Optimizing Gene Function in Plants

Linda Castle, Research Coordinator
Pioneer Hi-Bred International, Inc.

March 21, 2007
European Food Safety Authority
Parma, Italy

PIONEER.
A DuPont Company

DUPONT
The miracles of science™

Gene shuffling

A recombinant DNA technology that:

- Mimics the classical plant breeding process
- Elicits desirable phenotypic changes in months *vs.* years
- Rapidly generates high-quality genetic diversity
- Can use many parent genes at once
- Allows for evaluation of millions of progeny in each generation
- Is used with genetic engineering to introduce improved traits

Invented by Pim Stemmer in early 1990s:

- Maxygen, Inc. developed technology
- DuPont, including its wholly-owned subsidiary, Pioneer Hi-Bred International, Inc., has sole access for agricultural traits
- Used to develop glyphosate tolerance gene of Optimum™ GAT™ trait
- Currently in use in multiple traits including insect resistance, disease resistance, yield improvement, and nutritional traits

Natural genetic diversity makes every individual unique

Trait selection is gene variant selection



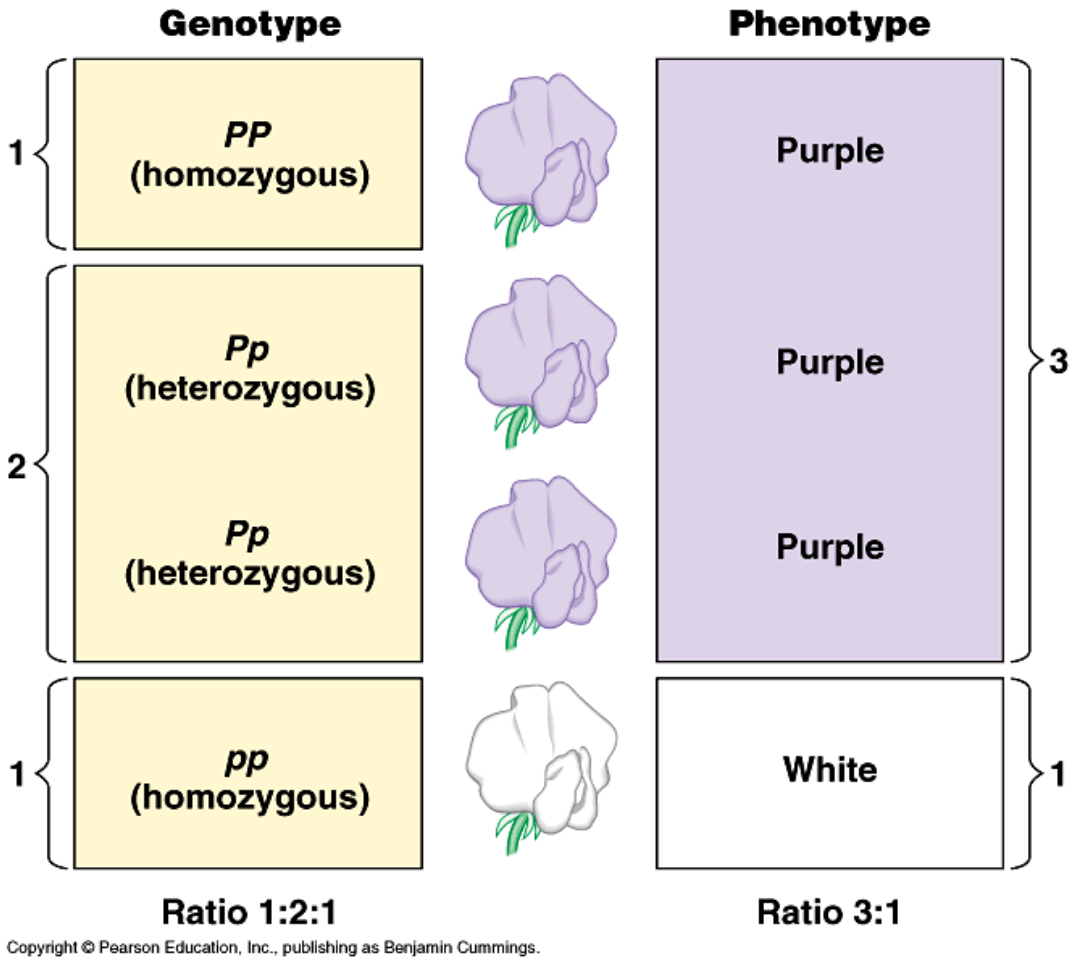








Genes are the blueprints for traits



- P gene variant function is to make purple pigment
- p gene variant fails to make pigment
- The same gene can have many variants (alleles)
- What would P1 or p2 do?

