Considerations from Conventional Plant Breeding Relative to the Safety Assessment of GM Crops Developed through Modification of Endogenous Plant Genes and Pathways

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Overview

I. **Genetic Modification and Crop Improvement**

   Natural genome evolution, selection, and genome plasticity

II. **Regulating Plant Genes and Pathways**

   Role of transcription factors and RNA interference

III. **Assuring Food, Feed, and Environmental Safety**

   Considerations for the safety assessment of improved crops with transgenic expression of transcription factors and RNA interference

Many of today's food crops bear no resemblance to their wild relatives

- Domesticated crops have been substantially changed compared to their wild relatives, including morphological, compositional, and physiological modifications
- These modifications have enabled dramatic improvements in crop abundance, productivity, and nutrition

Modern plant breeding and genetic modification builds on thousands of years of genetic improvement of crops

An Assyrian relief carving from 870 BC showing artificial pollination of date palms

http://www.colostate.edu/programs/lifesciences/TransgenicCrops/history.html

Genetic modification has been essential for improving food quality and abundance

- Genetic diversity also results from natural processes that ensure living organisms can adapt to their environment
- Plant genomes are not static; they are variable and change in response to biological and environmental factors

Barbara McClintock predicted the fluidity of the plant genome

"In the future, attention undoubtedly will be centered on the genome, with greater appreciation of its significance as a highly sensitive organ of the cell that … senses unusual and unexpected events, and responds to them, often by restructuring the genome."

"The significance of responses of the genome to challenge" 1984. *Science* 226: 792-801.

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Transcription factors & RNA interference have played important roles in crop domestication and improvement

Transcription Factors

- **Selection of variants has played an important role in plant breeding and in crop domestication**

  Green Revolution was due to transcription factor changes Peng et al. (1999) Nature 400: 256-261

  Domestication of modern maize was the result of a change in expression of the Teosinte branched1 (tb1) TxF Cubas et al. (1999) Plant Journal 18: 215-222

  Delayed ripening in commercial hybrid tomatoes is due to a mutation-induced change in TxF expression Vrebalov et al. (2002) Science 296: 343-346

RNA interference

- **New to science, ancient to nature**
- **Common defense mechanism found in plants, nematodes, insects and mammals**
- **Suppresses gene expression by either:**
  - Decreasing mRNA abundance directly (degradation)
  - Interfering with protein translation

Common themes for gene / pathway regulation by transcription factors or RNA interference

- Naturally occurring processes in plants
- Impact one or more genes / pathways
- Regulate endogenous genes / pathways

Transcription factors

- The active molecule is a DNA-binding protein
- The role of transcription factors in cells
    - Regulate endogenous genes to modulate existing pathways and plant products
- Mutations in transcription factors have provided major alterations in crop morphology during plant breeding and crop domestication
- Genes for transcription factors represent 6% of plant genomes

Riechmann, J.L., and Ratcliffe, O.J. A genomic perspective on plant transcription factors. Curr. Opin. Plant Biol. 2000. 3(5):423-34.

