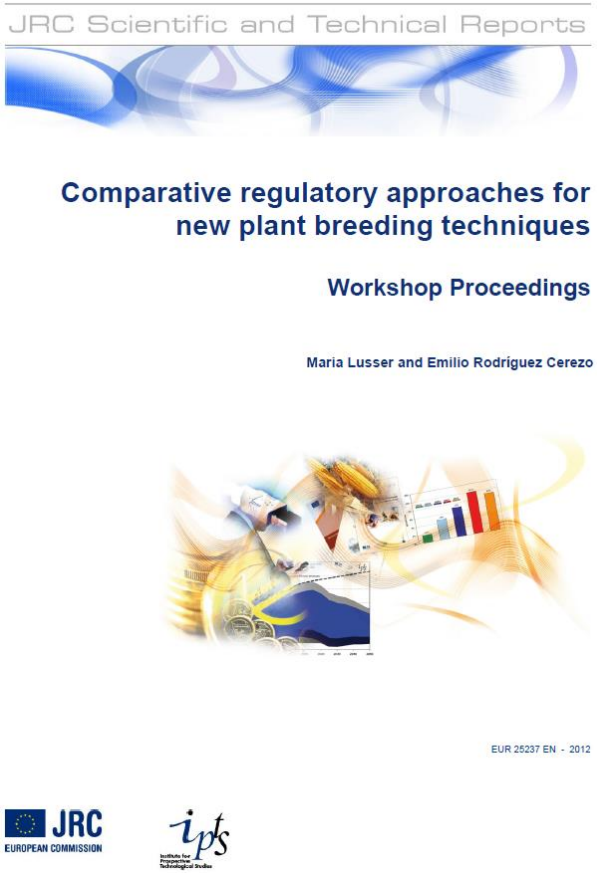
Conventional breeding

Traditional cross-breeding

Induced mutagenesis



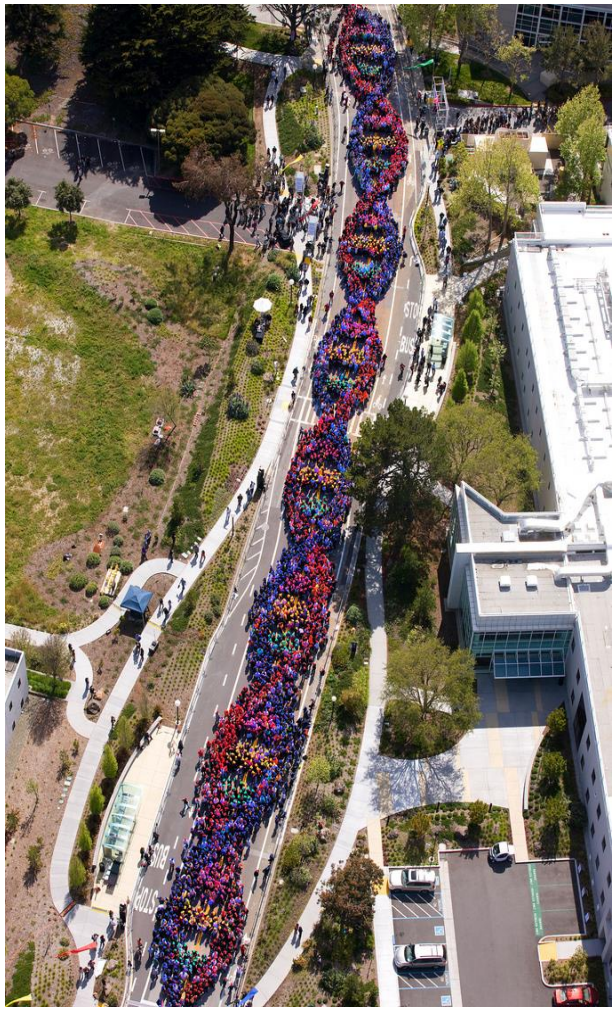
2011: first rumblings about new techniques



Are/should these NBTs be covered by current GM regulations?

2012: the momentum picks up

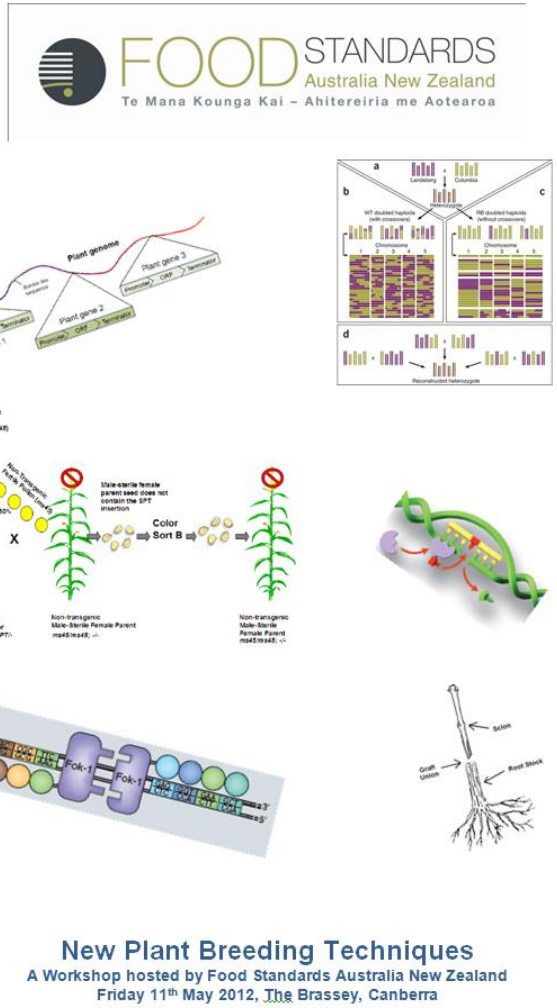
- Foods produced using a number of NPBTs [which became NBTs] were close to commercialisation
- FSANZ had received a number of enquiries in relation to some new NBTs
  - are they captured by the GM food Standard (Standard 1.5.2 Food produced using gene technology)?
- The Office of the Gene Technology Regulator – which issues licences for the growing of GM crops in Australia - had also received enquiries about NBTs
- Other countries began looking at the extent to which new breeding techniques were captured by their GM food legislation.



FSANZ Workshops on NBTs

- FSANZ hosted two technical workshops to discuss various new breeding techniques with a panel of invited experts in the fields of plant breeding and plant biotechnology.
- The aims of the workshops were to:
  - Improve FSANZ's understanding and knowledge of certain new plant breeding techniques.
  - Provide advice to FSANZ on whether there are safety concerns with food derived using certain new plant breeding techniques.
  - Provide a scientific opinion on whether derived food products should be regarded as GM food.

