Agriculture and Agri-Food Canada | Agriculture et Agroalimentaire Canada

Canada

Genome editing: a breeding tool for crop improvement in a changing world

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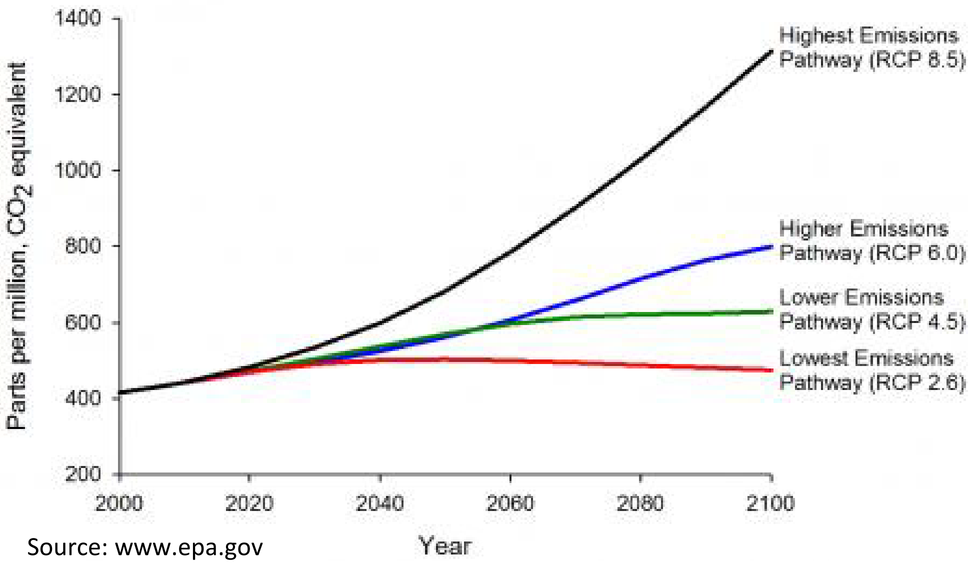
A changing world…..

1. Climate change

Projected atmospheric greenhouse gas concentrations for four emissions scenarios



Source: www.epa.gov

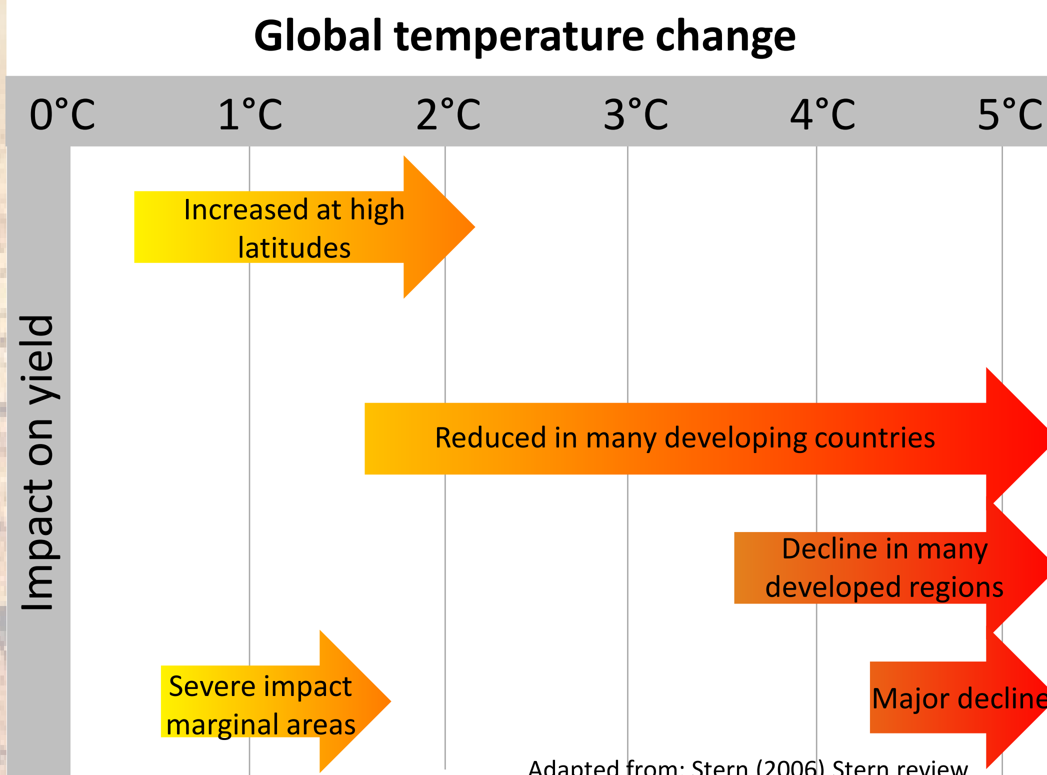
- Carbon dioxide (76%)
- Methane (16%)
- Nitrous oxide (6%)
- Fluorinated gases (2%)

