Development of an Agricultural Biotechnology Product

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NAICC January 29, 2016
Agenda/Content

- Why is Biotech necessary
- Stages in Product Development
- Elite Event Selection
- Safety Assessment
- Summary
World Population Projections

- Year: 1950, 1975, 2000, 2025, 2050, 2075, 2100
- Billions of people:
  - 1950: 3
  - 1975: 4
  - 2000: 8
  - 2025: 12
  - 2050: 16
  - 2075: 20
  - 2100: 24
The Challenge:

Feed More People With Current Resources

Global population rising

Arable Land Decreasing

Technology must provide solutions
Maize Yields Over Time

Minimum inputs vs. Best technologies

http://www.nass.usda.gov/QuickStats/PullData US.jsp.
Maize yields 1996 – 2007 Iowa vs. France + Italy

(Source: USDA and FAOSTAT, 2009)
Biotechnology is an Extension of Traditional Plant Breeding

**TRADITIONAL PLANT BREEDING**

Many genes are transferred

Desired Gene

Donor Plant

X

Commercial Plant Variety

New Plant Variety

**PLANT BIOTECHNOLOGY**

A single gene is transferred

Desired Gene

Donor

+ Desired Gene

Commercial Plant Variety

Improved Commercial Plant Variety
PRODUCT DEVELOPMENT

Phase 0
DISCOVERY PHASE – Product concept and gene discovery

Phase I
PROOF OF CONCEPT – Transformation and optimization

Phase II
EARLY DEVELOPMENT – Elite event identification

Phase III
ADVANCED DEVELOPMENT – Regulatory approval

Phase IV
LAUNCH – Product introduction and life cycle management
Phase 0: Discovery
Identifying The Trait

Balancing Act

- What do the growers want/need?
- What attributes can this science deliver
- Can value be captured?
- Consumer desires?
Grower’s Needs

- Land
- Seed
- Fertilizer
- Herbicides
- Pesticides
- Equipment
- Labor
- Fuel

BOTTOM LINE = YIELD

Iowa State University Jan. 2014
Phase 1: Gene Isolation
Scientifically what is possible

Single Gene → Phenotype

- Herbicide tolerance:
  - Modified mode of action (i.e., CP4 EPSPS)
  - Herbicide detoxifier (i.e., PAT)

- Insect Resistance:
  - Cry proteins
  - Vip proteins

- Disease resistance

More Complex Traits
- Metabolic pathways
- Yield enhancement
- Nutrient enhancement
Critical Steps in Transformation

- Getting the gene into the plant genome
- Getting the plant cell to turn into a plant…
  - …That expresses the gene
- Getting a transformed plant to be fertile
- Getting the progeny to express the phenotype…
  - …With good yield
Trait Introduction: Biolistics vs. Agro transformation

- Earliest events used biolistics (or similar brute force transformation)
- Protoplast transformation has also been used successfully
- Transformation using *Agrobacterium tumefaciens* is more precise
DOES IT WORK EVERYTIME?

NO!!!!

Why Not?
WHAT CAN GO WRONG?

Idea is bad
Trait is complex
Gene is not expressed
Gene is inserted in a poor location
• Harms plant (does not survive through tissue culture)
• Impacts plant metabolism unexpectedly
• Impacts plant phenotype
• Impacts plant fertility
Not inherited in a Mendelian fashion
Phase 2: Proof of Concept

- Phenotype correlates with expression of gene
- Works similarly in different germplasm backgrounds
- Meets product specifications
It’s a numbers game – start BIG

Many ideas to get the RIGHT one
Many cells transformed to get multiple events
Many events evaluated to get THE BEST One
### Average Duration

<table>
<thead>
<tr>
<th>Phase</th>
<th>Duration</th>
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<tbody>
<tr>
<td>Discovery</td>
<td>53.9 months</td>
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<tr>
<td>Phase I</td>
<td>27 months</td>
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<tr>
<td>Phase II</td>
<td>30 months</td>
</tr>
<tr>
<td>Phase III</td>
<td>37.2 months</td>
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<tr>
<td>Phase IV</td>
<td>48.8 months</td>
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</table>