The workshops were held on 11 May 2012 and 6 August 2013



..'regulators are struggling..'

14 APRIL 2016 | VOL 532 | NATURE | 147

Breeding controls

Scientists must help to inform regulators wrestling with how to handle the next generation of genetically engineered crops.

On 6 April, activists gathered in Paris to protest against an emerging class of genetically altered crops. Regulators often classify these as the product of 'new breeding techniques' (NBTs) that are sometimes distinct from classical — and historically controversial — genetically modified (GM) varieties. But some protesters, such as those who joined the Friends of the Earth demonstration in Paris last week, are unconvinced by that argument. They call the new plants 'hidden GMOs'.

Around the world, regulators are struggling to decide how to adapt the existing rules for transgenic technology to plant varieties that have been engineered using cutting-edge methods (see page 158). Many have found that their classical regulatory triggers rely on definitions of 'transgenic' or 'genetically modified organism' (GMO) that no longer apply. And they are questioning whether some NBT crops need to be regulated at all.

"There is room for a healthy debate as to how these crops are regulated."

THE CONVERSATION

Academic rigour, journalistic flair

Arts + Culture | Business + Economy | Education | Environment + Energy | Health + Medicine | Politics + Society | Science + Technology

All our food is ‘genetically modified’ in some way – where do you draw the line?

April 4, 2016 11.47pm AEST

“We shouldn't forget that there are also other promising novel… breeding technologies, post-GM, and we shouldn't make the mistake of regulating them to death as we have done with GM.” – Prof. Anne Glover (2013): [then] Chief Scientific Adviser to the President of the European Commission

There is strong evidence that the current regulatory regime does not adequately assess the safety of GMOs, particularly their long-term health impacts[46]. However, the current regulatory approach to GMOs should be the minimum requirement for these new GM techniques

http://emergingtech.foe.org.au/resources/gm-2-0-australian-regulators-engineering-the-truth/

What are these New Breeding Techniques?

- Agro-infiltration
- Cisgenesis/Intragenesis
- GM Rootstock Grafting
- RNA interference/RNA-directed DNA methylation

Transgenic assisted breeding**

- Speed breeding following early flowering
- Reverse Breeding
- Seed Production Technology (SPT) – a proprietary technique developed by Pioneer Hi-Bred

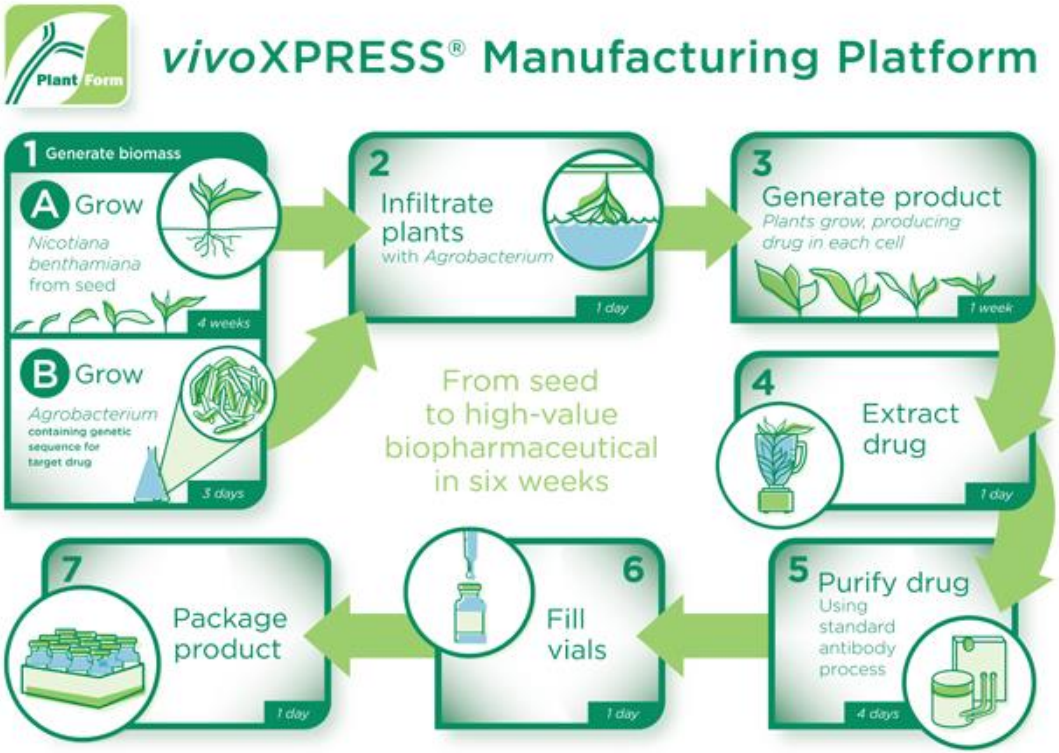
Gene editing**

- Site-directed nucleases – ZFN, TALEN, CRISPR/Cas9
- Oligo-directed mutagenesis (ODM)

Agro-infiltration

- Plant tissues are infiltrated with a liquid containing *Agrobacterium* with a desired gene
- If gene is transiently expressed in somatic cells, plant is non-GM
- If gene is incorporated into germ cells, the resulting seeds will be GM

