Environmental Risk Assessment for Stress–tolerant GM Crops: drought–tolerant maize

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The existing ERA paradigm is appropriate for stress-tolerant crops developed using biotechnology:

- Principles outlined in the recent literature on problem formulation provide the starting point for any risk assessment;
- Experience of the last 16 years, environmental risk assessment (ERA) of GM plants could be simplified without compromising environmental protection;
- ERA for GM plants should better integrate principles of pest risk analysis and experience with traditional breeding.
Assuming these foundational principles are still valid

- Genetic modification using modern biotechnology produces no novel risks has been a consistent conclusion of scientific expert panels
  - Plant breeding involving selection has rarely produced a problematic plant (never an environmentally harmful plant?)
  - Genomic changes that have occurred in traditional plant breeding may be greater than those observed in GM crops.
  - All GM events for agricultural use go through the process of selection in the course of breeding in addition to a data-rich risk assessment

- Comparative risk assessment is an appropriate approach for GM plants
  - Familiarity is an appropriate starting point for environmental risk assessment – starting with our knowledge of the crop, the trait and its likely use
  - The risks associated with the regulated GM plants approved to date are no greater than those posed by their conventional counterparts
Environmental Risk Assessment Paradigm

Problem Formulation is the process in which “harm” is defined, regulatory requirements are identified and an analysis plan is formulated.
New products under development can be classified into three groups

- **Familiar**
  - New HT/BT events and stacked combinations in familiar crops (cotton, maize, soybean)

- **Familiar crops with traits familiar to traditional breeding, but developed using GM**
  - Yield
  - Modified composition for foods/feeds
  - Stress tolerance in familiar crops

- **Less familiar GM plants or traits**
  - GM Switchgrass (*Panicum virgatum*)
  - Insecticidally active RNAi
Scoping for ERA – hazard identification focuses on two risk hypotheses

- **Toxicity Assessment**
  - Considers finding plausible mechanisms for harm to occur
    - the gene product has a history of safe use
    - Compositional analysis or anti-nutrients
  - May result in a recommendation to conduct laboratory testing

- **Plant Assessment**
  - Considers the invasive potential of the modified plant
    - history of safe use of the crop
    - phenotypic characteristics of the GM crop compared to its counterpart accounting for the trait
  - May result in field tests under controlled conditions

Hypotheses: the modified plant is no more toxic than its counterpart and the trait is nontoxic

Hypothesis: the plant is unchanged in its invasive potential compared to its counterpart

This hazard identification assumes that the two most important assessment endpoints are toxicity to nontarget organisms and increased weedyness.
Toxicity assessment scoping questions

- Is the gene product known to be toxic? yes/no
- Does the gene product have history of safe use? yes/no
- Have the levels of environmentally relevant anti-nutrients of endogenous toxins been elevated in the GM plant? yes/no

A “no” answer to these three scoping question supports a lack of toxic potential, and sufficient information to test the risk hypothesis, i.e., no further data needed.

A “yes” answer would trigger a higher tiered, experimental approach to the risk assessment.
Data to establish a lack of toxic potential

- History of safe use
- Acute toxicity information e.g., gavage study
- Bioinformatics
- Molecular analysis of the insert (e.g., codex)
  - Informs bioinformatics
- Targeted compositional analysis
  - Analyze known endogenous toxins and anti-nutrients
  - *de novo* synthesis of novel toxins is highly unlikely, there is no precedent from breeding

Composition and Molecular analyses are accepted by Codex as standards to assess unintended effects.
Plant assessment scoping questions

- Is the plant an invasive species anywhere in the world? – yes/no
- Is the plant related to a species known to be invasive anywhere in the world? – yes/no
- Is the novel trait likely to change the plant’s (or a sexually compatible relative’s) potential to become invasive under the conditions of use? – yes/no

A “no” answer to these three scoping question supports a lack of increased weediness potential, and sufficient information to test the risk hypothesis, i.e., no further data needed.

