Science For A Better Life

Development of an Agricultural Biotechnology Product

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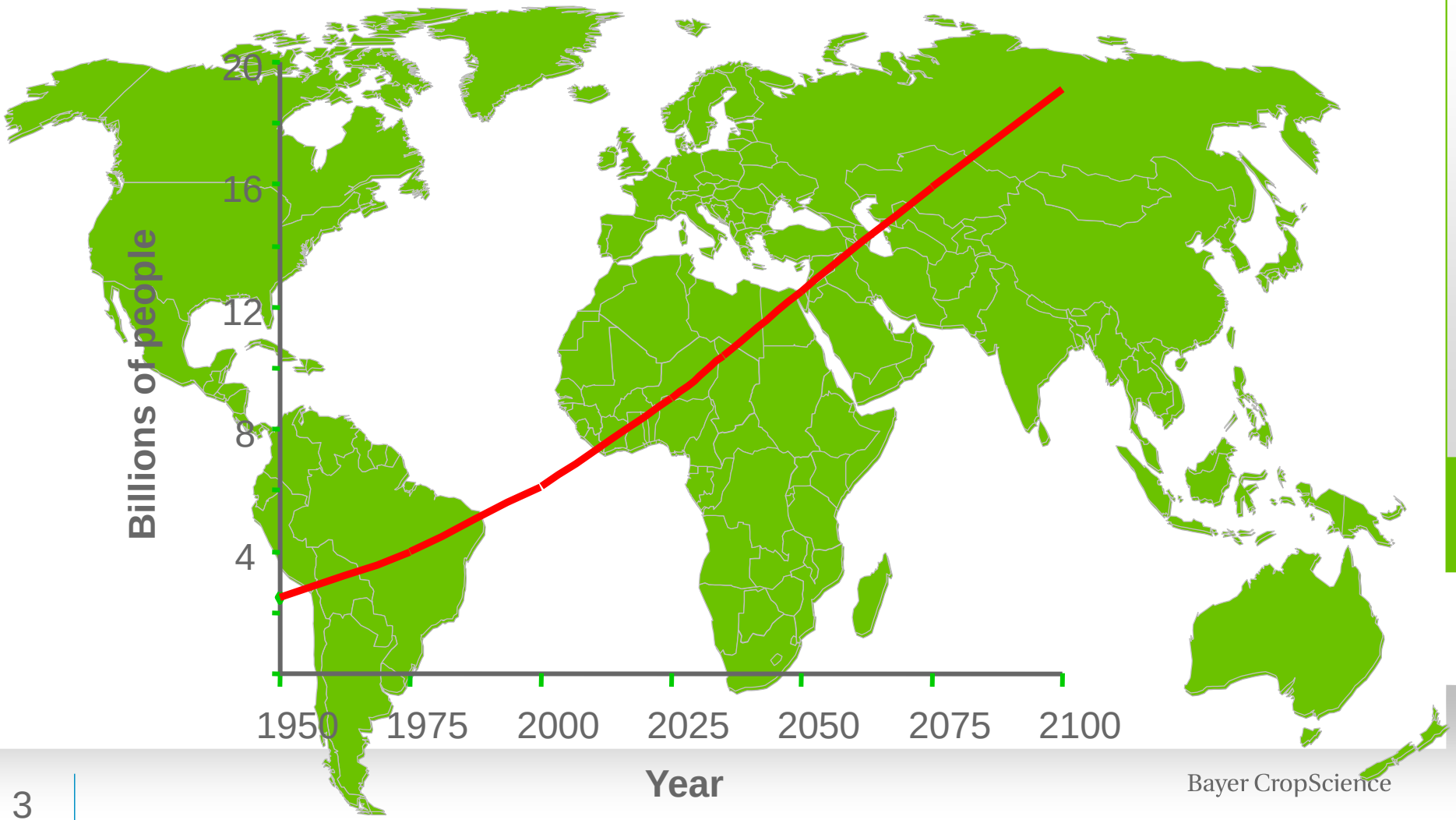
NAICC January 29, 2016

Agenda/
Content

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

World Population Projections



The Challenge:

Feed More People With Current Resources

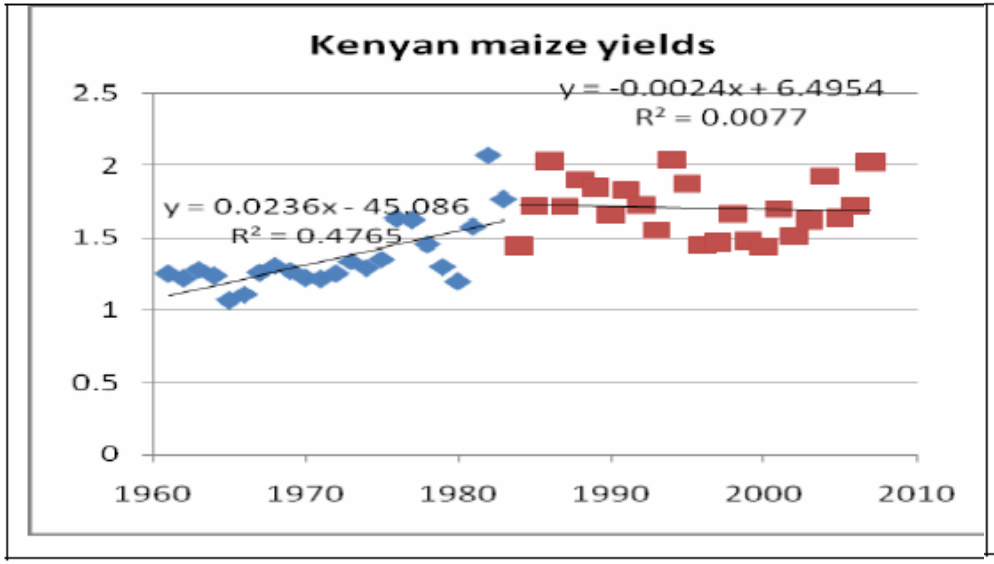
Global population rising

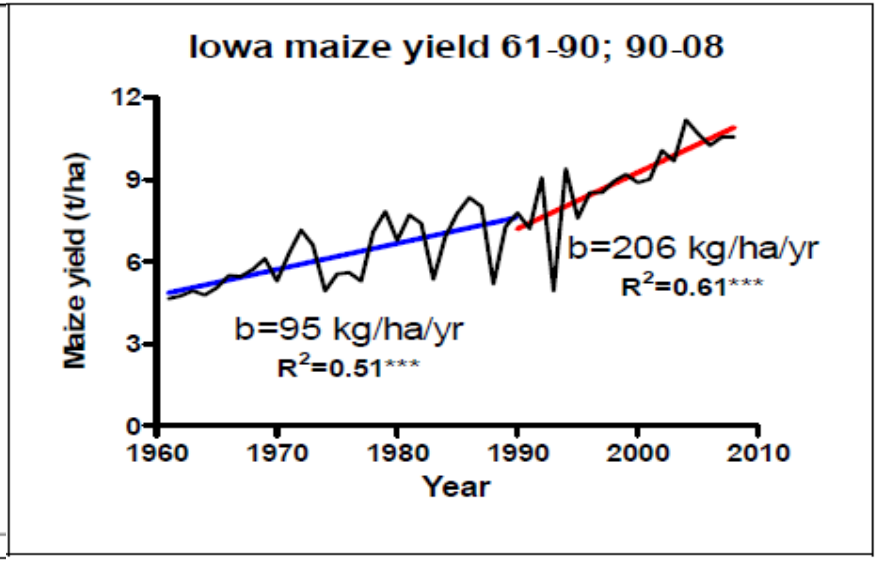
Arable Land Decreasing

Technology must provide solutions

Maize Yields Over Time

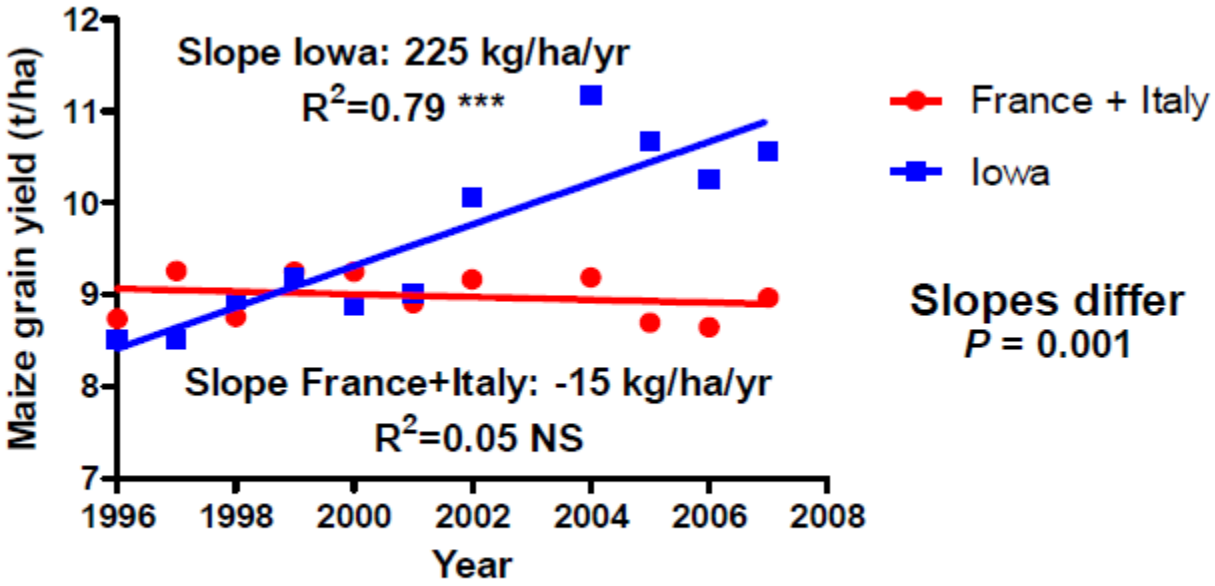
Minimum inputs vs. Best technologies





http: //www.nass.usda.gov/QuickStats/PullData US.jsp.

Maize yields 1996 – 2007 Iowa vs. France + Italy

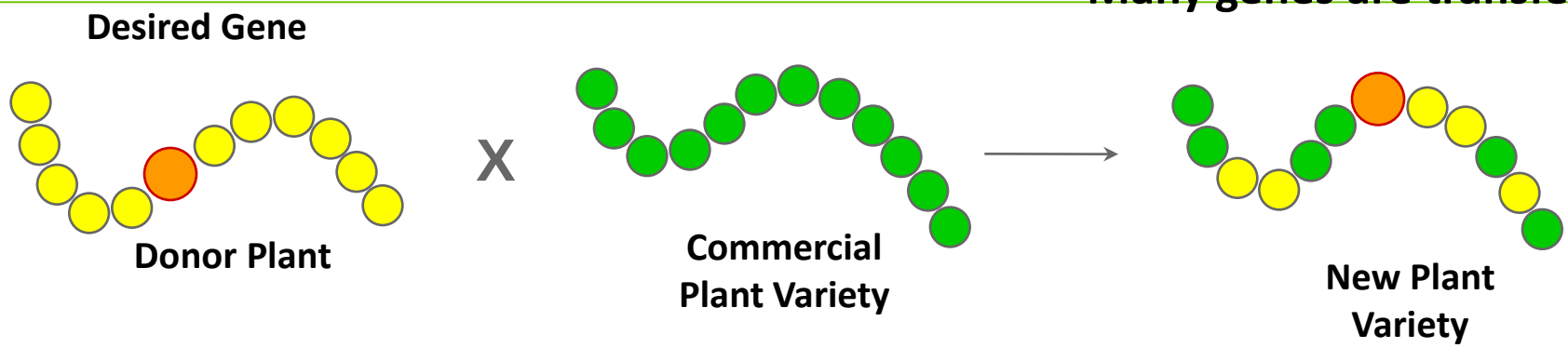


(Source: USDA and FAOSTAT, 2009)

