Canadian Food Inspection Agency | Agence canadienne d'inspection des aliments

Canadian Food Inspection Agency

Our vision:
To excel as a science-based regulator, trusted and respected by Canadians and the international community.

Our mission:
Dedicated to safeguarding food, animals and plants, which enhances the health and well-being of Canada's people, environment and economy.

Genetic basis and examples of potential unintended effects due to genetic engineering and breeding

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Canada

Outline

- Pre-market assessments, a comparative approach
- Insertional effects
- A comparison of insertional effects to other genetic changes that occur in plants
- Genetic changes and unintended effects
- Conclusions

Canadian pre-market assessments: A comparative approach

- In Canada, conducted for novel foods and novel feeds as well as for the environmental release of plants with novel traits (PNTs)
- The PNT, novel food or novel feed is compared to a counterpart
- Includes a molecular characterization and a comparison of composition and phenotype
- Defines novel trait and identifies any unintended traits
- Assesses the potential of these traits to:
  - Have harmful effects on the environment
  - Impact the safety and/or nutrition of the food
  - Impact the safety and/or efficacy of the feed

Can our pre-market assessments be refined?

- Increased familiarity with genetic engineering
- Growing body of scientific research on genetic engineering and GE plants, foods and feeds, especially at the molecular level
- As a first step, joint Health Canada/CFIA project to consider insertional effects
- In keeping with the comparative approach, compare insertional effects to other types of genetic changes in plants
- Consider:
  - Are there genetic changes that occur in plants that are similar to insertional effects?
  - What is the full extent of genetic change that occurs in plants?

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REVIEW

A comparative analysis of insertional effects in genetically engineered plants: considerations for pre-market assessments

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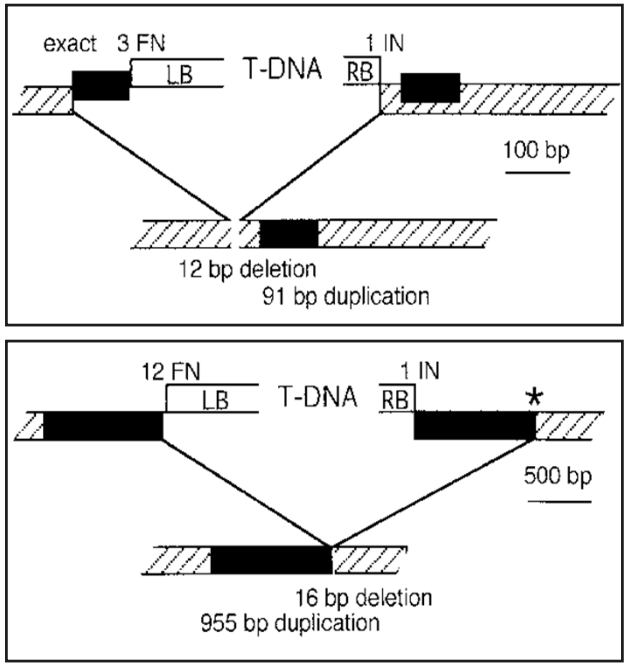
Insertional Effects

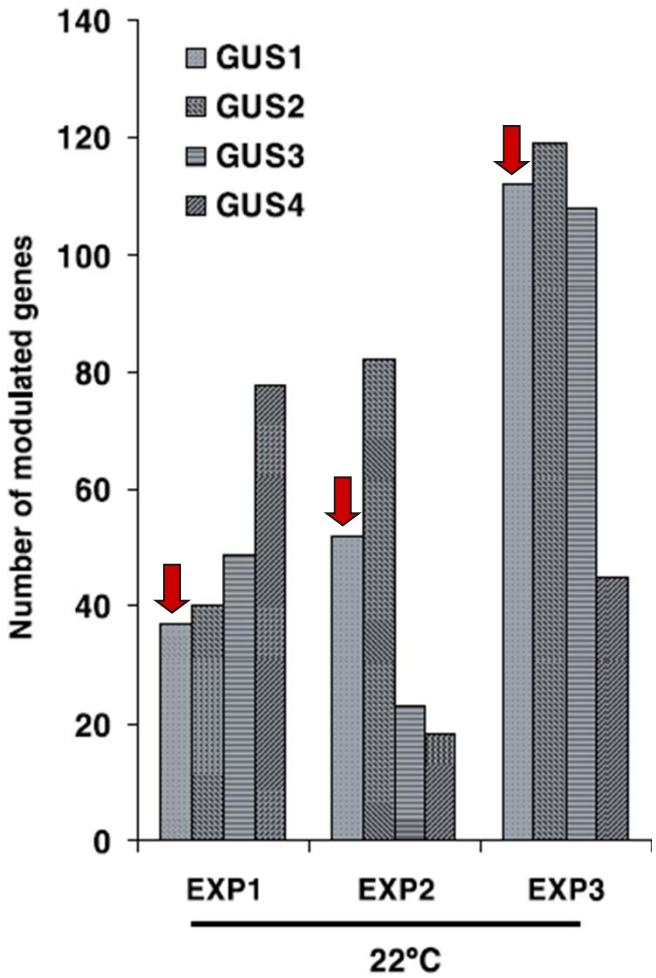
- Changes to a plant's genome that result from the process of inserting DNA by genetic engineering
- May include:
  - Insertion of "intended" DNA
  - Insertion of additional DNA
  - Deletions
  - Rearrangements
- Insertional effects can result in the expression of unintended traits