ICON GENETICS

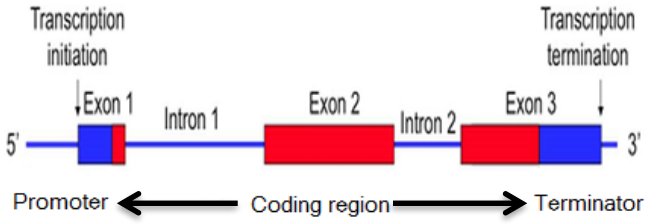


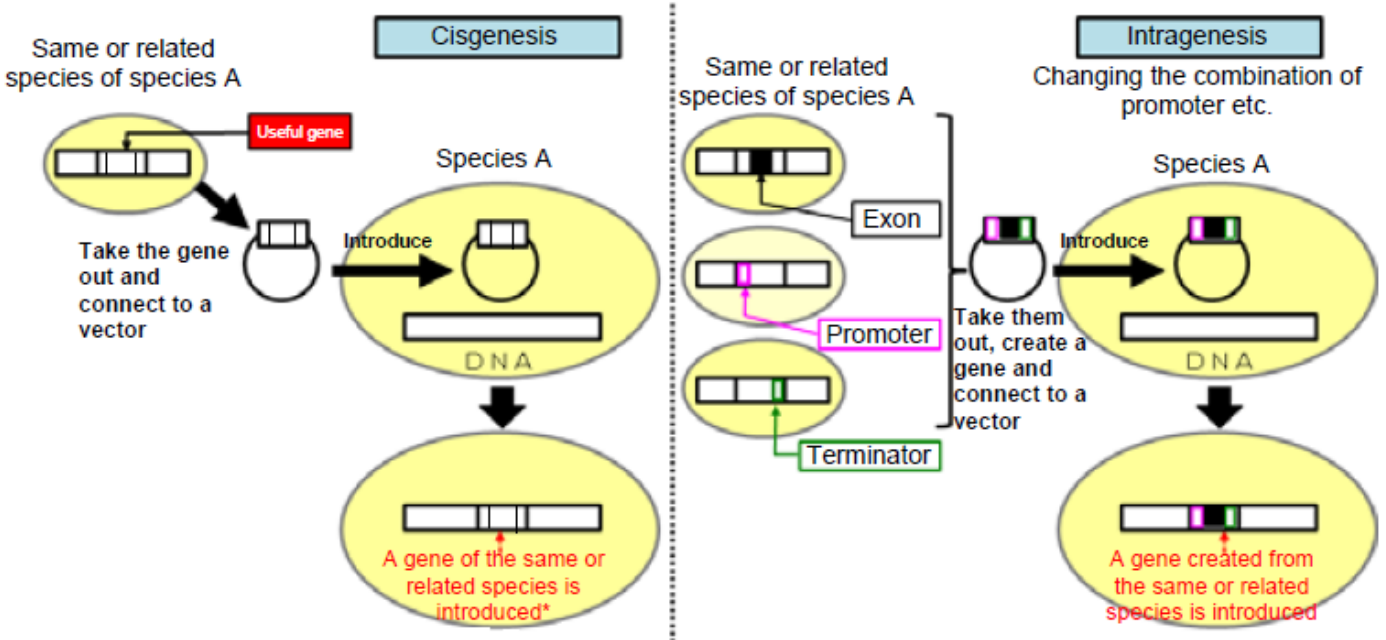
Plant Form

Cisgenesis and Intragenesis

Techniques are equivalent to transgenesis – involve using recombinant DNA techniques to introduce DNA into an undefined new site in the genome

Generalised gene structure



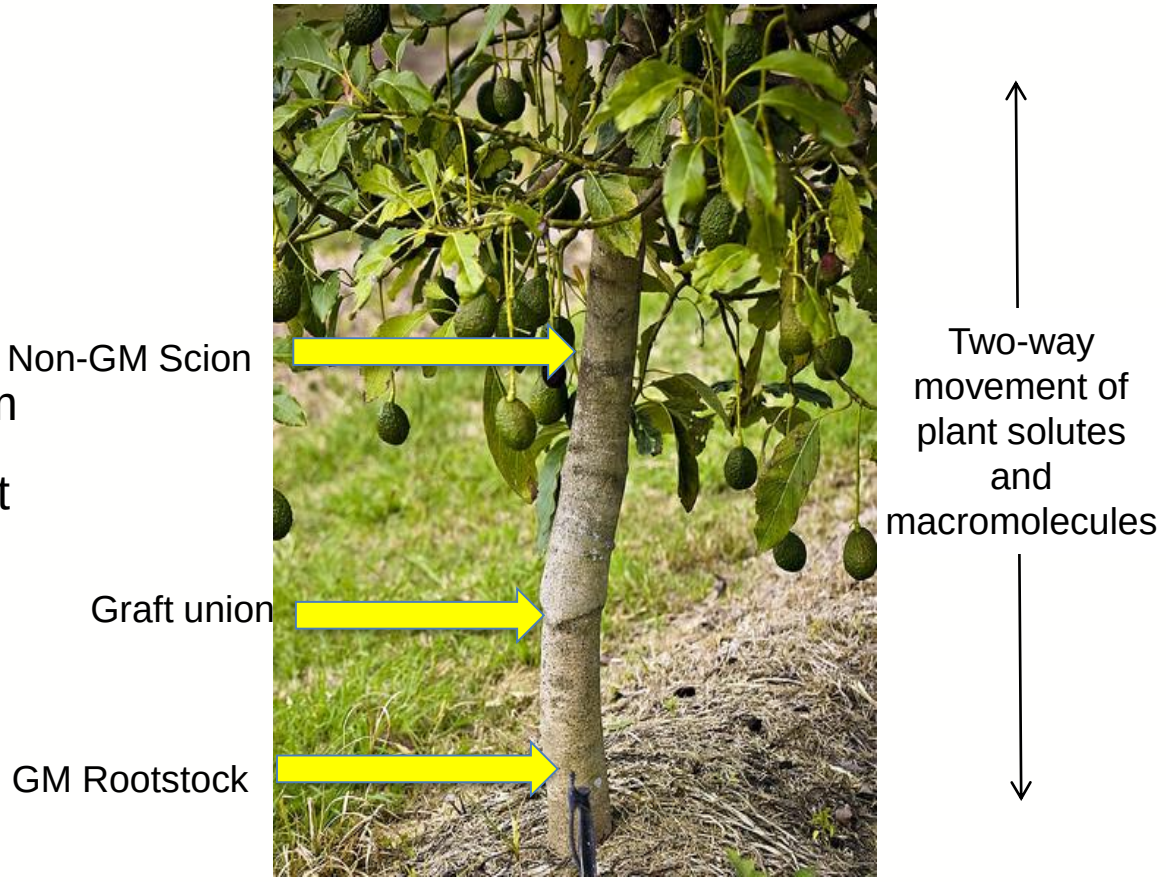


The inserted gene, associated introns, and regulatory elements are contiguous and unchanged

The inserted gene may have the introns removed, the regulatory elements (promoter and terminator) may come from different sources

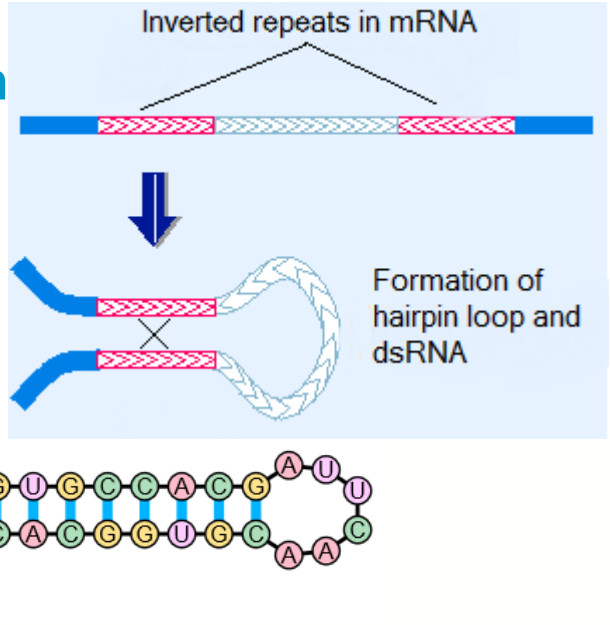
GM Rootstock Grafting

- Non-GM scion grafted on transgenic rootstock = a composite plant that is transgenic
- The scion and the rootstock share a single vascular system
- The non-GM scion/fruit will not contain any transgenic DNA, BUT it may contain traces of GM material (RNA or protein) and/or have altered characteristics as a result of the modification to the rootstock