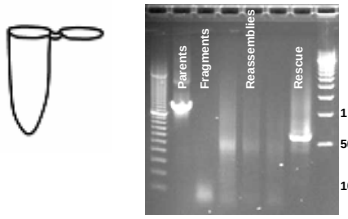
Gene shuffling brings long breeding cycles into the laboratory to speed them up

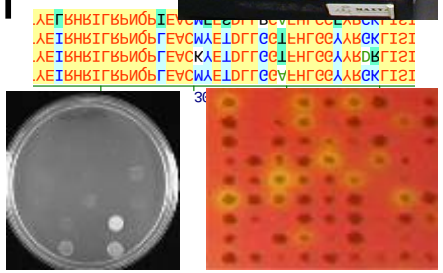
Parent selection

Breeding

Progeny selection

- Parent gene(s) selection
- Cloning
- Shuffling
- Library construction
- Quality check
- Gene expression
- Assay 10,000s for improvement

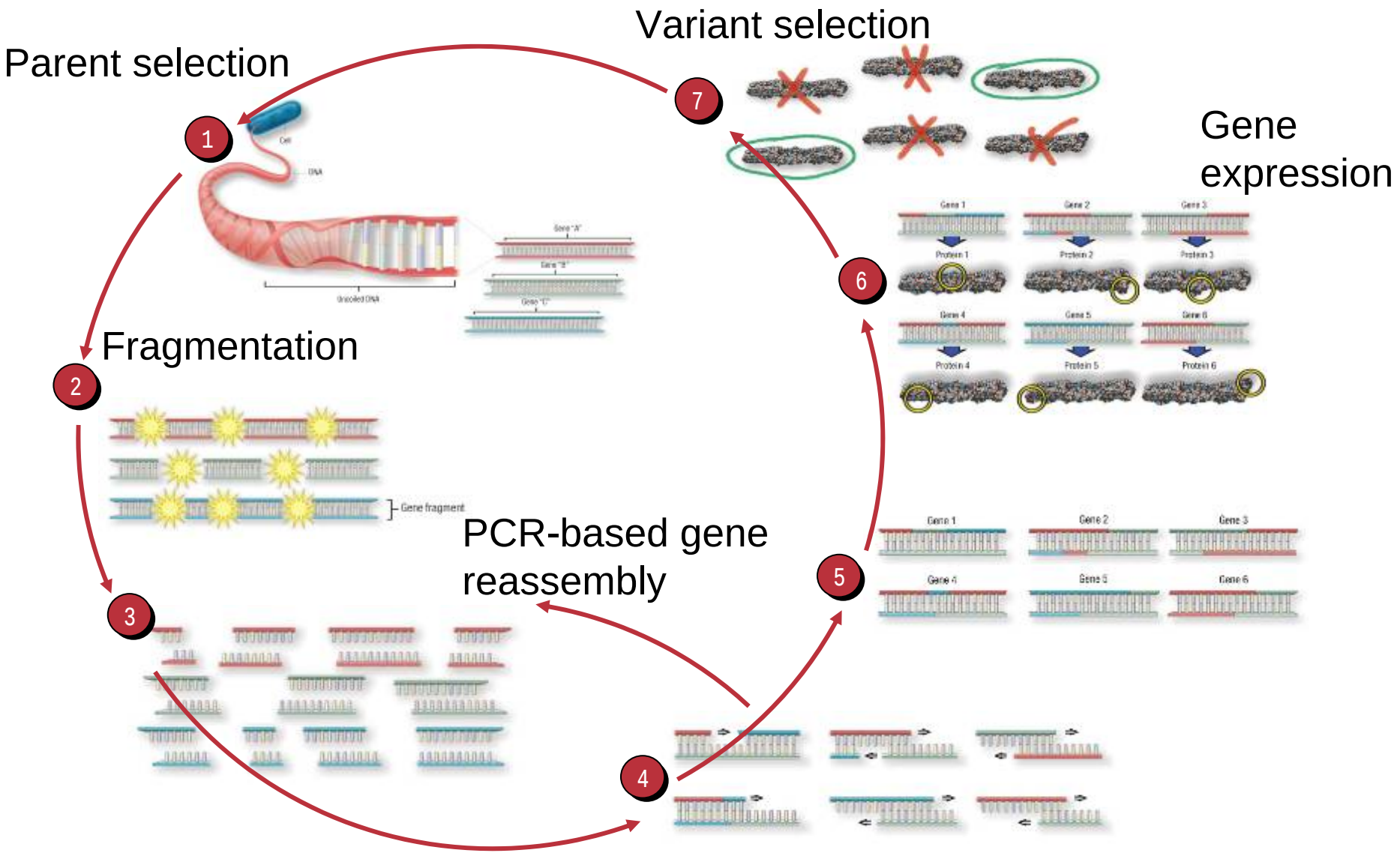




1-3 months

Introduce best gene into crop plants

Gene shuffling creates improved gene variants



Endogenous and novel traits can be improved

Endogenous trait examples

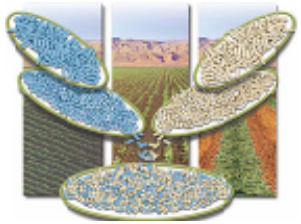
- Disease resistance
- Plant height
- Stalk strength
- Root architecture
- Oil composition
- Herbicide tolerance
- Nutritional composition

Novel trait examples

- Insect resistance
- Herbicide tolerance
- Nutritional composition

Development of an industry leading dual herbicide tolerance trait for soy and corn

- Glyphosate tolerance
  - Glyphosate is the number one selling agricultural herbicide.
  - Tolerance to glyphosate is a highly desirable trait in agriculture.
  - Current glyphosate tolerance trait relies on a target transgene coding for an enzyme that is not inhibited by glyphosate.
  - Pioneer strategy is to directly inactivate the glyphosate herbicide molecule within the plant.
- ALS-inhibitor tolerance
  - ALS inhibitors have been used as herbicides for the last 25 years