A “yes” answer would trigger a higher tiered, experimental approach to the risk assessment.
Data to establish the lack of increased weediness potential

- Comparative plant characterization based on familiarity with the conventional plant or another appropriate counterpart
  - Provides information on the nature and magnitude of the difference between the GM plant and its counterpart
- Familiarity information for the trait based on knowledge of breeding the crop.
  - Provide a historical context from breeding for the trait of interest and information as to whether the trait has caused in increased weediness in the crop. Example traits:
    - Yield (QTLs);
    - Modified composition (canola with low erucic acid);
    - Stress tolerance (short season B. napus).
Drought–tolerant Maize example

MON 12345
Drought tolerance is essential for corn yield improvement

- Drought stress is the major cause of yield reduction in corn
  - 15% average annual yield loss in temperate and tropical environments – may be greater depending on year
- Breeding and agronomic practices provide improvements
  - Breeding provides steady improvement over time (~1% per year)
  - Reduced tillage limits evaporation from the soil
- Biotechnology will make additional contributions
  - Moderate improvements in yield under drought conditions
  - Familiar phenotypes; still sensitive to stress
  - No expected change to corn production areas
Effects of water limitation on corn

Conventional corn

Water-limited during flowering  Well-watered during flowering
A Generic Construction of the Conceptual Model and Analysis Plan for GM Crops

Basic elements of the Conceptual Model and Analysis Plan for a GM crop link to assessment endpoints (what must we protect?).

<table>
<thead>
<tr>
<th>Basis of the Conceptual Model for a GM Crop</th>
<th>Consider:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>• The nature of the crop</td>
</tr>
<tr>
<td></td>
<td>• The nature of the trait</td>
</tr>
<tr>
<td></td>
<td>• The characteristics of the likely receiving environment</td>
</tr>
<tr>
<td></td>
<td>• The likely interactions between these elements</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Basic Elements of an Analysis Plan for a GM Crop</th>
<th>Product Characterization (define intended differences)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>• Molecular Analyses</td>
</tr>
<tr>
<td></td>
<td>• Expression Analyses (as appropriate[1])</td>
</tr>
<tr>
<td></td>
<td>Protein Characterization</td>
</tr>
<tr>
<td></td>
<td>• History of Safe Use</td>
</tr>
<tr>
<td></td>
<td>• Protein Allergenicity</td>
</tr>
<tr>
<td></td>
<td>• Protein Toxicity</td>
</tr>
<tr>
<td></td>
<td>• Protein Fate/Digestibility</td>
</tr>
<tr>
<td></td>
<td>Plant Characterization (detect meaningful phenotypic differences)</td>
</tr>
<tr>
<td></td>
<td>• Compositional Analyses</td>
</tr>
<tr>
<td></td>
<td>• Agronomic/Phenotypic Analyses</td>
</tr>
</tbody>
</table>

[1] Expression analysis must be appropriate to the nature of the inserted trait. Some traits do not express novel proteins or may be RNA-based.
Problem Formulation for Drought Tolerant Maize Guiding the Process

Assessment Endpoints

- Abundance of pests in maize fields
- Abundance of maize plants occurring outside fields
- Abundance of beneficial organisms in maize fields
- Microbial processes associated with maize production

Conceptual Model

- Familiarity with the biology and ecological interactions associated with maize
- Familiarity with the trait
- Product concept
- Familiarity with the intended environment for release and reasonable sites where release can occur.