Biotechnology is an Extension of Traditional Plant Breeding

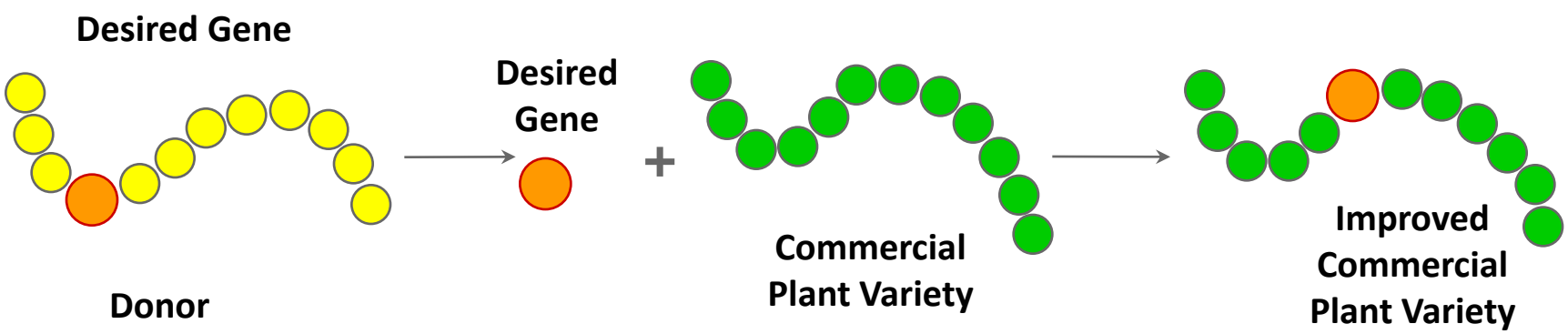
TRADITIONAL PLANT BREEDING

Many genes are transferred



PLANT BIOTECHNOLOGY

A single gene is transferred



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

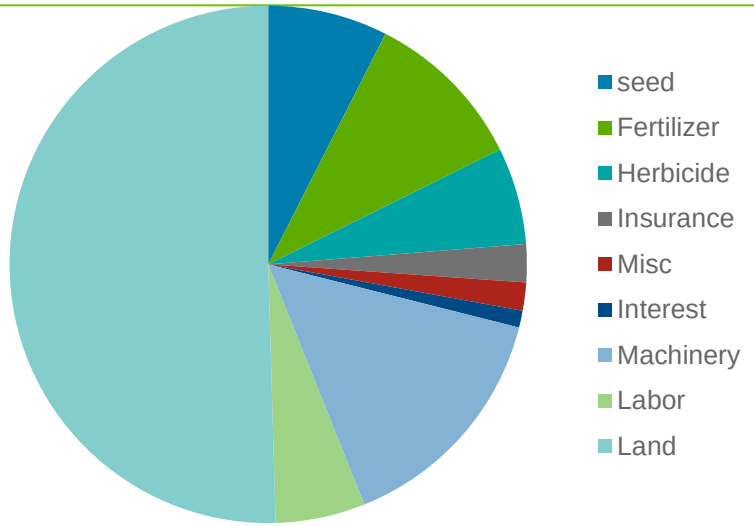
Balancing Act

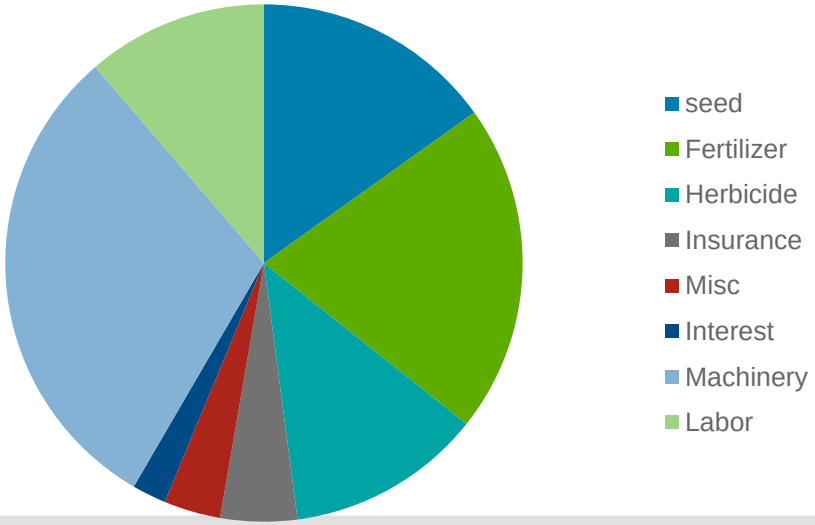
- What do the growers want/need?
- What attributes can this science deliver
- Can value be captured?
- Consumer desires?