  - Sulfonylureas (SUs) are one family of ALS inhibitor herbicides
  - DuPont is the leader in SU chemistry
  - HRA = “Highly resistant allele”, confers tolerance to ALS herbicides
  - HRA is the endogenous ALS from corn and soybean with two specific point mutations
  - SU products can be custom blended to meet grower needs
- Optimum™ GAT™ Trait
  - Molecular stack of *gat* and *hra* genes
  - Provides tolerance to two modes of action for weed management



Weed management issues are changing

"…it is not practical to expect growers to abandon the Roundup Ready technology. Hence, with continuous planting of Roundup Ready crops, the only practical resistance management strategy is to integrate other chemistry into the system."

From ***Southeast Farm Press***, an interview with three prominent weed scientists:

Alan York, North Carolina State University;

Stanley Culpepper, University of Georgia;

John Wilcut, North Carolina State University.

http://southeastfarmpress.com/news/060305-Glyphosate-resistance/

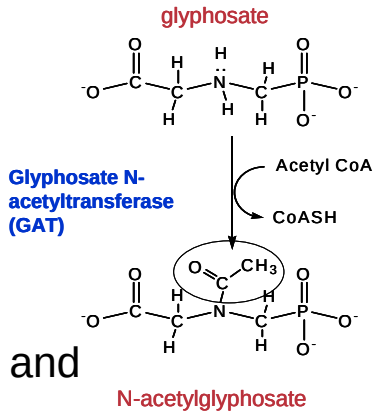
PIONEER. A DuPont Company

DUPONT The miracles of science™

Glyphosate N-acetyltransferase discovered in a bacteria

Activity found in *Bacillus licheniformis*

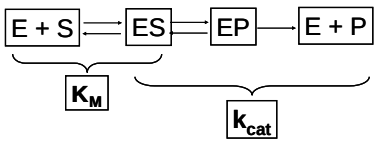
- Spore forming saprophytic bacteria
- Ubiquitous in soil
- Rich in metabolizing enzymes
- Industrial fermentation of GRAS proteases, amylases, antibiotics, and specialty chemicals for over a decade