### Mean Units Tested

<table>
<thead>
<tr>
<th>Regulatory Activities</th>
<th>Units</th>
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<tbody>
<tr>
<td>In silico screening</td>
<td>10,209</td>
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<tr>
<td>Preliminary Digestive Fate</td>
<td>511</td>
</tr>
<tr>
<td>Molecular Characterization</td>
<td>1,302</td>
</tr>
<tr>
<td>Full Regulatory Study Package</td>
<td>2</td>
</tr>
<tr>
<td>Data Defense</td>
<td>1</td>
</tr>
</tbody>
</table>
Many Ideas Don’t Make it Past the Discovery Stage – Regulators never see

- 10 – 15% don’t pass allergen/toxin homology screen
- May not be expressed in planta
- No efficacy
- Scientifically feasible, market cannot support

- Only the best performers with the correct attributes get promoted
- Economics also plays a role
- Some products providing good solutions can’t support the cost
Is this any different from Conventional Breeding?

- 80 -100 crosses are typical (although some breeders make 600).
- Similar for all crops.
- Utilizing contra-season nurseries, it takes about 6 - 7 years to develop a new cotton, soybean or wheat variety.
- Marker assisted breeding can assist in the development of multiple traits at one time.
Phase 2.
Selection of the ELITE Biotech Event

- Efficacy
- Agronomic Performance
  - Yield
  - Other agronomic parameters
  - Inheritance pattern
- Molecular characteristics
  - Single locus
  - Single gene inserted
  - Lack of vector backbone
  - Integrity of insert
  - Stability across generations
  - Insertion site
PHASE 3

- Safety assessment for plant biotech products is mandatory worldwide
- Considers human + animal health as well as environmental safety
- Approval only if authorities conclude:
  ➔ Genetically optimized plant is as safe as a conventionally bred plant
Safety Assessment is a multi-pronged undertaking

- **Crop Safety**
- **Gene(s)**
- **Protein(s)**
- **Crop Characteristics**
- **Food/Feed Composition**
- **Environmental Safety**

**Food / Feed and Environmental Safety**
Most Studied Food – Safety Reviewed by Agencies all Around the World

Production Countries (28)  Importing countries
Regulatory Studies are grouped in four categories:

- Molecular characterization
- Protein Characterization/Food/feed safety
- Agronomic and Compositional Equivalence
- Environmental safety
Molecular Characterization

- Number of DNA inserts, insert stability
- Number of copies
- Integrity of gene cassettes
- Presence of additional DNA (backbone)
- Sequence of genomic flanking DNA
- Sequence of the inserted DNA
Protein characterization, Food / Feed safety

- Gram quantity protein production and purification
- Establishing protein level in plant tissues
- Protein characterization and equivalence
- Allergenicity assessment
- Toxicity assessment
Protein expression levels: Tissues and Stages Considered

Crop and Trait Dependent

- Need to know how much is out there at any time throughout the growing season
- For a PIP this information belongs on the label as for any pesticide
- Contributes to exposure assessments – if not in the seed (and the seed is consumed) = no exposure
- If not in the pollen – reduces environmental exposure
Why proteins do not typically represent a hazard

- Proteins are relatively large and labile.
- Proteins are an essential part of the diet (avg. consumption 100 g/day).
- Digestive systems have evolved to convert the protein to its building blocks for incorporation. (very efficient only 6 – 12 g protein lost/day)
- The human body synthesizes approx. 300 g protein/day.
Protein Hazards

- Pathogenic bacterial toxins - botulinum, diptherium, active <100 μg/kg body weight.
- Plant toxins - ricin (0.5 mg MLD)
- Animal toxins - prions
- Allergens – Ara H2, β-lactoglobulin
- Antinutrients - trypsin inhibitors, some lectins
Weight of Evidence Approach

Two Tiers:

Basic Assessment

Supplementary Assessment
Basic Assessment

- History of safe use
- Bioinformatics sequence homology assessment
- Source of gene
- Mode of action
- Range and pattern of expression levels
- Stability of protein to temperature, pH, and digestive enzymes