- Escalating temperatures
- Changes in precipitation
- Increased salinization
- Rise in extreme weather

Effect on crop production

Global warming/climate change

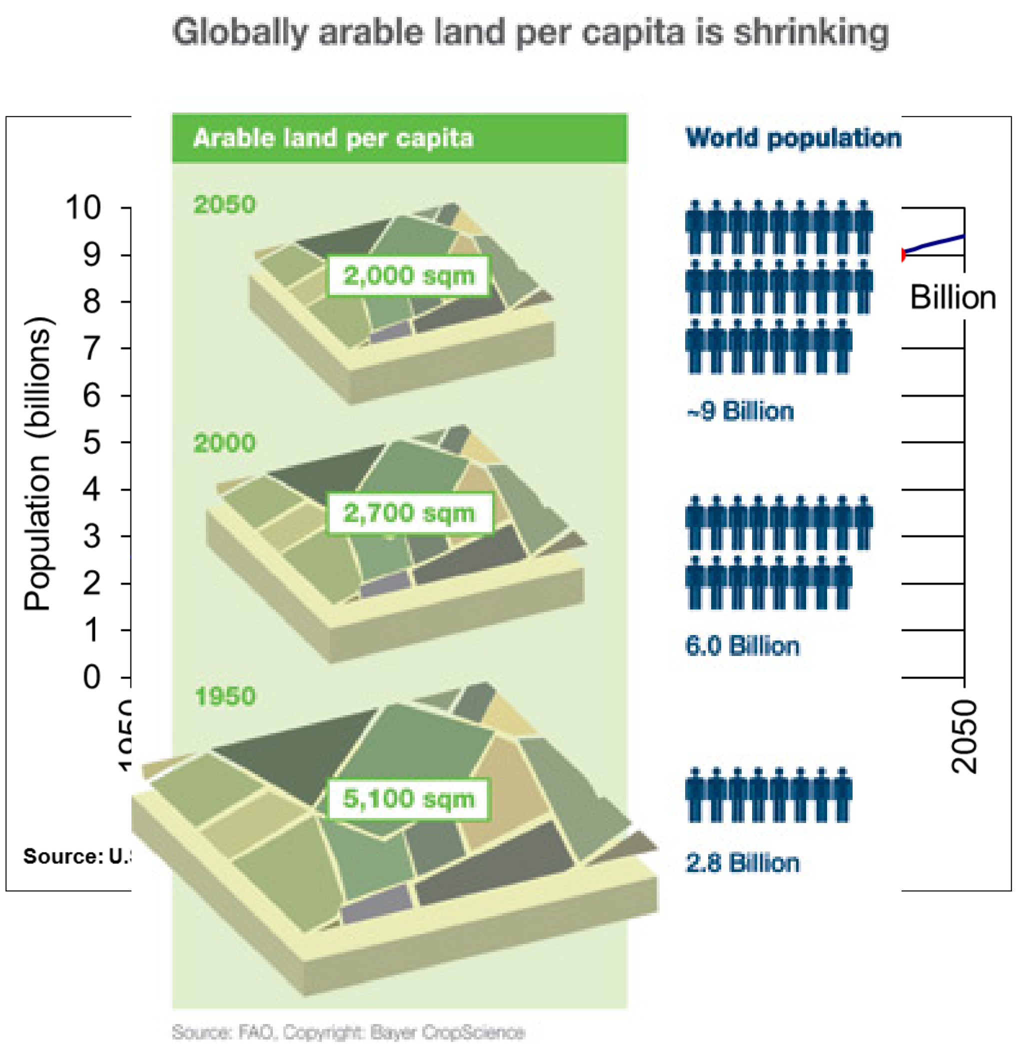
NEGATIVE IMPACTS	POSITIVE IMPACTS
Increased abiotic stress	Increased productivity
Rise in weed growth	Longer growing seasons
Proliferation of pests	Accelerated rates of maturation
Escalation in disease	New crops
Reduced quality	



