How GMOs Are Really Made:  
*Simple Precision or Messy Complexity?*

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Why does it matter?

1. GMO Risks to Human or Livestock Health or to the Environment arise from:
   - The inserted DNA itself (gene cassette)
   - The methods used to insert the gene cassette into the plant genome

2. Both can give rise to harmful unintended effects

3. These are not being adequately taken into account by regulators

4. Key Myth of Precision
What is a GMO?

How do you make a GMO?

Why do GMOs not behave as expected?
What is a GMO?
( Genetically Modified Organism )

(g) “Living modified organism” means any living organism that possesses a novel combination of genetic material obtained through the use of modern biotechnology;

--- Legal definition of a LMO (=GMO) of the Cartagena Protocol on Biosafety to the Convention on Biological Diversity, Montreal, 2000 (in force since 1993). The Convention is the main international instrument for addressing biodiversity issues.

What is a GMO? (Genetically Modified Organism)

An organism with one or more traits that have been created and introduced using genetic engineering techniques.

A few examples of Commercialized GMO Plants

<table>
<thead>
<tr>
<th>Herbicide Resistance Traits</th>
<th>Bt Pesticide Traits</th>
<th>Other Traits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Roundup Ready soybean</td>
<td>YieldGard Mon810 Bt Maize</td>
<td>Artic Apple</td>
</tr>
<tr>
<td>Liberty Link Rice</td>
<td>Bollgard Bt cotton</td>
<td>SunUp Papaya</td>
</tr>
<tr>
<td>Liberty Link Cotton</td>
<td></td>
<td>CZW-3 Squash</td>
</tr>
</tbody>
</table>

Genes: “DNA makes RNA makes Protein”
Birds and the GMO Food Web

Birds of Prey, Fish-eating Birds

Mammals, Reptiles, Amphibians, Fish

Birds

Invertebrate (soil, water, plant)

Microbes (bacteria, yeast)

Pollen (Nectar)

Seeds

Leaves

Stem, Sap

Roots, exudates

Plant Detritus
Midwestern GMO Maize Fields

- 2015: 400 million acres GMOs worldwide
- Most Bt corn and Herbicide Resistant Soybean
- ¾ of those acres were in US, Brazil and Argentina
Roundup Ready Soybeans in the Amazon: Before and After
How to Make a GMO

1. Prepare a gene cassette

2. Transfer the gene cassette into the plant genome

3. Select a successful GMO for commercialization
1. Prepare a DNA gene cassette

- Identify DNA that specifies the protein that results in the desired trait
- Identify regulatory sequences (eg. promoter, terminator, protein targeting sequences)
- Cut and paste them together using recombinant DNA technology
- Repeat if needed
DNA Gene-Cassette Used to Create RR2Yield’s Herbicide Resistance Trait

- Intervening DNA
- **Figwort Mosaic Virus** Enhancer (E)
- *A. thaliana* Promoter (P)
- *A. thaliana* 5’ Untranslated Leader (L)
- *A. thaliana* Intron (I)
- *A. thaliana* Chloroplast Transit Peptide (CTP)
- **Agrobacterium** Herbicide-Resistance Gene (CP4-EPSPS)
- *P. sativum* 3’ Untranslated Terminator (T)
Plasmid Vector and Gene Cassette

P= promoter, L= leader, I= intron, TS= Target sequence, CS= coding sequence, T= 3’ non-translated transcript term. Seq. and poly A signal, OR= origin of replication

Recap: Gene Cassettes are Complex

- Gene cassettes usually mix DNA from highly diverse organisms (e.g. viruses, bacteria, fungi) or different plant species

- “Promoters” and “terminators” are just names and are poorly understood
  - Viral Promoters specify proteins
  - Terminators that don’t terminate


Rang, Andreas, Bettina Linke, and Bärbel Jansen. "Detection of RNA variants transcribed from the transgene in Roundup Ready soybean." *European Food Research and Technology* 220.3-4 (2005): 438-443.
2. Transfer gene cassette DNA into the plant genome

Agrobacterium infection
or
A Gene Gun
Plasmid Vector and Gene Cassette for *Agrobacterium*-mediated DNA Transfer

**Diagram**

- **PV-GMGOX20**: 9664bp
- **CS-aadA**: Resistance cassette for aadA
- **CS-cp4epsps**: Expression cassette for cp4epsps
- **CS-rop**: Expression cassette for rop
- **OR-ori-PBR322**: Origin of replication for PBR322
- **OR-oriV**: Origin of replication
- **RB**: Replication origin
- **L-Tsfl**: Large fragment
- **L-Tsfl**: Large fragment
- **I-Tsfl**: Intermediate fragment
- **TS-CTP2**: Target sequence
- **P-FMV/Tsfl**: Promoter

**Annotations**

- P=promoter, L=leader, I=intron, TS=target sequence, CS=coding sequence, T=3’ non-translated transcript term. Seq. and poly A signal, OR=origin of replication

**Data**

Agrobacterium-mediated DNA Transfer

1. **Gene cassette** integration into the chromosome of Agrobacterium with a plasmid carrying the gene cassette.