**REQUIRE SMALL AMOUNTS OF PROTEIN**
Supplementary Assessment

- Acute toxicity testing with recombinant transgenic proteins
  14 day mouse study – single dose 2000 mg/kg body weight

- 28 day repeat dose toxicology testing at 1000 mg/kg body weight DAILY

- Hypothesis-based testing to be determined on a case-by-case basis

REQUIRE LARGE AMOUNTS OF PROTEIN TO CONDUCT
Proteins are tested at levels equal to a 75 kg man eating 50 tons of corn for lunch.
Bridging Study to Show Equivalence

1. Is the plant producing what we expect it to produce?
2. Is the test substance used in toxicological studies the same as what the plant produces?

- Size (SDS-PAGE, MALDI-TOF)
- Immunoreactivity (western analysis, ELISA)
- Functionality (enzymatic assay, bioassay)
- Post-translational modification (mass spectrometry, others)
- N-terminal amino acid sequence (Edman degradation)
Agronomic and Compositional Equivalence

- Agronomic / phenotypic assessments
- GM plant samples from multi-location, multi-year, replicated field trials
- Nutrient / antinutrient analyses on grain and processed products
- Animal feeding studies
- Weediness assessment
### Agronomic Parameters

<table>
<thead>
<tr>
<th>Corn</th>
<th>Cotton</th>
<th>Soybean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yield</td>
<td>Yield</td>
<td>Yield</td>
</tr>
<tr>
<td>Germination</td>
<td>Days to Emergence</td>
<td>Germination</td>
</tr>
<tr>
<td>Days to Silking</td>
<td>Days to Flowering</td>
<td>Seedling vigor</td>
</tr>
<tr>
<td>Days to pollen shed</td>
<td>Days to Open Bolls</td>
<td>%Plant stand</td>
</tr>
<tr>
<td>Disease</td>
<td>Plant Height</td>
<td>Days to 50% full bloom</td>
</tr>
<tr>
<td>Weediness</td>
<td>Vigor</td>
<td>Days to harvest</td>
</tr>
<tr>
<td># seed/ear</td>
<td>Disease</td>
<td>Stay green</td>
</tr>
<tr>
<td>% moisture</td>
<td>Uniformity</td>
<td>Plant height</td>
</tr>
<tr>
<td>Emerged plants</td>
<td>Stand Count</td>
<td>Disease</td>
</tr>
<tr>
<td>Test weight</td>
<td>Lodging</td>
<td>Insect damage</td>
</tr>
<tr>
<td>Plant height</td>
<td>Boll Type</td>
<td>Soil ecology</td>
</tr>
<tr>
<td>Ear height</td>
<td>Percent Open Bolls</td>
<td>Earthworms</td>
</tr>
<tr>
<td>Lodging</td>
<td>Total Harvest weight</td>
<td>Soil microbes</td>
</tr>
<tr>
<td>Stay green</td>
<td>Insect damage</td>
<td></td>
</tr>
<tr>
<td>Dropped ears</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
## Analytes

<table>
<thead>
<tr>
<th>Corn</th>
<th>Cotton</th>
<th>Soybean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proximates</td>
<td>Proximates</td>
<td>Proximates</td>
</tr>
<tr>
<td>Fatty Acids</td>
<td>Fiber</td>
<td>Fatty Acids</td>
</tr>
<tr>
<td>Minerals</td>
<td>Fatty Acids</td>
<td>Minerals</td>
</tr>
<tr>
<td>Vitamins</td>
<td>Minerals</td>
<td>Vitamins</td>
</tr>
<tr>
<td>Amino acids</td>
<td>Vitamins</td>
<td>Amino acids</td>
</tr>
<tr>
<td>Ferulic acid</td>
<td>Amino Acids</td>
<td>Lectins</td>
</tr>
<tr>
<td>Coumaric acid</td>
<td>Gossypol</td>
<td>Isoflavones</td>
</tr>
<tr>
<td>Phytate</td>
<td>Phytate</td>
<td>Lecithins</td>
</tr>
<tr>
<td>Xanthophylls</td>
<td>Malvalic Acid</td>
<td>Phytate</td>
</tr>
<tr>
<td>Starch</td>
<td>Sterculic Acid</td>
<td>Trypsin inhibitor</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Urease activity</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ADF &amp; DNF</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Raffinose &amp; Stachyose</td>
</tr>
</tbody>
</table>