Adapted from: Stern (2006) Stern review

A changing world.....

2. A growing population



NEGATIVE IMPACTS ON CROP PRODUCTION

- Increased demand
  - food
  - feed
  - biofuel/industrial

- Urbanization of agricultural land
  - less arable land

- Expansion of growing regions
  - higher latitudes
  - marginal land

- Increased pollution

What can we do about it?

- Urgent need to increase agricultural productivity in unfavourable conditions
- Develop cultivars with improved performance traits

Traits:

- Nutrient use
- Water use
- Abiotic stress tolerance
- Disease resistance
- Yield/biomass
- Persistence
- Competitiveness

Source: perspectives.devalt.org

Quality traits

Traits

- Livestock performance and health

→

- Digestibility
- Soluble carbohydrate content
- Protein content, quality and stability
- Secondary compounds

- Human nutrition
- Aesthetics

→

- Fatty acid composition
- Antioxidant levels
- Vitamin content
- Non-browning

- Environmental well-being

→

- Leaf lipid content
- Protein/carbohydrate ratio
- Secondary compounds
- Carbon capture

How can we achieve this?

Conventional breeding

'GM'

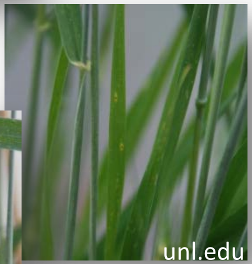
Genome editing

All based on genetic alterations of the original genotype

Conventional breeding:

- Selection of natural or induced mutations
  - based on random mutations
    - Development of low erucic acid and glucosinolate canola
    - Development of semi-dwarf rice

- Interspecific and intergeneric breeding
  - allows the leveraging of gene pools
    - Rust resistance acquired from wild relative of wheat



Pros:
- Public acceptance
- Ease of regulation
- New techniques are enabling further progress