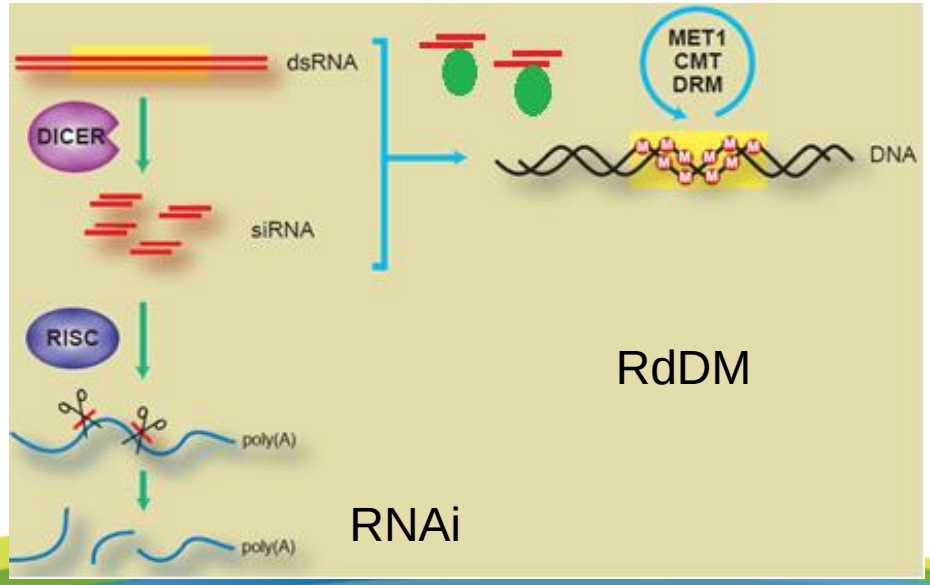
RNA interference/RNA-directed DNA methylation

Transgenic plant is generated using an inverted repeat that, when transcribed, results in the formation of dsRNA.



RNAi

- Targets RNA of a specified gene and leads to gene silencing.
- Requires the RNAi construct to be stably inherited.



RdDM

- Targets (promoter) sequence and leads to methylation that then inhibits transcription of the gene.
- Intention is that the RdMD construct be segregated away but the methylation pattern/gene silencing is inherited. This is known as an ***epigenetic*** modification (i.e. a heritable change without alteration to the gene sequence).

Journal of Cell Science 117(21)

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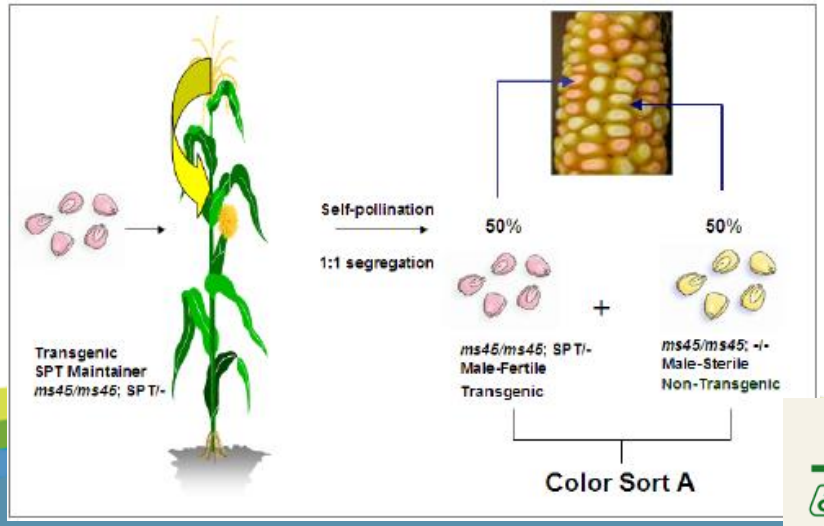
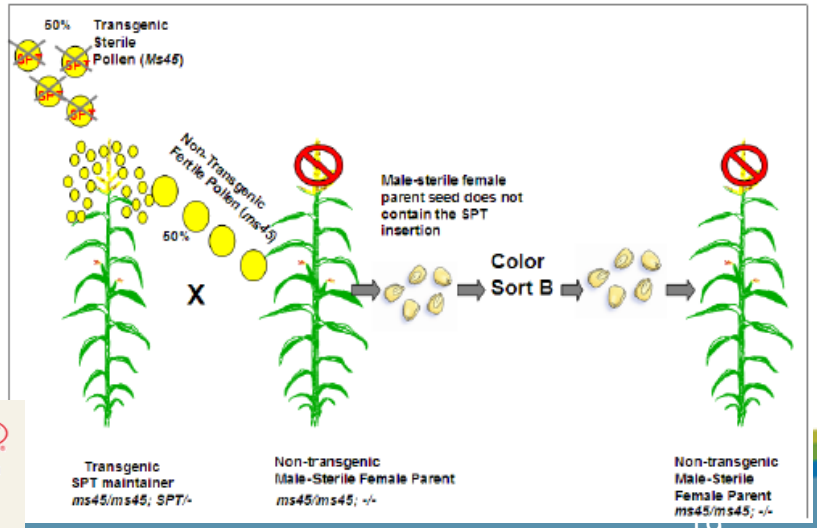
Transgenic assisted breeding (transient transgenics)

- These techniques have in common a transgenic stage the outcome of which will confer a trait facilitating the conventional breeding that then follows e.g. induction of early flowering, reverse breeding, Pioneer SPT.
- In all cases, once the introduced genes have done their job, and are no longer needed they are segregated away during the subsequent conventional breeding stages.
- The final product which bears the food contains no evidence of the genetic material that was initially introduced. It is referred to as a ***null segregant*** and is several generations removed from the transgenic progenitor.

