A New Glyphosate Resistant Soybean

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Steven R. Paszkiewicz, Ph. D.
Research Scientist
Pioneer Hi-Bred International, Inc.
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Outline

- Herbicide tolerant crops
- Glyphosate N-acetyltransferase (GAT) development
- Introduction to HRA and ALS-inhibitor herbicides
- Combining GAT/HRA with Blends Technology
- Projected product timeline

Herbicide tolerance dominates the transgenic market

% transgenic of total acres in 2004

	global	US	Canada
Soybean	56%	85%	48%*
Corn	14%	45%	58%*
Cotton	28%	76%	-
Canola	19%	84%*	77%

* 2003

% of global transgenic acres by trait

Herbicide tolerant Soybean	60%
Bt Corn	14%
Herbicide tolerant Canola	5%
Bt/herbicide tolerant Corn	5%
Herbicide tolerant Corn	4%
Bt Cotton	5%
Bt/herbicide tolerant Cotton	4%
Herbicide tolerant Cotton	2%

% 2003 transgenic glyphosate tolerant acres	global	US	Canada
Soybean	99%	99%	
Corn	32%	31%	
Cotton	56%	97%	-
Canola			47%
Total	72%		

Sources: Clive James, ISAAA Brief No. 32 – 2004; Agrow, No. 452, 2004; www.ers.usda.gov

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Glyphosate - Herbicide tolerance

- Glyphosate operates by inhibiting the enzyme enolpyruvyl-shikimate-3-phosphate synthase (EPSPS) that leads to the biosynthesis of essential aromatic amino acids.
- The inhibition does not allow plants to survive.
- Researchers have found some microbial EPSPS enzyme variants that are not inhibited by glyphosate.
- Plants that contain these enzyme variants can survive in the presence of high concentrations of the glyphosate herbicide. Makes minor modifications to the molecular structure of EPSPS enzymes – glyphosate will not bind.
- However, glyphosate remains in the plant.