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

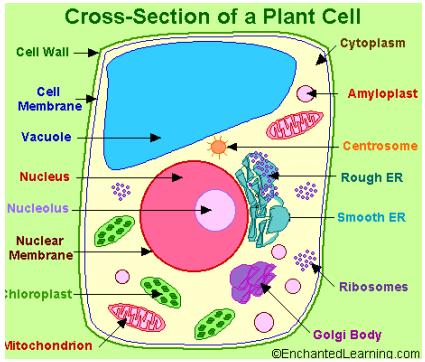
Single Gene → Phenotype

- Herbicide tolerance:
  - Modified mode of action (i.e., CP4 EPSPS)
  - Herbicide detoxifier (i.e., PAT)
- Insect Resistance:
  - Cry proteins
  - Vip proteins
- Disease resistance

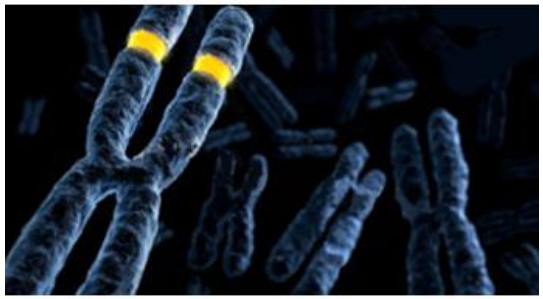
More Complex Traits

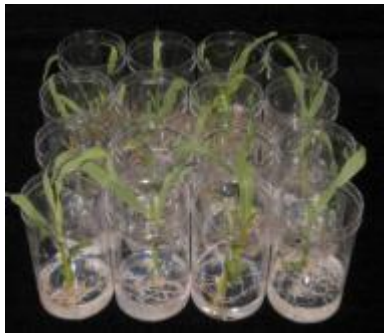
- Metabolic pathways
- Yield enhancement
- Nutrient enhancement

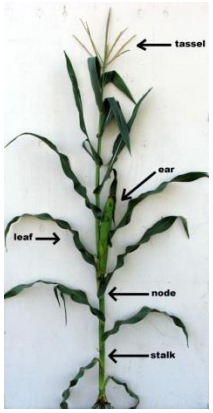
Critical Steps in Transformation



- 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

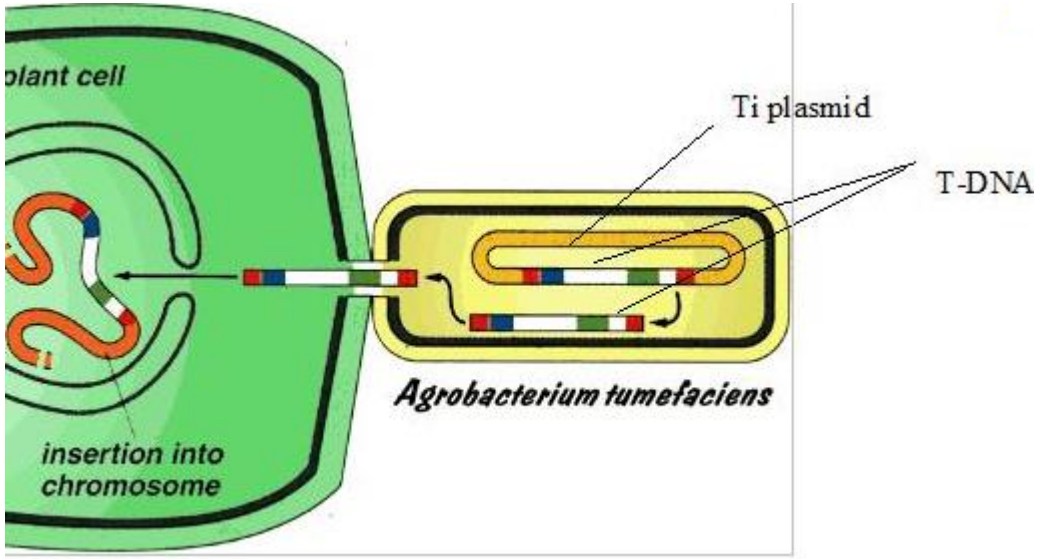






Trait Introduction: Biolistics vs. Agro transformation

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

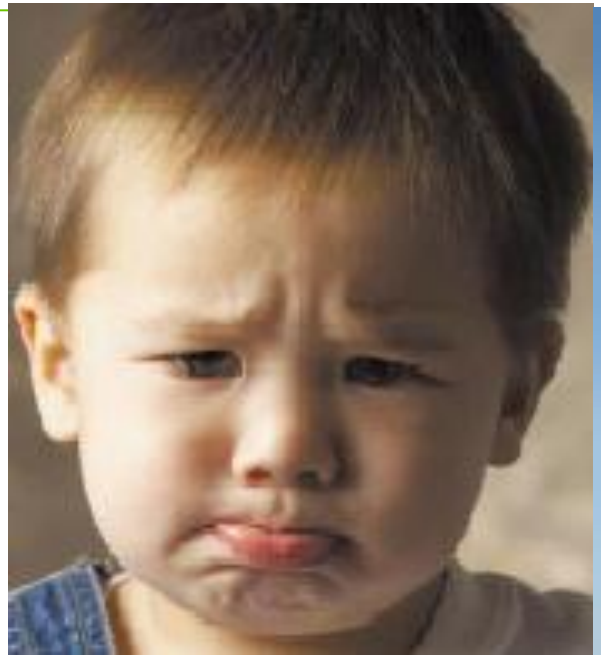


PSD-1000/He Particle Delivery System

DOES IT WORK EVERYTIME?

NO!!!!

Why Not?



WHAT CAN GO WRONG?