Glyphosate N-acetyltransferase is a member of the N-acetyltransferase super-family

- Present in all organisms
- Diverse functions
- High sequence diversity
- Similar structures

Native glyphosate N-acetyltransferase enzymes have weak activity on glyphosate

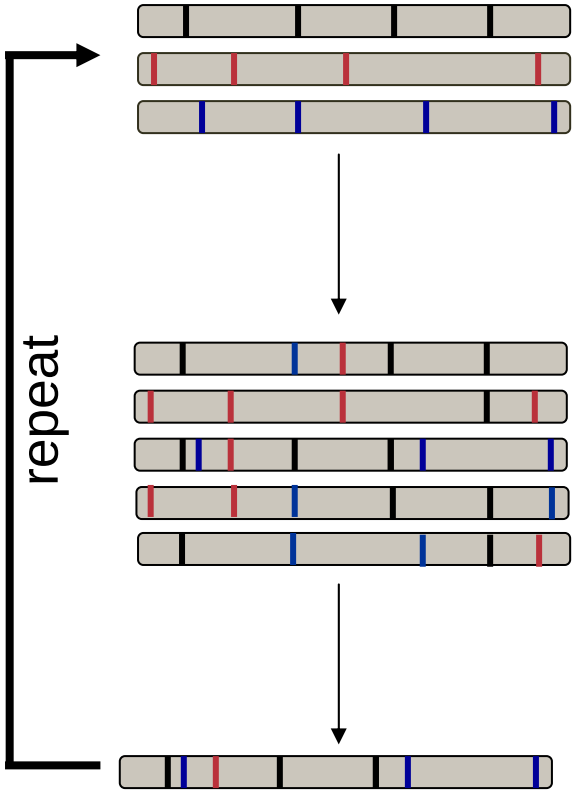


- Low turn-over rate
- Low affinity for glyphosate
- Native genes are inadequate and not sufficient to confer glyphosate tolerance in bacteria or plants