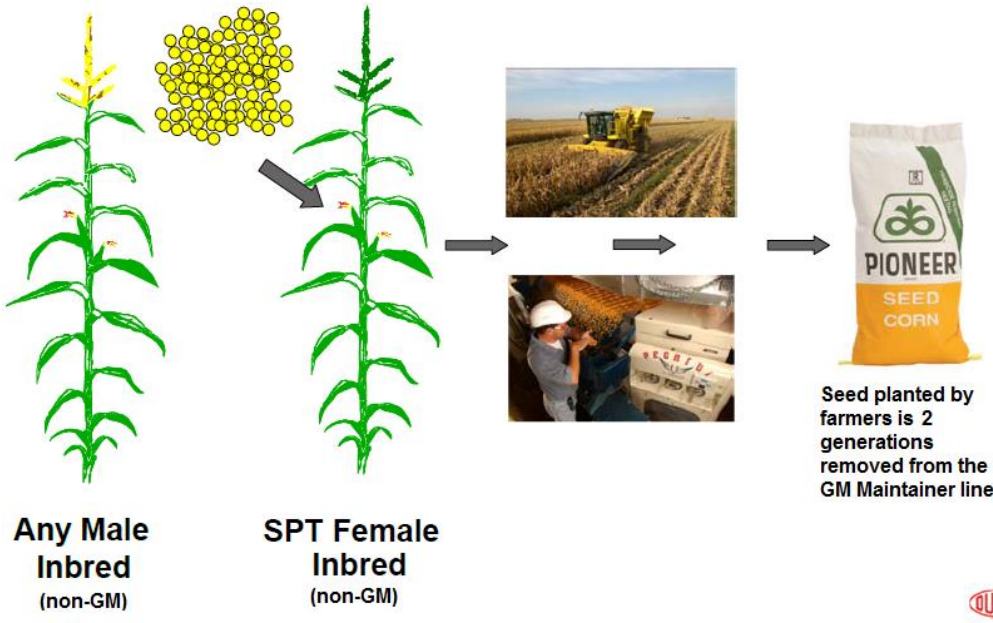




DUPONT PIONEER

Pioneer SPT corn is already being grown in the USA

Field showing commercial corn hybrids produced using the SPT process. It is a non-invasive process that leaves corn plants undamaged during hybrid seed production.



Plant Biotechnology Journal

Plant Biotechnology Journal (2016) **14**, pp. 1046–1054 doi: 10.1111/pbi.12477

Development of a novel recessive genetic male sterility system for hybrid seed production in maize and other cross-pollinating crops

Yongzhong Wu†, Tim W. Fox, Mary R. Trimnell, Lijuan Wang, Rui-ji Xu‡, A. Mark Cigan, Gary A. Huffman§, Carl W. Garnaat¶, Howard Hershey and Marc C. Albertsen*

DuPont Pioneer, Johnston, IA, USA

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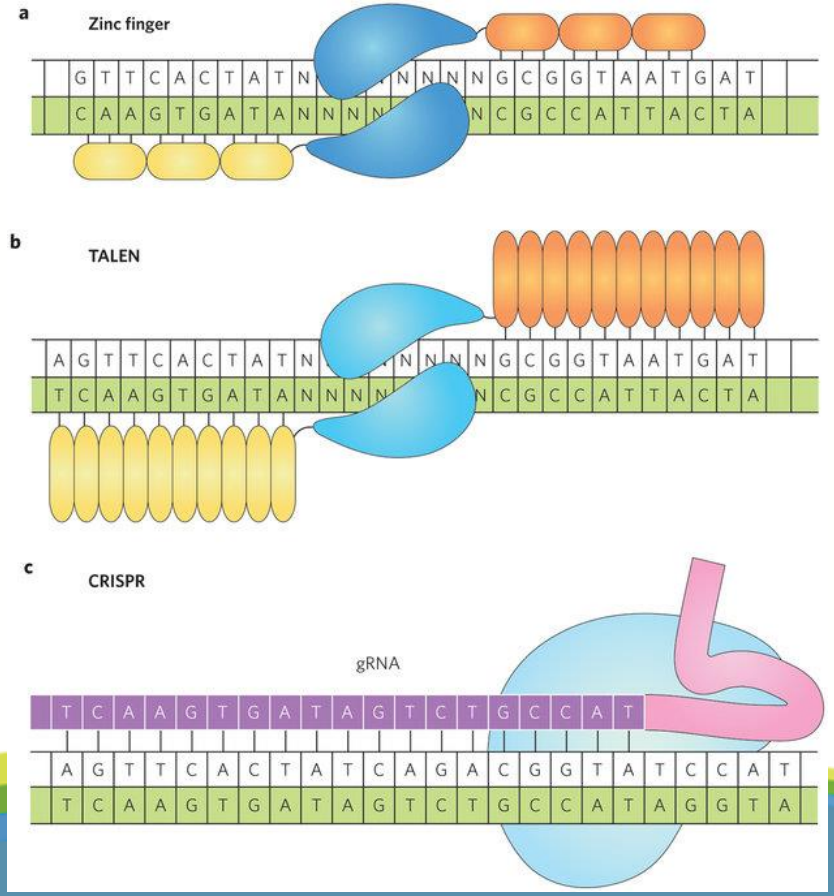
Site directed nucleases (SDNs)

There are two key components to each SDN:

- A DNA recognition code (protein-based or RNA) that finds a specific site in the genome
- A nuclease (protein) that cuts the double stranded DNA at that specific site



These components can be added to the host :

- directly as the proteins or RNA
- via a transgenic step

If by transgenesis, once the component genes have done their job, the idea is that they are segregated away during subsequent breeding.

Site directed nucleases (SDNs)