Forsbach et al. 2003. Plant Mol Biol 52: 161-176

Do Insertional effects = unintended traits?

- In a large scale T-DNA insertional mutagenesis study in rice, 22,665 field-grown T1 lines were examined, but only 4,065 had a visible mutant trait (Chern et al. 2007. Plant Mol Biol 65: 427-438)
- Introduction of *uidA*/*nptII* in *Arabidopsis* does not reproducibly impact the transcriptome

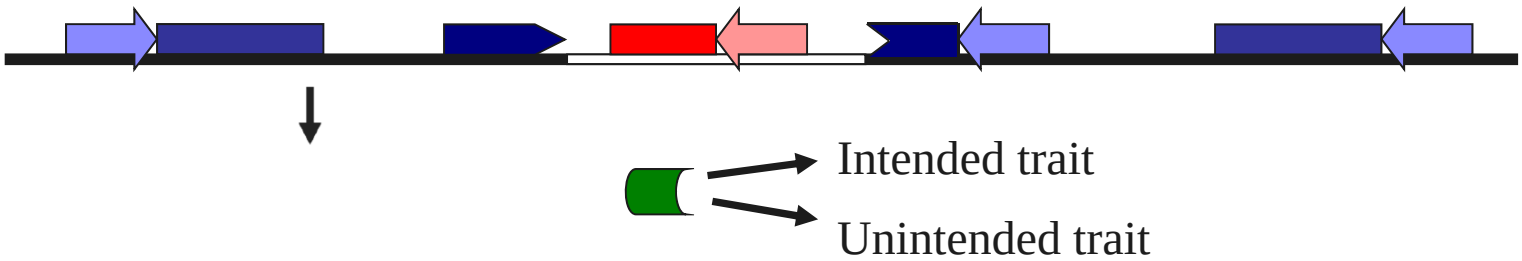


El Ouakfaoui and Miki 2005. Plant J 41:791-800

Insertional effects are not….

Pleiotropic Effects

- Single gene within the inserted DNA confers multiple traits



Position Effects

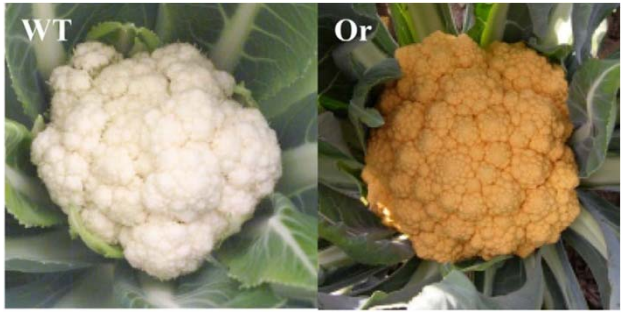
- Variations in the expression of genes within the inserted DNA that are dependent on the site of insertion

How do insertional effects compare to other genetic changes in plants?

- Plant genomes change over time in response to natural selection
- Humans shape crop genomes through conventional breeding
- There is a history of safe use of plants in which genetic changes occur
- Consider:
  - Are there genetic changes that occur in plants that are similar to insertional effects?
  - What is the full extent of genetic change that occurs in plants?