- "ADF" and "DNF" represent Acid Detergent Fiber and Degradable Nitrogen Fraction.
Typical Processed Fractions

**Cotton**
- Ginned seed
- Linters
- Delinted seed
- Hulls
- Meals
- Crude oil
- RBD oil

**Canola**
- Seeds
- Presscake
- Toasted Meal
- Solvent extracted meal
- Crude oil
- RBD oil

**Soybean**
- Seeds
- Meal
- Hulls
- Crude oil
- RBD oil
Animal Wholesomeness Studies

- Rat 90 day subchronic study with grain
- 42 Day Poultry feeding study
- Catfish study
- Swine study
- Dairy cattle study
- Beef cattle study
Environmental Safety

• Environmental safety and fate

• Interactions with target and nontarget organisms

• Interactions with the abiotic environment and soil
For Pesiticidal Plants
Ecotoxicological Studies:

- Collembola
- Honeybee
- Fish
- Daphnia
- Avian
- Ladybeetle
- Soil Stability
- Earthworm
- Lacewing
- Resistance management
US Stack Percentage

STEWARDSHIP

• Need for confinement until launch
• Not all geographies grant approvals at the same time - asynchrony
• Not all geographies want to enjoy the benefits of these technologies
• These products represent options
• Need for co-existence

• Hence the need to preserve genetic integrity
**BIOTECH CROPS [PLANTED OR APPROVED* IN 2014] AND BENEFITS:** 2014 ISAAA Global Status Report Updates

**UNITED STATES: INNATE™ POTATO APPROVED**
- 4th most important food crop in the world
- Will not discolor when peeled
- Has fewer bruising spots
- Increases shelf-life
- Decreases potential for producing acrylamide [potential carcinogen] when potatoes are cooked at high temperatures

**INDONESIA: DROUGHT-TOLERANT SUGARCANE APPROVED**
- Increases availability of valuable food source
- Indonesia is the second-largest sugar importer in the world, importing 2.4M tonnes at $1.6B annually
- Decreases dependency on imported sugar
- In field trials, yield increased markedly
- Public-private partnership with Ajinomoto

**BANGLADESH: TIMELY COMMERCIALIZATION OF Bt BRINJAL (Eggplant)**
- Less than 100 days post-approval
- One of the most nutritious and important vegetables in the country
- Significant opportunity for resource-poor farmers
- 70 to 90% decrease in insecticide sprays on a food crop

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The International Service for the Acquisition of Agri-biotech Applications (ISAAA) is a not-for-profit organization with an international network of researchers designed to contribute to the alleviation of hunger and poverty by sharing knowledge and crop biotechnology applications. Clive James, Emeritus Chairman and Founder of ISAAA, has lived and/or worked for the past 30 years in the developing countries of Asia, Latin America and Africa, devoting his efforts to agricultural research and development issues with a focus on crop biotechnology and global food security.
Very Useful Websites

- http://www.cera-gmc.org/
  - Database on all approved biotech events
  - Database of applications and approvals
- https://gmoanswers.com/
  - Council for biotechnology information
- http://www.isaaa.org/
  - Conducts annual surveys on acceptance/adoption of biotech products
Summary

- Biotechnology solutions are critical for sustainable agriculture and feeding the growing population.
- It takes a lot of time and effort to produce the single ELITE event.
- Registerability considerations begin early in the R&D process.
- Efficacy is the most important elite event selection criteria.
- Evaluation of plant performance eliminates events in which the insertion site was unacceptable.
- The event selection process is similar to conventional breeding.
- Many events are not advanced due to properties NOT associated with safety.
- Reviewers around the world evaluate the safety packages – most highly studied food/feed consumed.
- Approval is granted only when the GM crop has been shown to be as safe as conventional counterpart.
Thank you!

Science For A Better Life