Risk hypotheses –

- Maize with tolerance to water stress will not persist and invade natural plant communities and adversely alter the structure of those communities.
- Maize with tolerance to water stress will not produce novel or new levels of nutrients and antinutrients that could affect exposed organisms.
Analysis Plan for Drought Tolerant Maize

<table>
<thead>
<tr>
<th><strong>Appropriate product characterization</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>molecular and expression analysis (as appropriate)</td>
</tr>
</tbody>
</table>

**Detailed characterization of the introduced protein**

- lack of toxic and allergenic potential

**Detailed compositional analyses on field produced samples**

**Detailed plant characterization**

- water stress and non-stress conditions; plant-pest interactions at multiple locations over 2 years

**Field studies assessing survival and competition in non-crop environment**

**Confirmatory studies with animals and microorganisms confirm risk hypothesis of lack of toxicity**

- confirmatory because of our knowledge of the trait and crop
CSPB: an RNA chaperone improves yield under drought conditions

Reduced yield loss under water-limited conditions

Improved physiological capacity during drought allows greater yields

Physiological functions are less impacted during water limitation

General RNA Chaperone Activity of CSPB proteins in MON 12345

CSPB binds RNA, but not dsDNA, and unfolds RNA secondary structures consistent with bacterial CSPs and plant CSD-containing proteins

Accumulates in rapidly growing and developing regions of leaves and reproductive organs in MON 12345

On sub-cellular level CSPB localized to cytoplasm and nucleus

Accumulation and localization pattern is similar to what is known for other CSD-containing proteins found in plants

Under water-limited conditions plants expressing CSPB exhibit improved vegetative productivity: Improvements in:
• CO₂ assimilation
• Stomatal conductance
Trends toward improved:
• Leaf extension rate
• Overall growth rate
Minimal effect on final plant height

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Molecular and Expression characterization

- Developed by *Agrobacterium*–mediated transformation
- Single T–DNA insertion
- Single copy of *cspB* and *nptII* cassettes
- No additional elements or bacterial sequence detected
- *nptII* cassette is flanked by *lox–P* sites
  - Molecular characterization data demonstrate generational stability
  - Several commercial products contain *nptII*
  - *nptII* is safe for food, feed, and environmental uses
- Inserted genes segregate following expected pattern
- Expression determined in multiple tissue types at 3 time points throughout the season
No yield advantage under well-watered conditions

<table>
<thead>
<tr>
<th>Phenotypic characteristic</th>
<th>Units</th>
<th>MON 12345</th>
<th>Control</th>
<th>Min</th>
<th>Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seedling vigor</td>
<td>0-9 scale</td>
<td>4.9</td>
<td>4.7</td>
<td>4.3</td>
<td>6.0</td>
</tr>
<tr>
<td>Early stand count</td>
<td>#/plot</td>
<td>76.1</td>
<td>73.0</td>
<td>71.0</td>
<td>80.0</td>
</tr>
<tr>
<td>Days to 50% pollen shed</td>
<td>Days</td>
<td>66.8</td>
<td>66.7</td>
<td>65.0</td>
<td>74.3</td>
</tr>
<tr>
<td>Days to 50% silking</td>
<td>Days</td>
<td>65.2</td>
<td>65.3</td>
<td>62.7</td>
<td>71.0</td>
</tr>
<tr>
<td>Stay green</td>
<td>0-9 scale</td>
<td>2.4</td>
<td>2.9</td>
<td>1.0</td>
<td>6.7</td>
</tr>
<tr>
<td>Ear height</td>
<td>in</td>
<td>55.9</td>
<td>52.8</td>
<td>46.1</td>
<td>69.1</td>
</tr>
<tr>
<td>Plant height</td>
<td>in</td>
<td>101.1</td>
<td>99.0</td>
<td>94.4</td>
<td>116.4</td>
</tr>
<tr>
<td>Dropped ears</td>
<td>#/plot</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Stalk lodged plants</td>
<td>#/plot</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Root lodged plants</td>
<td>#/plot</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Final stand count</td>
<td>#/plot</td>
<td>75.2</td>
<td>74.0</td>
<td>71.3</td>
<td>79.3</td>
</tr>
<tr>
<td>Grain moisture</td>
<td>%</td>
<td>14.8</td>
<td>15.2</td>
<td>10.1</td>
<td>20.2</td>
</tr>
<tr>
<td>Test weight</td>
<td>lbs/bushel</td>
<td>56.4</td>
<td>55.8</td>
<td>54.0</td>
<td>61.2</td>
</tr>
<tr>
<td><strong>Yield</strong></td>
<td>bu/a</td>
<td><strong>220.7</strong></td>
<td><strong>220.0</strong></td>
<td><strong>166.7</strong></td>
<td><strong>248.4</strong></td>
</tr>
</tbody>
</table>

Note: No statistically significant differences were detected (p<0.05) between the test and control substance.