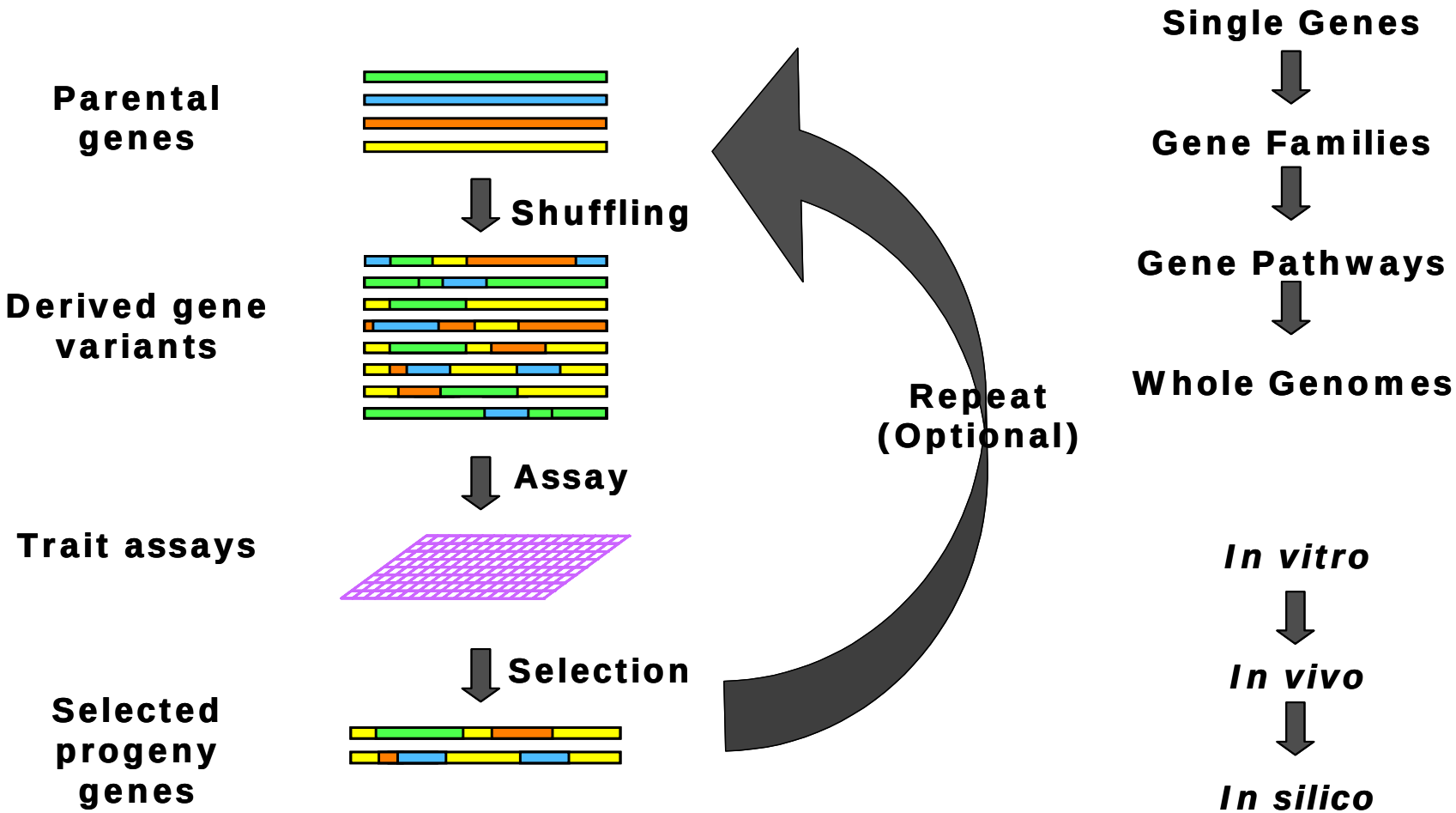
Glyphosate - Herbicide tolerance

- Researchers from Pioneer Hi-Bred, International, Inc. and Verdia Inc. in Redwood City searched for a method to detoxify glyphosate.
- The advantage of detoxification is that the glyphosate is transformed into a substance that does not harm the plant.
- Glyphosate N-acetyltransferase (GAT) enzymes converts glyphosate to N-acetylglyphosate, which is no longer herbicidal or of toxicological relevance.

Source of Glyphosate N-acetyltransferase (GAT) enzyme

- GAT found in Bacillus licheniformis (ATCC 14580)
  - Spore forming saprophytic bacteria
  - Ubiquitous in soil
  - Rich in metabolizing enzymes
- GAT is a member of the N-acetyltransferase super-family
  - Present in all organisms
  - Diverse functions
  - High sequence diversity
  - Similar structures
- Native GAT enzymes have weak activity on glyphosate
  - Low turn-over rate
  - Low affinity for glyphosate
  - Do not confer glyphosate tolerance in bacteria or plants

Gene Shuffling



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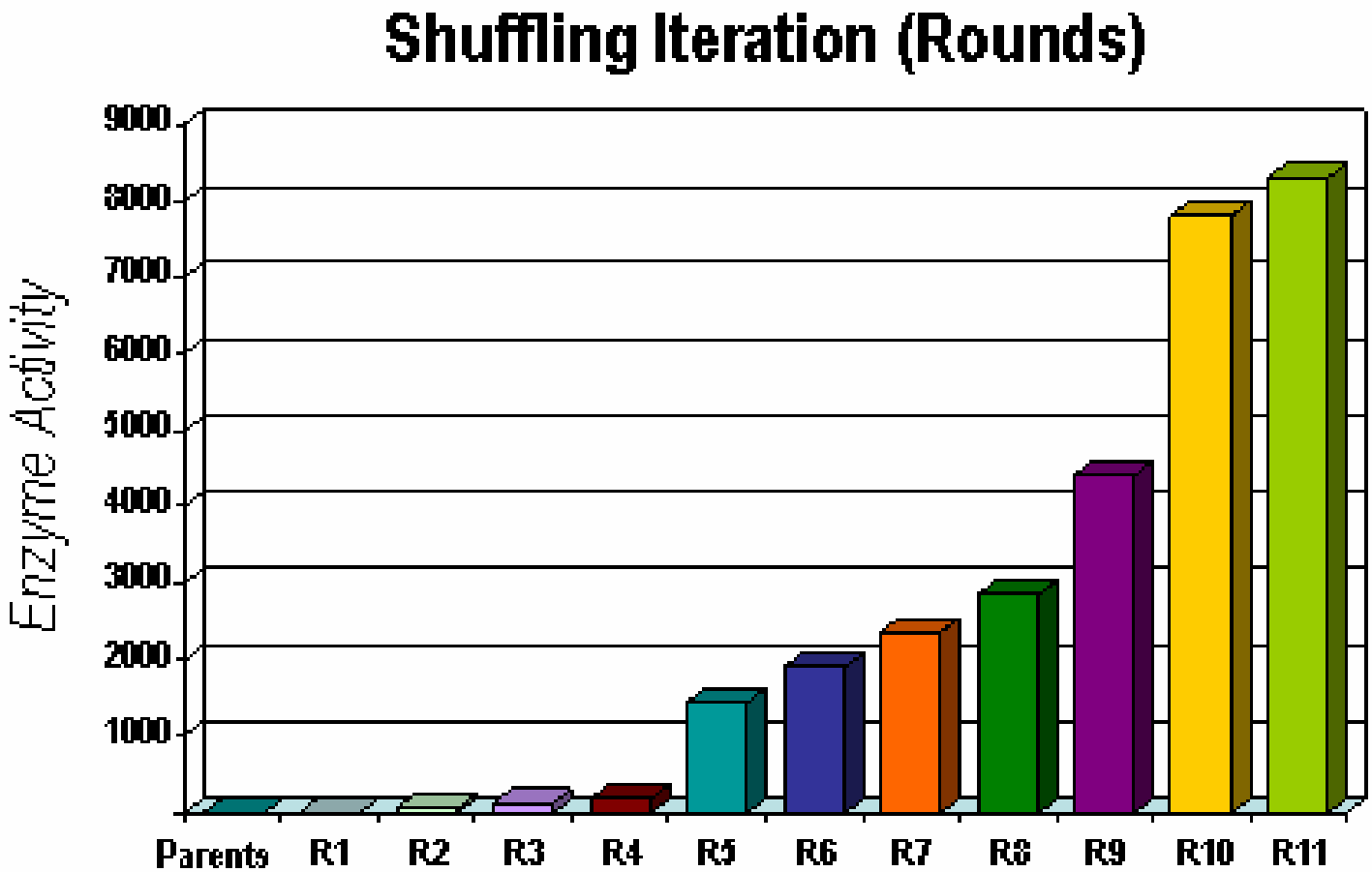
Gene Shuffling



