Food and feed safety assessment principles to evaluate new applications for agricultural biotechnology

ILSI IFBiC Taskforce 8

The University of Georgia

Wayne Parrott
Department of Crop and Soil Sciences
Institute for Plant Breeding, Genetics & Genomics

Actors & Outcome

- Taskforce 8:
  - Wayne Parrott, Bruce Chassy (Univ of IL), Bryan Delaney (Pioneer), Kathy Musa-Veloso (Cantox Health Sciences International), Jim Ligon (BASF), Linda Meyer (Syngenta), Jay Petrick (Monsanto), Junguo Zhou (Bayer), Rod Herman (Dow), Marci Levine (ILSI)
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Review

Application of food and feed safety assessment principles to evaluate transgenic approaches to gene modulation in crops

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New opportunities for agricultural biotechnology

- Transcription factors
  - Genes that alter gene expression within a plant
    - Approximately 10% of genes in a plant genome
  - Many traits can be affected as a consequence
- Gene suppression/RNA interference (RNAi)
  - Turn off expression of genes that are normally turned on
- Other modulations of gene expression
- Began with a review of the role of TF and gene-silencing in plant domestication & breeding
  - Some examples follow

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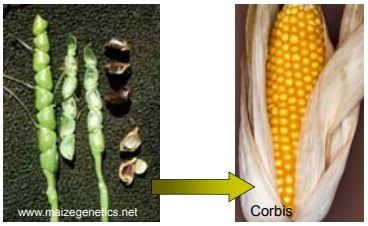
Naked grains of maize



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

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

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Fruit size in tomato

Frary et al., 2002. Fw2.2: a quantitative trait locus key to the evolution of fruit size in tomato. *Science* 289: 85-88

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Green Revolution wheats

- The basis for the Green Revolution was shorter plants due to TF mutation
  - Better response to fertilizer
  - Less lodging

The Harvesters, by Pieter Bruegel, 1565

Peng et al. 1999. 'Green Revolution' genes encode mutant gibberellin response modulators. Nature 400, 256–261.

Example of tall (L) and dwarf (R) wheats

TF's also involved in stress tolerance

- 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

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Summary - *Transcription factors*

- Mutations in TFs have provided major alterations in crop morphology during crop breeding and crop domestication
  - Control plant development
  - Control plant response to stress
- Changing TFs can allow engineering for
  - Stress resistance
  - Yield
  - Improved nutritional composition
- There are plenty of examples in nature that can be studied to help guide safety assessments

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RNA interference

Gene silencing

- The unifying mechanism behind
  - Natural & engineered virus resistance
  - Other naturally occurring traits

Low glutelin rice

News and Views

Nature **423**, 390 (22 May 2003) | doi:10.1038/423390b

RNA interference: Cereal adultery

Christopher Surridge

For people who must restrict their protein intake — such as patients with kidney failure — a mutant rice that is naturally low in proteins called glutelins is beginning to be used as a dietary therapy. Makoto Kusaba and colleagues have now discovered how this mutant achieves low glutelin levels (*Plant Cell* doi:10.1105/tpc.011452; 2003). The answer involves the increasingly well-known biological phenomenon of RNA interference.

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

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Historical use of intentional gene silencing in agriculture

- 1930's - Farmers intentionally inoculate their orchards with a mild virus
  - Become resistant to more virulent strains
  - Became the incentive to engineer plants with virus coat protein genes
- Turns out to silence virus genes via RNAi

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

Virus-resistant Hawaiian papaya

Non-transgenic Transgenic

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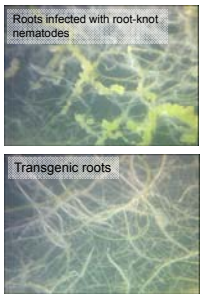
Gene-suppresion

- Some original GM products are now understood to have resulted from RNAi
  - Flavr Savr tomato

• Krieger E, Allen E, Gilbertson L, Roberts J, Hiatt W, Sanders R. 2008. The Flavr Savr tomato, an early example of RNAi technology. HortScience. 43: 962-964
• Sanders RA, Hiatt W . 2005. Tomato transgene structure and silencing. Nature Biotechnology 23: 287-289

How can RNAi be used in agriculture?

- Virus resistance
- Altered ripening
- Specialty oils
- Pathogen resistance
- Insect control
- Flower color



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

RNAi summary

- Is a natural phenomenon
  - Explains many traits in crop plants
- Is a unifying mechanism that explains many of the observations seen with genetically modified plants
  - Co-suppression
  - Anti-sense RNA
  - Virus resistance by cross protection

Safety

Are plants produced via TFs and RNAi safe for food/feed?

- First set of GM crops produced by introducing a gene that **makes a protein**
  - Insect and herbicide resistance
- Safety assessment geared towards safety of the protein
  - Allergenicity
  - Toxicity
- Safety assessment also uses compositional analysis

Transcription factors

Food/feed safety issues

- TFs only modulate **existing** pathways and metabolites within a species
  - Relative quantities/timing of plant compounds can be changed, but
  - No novel compounds produced
  - No new allergy or toxin concerns

Therefore, a targeted compositional analysis may be performed to ensure safety of elevated levels of given compounds

High anthocyanin tomato

Gonzali et al., 2009 Purple as a tomato: towards high anthocyanin tomatoes Trends in Plant Science 14:237-241

RNAi – Assessing safety

- RNA is ubiquitous and an innocuous part of the diet
  - Tens of thousands in each plant cell
  - Many are perfect complements for essential human genes
    - No indication that a plant-produced siRNA has ever silenced a human gene
- No proteins are produced
  - No concerns with toxins or allergens
    - If ensure no start codons are present
- Is specific only to target gene sequence
  - No concerns over effects to non-targets

Picottee patterns in flowers are due to RNAi

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Overall Recommendations

- The current comparative assessment process is appropriate to evaluate TF/RNAi transgenics
- The currently employed targeted analytical composition methodologies are fully capable of detecting biologically significant unintended effects
  - Unintended ≠ dangerous

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Specific Recommendations

- The TFs engineered into plants may come from a source organism with a history of safe use
  - When a history of safe use cannot be established, a Tier 1 protein safety assessment should be applied to assess safety of the TF protein.
- The safety assessment of crops developed by the modulation of endogenous plant gene expression should focus on a comparative assessment/compositional analysis to the non-transgenic version
  - In the case of a stress-tolerance trait, the comparator should also be grown, to the extent possible, under conditions that the crop has been engineered to tolerate, to ensure that no adverse compositional changes affecting safety for consumption have occurred

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Bon appetit!