Humans have modified plant DNA for thousands of years

Crop domestication
Teosinte → Maize



Selective breeding
Orange cauliflower

Mutagenesis
Semi-dwarf rice

Genetic engineering
Non-browning apples

Fedoroff 2003. Science 302: 1158-1159
Monna 2002. DNA Research 9:11-17

Li 2006. Phytochemistry 67: 1177-1184
Biofortified.org

Are there genetic changes that occur in plants that are similar to insertional effects?

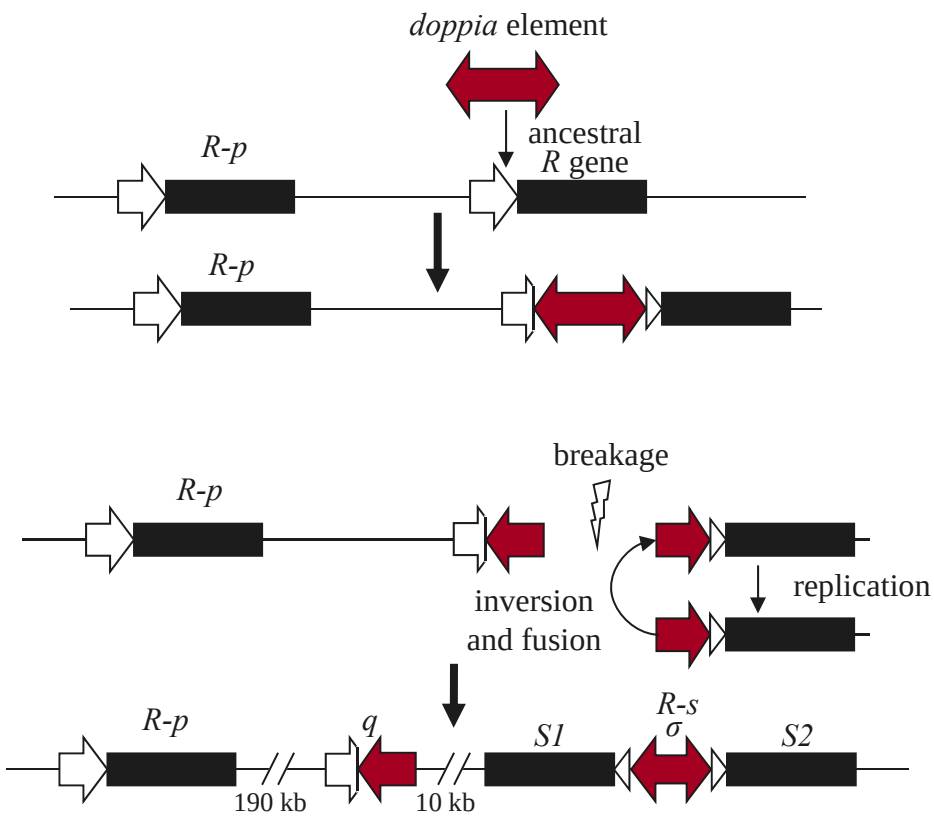
1. The movement of transposable elements
2. Repair of double-strand breaks by non-homologous end-joining (NHEJ)
3. Intracellular transfer of organelle DNA

1. The movement of transposable elements

- Transposable elements are found in the genomes of all plants
- They can cut or copy themselves and insert into new positions in the genome
- At the site of excision of DNA transposons, deletions, inversions and insertions can be found
- Rearrangements may result from aberrant transposition
- One new insertion of *nDart* in every four plants identified in backcrosses with the rice cultivar Hoshinoyume

Fujino et al. 2011. Mol Genet Genomics 273: 150-157
Bennetzen 2000. Plant Mol Biol 42: 251-269
Casacuberta and Santiago 2003. Gene 311: 1-11

The *R-s* subcomplex of the *R-r:std* allele formed as a result of the insertion of a *doppia* transposable element



May and Dellaporta 1998. Plant J 13: 241-247
Walker et al. 1995. EMBO J 14: 2350-2363

2. Repair of double-strand breaks by non-homologous end-joining (NHEJ)

- Double-strand breaks in DNA are caused by:
  - Mechanical stress
  - DNA replication across a nick
  - Reactive oxygen species
  - Ionizing radiation
- Most double-strand breaks are repaired by NHEJ
- NHEJ is error-prone:
  - Deletions
  - Insertions
  - Rearrangements
- A hallmark of NHEJ is the presence of microhomologies at the site of repair