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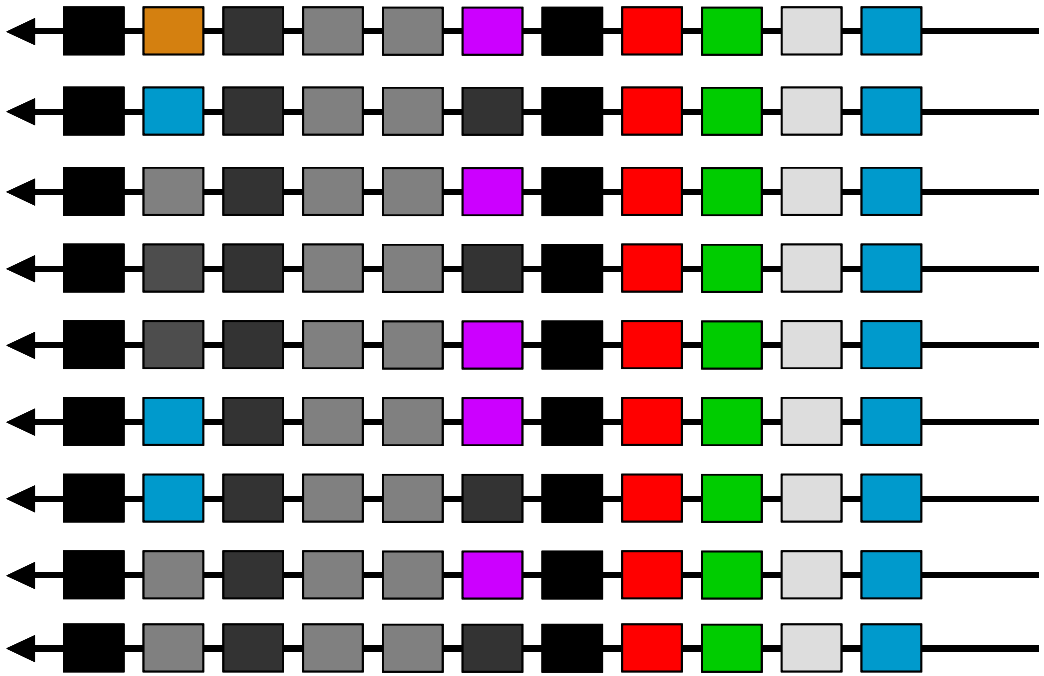
Gene Shuffling

Diversity of Shuffled Progeny Proteins



High Throughput Screen for best functionality

Best Variant

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ALS-inhibitor herbicides and HRA

- Acetolactate synthase, or ALS, is a key enzyme in amino acid biosynthesis
- ALS inhibitors have been used as herbicides for the last 25 years.
- Sulfonylureas (SU's) are one family of ALS inhibitor herbicides.
- HRA = "Highly resistant allele", confers resistance to ALS herbicides
- HRA is a gene from corn and soybean, with specific introduced substitutions.