Idea is bad

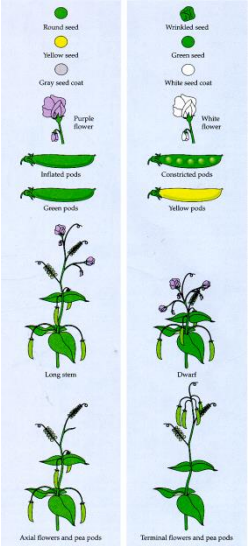
Trait is complex

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

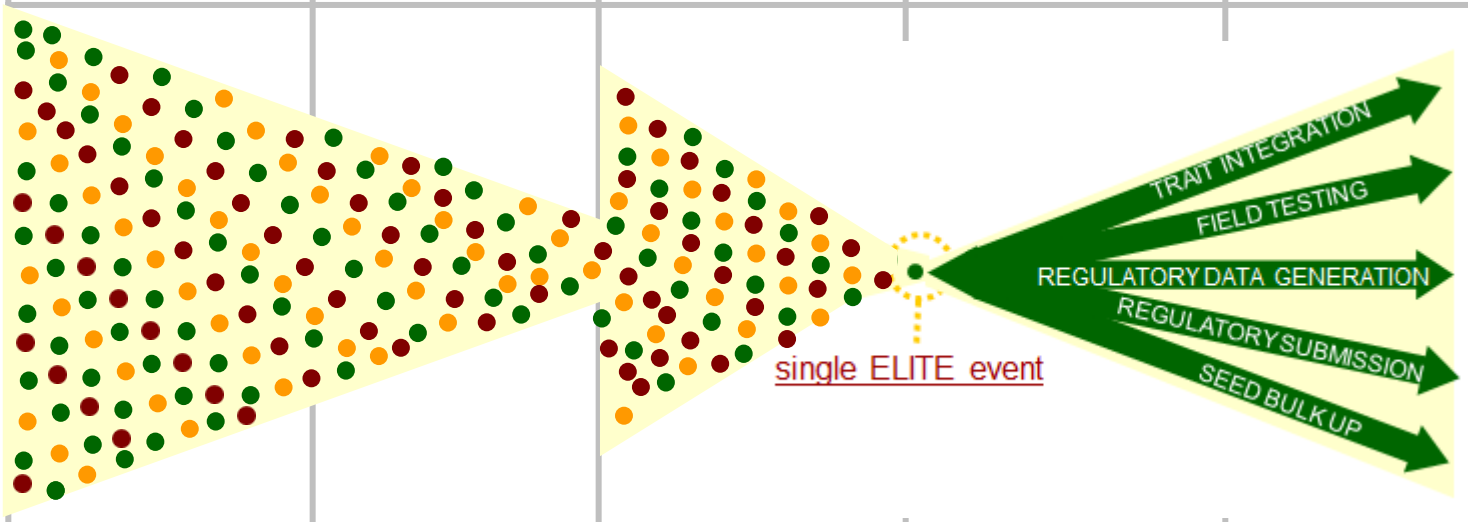
Many ideas to get the RIGHT one

Many cells transformed to get multiple events

Many events evaluated to get THE BEST One

http:www.croplife.org/PhillipsMcDougall Study

	DISCOVERY Gene/Trait Identification	PHASE I Proof Of Concept	PHASE II Early Development	PHASE III Advanced Development	PHASE IV Pre-launch
AVERAGE DURATION[1]	53.9 MONTHS	27 MONTHS	30 MONTHS	37.2 MONTHS	48.8 MONTHS
Mean units Tested	10209	511	1302	2	1
Regulatory Activities	*In silico* screening	Preliminary Digestive Fate	Molecular Characterization	Full Regulatory Study Package	Data Defense



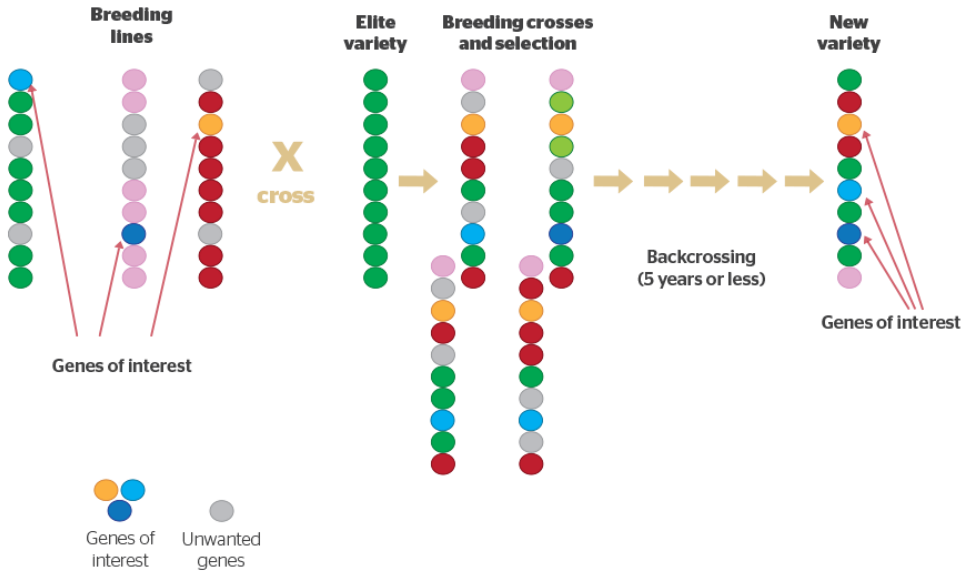
Many Ideas Don't Make it Past the Discovery Stage – Regulators never see

- X 10 – 15% don't pass allergen/toxin homology screen
- X May not be expressed in planta
- X No efficacy
- X 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

Once EVENT identified – Safety assessment conducted.