1 Reference range was calculated from the 3 sites exhibiting a water treatment effect among the 12 reference corn hybrids.

2 Seedling vigor rating scale: 0 = dead and 9 = above average vigor.

3 Stay green rating scale: 0 = entire plant is dried and 9 = entire plant is green.

4 No statistical comparisons were made due to lack of variability in the data. The test was considered effectively not different from the control because the test and control mean values were identical.
Yield advantage under water–limited conditions

<table>
<thead>
<tr>
<th>Phenotypic characteristic</th>
<th>Units</th>
<th>Mean</th>
<th>Reference Range¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>MON 12345</td>
<td>Control</td>
</tr>
<tr>
<td>Seedling vigor</td>
<td>0-9 scale²</td>
<td>5.0</td>
<td>4.8</td>
</tr>
<tr>
<td>Early stand count</td>
<td>#/plot</td>
<td>76.8</td>
<td>75.7</td>
</tr>
<tr>
<td>Days to 50% pollen shed</td>
<td>Days</td>
<td>67.4</td>
<td>68.1</td>
</tr>
<tr>
<td>Days to 50% silking</td>
<td>Days</td>
<td>67.3</td>
<td>66.8</td>
</tr>
<tr>
<td>Stay green</td>
<td>0-9 scale³</td>
<td>4.3</td>
<td>4.7</td>
</tr>
<tr>
<td>Ear height</td>
<td>in</td>
<td>48.0</td>
<td>45.1</td>
</tr>
<tr>
<td>Plant height</td>
<td>in</td>
<td>83.9</td>
<td>78.1</td>
</tr>
<tr>
<td>Dropped ears⁴</td>
<td>#/plot</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Stalk lodged plants²</td>
<td>#/plot</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Root lodged plants²</td>
<td>#/plot</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Final stand count</td>
<td>#/plot</td>
<td>76.7</td>
<td>75.1</td>
</tr>
<tr>
<td>Grain moisture</td>
<td>%</td>
<td>19.5</td>
<td>21.3</td>
</tr>
<tr>
<td>Test weight</td>
<td>lbs/bushel</td>
<td>56.7</td>
<td>56.0</td>
</tr>
<tr>
<td>Yield</td>
<td>bu/a</td>
<td>114.5*</td>
<td>86.7</td>
</tr>
</tbody>
</table>

* Indicates statistical difference between the test and the control (p≤0.05).

¹ Reference range was calculated from the 3 sites exhibiting a water treatment effect among the 12 reference corn hybrids.
² Seedling vigor rating scale: 0 = dead and 9 = above average vigor.
³ Stay green rating scale: 0 = entire plant is dried and 9 = entire plant is green.
⁴ No statistical comparisons were made due to lack of variability in the data. The test was considered effectively not different from the control because the test and control mean values were identical.
# Dormancy and Germination

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Characteristic</th>
<th>Mean %</th>
<th>Reference Range</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>MON 12345</td>
<td>Control</td>
</tr>
<tr>
<td>5°C</td>
<td>Dead</td>
<td>3.9</td>
<td>4.5</td>
</tr>
<tr>
<td></td>
<td>Germinated</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>Viable Firm Swollen</td>
<td>96.1</td>
<td>95.5</td>
</tr>
<tr>
<td></td>
<td>Viable Hard†</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>10°C</td>
<td>Dead</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>Germinated†</td>
<td>89.8</td>
<td>90.0</td>
</tr>
<tr>
<td></td>
<td>Viable Firm Swollen†</td>
<td>9.2</td>
<td>9.0</td>
</tr>
<tr>
<td></td>
<td>Viable Hard†</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>20°C</td>
<td>Dead</td>
<td>0.6</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>Germinated</td>
<td>99.4</td>
<td>99.8</td>
</tr>
<tr>
<td></td>
<td>Viable Firm Swollen†</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>Viable Hard†</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>30°C</td>
<td>Dead</td>
<td>1.2</td>
<td>1.7</td>
</tr>
<tr>
<td></td>
<td>Germinated</td>
<td>98.8</td>
<td>98.3</td>
</tr>
<tr>
<td></td>
<td>Viable Firm Swollen†</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>Viable Hard†</td>
<td>0.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