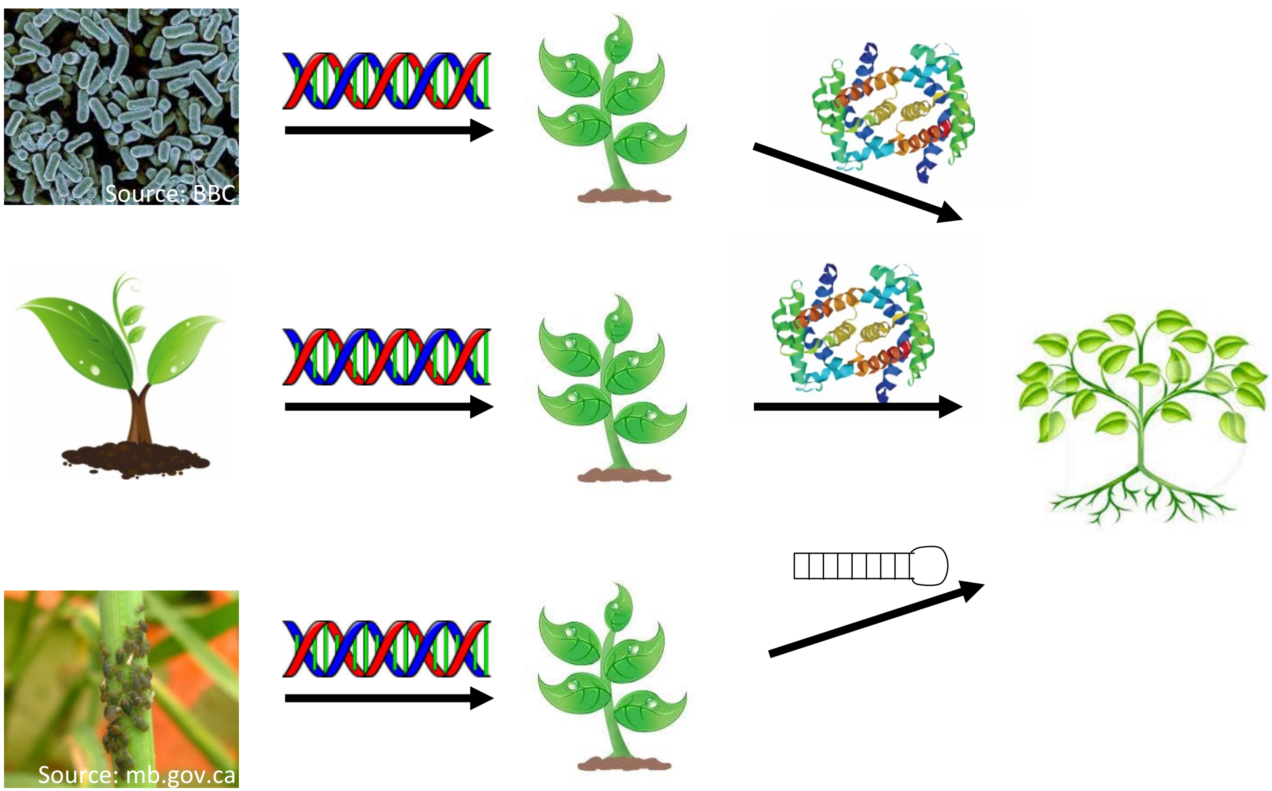
Cons:
- Limited genetic diversity
- Unanticipated negative traits
- Time

‘GM crops’

Transgenic
‘Foreign’ protein-coding gene

Cisgenic
Protein-coding gene from close relative

RNAi
Non-protein-coding DNA



'GM': successes and challenges





Source: brittanica.com

- The four major GM crops produced worldwide are soybean, maize, cotton and canola
- Predominant traits have been herbicide and insect resistance

Pros:
- Expansion of gene pool
- Fast, easy, specific
- No introgression of negative traits
- Spatiotemporal expression
- Ease of trait stacking

Cons:
- Random integration
- Public acceptance
- Regulatory impediments
- Potential environmental issues
- Requires knowledge of genetic basis

Genome editing: the new frontier

- **A new breeding technology – a tool that has the potential to take breeding to a new level**

Benefits

- Targeted mutations can be introduced into a plant genome in a highly specific manner with great precision
- Can be used to leverage vast amounts of ‘omics’ data
- Expands genetic diversity
- No introgression of negative traits
- Fast and often simple, ability to multiplex

