Safety Assessment of Food and Feed from Biotechnology-Derived Crops with Modifications that Regulate Endogenous Gene Expression

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Abstract

Agricultural biotechnology traits in development include those using tools such as transcription factors and RNA interference (RNAi) to modulate endogenous plant gene expression for the improvement of yields, tolerance to biotic and abiotic stresses, and nutritional quality. Biotechnology-derived crops are presently evaluated for food and feed safety relative to a conventional comparator according to an internationally-accepted safety assessment paradigm. This robust and comprehensive approach incorporates basic concepts from food safety, toxicology, nutrition, molecular biology, and plant breeding and has been used effectively by scientists and regulatory agencies for nearly 15 years. The main aspects of the comparative safety assessment process are reviewed and recommendations are provided for scientifically sound principles to evaluate the safety of biotechnology-derived crops developed using biological approaches that modify endogenous plant gene expression. Key considerations for applicability of the existing safety assessment process include: 1) The history of safe consumption of RNAi-mediating small RNAs (e.g., miRNAs) and regulatory proteins such as transcription factors; 2) The growing scientific literature describing the central role that RNAi and transcription factors have played in plant domestication and conventional breeding; 3) Crops engineered using RNAi constructs are not expected to produce heterologous proteins; 4) Modulation of plant gene expression may result in quantitative differences in levels of endogenous plant components (but not *de novo* components) that can be assessed through compositional analysis.

Introduction: Emerging Technologies in Agricultural Biotechnology

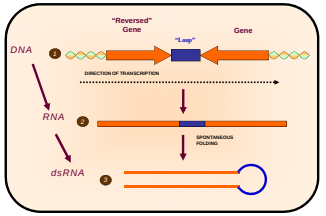
- New crop varieties containing traits such as enhanced nutritional profiles, increased yield, and drought tolerance are being developed.
- In some cases, these traits are dependent on small RNAs or regulatory proteins such as transcription factors (TXFs) that modify expression of endogenous plant genes.
- To date, food and feed safety of biotechnology-derived crops has been assessed by the application of a set of internationally accepted procedures for evaluating their safety.
- This paper reviews the main aspects of the current safety assessment paradigm and recommends this paradigm as a scientifically sound approach for assessing the safety of crops developed through modification of endogenous plant gene expression.

- Key considerations for such a safety assessment include the following:
  - (1) RNA and TXFs have a history of safe consumption
  - (2) Genes encoding RNAi-mediating small RNAs/dsRNAs and regulatory proteins such as TXFs are an important component of the plant genome
  - (3) Crops with RNA-based modifications (e.g., RNAi) can be designed such that heterologous proteins are not produced
  - (4) Modulating TXFs may result in quantitative differences in endogenous plant components, which can be assessed through the existing paradigm for safety evaluation of biotechnology-derived crops.

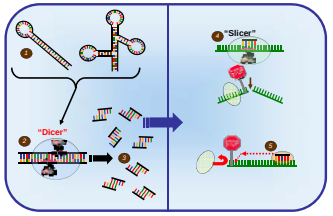
Current Safety Assessment Paradigm

- An internationally accepted comparative safety assessment paradigm is utilized to compare the biotechnology-derived crop to an appropriate conventional comparator with a history of safe use.
- This assessment has focused primarily on the safety of the expressed transgenic proteins and the evaluation of any possible unintended changes in the crop plant or its derivatives (e.g. grain).
- Key components of the safety assessment include: agronomic evaluation, compositional analyses, evaluation of the inserted DNA/insert site, evaluation of the potential toxicity/allergenicity of the expressed protein (e.g., bioinformatics/digestibility), and on a case-by-case basis, specific toxicology and animal feeding studies.
- This assessment paradigm was developed with so-called "input traits" in mind, such as herbicide tolerance and insect resistance, but has also been successfully applied to nutritionally-enhanced crops.
- The next generation of crops developed through modulation of endogenous plant gene expression using methods such as RNAi or other non-coding RNA, TXFs or other regulatory proteins is not expected to present any novel hazards.
- The current safety assessment paradigm is applicable to crops produced using RNAi and TXFs and any additional safety studies should be considered on a case-by-case basis.

RNA Interference -Background



1) Model DNA construct to produce RNAi
2) mRNA product from construct
3) mRNA loop folds to form dsRNA precursor that is used to yield RNAi



1) dsRNA precursor
2) DICER nuclease activity
3) 21-24 nt small RNAs (siRNAs)
4) siRNAs + RISC complex = mRNA cleaved
5) siRNAs can suppress translation to protein

- Small RNAs are endogenous regulators of growth and development in plants, animals, and humans. RNA interference is thus a natural process that can be harnessed to introduce traits in plants.
- Small RNAs are involved in plant disease resistance (e.g., virus resistance).
- Modifications in RNA-based gene regulation have been made during crop domestication and breeding.
- Small RNAs can regulate gene expression via reducing mRNA levels and suppressing translation.
- Gene expression can also be regulated via small RNA induced changes in DNA methylation.
- The universality of small RNAs in biology underscores that these sequences are natural and have a history of safe consumption.