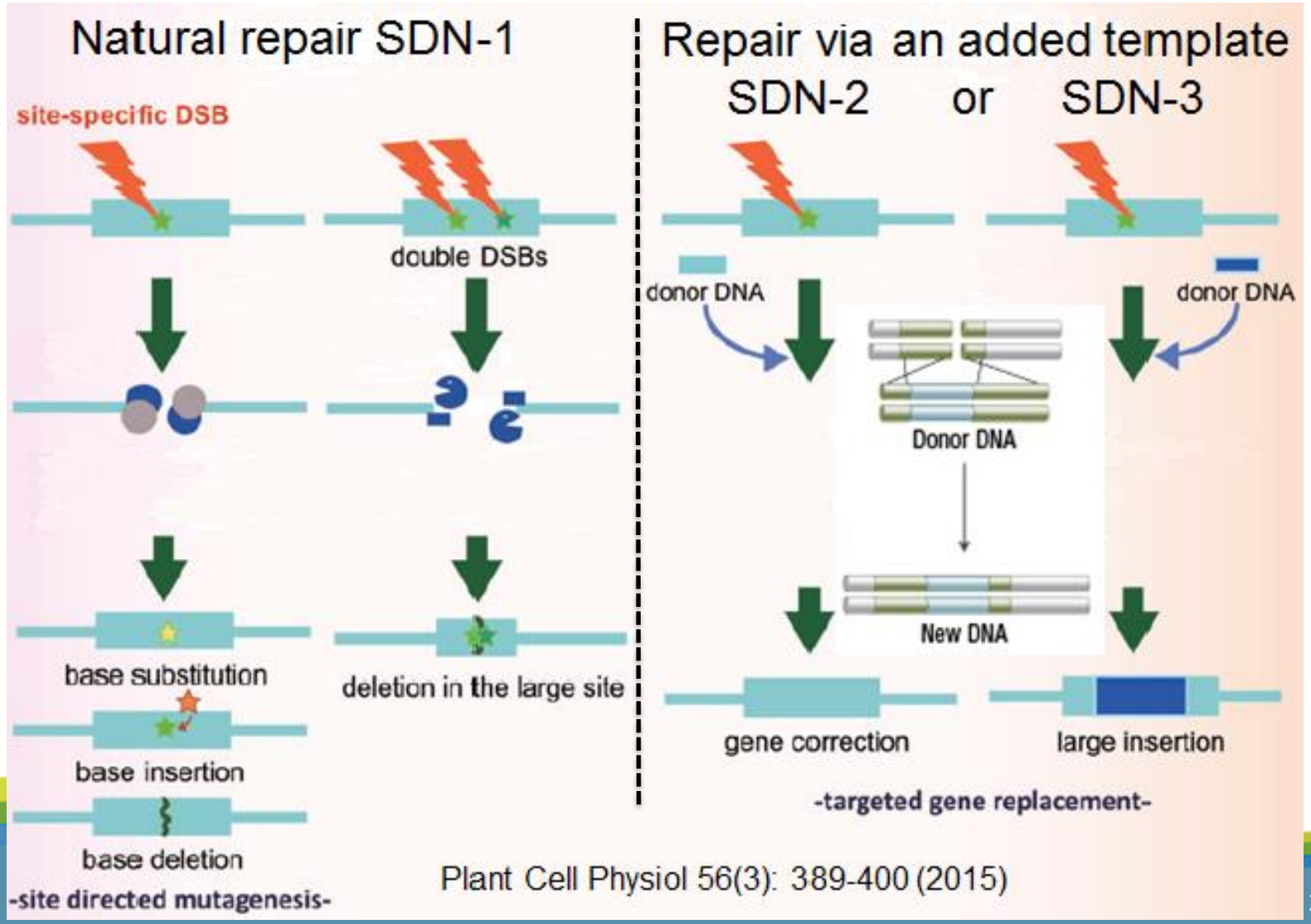
There are three classes of SDNs

- Zinc-finger nuclease (ZFN) – uses an engineered zinc finger protein + nuclease dimer (Fok1)
- Transcription activator-like effector nuclease (TALEN) – uses a designer TALE protein + nuclease dimer (Fok1)
- Clustered, regularly interspaced, short palindromic repeats (CRISPR) – uses a guide RNA + nuclease (Cas9)

Method	Recognition	Cleavage
ZFN	ZFN Protein-DNA	Fok1
TALEN	TALE Protein-DNA	Fok1
CRISPR	CRISPR RNA-DNA	Cas9

Site directed nucleases (SDNs)

There are three ways in which each SDN technique can be used



Site directed nucleases (SDNs)

SDN food products are already non-regulated in the USA

1. TALEN (SDN-1, gene disruption, null segregant) wheat

USDA clears mildew-resistant biotech wheat

Published on March 15, 2016 12:08PM
Last changed on March 16, 2016 9:29AM

MLO_KO Wheat has improved disease resistance to powdery mildew attributable to the knockout of the Mildew Resistance Locus gene achieved through transient expression of a Transcription Activator-Like Effector Nuclease (TALEN®).

2. CRISPR/Cas9 (SDN-1, gene deletion, null segregant) waxy corn

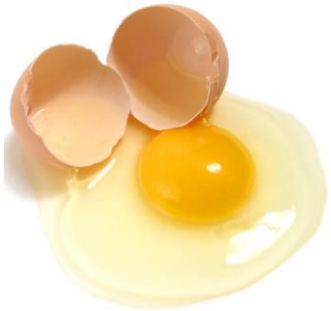
April 18, 2016, JOHNSTON, Iowa – DuPont Pioneer today announced waxy corn hybrids as its first commercial agricultural product developed through the application of CRISPR-Cas enabled advanced breeding technology. This next generation of elite waxy corn hybrids is expected to be available to U.S. growers within five years, pending field trials and regulatory reviews.

The United States Department of Agriculture (USDA) recently **(USDA) recently published its response** to **Pioneer's "Regulated Article Letter of Inquiry"** stating that it does not consider next-generation waxy corn developed with CRISPR-Cas enabled advanced breeding technology as regulated by USDA Biotechnology Regulatory Services.

..and we can't forget that agricultural animals are in the wings

Generation of gene-modified goats targeting *MSTN* and *FGF5* via zygote injection of CRISPR/Cas9 system

SCIENTIFIC REPORTS | 5:13878 | DOI: 10.1038/srep13878



Targeted gene knockout in chickens mediated by TALENs. *PNAS 2014 111 (35) 12716-12721*

Transgenic Res (2015) 24:147–153
DOI 10.1007/s11248-014-9832-x

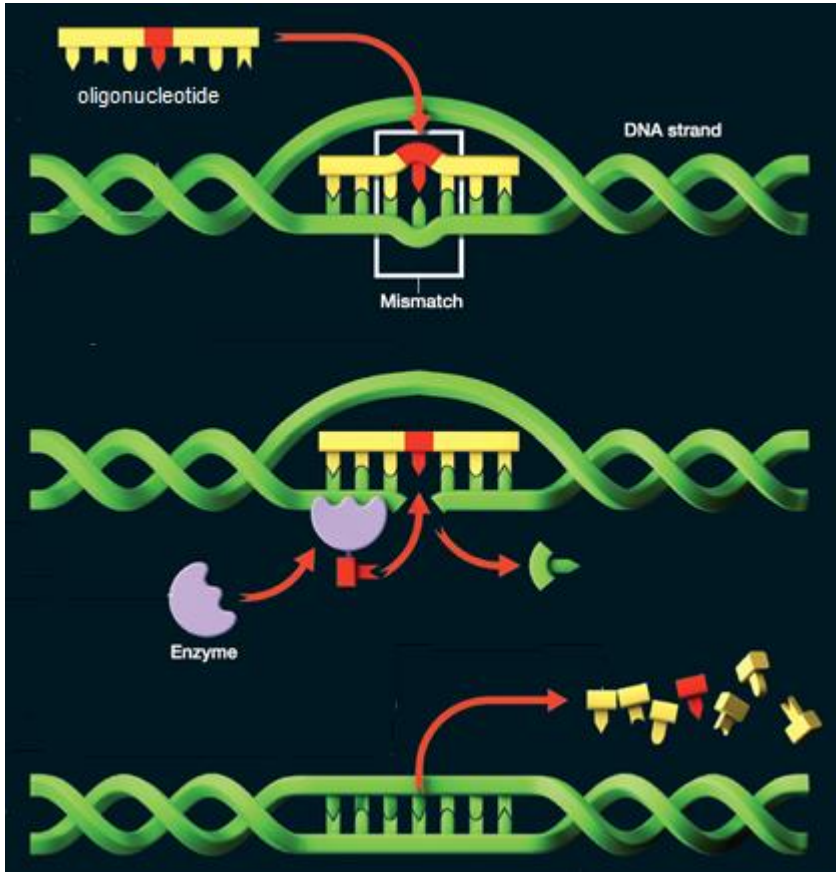
ORIGINAL PAPER

Genome edited sheep and cattle

Oligonucleotide-directed mutagenesis (ODM)