Genome editing: the new frontier

Possible disadvantages

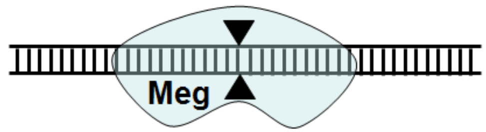
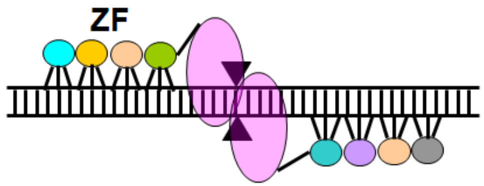
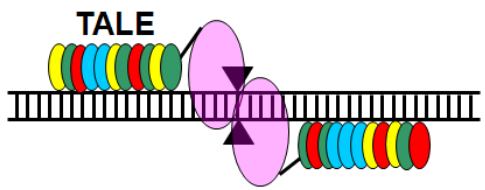
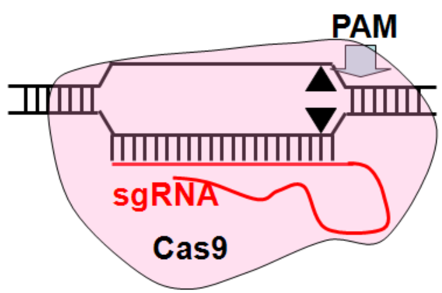
- Off-target mutations
  - risk is low
  - new technologies being developed to reduce risk
  - advances in sequencing allow us to assess

- Multiplexing of traits could result in unanticipated outcome
  - e.g. loss of fitness

Comparison of techniques

	Induced mutagenesis	Transgenic	Genome editing
Genetic modification	Random mutations	Random insertion of known transgene	Precise mutation
Precision	Imprecise	Precise/imprecise	Precise
Genes affected?	Unknown	1	Chosen number
Foreign DNA?	No	Yes	No*
Requires human intervention?	Yes	Yes	Yes
Use in polyploid plants	Difficult	Useful	Useful
Risk of unanticipated effects	High	Moderate	Low
Risk of spread of genetic modification	High	High	High
Stringency of regulatory testing	Low	High	?
Time to develop	High	Low	Low

Genome editing: site-specific nucleases

Meganuclease		14-40 bp	Monomer
Zinc finger nuclease (ZFN)		18-36 bp/ZFN pair	Dimer
Transcription activator-like effector nucleases (TALENs)		28-40 bp/TALEN pair	Dimer
Clustered regularly interspaced short palindromic repeats (CRISPR-Cas9)		20 bp guide + 2 bp PAM	Monomer + sgRNA

Source: intechopen.com

All result in the formation of a double strand break in their target DNA sequence