Small RNA: History of Safe Consumption

- RNA is a normal component of the diet and is therefore generally recognized as safe.
- Small RNAs and their longer dsRNA precursors are ubiquitous in plants and animals used for food.
- Small RNAs with homology to important human genes are consumed in staple food crops.
- Small RNAs are responsible for phenotypes in foods with a history of safe consumption, such as the buff seed coat color in soybeans and low-glutelin rice (a low protein variety).
- RNAi is a unifying mechanism for co-suppression and antisense gene suppression.
- RNAi is the mechanism for several traits in several approved biotechnology-derived crops: virus resistance in plum, squash, and papaya, delayed ripening in tomato, altered fatty acid composition in soybean oil, and altered starch content in potato.

Crops with RNA-based Modifications: Food Safety

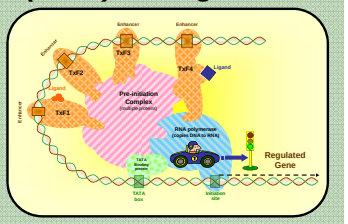
- Noncoding RNA constructs (e.g., RNAi) are not translated into proteins and the focus should therefore be on the safety of the RNA molecules and any unintended effects (via the existing paradigm).
- Absorption of dietary RNA is unlikely due to nucleases in the saliva, acidic pH of the stomach, and the action of pancreatic nucleases in the intestine.
- Biodistribution of RNA is highly unlikely due to nucleases in the saliva and blood.
- Uptake of any remaining intact RNA into cells is not expected due to membrane barriers.
- Delivery is the key challenge to use of RNA-based drugs (e.g. siRNAs), requiring specialized delivery agents and extensive chemical modifications. This illustrates that oral activity of naked RNA molecules is very unlikely.
- Pharmaceutical studies demonstrate that naked siRNAs are not active by the. i.v. route (at 50 mg/kg).

Key Conclusions for RNA-based Crops

- **The existing paradigm for safety evaluation of biotechnology-derived crops is applicable to those produced using RNA-based modifications.**
- **This is supported by the history of safe consumption for RNA (including dsRNA/siRNAs/miRNAs) and the weight of evidence suggesting a lack of oral RNA activity.**
- **Any additional safety studies should therefore be considered only on a case-by-case basis**

Transcription Factors (TXFs) -Background

- Transcription factors (TXFs) are proteins that bind DNA and other proteins.
- TXFs facilitate transcription at the basal transcriptional complex.
- TXFs have played a key role in plant domestication and breeding
  - Domestication of modern maize from teosinte
  - Reduced shattering in rice
  - Reduced wheat stature (reduces lodging)
  - Change from 2-row to 6-row barley



Crops with TXF Modifications: Food Safety

- TXF proteins have a history of safe consumption, as they are found in all nucleated cells and are therefore present in all foods.
- TXFs are proteins and their safety can be assessed using the existing protein safety paradigm.
  - To date, there are no known toxic or allergenic transcription factors.
- TXF proteins tend to be expressed at low levels and will not likely pose a safety hazard.
- New crop varieties containing traits such as enhanced nutritional profiles, increased yield, and drought tolerance are being developed and some of these may rely on TXF based approaches.
- Due to various barriers to functionally active proteins, it is extremely unlikely that ingested transcription factors could impact gene expression in the consuming organism.
- Heterologous TXF proteins can only modulate existing plant genes and are thus highly unlikely to cause formation of new plant metabolites or toxic substances.
- Safety of crops produced using TXF modifications should therefore focus on protein safety and any unintended effects in the biotechnology-derived crop.

Overall Conclusions

1) **Crops with RNA-based traits (e.g., RNAi), expression of heterologous transcription factors (TXFs), or altered levels of endogenous TXFs are being developed.**
2) **Both RNAi- and TXF-associated pathways have been altered in the process of domestication and breeding of conventional crops.**
3) **Small RNAs and their dsRNA precursors and TXFs are ubiquitous in plant and animal genomes; therefore there is a long history of safe consumption.**
4) **Crops with RNA-based modifications (e.g. RNAi) can be designed such that heterologous proteins are not produced. This makes a protein safety assessment unnecessary.**
5) **Safety of TXF-based modifications should assess safety of the heterologous protein (present at very low levels).**
6) **When a history of safe use for the protein cannot be established, an appropriate protein safety assessment should be conducted.**
7) **The safety assessment of crops developed by the modulation of endogenous plant gene expression (e.g., RNAi and TXFs) should focus on the existing paradigm of comparative assessment/compositional analysis relative to the non-transgenic version.**
8) **Engineering crops for the production of small RNAs or for the expression of TXFs does not present any novel hazards such that the current safety assessment paradigm provides a high level of assurance that these engineered crops will be safe for food and feed use.**
9) **Additional safety studies should only be conducted as needed, on a case-by-case basis.**