The chemically synthesised oligonucleotide (20 – 100 bp) has homology with the target gene except for the nucleotide(s) to be changed.

- oligonucleotides are added directly and hence do not involve a transgenic stage
- oligonucleotides themselves are not “heritable material“
- oligonucleotides act like a mutagen, but are sequence specific
- the obtained mutations cannot be distinguished from spontaneous mutations (natural variation) or mutations induced by classical mutagenesis



ODM product already commercially available

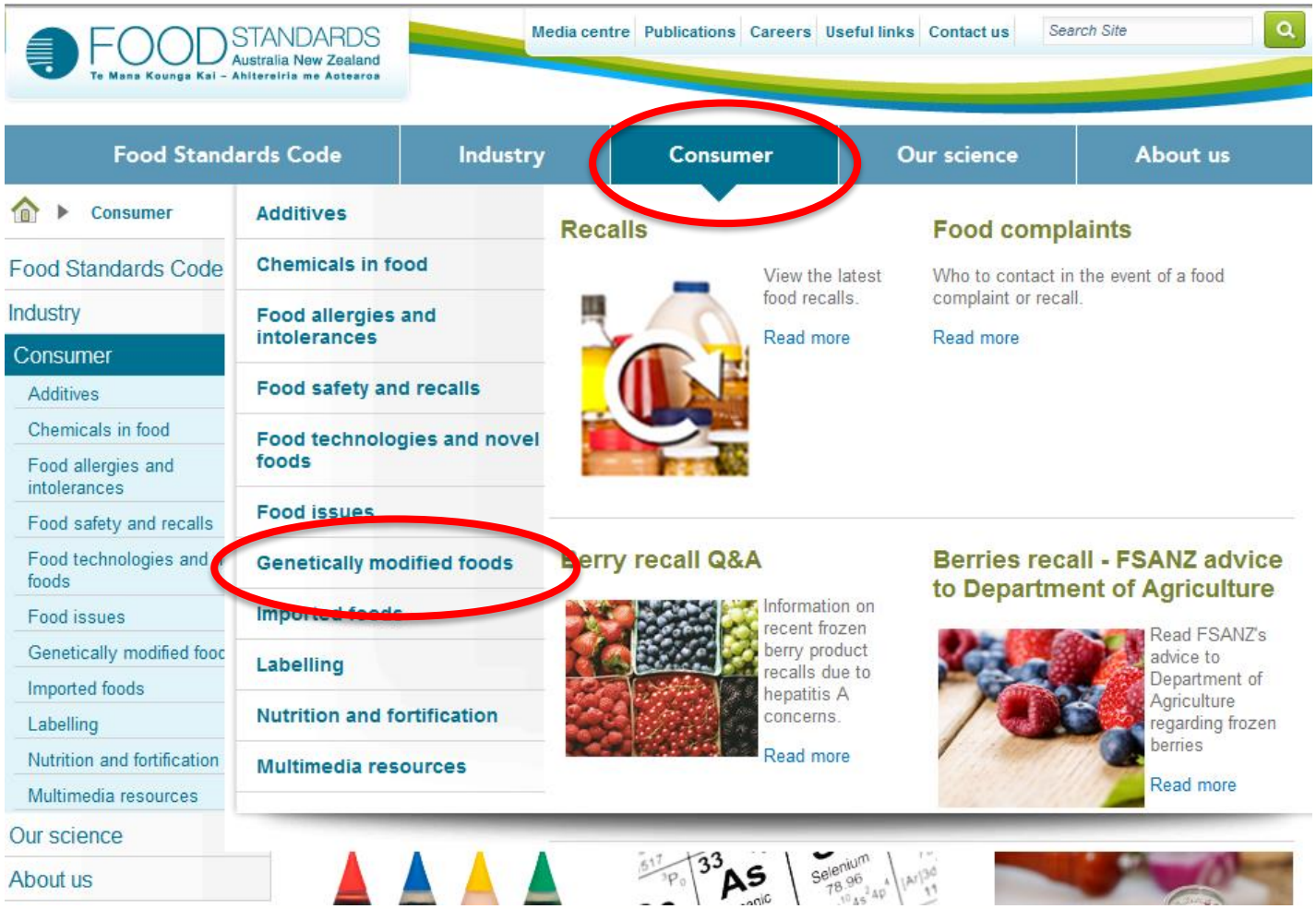
Cibus Announces Approval of First Commercial Product SU Canola™ in Canada

San Diego (March 18, 2014) — Cibus, a cutting-edge precision gene editing firm, announced today that its first commercial product SU Canola (Sulfonylurea Tolerant) has received Plant Novel Trait (PNT) approval by the Canadian Food Inspection Agency (CFIA) and Health Canada (HC). With this regulatory approval, Cibus is permitted to fully commercialize this product in Canada. Cibus SU Canola offers Canadian farmers a new alternative for weed control that will provide Canadian farmers with sound stewardship options to deal with weed resistance resulting from overuse of current herbicide tolerant GM varieties.

Cibus, a San Diego-based company, produces plants with new traits using a gene editing platform. For instance, the company's SU Canola™ is a non-transgenic sulfonylurea herbicide tolerant canola available in the United States. The USDA regards Cibus' technology to be a modern form of mutagenesis, which should not be regulated by US agencies.