Genome editing: comparison of nucleases

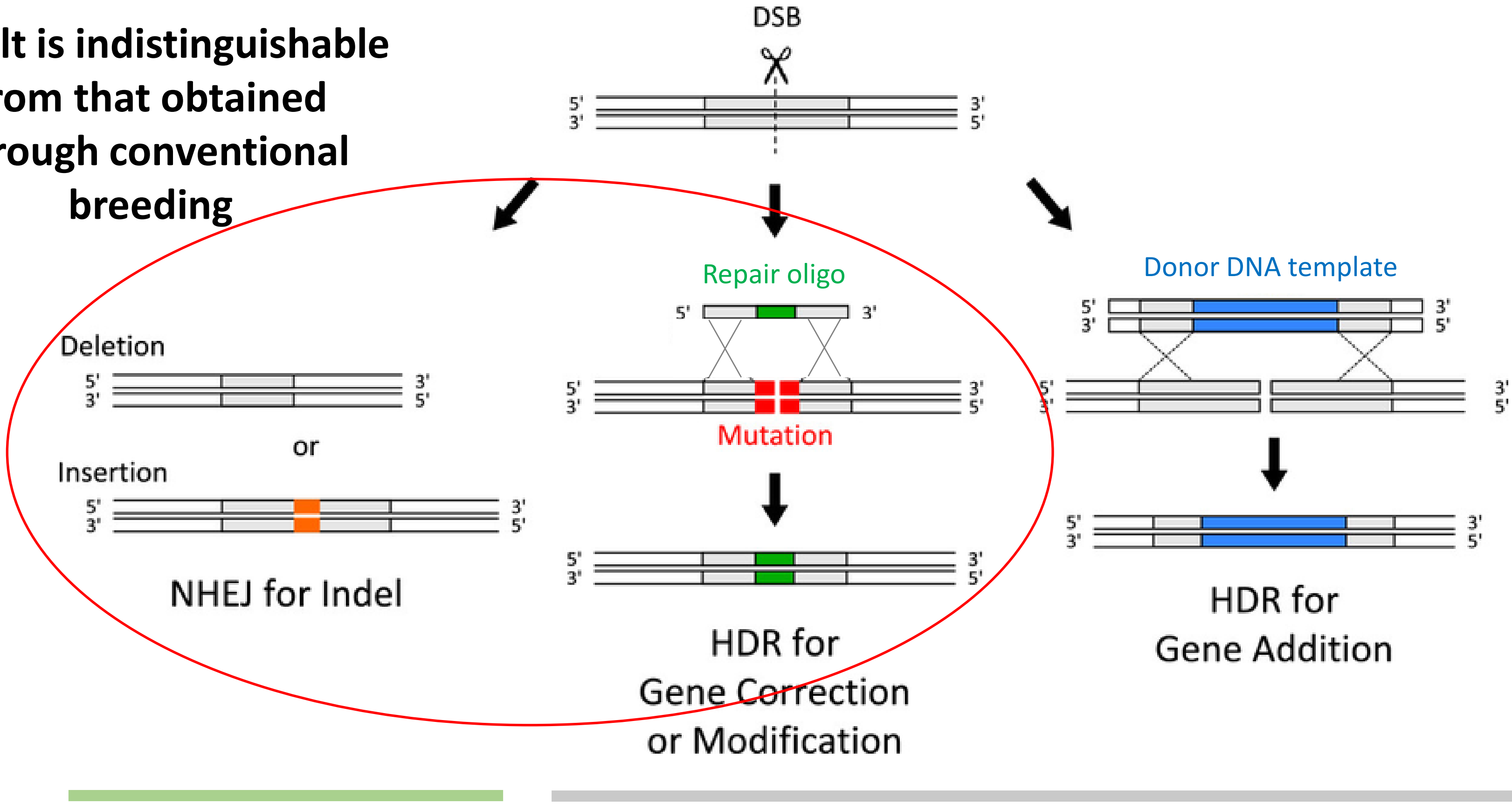
	MegaN	ZFN	TALEN	CRISPR
Cleavage efficiency	Low	Low	High	Very high
Risk of off target effects	Moderate	Moderate	Low	Target-dependent
Targeting	Low efficiency for novel targets	Non-GC-rich targets difficult	5’ base must be T	Must precede a PAM
Ease of design	Very difficult	Very difficult	Difficult	Easy
Cost	High	High	Moderate	Low
Ease of multiplexing	Very difficult	Very difficult	Very difficult	Easy

Adapted from: Abdullah et al. (2015) GM Crops & Food

Genome editing: eliciting mutations

Mechanisms of repair

Result is indistinguishable from that obtained through conventional breeding



Non-homologous end joining (NHEJ)

Homology directed repair (HDR)
Requires oligo or DNA template

Non-GMO?

Adapted from: Araki and Ishii (2014) Rep Biol Endocrinol

Genome editing: component delivery

- **Has been a bottleneck due to plant cell walls**

- Delivery in form of plasmid DNA
  - stable or transient
  - can segregate out
  - fragments often left behind in genome

Introduction of 'foreign' DNA

- Delivery in form of RNA
  - transient, quickly degraded

DNA-free, but very low efficiency

- Delivery in form of protein or Cas9 protein/sgRNA complex
  - transient, degraded within cell

DNA-free, nothing left behind, but lower efficiency

Genome editing: DNA-free delivery

- **Direct delivery of protein or protein/RNA complex is a promising method of delivery**

- PEG-mediated
  - protoplasts
  - tobacco, rice, soybean, wheat, lettuce (e.g. Luo et al. 2015; Woo et al. 2015)
  - frequencies up to 46%, but usually between 1-8%

- Particle bombardment
  - immature embryo cells
  - wheat (Liang et al. 2017)
  - frequency of approximately 0.2%

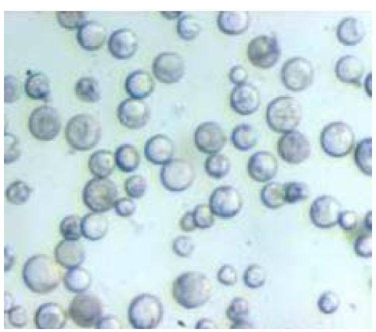
Any other way?