PIONEER. A DuPont Company

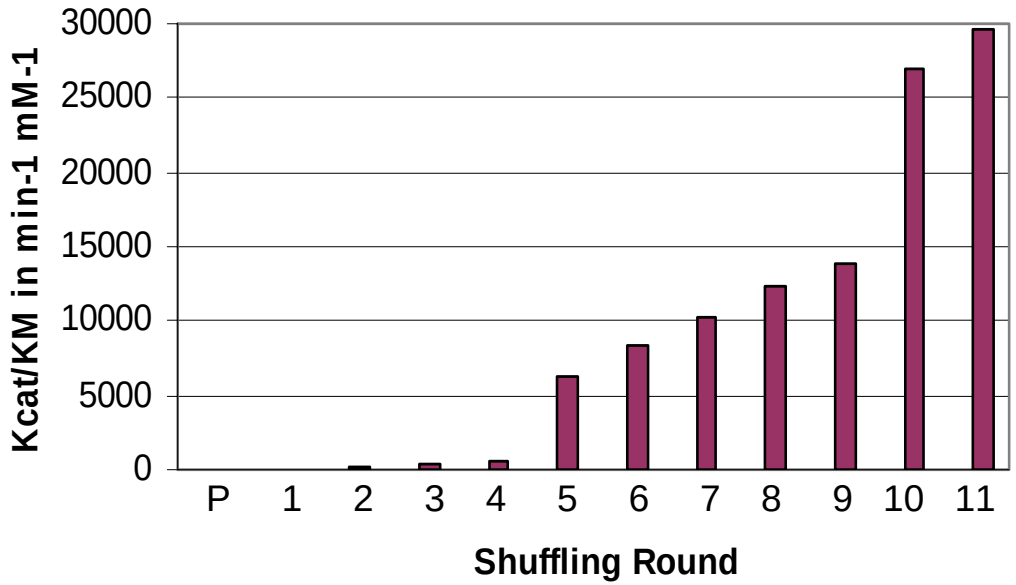
DUPONT The miracles of science™

Parent genes have natural diversity and high potential



Parent genes

Shuffled recombinant progeny



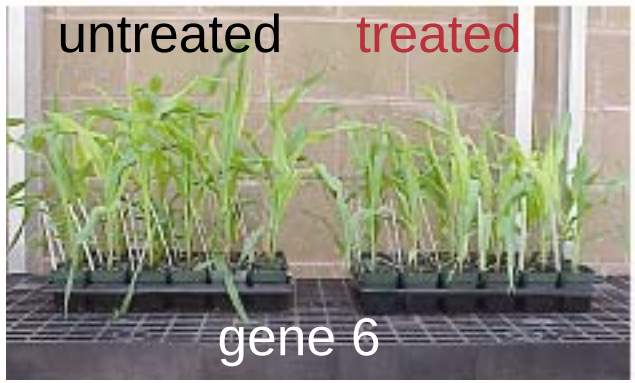
Individuals assayed for greater activity on glyphosate by product accumulation and enzyme kinetics

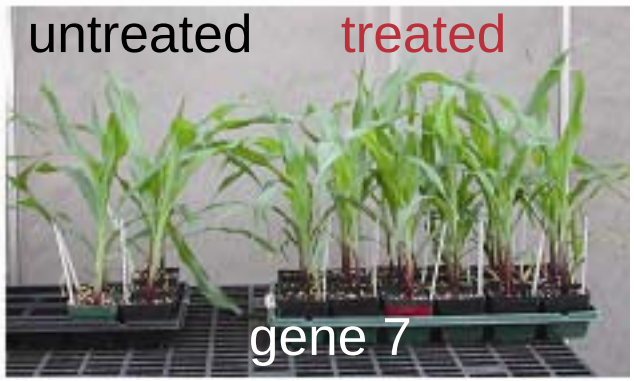
Glyphosate resistance in plants improved by shuffling

Glyphosate damage

Glyphosate affect

Glyphosate affect





No difference between glyphosate treated and untreated

14 Days after spray application of glyphosate and an ALS-inhibitor herbicide on soy and corn

Soybean

conventional soybean

Soybeans with the Optimum™ GAT™ Trait

Corn

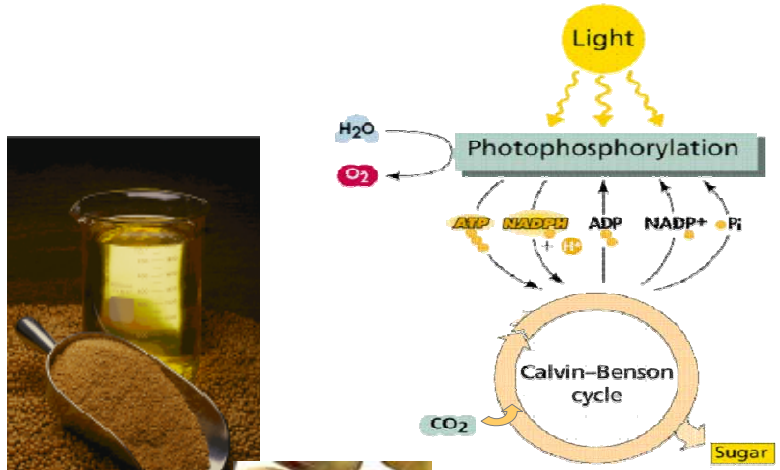
Corn with the Optimum™ GAT™ trait

conventional corn

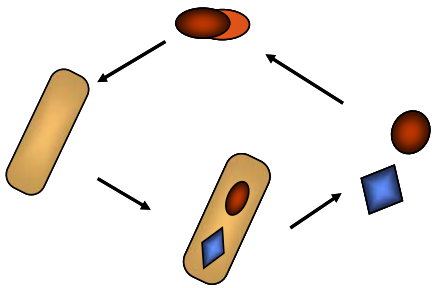
Combining ALS-inhibitor and glyphosate herbicide tolerances in soybean and corn will give growers flexibility in their weed management program.