The origin of naked grains of maize

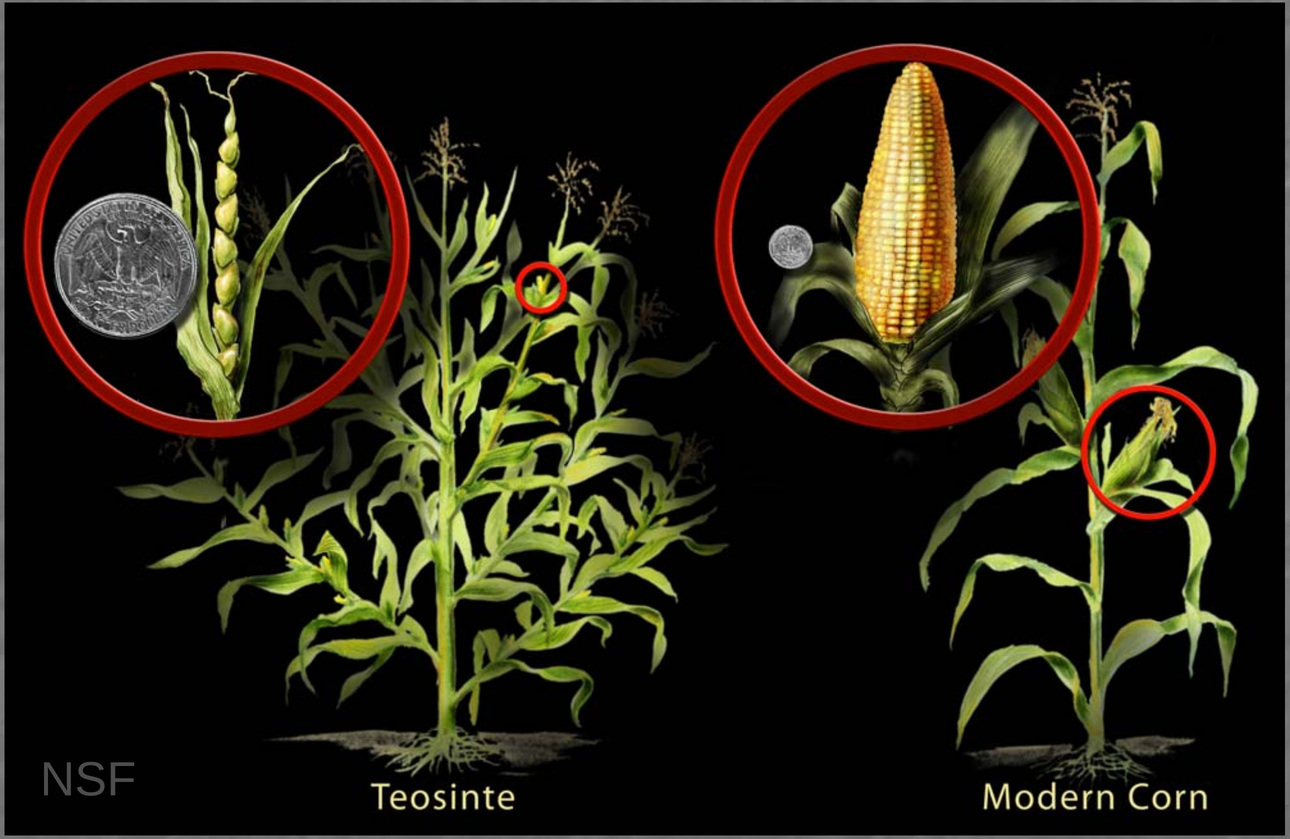
www.maizegenetics.net

Corbis

- The tga1 gene in maize
- SBP-domain transcription factor

Wang et al., 2005
Nature 436: 714-719

The evolution of apical dominance in maize



Tb1 gene

Doebley, Stec, & Hubbard, 1997
Nature, 486-488

The domestication of rice by reducing shattering

- qSH1 gene
  - BEL1-type homeobox gene with a 1 base pair change
- sh4 gene
  - 1 amino acid substitution in the DNA-binding domain

"An SNP caused loss of seed shattering during rice domestication"
Konishi et al, 2006
Science, 312: 1392-1396

"Rice domestication by reducing shattering"
Li, Zhou & Sang, 2006
Science, 311: 1936-1939

Cold tolerance in wheat


Corbis

- CBF transcription factors

"The expression of several Cbf genes at the Fr-A2 locus is linked to frost resistance in wheat"
Vágújfalvi et al, 2005
***Mol. Gen. Genomics* 274: 506-514**

"A cluster of 11 CBF transcription factors is located at the frost tolerance locus Fr-A^{m}2 in Triticum monococcum"
Miller, Galiba & Dubcovsky, 2006
***Mol. Gen. Genomics*, 275: 193-203**