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

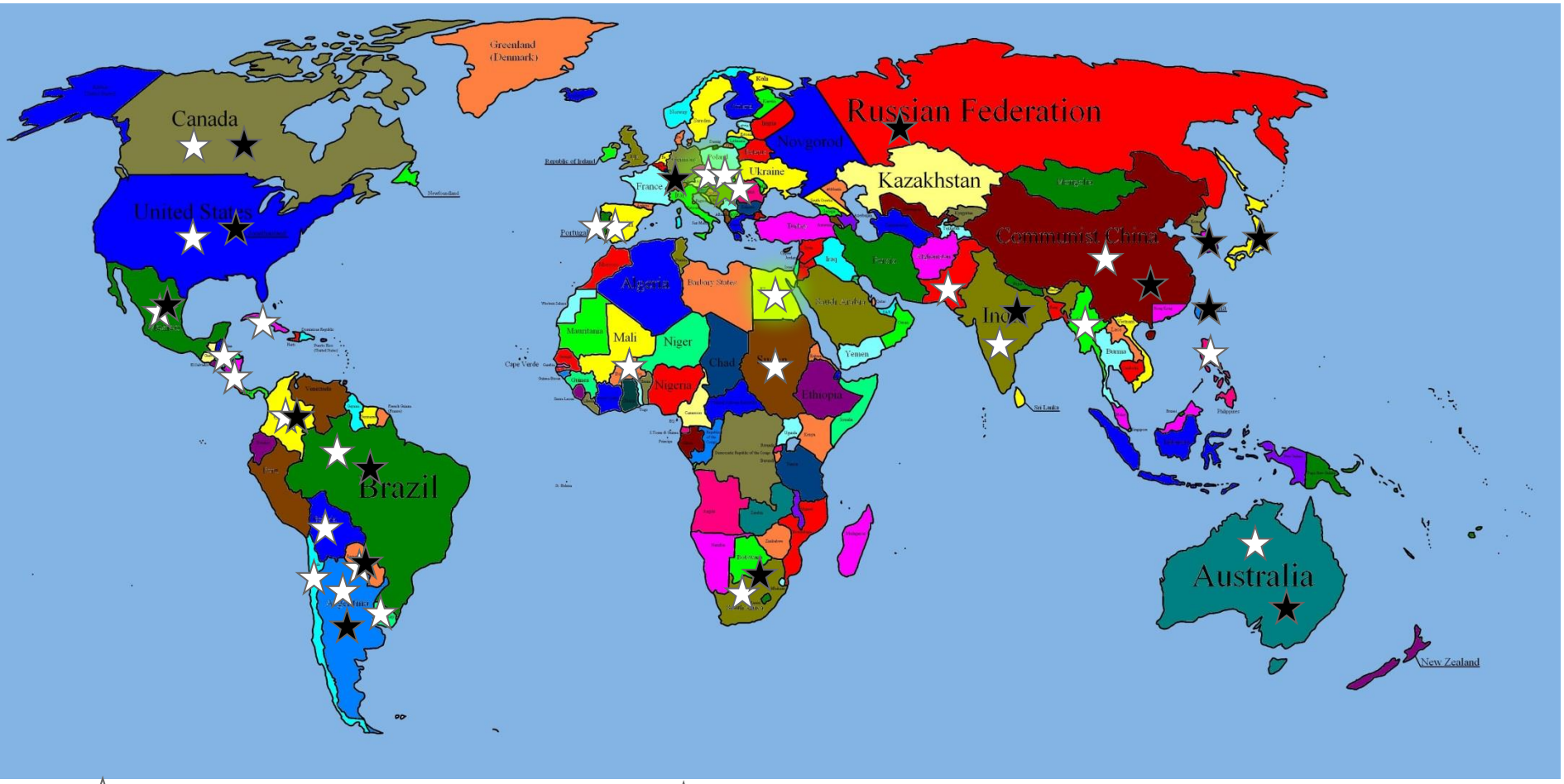
Crop Safety

Gene / Protein

Food / Feed and Environmental Safety

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

Most Studied Food – Safety Reviewed by Agencies all Around the World



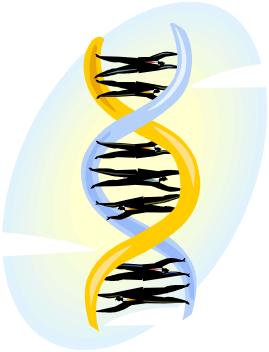
☆ Production Countries (28) ★ Importing countries

Regulatory Studies are grouped in four categories:

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

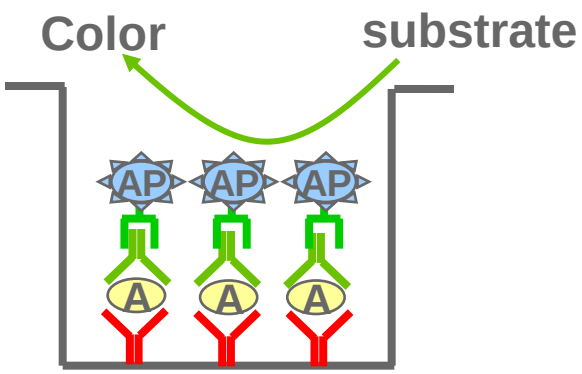
Molecular Characterization

- **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

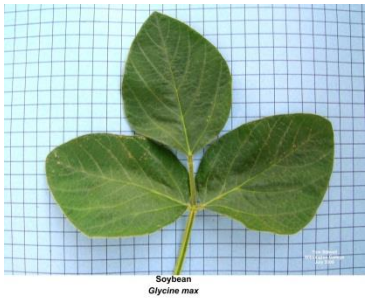
- **Gram quantity protein production and purification**
- **Establishing protein level in plant tissues**
- **Protein characterization and equivalence**
- **Allergenicity assessment**
- **Toxicity assessment**