Potential future shuffled-gene traits

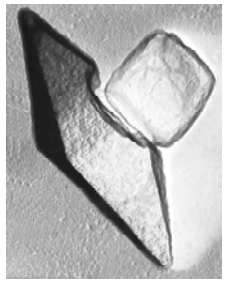
- Insect resistance
- Nematode resistance
- Disease resistance
- Increased yield
- Nitrogen utilization
- Nutritional composition
- Oil composition



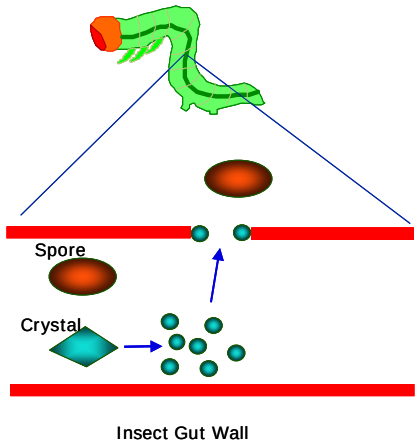
Insecticidal proteins can be improved by shuffling



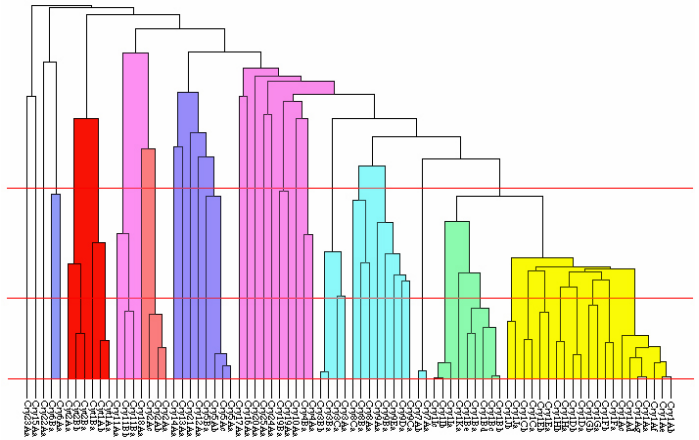
Bacteria produce spores and protein crystals.

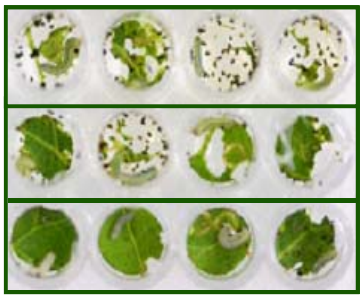


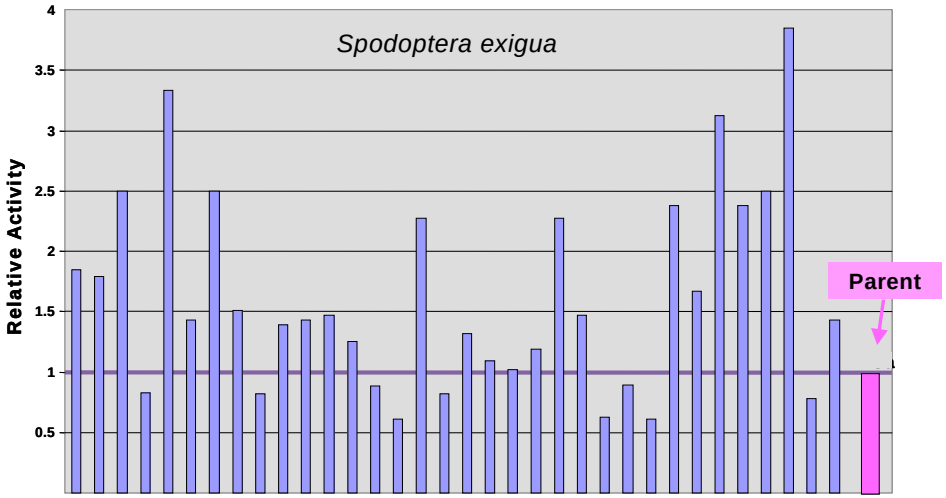
Sharpe, E. S. and F. L. Baker (1979) J. Invertebr. Pathol. **34**: 320-322



Crystals dissolve and the insecticidal proteins bind the gut wall making pores and allowing infection of the bacterial spore.