Glyphosate and ALS-inhibitors have different modes-of-action and complementary characteristics

Glyphosate		ALS Herbicide Family
▪ Broad spectrum control, but better on grass weeds and annual broadleaves	⟺	▪ Broad range of weed spectrums – grass, small-seed/large-seeded broadleaves
▪ Post-emergence use only	⟺	▪ Pre- or post-emergence use
▪ No soil residual activity	⟺	▪ Wide range of soil residual activities
		▪ Very low usage rates (oz/acre)

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

Non GAT-HRA soybean

GAT-HRA soybean

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

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Integrating technologies to create a tool for weed management

GAT/HRA soybeans

+

Crop Protection Blends Technology

- Blends Technology = DuPont system for producing homogeneous blends of formulated crop protection chemicals that enables delivery of customized mixtures designed to solve specific grower problems.

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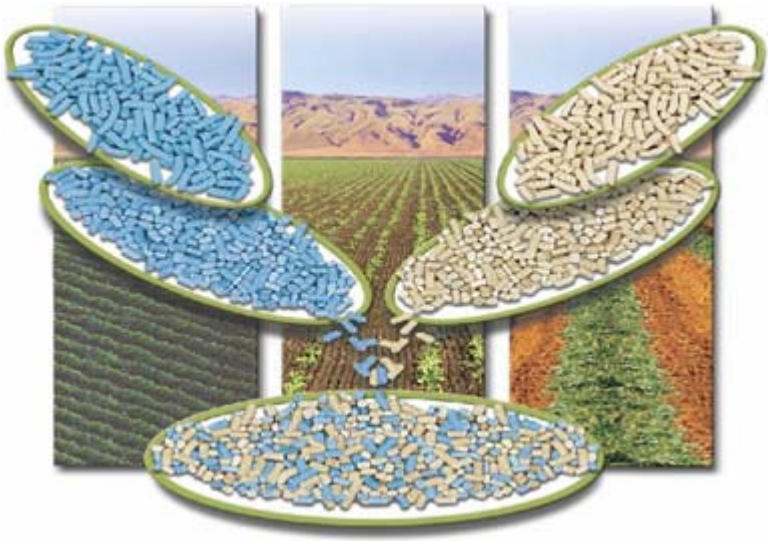
DuPont Homogeneous Blends Technology



Granule Blends

- Granulate (extrude) single herbicide active ingredient formulations,
- Can blend different active ingredients (2,3, 4+ actives possible),
- Blend granules to give desired ratio to treat weed populations as needed.

Why would farmers want GAT/HRA and Blends technology together?

- Usually, escaping weed species occur **regionally and as weed populations**, not individually and differently by farm,

Blends can be designed for regional and population needs, and farmers won't use or pay for herbicides they don't need.

- Glyphosate does not offer residual efficacy,

A Blend can do so <u>if needed</u>, for regionally required weed control

- Broad spectrum activity
- ALS chemistry can be applied both pre- and post- emergence
- Filling efficacy holes in both glyphosate and ALS herbicide programs
- Weed resistance management – 2 or more modes-of-action

Stewardship Plan

- Pioneer/DuPont Philosophy
  - We have committed to minimizing and managing any safety, health or environmental risks of our products, a commitment embedded in our "long look" philosophy.
    - Train
    - Educate
    - Promote
    - Monitor
  - We have developed a cross-functional team across Pioneer and Crop Protection Products to develop herbicide resistance management practices for GAT/HRA products.
    - Regulatory Science, Registration, Stewardship, Product Development

Timeline

- Pioneer anticipates U.S. commercialization of its proprietary glyphosate plus SU resistance trait within the next five years.
- Efficacy trials of lines containing genes from several shuffling iterations are underway in field and commercial levels of this glyphosate tolerance.
- Moving forward with necessary regulatory approvals in the United States and other world markets.

Summary

- Glyphosate and ALS-inhibitor chemistries are complementary (different modes-of-action, broad spectrum activity, etc.).
- Together with the Blends technology, GAT/HRA crops can tackle weed problems in a *regionally specific* manner.
- Adopting tactics such as blended offerings, rotations and other cultural practices in an integrated weed management system will reduce selection pressure for resistant weeds, and the value of these herbicide technologies will be preserved.

Summary

- Patented gene shuffling process developed by Verdia, a business purchased in 2004 by DuPont. Prior to the purchase, Pioneer and Verdia had a joint research program.
- GAT is the first-ever agricultural trait developed through gene shuffling.
- The gene shuffling technology should also help Pioneer identify and develop a number of new traits to help plants survive environmental stress, including drought.
- Pioneer has the exclusive right to use gene shuffling for agricultural purposes.

Next Generation Weed Control - GAT

- Developed a new proprietary glyphosate-resistant trait technically referred to as GAT – glyphosate N-acetyltransferase.
- GAT is an enzyme that inactivates glyphosate. GAT binds with glyphosate, transforming it into a substance that does not harm the plant.
- Proprietary offering
- Combining GAT with SU
  - Resistant weed management solution
  - Residual weed control

Glyphosate treated without GAT gene

Glyphosate treated GAT gene protected

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