Genome editing: delivery using CPPs

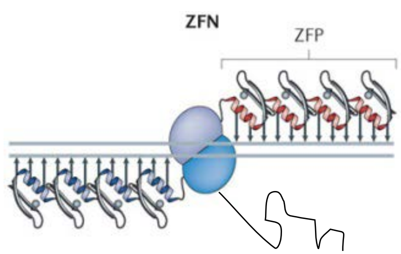
- **Cell penetrating peptides (CPPs)?**

- Small peptides (< 30 amino acids)
- Often cationic
- Able to enter cells
- Are not cell specific
- Do not cause significant membrane damage

- Have been used to transfer purified, DNA-free genome editing machinery into human cell lines
- Also recently been shown to be capable of transferring various other proteins into plant cells



Microspores (haploid predecessors of male gametes)



ZFN protein covalently linked to CPP

Genome editing at the single cell level?

Dr. Andriy Bilichak, Dr. Francois Eudes
AAFC, Lethbridge Research and Development Centre

Genome editing: what could we improve?

- Herbicide resistance
- Abiotic stress tolerance
  - drought, flooding
  - salinity
  - frost, heat
- Yield
  - seed yield
  - biomass yield
- Nutritional and aesthetic quality
  - oil content/composition
  - reduction of antinutritional compounds
  - non-browning
- Environmental footprint
  - forage crops for more efficient rumen fermentation
  - increased nutrient use capacity
- Disease resistance
  - particularly effective for production of durable resistance due to ability to multiplex

Particularly useful for eliminating or reducing the effects of genes/alleles that limit productivity, aesthetics and nutrition

Source: pureprairie.ca

Genome editing: commercialization

- Patents on technologies
  - confusion as to ownership
  - cost can be prohibitive
  - can stifle innovation
  - partnerships with industry

Source: wsuventures.org

- Unpredictability of regulatory system
  - risk that products might not be de-regulated
  - innovation requires investment, which is less likely if uncertainty about outcome

- Regulatory cost
  - could exclude public sector, small organizations, and producers from contributing to innovation
  - length of process can stall productivity

- Asynchrony of regulatory processes
  - current source of uncertainty
  - can impede trade

Genome editing: regulatory status

Increasing number of crop species altered using genome editing that are de-regulated or that USDA claims will not be regulated

(Pacher and Puchta (in press) Plant J)

Examples:

CRISPR-Cas9 + oligo	TALEN	ZFN	CRISPR-Cas9	TALEN	TALEN
Herbicide resistant	High oleic	Low phytate	Non-browning	Powdery mildew resistant	Low acrylamide
Cibus	Calyxt	Dow AgroScience	Penn State	Calyxt	Cellectis

However, discussions are ongoing and there is a lot of uncertainty globally

Genome editing: likelihood of regulation

In many cases, genome editing yields genetic alterations that are indistinguishable from those obtained through conventional breeding approaches

Two main models of GM food regulation:

- a product-focused model (e.g. US, Canada, South America)
  - decisions based upon the new trait
- a process-based model (e.g. EU countries)
  - decisions based upon how the trait was generated

Anticipated degree of regulatory scrutiny

Delivery / Mutation type	Protein/RNA/ transient	Stable introduction/null-segregants	Stable introduction
NHEJ (indel)	Low	Moderate	High
HDR (one or very few nucleotides)	Case-by-case	Case-by-case	High
Site-directed transgene insertion	N/A	N/A	High

Adapted from: Wolt et al. (2015) Plant Biotechnol J

Genome editing: ethics and biosecurity

Benefit

- Applications should benefit producers, consumers, and the environment

Accessibility

- Products need to be accessible to developing countries

Transparency

- Find ways to increase transparency

Traceabiliity

- For monitoring purposes

Genome editing: public engagement

How can we prevent the polarization that surrounds transgenic crops?