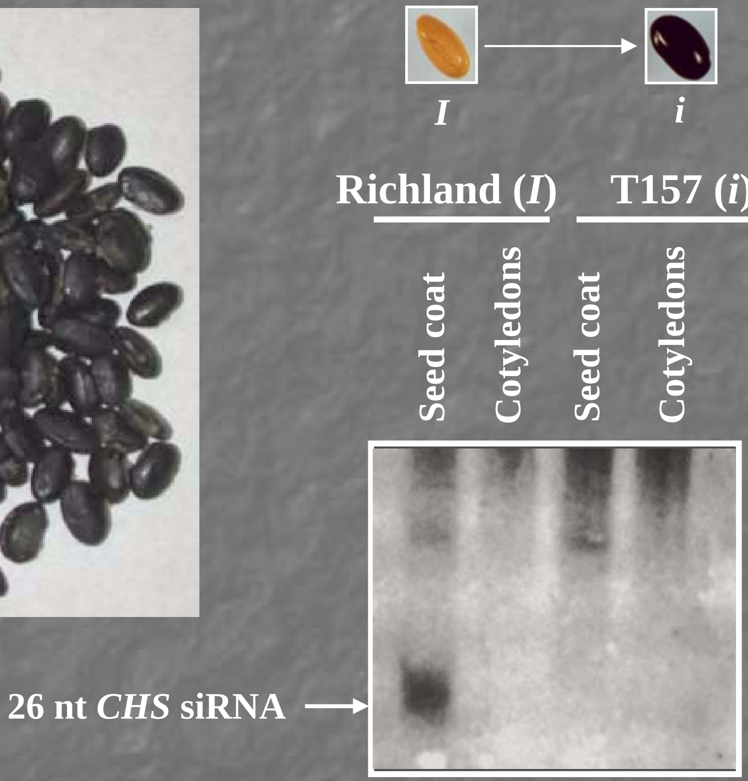
Summary – Transcription factors

- Naturally occurring and common in plants
- Played an important role in plant breeding and in crop domestication
- Regulate plant development and response to stress
- Will be important for modifying complex and multi-genic traits
  - Stress resistance
  - Improved nutritional composition
  - Intrinsic yield gain

RNA interference (RNAi)

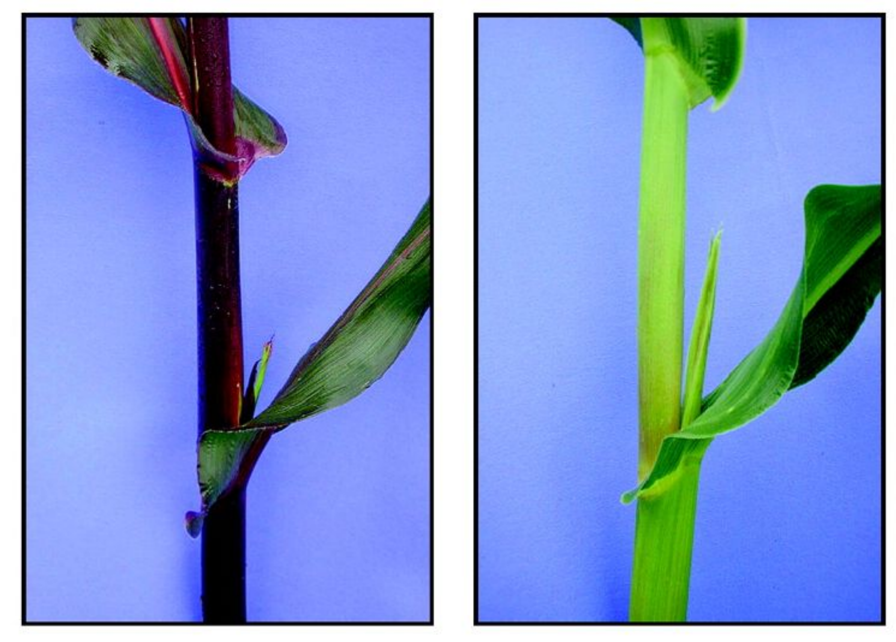
- The active molecule is an RNA rather than a protein
- The role of RNA interference in cells
  - Down-regulate endogenous genes (e.g. TxF mRNA)
  - Regulate chromatin structure via DNA methylation and histone modifications
  - Down-regulate exogenous genes (e.g. viral RNA)