Gorbunova and Levy 1999. Trends Plant Sci 4: 263-269

NHEJ is also involved in…

- The repair of DNA transposon excision sites
- Genetic engineering using both *Agrobacterium*-mediated and particle bombardment methods

	Microhomology	Insertions	Deletions	Rearrangements
Genetic engineering	66/186[1] 1-8 bp	109/186[1] 1-1700 bp	64/73 1-300 bp	3/73 20 bp, translocations
Double-strand breaks	13/17 1-15 bp	18/28 4-2355 bp	27/28 1-1322 bp	0/28 N/A
Mutagenesis	16/21 1-8 bp	1/21 1 bp	21/21 1-129700	2/21 1284.8, 3208.5 kbp

[1]Sequences at the left border and right border of the T-DNA insertions were analyzed separately.

Morita et al. 2009. Genes Genet Syst 84: 361-370
Forsbach et al. 2003. Plant Mol Biol 52: 161-176
Saloman and Puchta 1998. EMBO J 17: 6086-6095

Intracellular transfer of organelle DNA

- There are non-coding sequences in plant nuclear genomes that are homologous to plastid and mitochondrial sequences
  - These are known as nuclear organelle DNAs
- They are there because organelle DNAs have repeatedly been inserted into the nuclear genome
- Microhomologies, deletions and insertions are found at the sites of insertion, suggesting the involvement of NHEJ
- Transfer of a marker gene from the chloroplast to the nucleus in tobacco was observed at a rate of 1 in every 16,000 progeny

Huang et al. 2003. Nature 422: 72-76

What is the full extent of genetic change that occurs in plants?

- A comparison of 36 maize lines revealed high variation in DNA sequences
  - Single nucleotides varied every 60.8 bp
  - Insertions/deletions every 126 bp, ranging in size from 1 bp to 400 bp
- A comparison of two rice varieties found differences in the total number of genes
  - 91 genes had different copy numbers
  - 82 genes were present in one variety and absent in the other

Ching et al. 2002. BMC Genet 3:19
Ding et al. 2007. BMC Genomics 8: 154

Spontaneous genetic changes

1. Spontaneous mutations
   - In Arabidopsis, occur at a rate of 1.75 mutations per generation per diploid plant
2. Transposable elements
   - Amplify genomic DNA sequences, create new genes
   - In rice, the *Mutator*-like DNA elements (MULEs) contain gene fragments, often from different chromosomal loci
3. Homologous recombination
   - Mutations can be caused by homologous crossing over between repeated sequences
   - Copy number variants from meiotic recombination may be generated as often as 1 out of every ~700 seeds in *Arabidopsis*

Ossowski et al. 2010, Science 327: 92-94
Gaut et al. 2007, Nature Rev Genet 8: 77-84
Jiang et al. 2004, Nature 431: 569-573
Jelesko et al. 2004, Genetics 166: 947-957

Genetic changes from conventional breeding

Selective Breeding

- Via homologous recombination
- The desired trait is often accompanied by undesired traits, known as linkage drag
  - The *Mi* locus from *Lycopersicon peruvianum* was accompanied by dozens to hundreds of genes when transferred to tomato
- As the phylogenetic distance between two species becomes wider, process may be facilitated by ionizing radiation, somatic hybridization or chromosome doubling