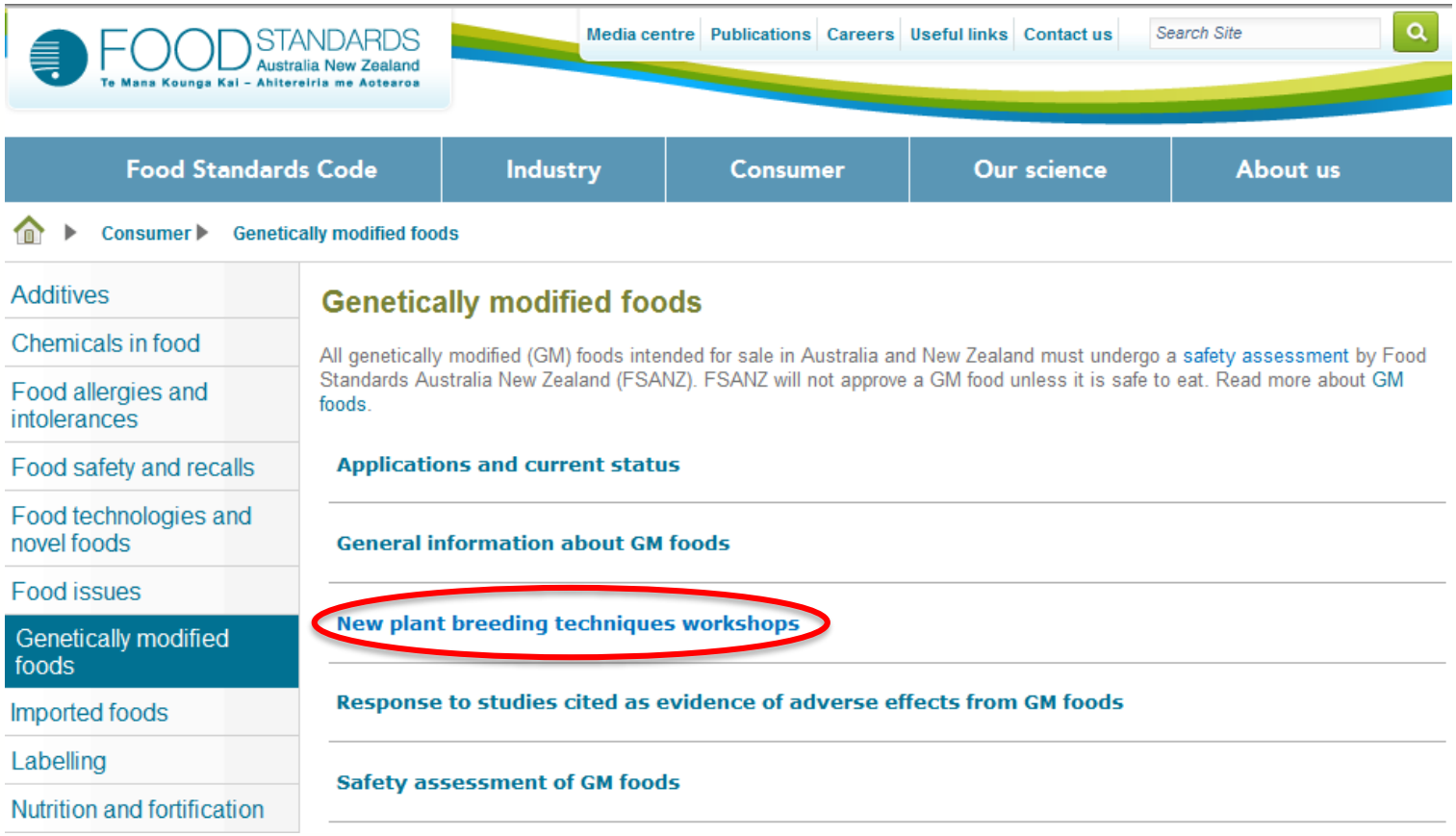
ISB NEWS REPORT AUGUST / SEPTEMBER 2015

Our website - http://www.foodstandards.gov.au



Workshop Reports

http://www.foodstandards.gov.au/consumer/gmfood/Pages/New-plant-breeding-techniques-in-the-spotlight.aspx



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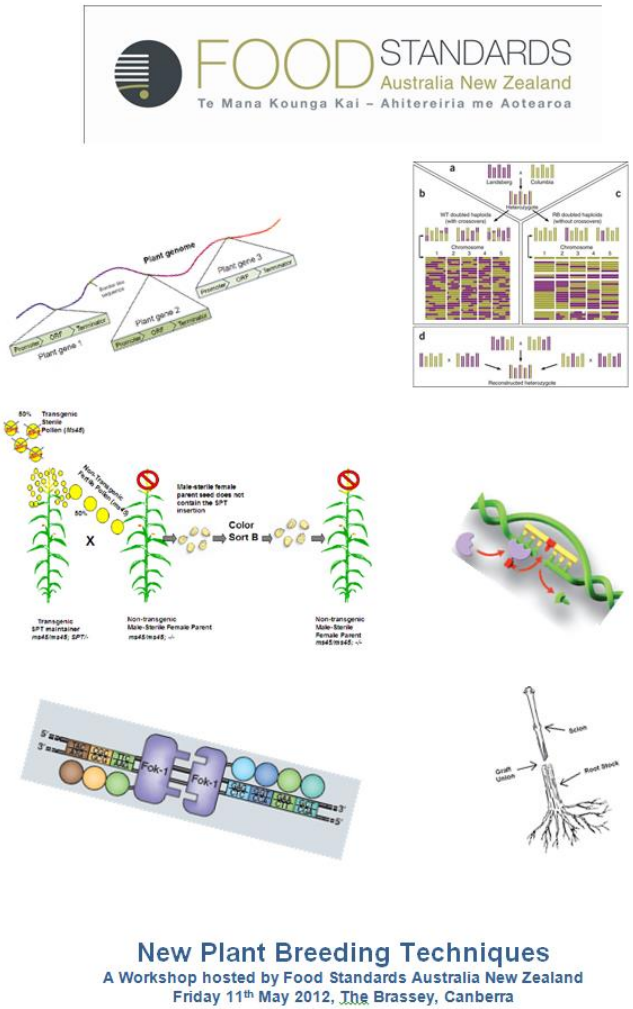

Conclusions from FSANZ Workshops on NBTs

Agro-infiltration	• Limited applicability to food; any food products will be purified proteins • Depends on use and whether the plants from which they are derived are considered to be a GMO – a matter of interpretation
Cisgenesis/Intragenesis	• No distinction between cisgenesis, intragenesis and transgenesis
GM rootstock grafting	• The grafted plant is a single organism and is therefore GMO • The food may contain novel RNA and/or protein and may also have altered composition or other characteristics
Transgenic-assisted breeding	• Where null segregants are the final food producing lines, these are comparable to those developed using conventional plant breeding techniques
Gene editing techniques – SDN & ODM	• Small nucleotide changes - final food producing lines comparable to those developed using conventional mutagenic techniques • Added gene(s) remaining or gene replacement – equivalent to transgenesis

FSANZ 25th birthday cake - August 2016

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