Helicoverpa zea

Spodoptera exigua

PIONEER. A DuPont Company

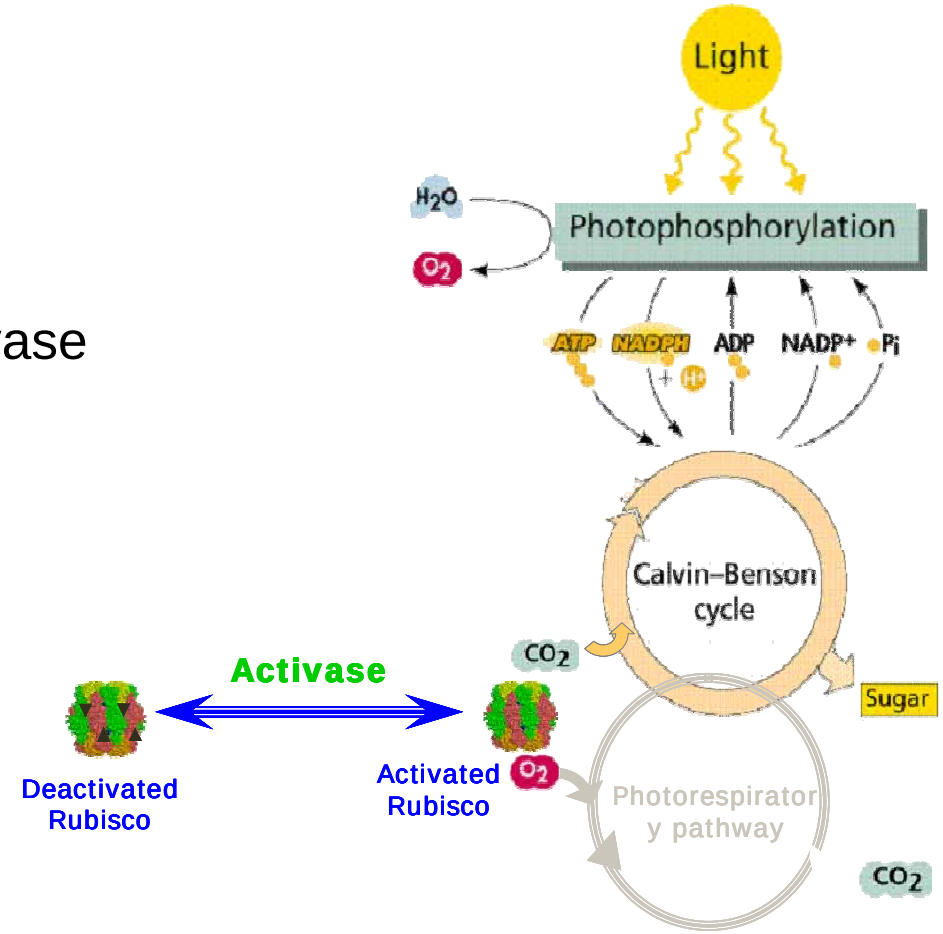
DUPONT The miracles of science™

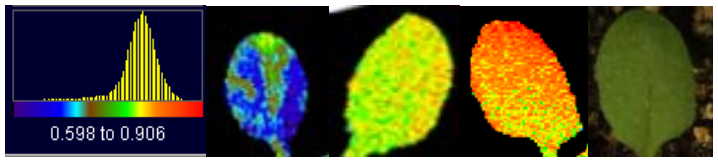
Shuffling improves photosynthesis

- Increase carboxylase activity
- Decrease oxygenase activity
- Increase heat tolerance of activase

WT Round 1 Round 2

Arabidopsis plants subjected to 4 hours heat/day





PIONEER. A DuPont Company

DUPONT The miracles of science™

Summary

Gene shuffling:

- Uses natural genetic diversity
- Mimics classical breeding
- Enables trait improvement and novel trait development
- Is a proprietary Pioneer technology
- Is being implemented in several key trait areas

Optimum™ GAT™ Trait:

- Will provide growers with more choices
- Provides robust herbicide tolerance

The miracles of science™