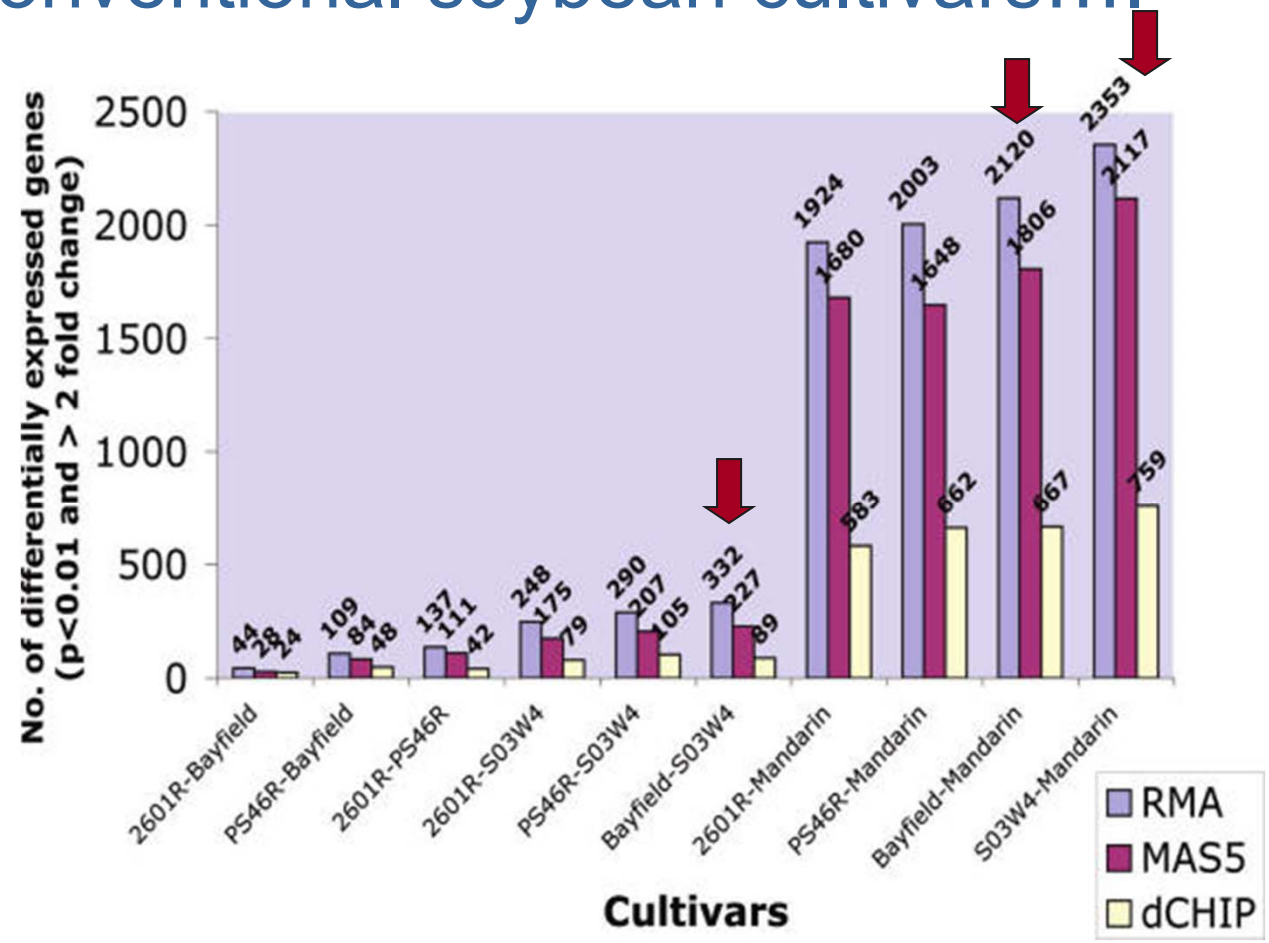
Ho et al. 1992. Plant J 2: 971-982

Genetic changes from conventional breeding

Mutagenesis

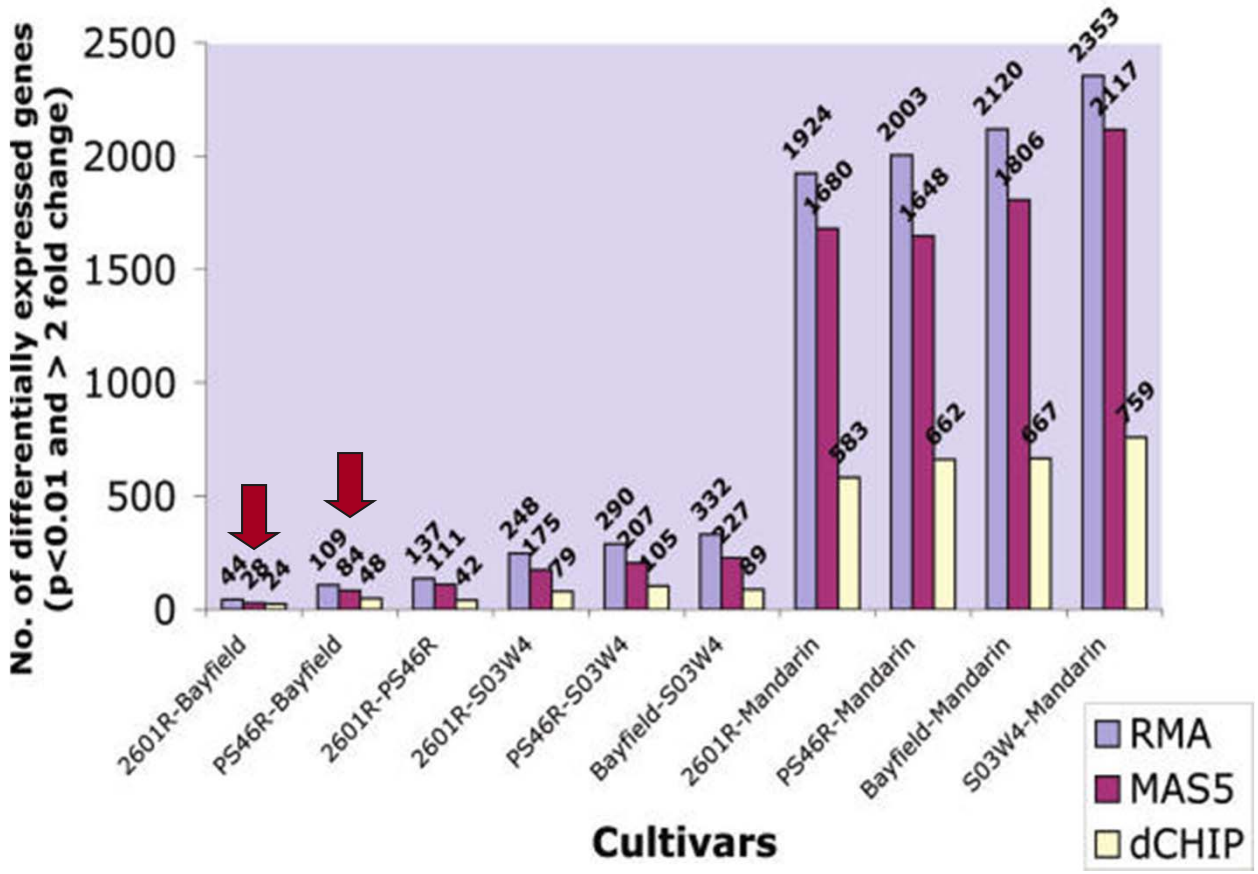
- Ionizing radiation induces double-strand breaks and single base substitutions
- Chemical mutagens typically induced single base substitutions
- The FAO/IAEA Mutant Variety Database currently lists 3218 mutant plant varieties released worldwide

Gene expression differs more between two conventional soybean cultivars….



Cheng et al 2008. J Agric Food Chem 56: 3057-3067

…than between genetically engineered plants and the closest conventional cultivar



Cheng et al 2008. J Agric Food Chem 56: 3057-3067

Genetic changes and unintended traits

- Genetic change ≠ unintended trait ≠ hazard
- In many cases, genetic changes will not give rise to discernible changes in a plant's phenotype
- There are a limited number of unintended traits that have the potential to be hazardous
- The potential for a new toxin, anti-nutrient or allergen to be introduced is low
- Similarly, the potential to introduce a trait that can contribute to weediness is low

Bradford et al. 2005. Nat Biotech 23: 439-444
Weber et al. 2012. Plant Physiol 160: 1842-1853
Stewart and Warwick 2005. Crop Ferality and Volunteerism, p. 9-30

Conclusions

- Genetic changes similar to insertional effects occur during:
  - The movement of transposable elements
  - The repair of double-strand breaks by NHEJ
  - The transfer of organelle DNA to the nucleus
- Insertional effects are only one of the many types of genetic changes that can occur in plant genomes
- Since insertional effects are similar to other genetic changes that occur in plants, they should present a similar level of risk.

Application to pre-market assessments

- With nearly 20 years of experience and scientific research, can we re-evaluate some of our early concerns and put them in perspective?
- Can we use these conclusions to re-evaluate the criteria required for the pre-market assessment of genetically engineered plants and the foods and feeds derived from genetically engineered plants?
- There is a history of safe use of crops developed using conventional breeding and its associated genetic changes
- Unintended traits are an outcome of all forms of plant breeding; we need to have the correct perspective for those arising from genetic engineering

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