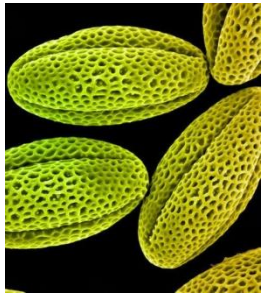


Protein expression levels: Tissues and Stages Considered

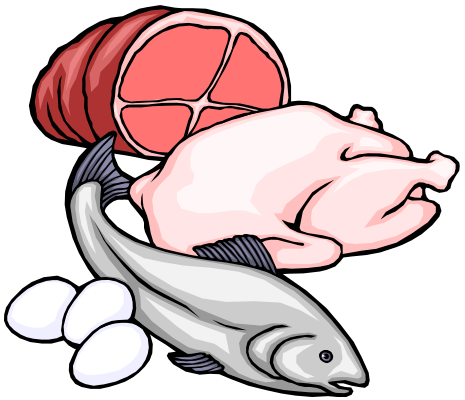
Crop and Trait Dependent

- 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

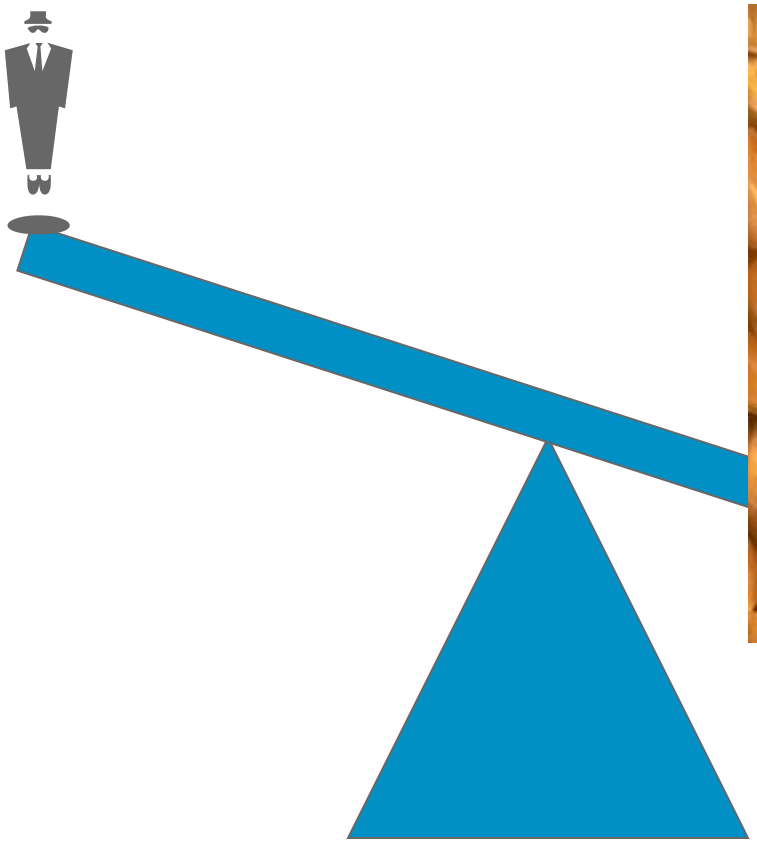
- Acute toxicity testing with recombinant transgenic proteins
  14 day mouse study – single dose 2000 mg/kg body weight

- 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**

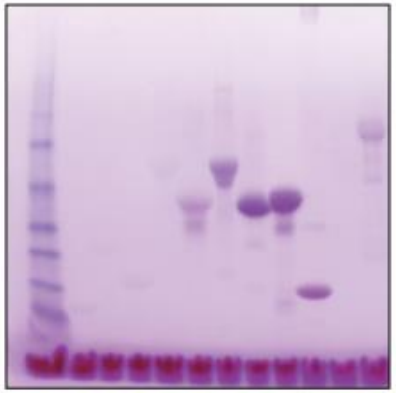


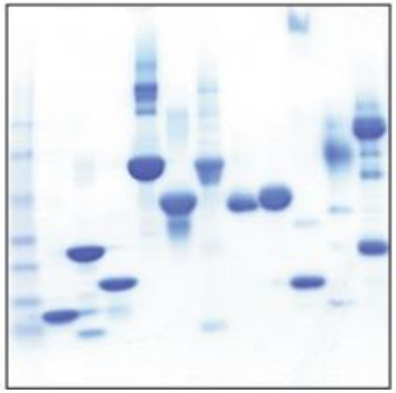
of corn for lunch.

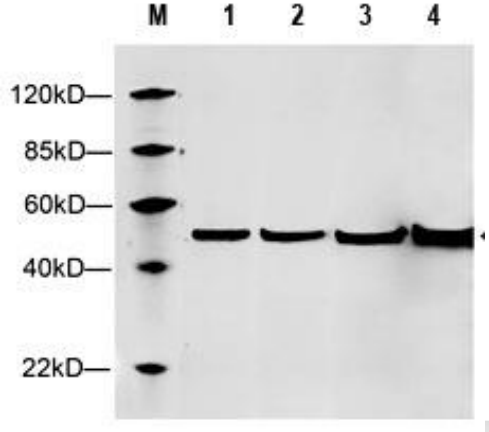
Bridging Study to Show Equivalence

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)**





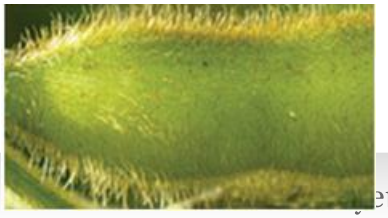


Agronomic and Compositional Equivalence

- **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

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

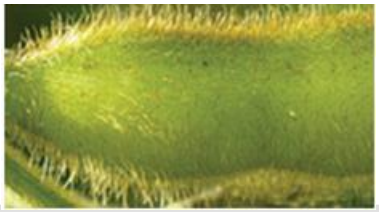


Analytes

<u>Corn</u>
Proximates
Fatty Acids
Minerals
Vitamins
Amino acids
Ferulic acid
Coumaric acid
Phytate
Xanthophylls
Starch

<u>Cotton</u>
Proximates
Fiber
Fatty Acids
Minerals
Vitamins
Amino Acids
Gossypol
Phytate
Malvalic Acid
Sterculic Acid

<u>Soybean</u>
Proximates
Fatty Acids
Minerals
Vitamins
Amino acids
Lectins
Isoflavones
Lecithins
Phytate
Trypsin inhibitor
Urease activity
ADF & DNF
Raffinose & Stachyose