Buff seed coat in soybean

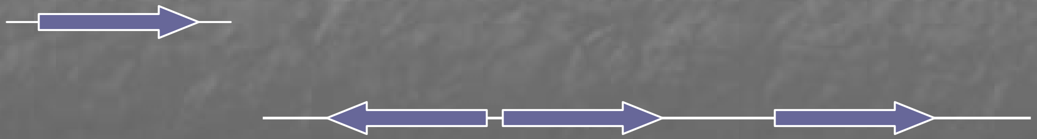


Tuteja et al., 2004. Plant Cell 16:819-835

Green stalks/foliage in maize

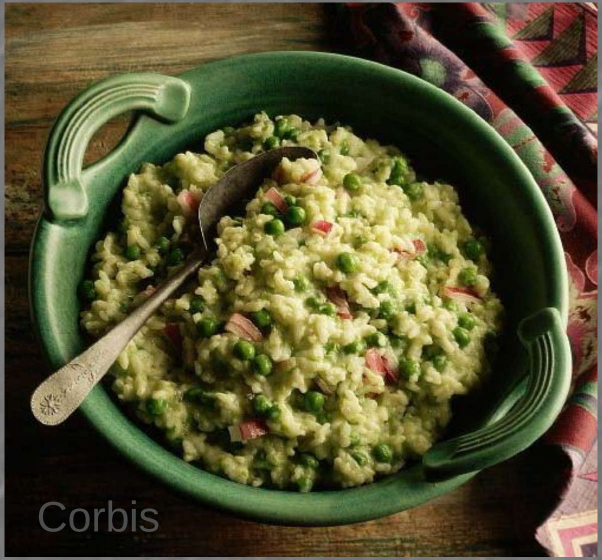


C2/C2 C-Idf/C2



Della Vedova et al 2005. Genetics 170:1989-2002

Low glutelin rice



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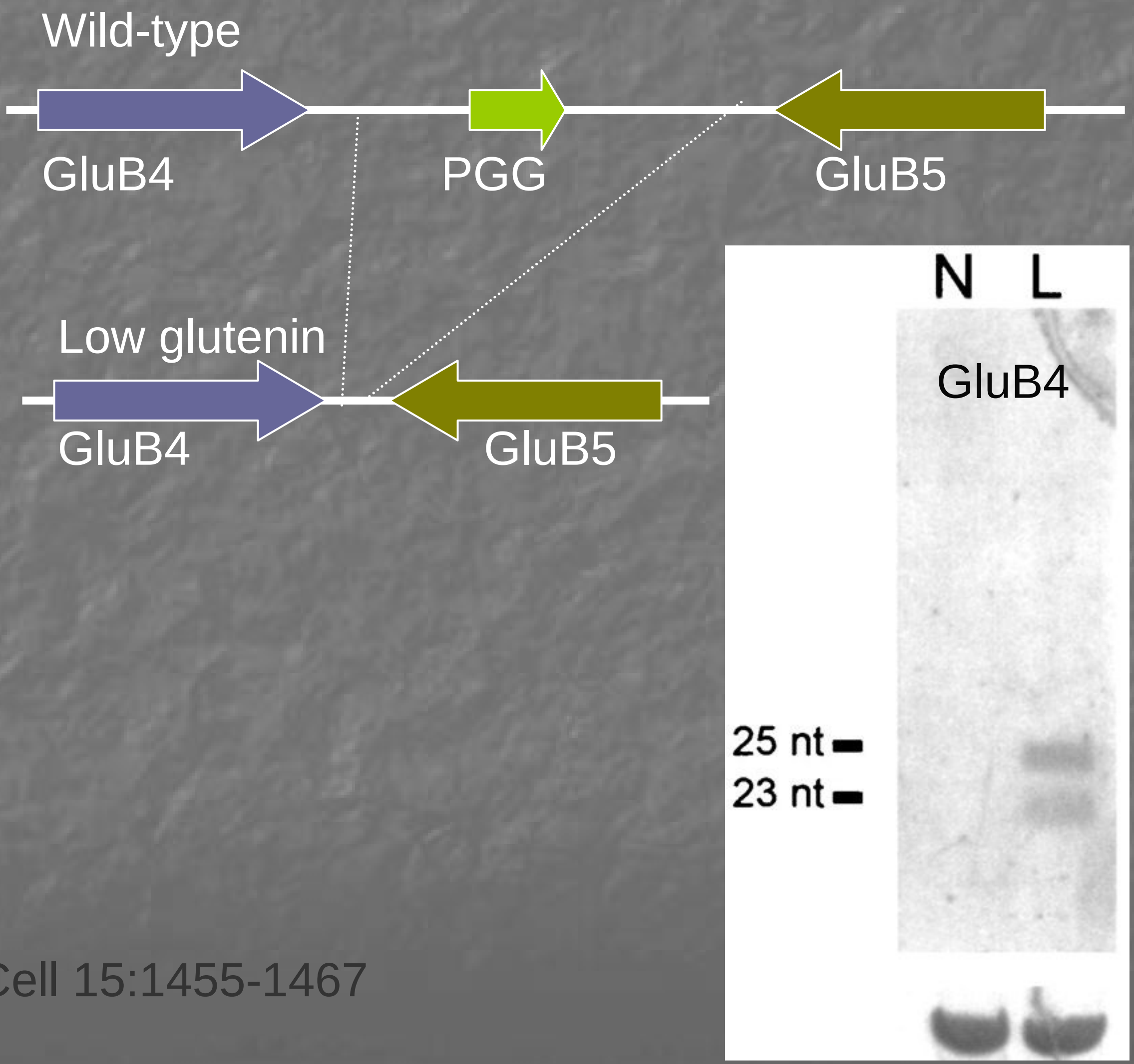