N = 400 seed (100/rep)
Volunteer Potential

Seed planted in fall 2006 and assessed for volunteer establishment in fall and spring 2007 at 3 locations.

Results
No volunteers were observed at any site.
Survival Outside Cultivation

Purpose
Conducted to assess whether the drought trait meaningfully changes the ability of maize to survive, persist or establish outside cultivation.

Replacement Value
Ability of a plant population to “replace itself.”
In the environment tested:
- R>1 may mean population is expanding
- R<1 may mean population is in decline

Comparative Assessment
Watchout: large significant differences where R>1
Some crop species have R>1
Multiple lines of evidence support a conclusion that MON 12345 poses no increased environmental risk

- MON 12345 is unlikely to persist in the environment
- MON 12345 grain is unlikely to overwinter and volunteer in subsequent seasons
- MON 12345 does not exhibit altered dormancy compared to conventional corn
- MON 12345 does not exhibit altered susceptibility or tolerance to arthropod and disease stressors
- MON 12345 does not alter the abundance of pest or beneficial arthropods
- Pollen viability of MON 12345 does not differ from conventional corn
Improved Harmonization with Pest Risk Analysis

Looking forward
Pest Risk Analysis and invasive plant assessment – relevance for for GM crops

- ERA could be more efficient if we focused on basic questions e.g., could maize be invasive and under what conditions?
  - “lists of species that have become naturalized elsewhere is time honored manner of [predicting invasiveness]” (Mack, 1996)
  - few traits by themselves are good predictors of invasiveness (Monaco, 2006)
  - Slight changes is “fitness” are unlikely to result in meaningful changes to invasiveness of most domesticated crops, e.g., yield.

- For plants with less familiarity, those where one cannot answer “no” to the scoping questions, more uncertainty may exist e.g., phenotypic plasticity (Sultan, 2004).
Challenges going forward

- Acceptance of the scoping phase approach
  - Recognizing the reality that the perceptions of risk associated with the process of GM are inconsistent with experience;
  - Recognizing that determinations of risk may be different for a food & feed assessment versus an environmental assessment;
  - and, the vocal anti-GM activist will strongly object.

- Conduct regulatory–required ERA as a directed process versus a knowledge discovery approach
  - Regulation must be directed by protection goals and assessment endpoints whereas research is a discovery activity
  - Perceptions of “complexity” and “novelty” should be addressed case–by–case and within a context of experience; where theory is weighed against expert judgment of plausibility.
Summary

- 20 years of experience with GM crop development and breeding
- ERA currently in practice is robust, possibly too robust tending toward basic research
  - Newer GM traits may require testing novel hypotheses and pose experimental challenges that are manageable with existing conceptual approaches
- Comparative risk assessment is appropriate for any crop and trait combination
  - Concept of familiarity is the appropriate starting point
- Recommend that ERA begin with a scoping step akin to the initiation step in ISPM 11 and consistent with the principles of problem formulation
  - Recognition that data requirements should be proportionate to risk, and much is often known at the outset of the ERA to guide data requirements based on knowledge of the crop and trait.
- The Drought–tolerant maize case study will enable a rich discussion on these points
Key literature – ERA concepts

- **GMO’s in general**

- **GM Plants**

- **Pest Risk Analysis**

- **Drought-tolerant Maize**