Typical Processed Fractions

Cotton

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

Canola

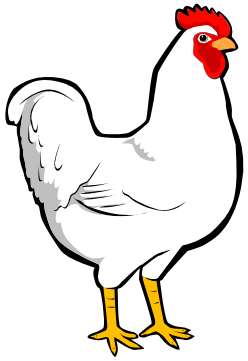
- Seeds
- Presscake
- Toasted Meal
- Solvent extracted meal
- Crude oil
- RBD oil

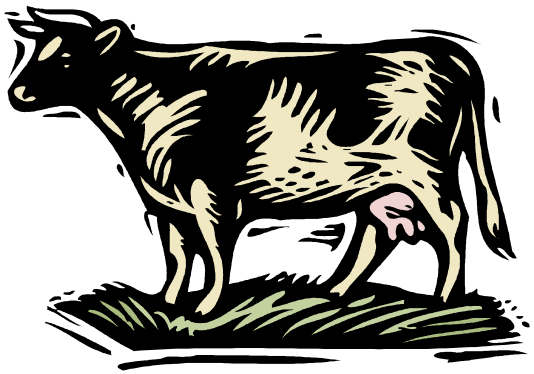
Soybean

- 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





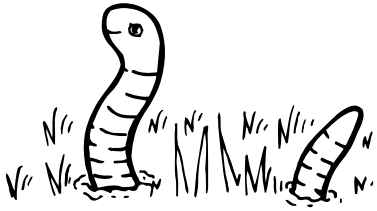
Environmental Safety

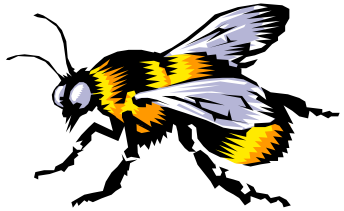
- **Environmental safety and fate**
- **Interactions with target and nontarget organisms**
- **Interactions with the abiotic environment and soil**



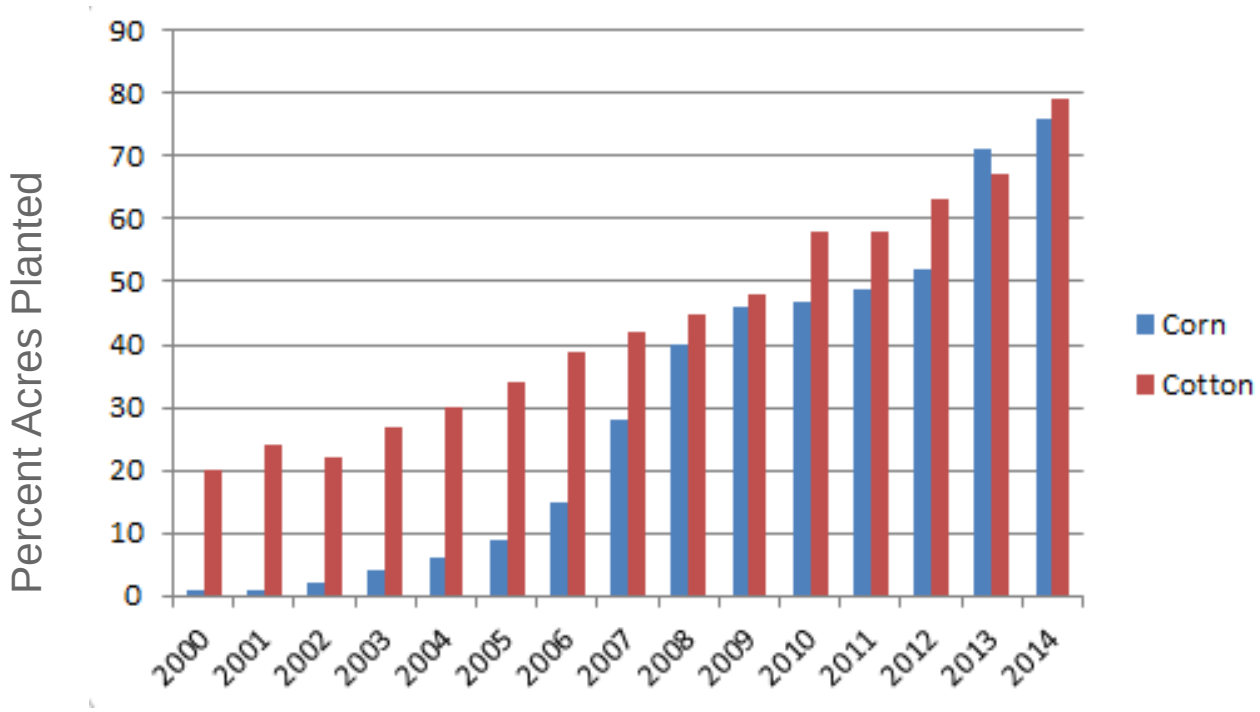
For Pesiticidal Plants Ecotoxicological Studies:

- **Collembola**
- **Honeybee**
- **Fish**
- **Daphnia**
- **Avian**
- **Ladybeetle**
- **Soil Stability**
- **Earthworm**
- **Lacewing**
- **Resistance management**





US Stack Percentage



http://www.ers.usda.gov/data-products/adoption-of-genetically-engineered-crops-in-the-us.aspx

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

STEWARDSHIP

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

*Approved in 2014: potato and alfalfa in the U.S., sugarcane in Indonesia, and maize in Vietnam. Approved prior to 2014, pending planting in 2016: virus resistant dry beans and HT soybean in Brazil.

The International Service for the Acquisition of Agri-biotech Applications (ISAAA) is a not-for-profit organization with an international network of centers 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.

ISAAA
INTERNATIONAL SERVICE FOR THE ACQUISITION OF AGRI-BIOTECH APPLICATIONS

Very Useful Websites

- http://www.cera-gmc.org/
  - Database on all approved biotech events
- http://www.foodstandards.gov.au/consumerinformation/gmfoods/gmcurrentapplication1030.cfm
  - 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

Science For A Better Life

Thank you!