2. **Plant Cell** transformation with the T-DNA (Trans Transfer DNA) transferred during infection.

3. Put cells onto selection media (with glyphosate and antibiotics).

4. **Transformed Cell** selected for transgene expression.

5. **Plant cells with transgene multiply in cell culture.**

6. Move to root and shoot regeneration media.

7. **Transgenic Plant** resulting from cell culture.

8. **Plantlet** formation.

http://2010.igem.org/Team:Nevada/Agrobacterium_Transformations
Agro Gene Cassette Transfer Process “Up Close”

http://2010.igem.org/Team:Nevada/Agrobacterium_Transformations
Gene Gun-mediated DNA Transfer (Particle Bombardment)

1. **Isolate** large amounts of gene cassette DNA

2. **Coat** DNA onto tiny gold or tungsten particles

3. **Shoot** particles into plant cells using a Gene Gun
Gene Gun (particle bombardment)

1. **Load** DNA covered pellets into gene gun.
2. **Blast** pellets into plant cells using compressed gas.
3. Grow cells on **selective cell culture media** (glyphosate).
4. Transfer cells to root and shoot **regeneration media**.

Inside cell-- DNA floats off pellets and can integrate into plant DNA.
Inside Cell Nucleus after Gene Gun Bombardment

Examples of Gene Cassette Insertion Sites

Gene Cassette

Plant Chromosomal DNA

Precise Insertion

Actual Insertions (deletions, duplications, rearrangements, insertions)

Recap: Gene Transfer is Complex and Messy

- Can’t choose site of transgene insertion
  - *Agrobacterium* insertion used by researchers as a mutagen to disrupt genes intentionally

- Can’t predict or prevent damage at site of transgene insertion

- Numerous Genome-wide mutations
  - Superfluous DNA insertion around the genome
  - Tissue culture, *Agro* infection, gene gun – mutagenic

What does this mean for the resulting GMOs -- and the Biotech Companies?
3. Select a GMO for commercialization

Selecting a Glyphosate Resistant GMO Wheat for Commercialization

A. Infect/bombard many independent “explants” with gene cassette
   (explants = e.g. pieces of leaf, stem or embryo)
   (Total for Monsanto’s GMO wheat: >98,800)

B. Select many independent candidate GMO plants
   (Total for Monsanto’s GMO wheat: >4000)

C. Carry out several generations of testing and selection
   (for candidates with good GMO trait expression and acceptable crop characteristics)

Select a GMO for commercialization

Glyphosate Resistant GMO Wheat: Four Generations of Selection

Table 3 The number of transgenic events derived from Agrobacterium transformation and biolistic transformation of wheat and the percentage advanced by evaluation at different generations (ae acid equivalent)

<table>
<thead>
<tr>
<th>Generation</th>
<th>Agrobacterium Transformation</th>
<th>Biolistic Transformation</th>
<th>Criteria for advancement</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of events</td>
<td>Percentage</td>
<td>Number of events</td>
</tr>
<tr>
<td>T_0 to T_1</td>
<td>773</td>
<td>100</td>
<td>597</td>
</tr>
<tr>
<td>T_1 to T_2</td>
<td>131</td>
<td>17</td>
<td>110</td>
</tr>
<tr>
<td>T_2 to T_3</td>
<td>19</td>
<td>2.5</td>
<td>16</td>
</tr>
<tr>
<td>T_3 to T_4</td>
<td>11</td>
<td>1.4</td>
<td>7</td>
</tr>
<tr>
<td>T_4 to T_5</td>
<td>6</td>
<td>0.8</td>
<td>1</td>
</tr>
</tbody>
</table>

1. Selection for “normal” looking candidate plants with good GMO trait expression
   (>1300 T_0 selected for further testing/~2700 T_0 discarded)
2. More intensive selection for good GMO trait expression
   (241 T_1 selected for further testing/~1060 T_1 discarded )
3. Even more intensive selection for good GMO trait expression AND >80% fertility
   (35 selected for further testing/ 206 T_2 discarded )
4. One copy of gene cassette inserted, Homozygous, good agronomic performance
   (17 T_3 selected for further testing/ 18 T_3 discarded)
5. Molecular analysis, further agronomic tests
   (7 T_4 selected / 10 T_4 discarded)

1 candidate (#33391) was chosen as a Roundup Ready wheat commercial candidate.
Does Selection Ensure GMOs Behave As Expected?