Wild-type

GluB4

PGG

GluB5

Low glutenin

GluB4

GluB5

Kusaba et al. 2003. Plant Cell 15:1455-1467

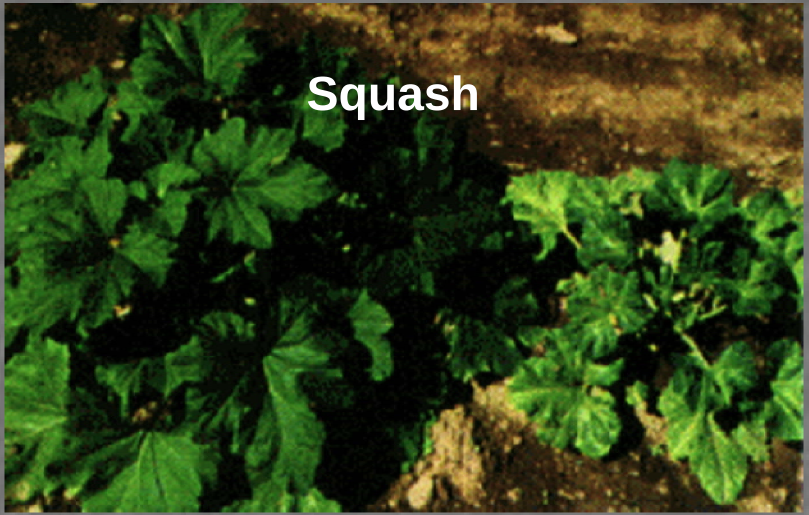
Viral cross protection

- Farmers intentionally inoculated their orchards with a mild virus
  - Become resistant to more virulent strains
  - Discovered in the 1930’s
- Became the incentive to modify plants with virus coat protein genes