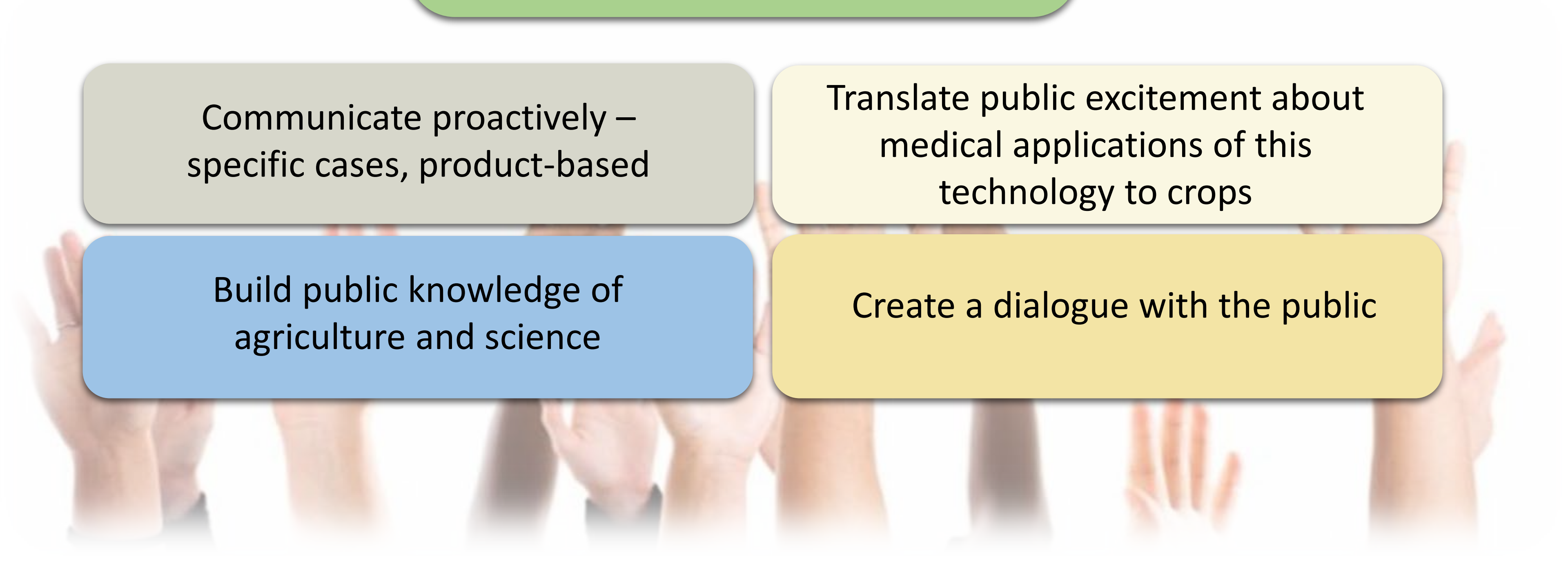
Plain language, transparent discussions about the technology, why we need it, and how it is regulated.
We need to build trust!

Communicate proactively – specific cases, product-based

Translate public excitement about medical applications of this technology to crops

Build public knowledge of agriculture and science

Create a dialogue with the public



Closing statements:

- Although still in its infancy, genome editing technology is developing quickly and is set to change the agricultural landscape
- A powerful tool that has the potential to create traits that could not be achieved through conventional breeding and increase diversity in modern cultivars
- Should substantially improve the pace of innovation
- Need to focus on improving traits that will benefit producers, the public and the environment
- Regulatory issues need to be solved and should be based on risk and science
- Public engagement should be undertaken sooner rather than later

Source: Iastate.edu

Together with conventional breeding, genome editing technology has the potential to be instrumental in helping to secure the world's food supply in a changing world

Thank you!

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