NO

Numerous unexpected effects have been documented in commercial lines

e.g. RR 2 Yield Soybean is 5% shorter than the non-GMO parent
# Unintended Consequences Are Important

<table>
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<tr>
<th>Commercial Transgenic Events</th>
<th>Unintended Consequence</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>RR Soybean (Glyphosate\textsuperscript{R}) 40-3-2</strong></td>
<td>7-11% Yield Decrease (&gt; 210 Million Bushels Lost per annum from 3 Billion per annum US Soybean Crop)</td>
<td>USDA 93-258-01p, Elmore et al. 2001, Nelson et al. 2002</td>
</tr>
<tr>
<td><strong>RR Rice (Glufosinate\textsuperscript{R})</strong></td>
<td></td>
<td>USDA 06-234-01p, USDA 98-329-01p, USDA 06-234-01p</td>
</tr>
<tr>
<td>LLRice601</td>
<td>3-7% Yield Decrease</td>
<td>USDA 06-234-01p, USDA 98-329-01p, USDA 06-234-01p</td>
</tr>
<tr>
<td>LLRice06</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LLRice62</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Bt+ Paymaster Cotton 1560 BG</strong></td>
<td>Loss of Nematode Resistance</td>
<td>Colyer et al. 2000</td>
</tr>
<tr>
<td><strong>Bt+ Atlantic Newleaf Potato</strong></td>
<td>Loss of Golden Nematode Resistance</td>
<td>Brodie 2003</td>
</tr>
</tbody>
</table>
| **Squash CZW-3** | ▪ Iron 87% of control  
▪ Fat 50% of control  
▪ Beta-Carotene 1.5% of control  
▪ Vitamin A increased 2-fold  
▪ Sodium increased 4-fold | USDA 95-352-01p |
## Documented Unintended Effects of Genetic Engineering with Health or Agronomic Implications

<table>
<thead>
<tr>
<th>Harmful or Potentially Harmful Unintended Effects</th>
<th>Some Commercial Examples from the Scientific Literature (references available)</th>
</tr>
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</table>
| **Altered Nutrition** *(nutrients: e.g. vitamins, minerals, antioxidants or antinutrients: e.g. lectins, protease inhibitors)* | • Squash (Virus-R): e.g. 67.6 times less Beta Carotene  
• Maize (and other commercial Bt crops): higher lignin |
| **Increased Toxicity** *(novel or increased toxins or allergens, high pesticide residues)* | • Soybeans now routinely contain extreme levels of Roundup and AMPA (its breakdown product)  
• Bt maize: systemic expression of (Bt toxin +neonic) + additional sprays  
• Maize (Bt) Mon810: newly expressed allergen |
| **Non-target effects** *(organisms in bird food chain are harmed due to unintended effects of genetically engineered crop)* | • Maize (Bt) harmful effects on earthworm, beetles  
• Various GMO crops used in animal feeding studies show harmful effects (Krimsky review 2015) |
| **Agronomic effects** *(Can lead to higher pesticide or herbicide use by farmers)* | • Bt Cotton and Bt potato: lost nematode resistance  
• Bt Maize increased susceptibility to aphids and decreased mycorrhizal levels  
• RR2Yield Soybean: decreased height  
• RR Soybean: decreased yield, Manganese deficiency |
Why are GMOs Especially Prone to Harmful UEs?

<table>
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<tr>
<th>GE Technique or Trait</th>
<th>Risk (well-documented – refs. available)</th>
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<tbody>
<tr>
<td><strong>Plant transformation:</strong> <em>Agrobacterium</em> infection or Gene Gun; random transgene insertion; cell culture</td>
<td>All are highly mutagenic procedures</td>
</tr>
<tr>
<td>Transferred genes are often composed of DNA from other species (bacterial, viral, fungal)</td>
<td>Different mRNA or protein processing can alter the transgene product’s toxicity, allergenicity or behavior</td>
</tr>
</tbody>
</table>
| **Viral promoters are widely used** | 1. High expression leads to imbalances (eg. Altered protein profile)  
2. Viral promoters encode viral proteins (potential toxins) |
| **RNAi technology:** Suppress expression of genes in crop plant or in targeted “pest” organism | 1. Imprecise (off-target effects)  
2. The ds-RNAs produced are PAMPs known to stimulate vertebrate immune responses |
| **Herbicide tolerance and Pesticidal traits are most common** | 1. Increased exposure of consumers to herbicide and pesticide toxins  
2. Result in altered composition (biochemistry) of the GE plant |
Conclusion

1. The methods used to make a GMO are imprecise, unpredictable and highly mutagenic.

2. Commercial GMOs are highly prone to showing unintended effects.

3. These risks are ignored by GMO safety regulators and have urgent implications for
   • Food safety
   • Crop failure
   • Biodiversity loss

For more examples of unintended effects of GMOs collected from the scientific literature see: Nature Institute Website http://natureinstitute.org/nontarget/report_class.php