L. Navarro, Instituto Valenciano de Investigaciones Agrarias, www.forestryimages.org

Genetically-modified virus resistance


Potato


Squash


Papaya

Other examples

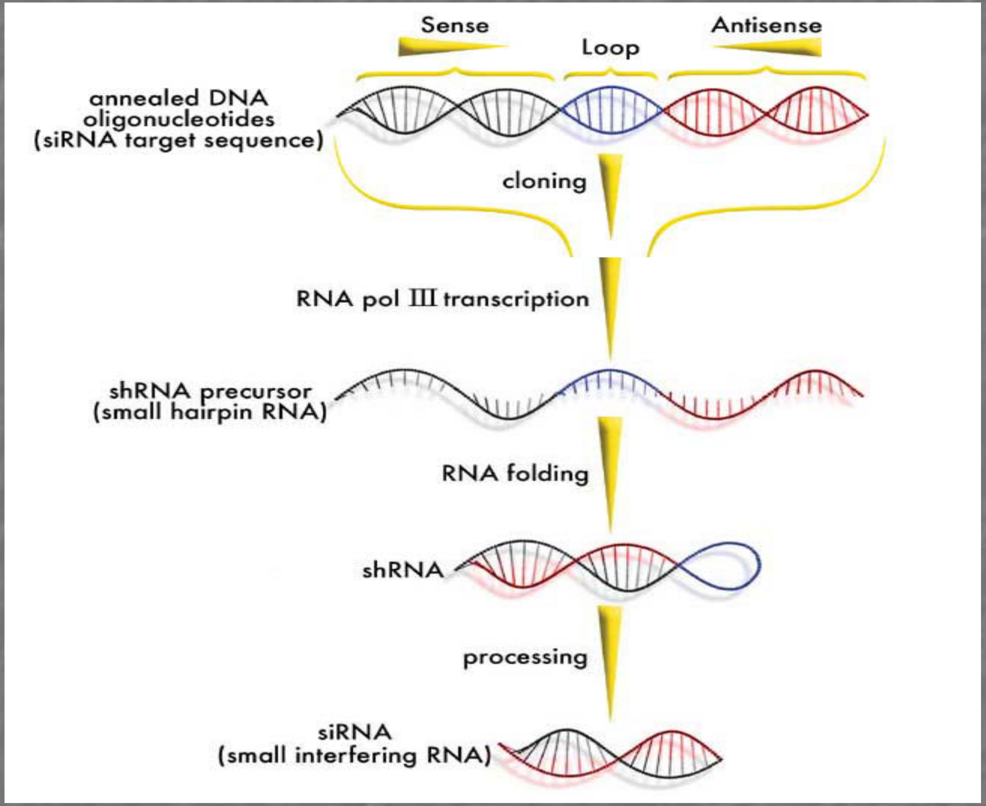
Co-suppression
Expression of an extra copy of a gene turned all expression off

Anti-sense RNA
Expression of an inverted DNA segment turned expression off

But researchers discovered that …

- Co-suppression and anti-sense approaches likely were not mediated by proteins, since most resistant plants did not express the expected protein
- Suggested an RNA-mediated mechanism, rather than a protein-mediated one as the basis for resistance

In time, the RNA interference mechanism was more fully described and understood

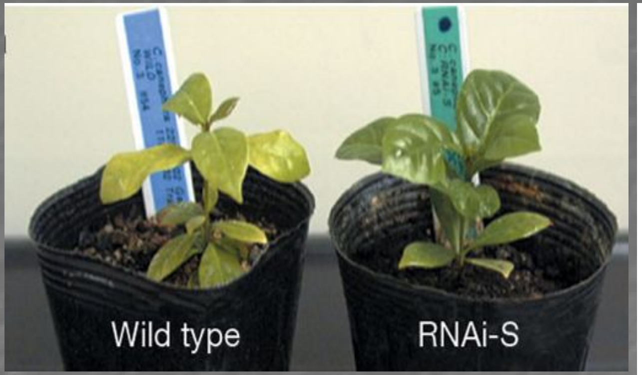


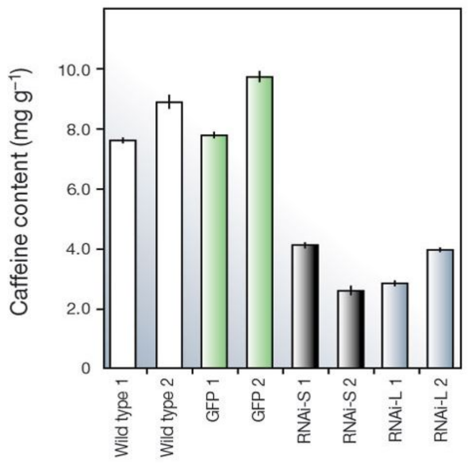
This enabled researchers to contemplate new plant improvements utilizing RNA interference

Diagram by Millipore

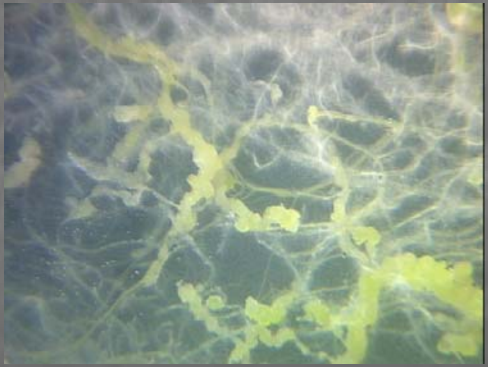
Possible applications of RNAi in agriculture

- Virus resistance
- Altered ripening
- Flower color
- Pathogen resistance
- Nutritional composition
- Specialty oils

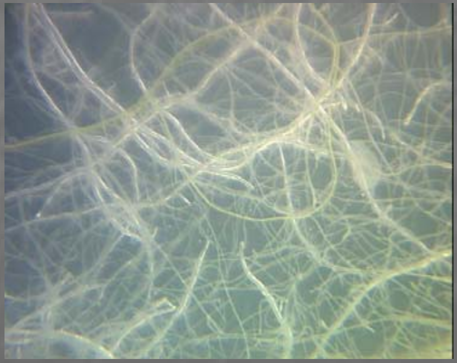




Ogita et al. 2003. Nature 423: 823



Roots infected with root-knot nematodes



Transgenic roots

Huang et al. 2006. PNAS 103: 14302-14306

Incomplete suppression

Complete suppression

Deroles et al., 1998. Molecular Breeding 4: 59-66

Summary – RNAi

- Is a natural process
- Basis of many traits in conventionally bred crop plants
- Also explained the phenotype of some transgenic plants
  - Co-suppression
  - Anti-sense RNA
  - Virus resistance by cross protection
- Has many possible uses for improving crops using genetic modification

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Robust safety assessment approach

Gene / Protein Characterization

Food / Feed Safety

Product/Crop Characterization

- Information relating to the genetic modification, e.g.
    - Methods used
    - Nature and source of the vector
    - Intended function of the insert
    - Description of the trait and its expression

- Information relating to the GM plant, e.g.
    - Description of the trait and its expression
    - Inserted sequences/stability
    - Composition/Phenotype
    - Reproductive/dissemination/ survival ability
    - Anticipated intake/use
    - Any toxic/allergenic/harmful effects
    - Nutritional assessment

Safety assessment of GM plant products derived using TxF and RNAi is essentially the same as for "first generation" products

- Conduct a comparative safety assessment of the GM plant with its conventional counterpart
- Establish the safety of the defined differences
  - The newly expressed gene(s) or gene product(s) and any other defined differences (focused on the introduced trait)
  - Explain how the safety assessment approach takes into consideration all changes that may be associated with expression of a TxF or RNAi
- Inform the scientific community and public about the natural function and use of TxFs and RNAi in plants and animals

Safety considerations for TxF products

- Played a major role in crop domestication and crop improvement
- Have been consumed safely from both plant and animal sources
- Are not active by themselves
- Can only regulate genes already present in a crop genome

RNA is generally regarded as safe for consumption

- RNAi is ubiquitous in plants and animals
- Consumed daily from plant and animal sources
- Mechanism of action is known
- RNAi serves a variety of functions within living cells including down-regulation of viral genes and regulation of metabolic pathways
- Products utilizing RNAi already have been confirmed to be safe for consumption
  - Flavr-Savr Tomato with an anti-sense RNA
  - High Oleic Acid Soybean with co-suppression
  - Virus resistant papaya and potatoes

Considerations for environmental safety assessment of TxF and RNAi products

- Current comparative environmental risk assessment process is appropriate and applicable to improved crops with expressed TxF and RNAi
- Consideration of the trait as well as the technology is important
- Adjustments to the environmental risk assessment may be considered on a case-by-case basis

Summary

- Breeding has produced large and important morphological changes over the centuries
- These modifications are regarded as safe and have enabled dramatic improvements in crop yield and nutrition
- Selection of transcription factor variants has played an important role in plant breeding and in crop domestication
- Transcription factors regulate endogenous genes to modulate existing pathways and plant products
- RNA interference also is a naturally occurring process that modulates gene and phenotypic expression in plants
- Transcription factors and RNA interference are expected to play key roles in the next generation of GM crops

Acknowledgement

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