Incorporating Genetic Diversity for Crop Improvement

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Regulatory Scientific Affairs
Bayer Crop Sciences

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Key Take home Messages

- Plants are not animals: plant breeders can throw away plants.
- Plant genomes are labile in nature.
- Three concepts used to incorporate genetic diversity into a variety: backcrossing, cell division and selection.
- Breeding methods are used to introduce traits from technologies.
Plants are not Animals: You can grow many plants, discard progeny and focus on what you want.

The process of field testing is a powerful tool used by breeders to eliminate off types.
Plant Genomes Have More Plasticity than Mammal Genomes

Genetic changes are common in plants while one chromosome change in humans can be lethal
Naturally Occurring Genetic Changes Are Common in Plants

<table>
<thead>
<tr>
<th>Genetic Change</th>
<th>Genotypic/Phenotypic Example</th>
<th>Reference</th>
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</thead>
<tbody>
<tr>
<td>Transposable elements (transposons)</td>
<td>White grapes, blood oranges</td>
<td>Lisch (2013)</td>
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<td></td>
<td>&gt;25K unique insertions detected across 31 varieties of soybean</td>
<td>Tian et al. (2012)</td>
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<td>Yellow maize</td>
<td>Palaisa et al. (2003)</td>
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<td>&gt;50 new inserts of a transposon per rice plant per generation</td>
<td>Naito et al. (2006)</td>
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<td>Elongated tomato fruit</td>
<td>Xiao et al. (2008)</td>
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<td>Round or wrinkled peas (Mendel)</td>
<td>Ellis et al. (2011)</td>
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<td>2 million transposons exchanged between higher plants</td>
<td>El Baidouri et al. (2014)</td>
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<td>Organellar DNA in nuclear DNA</td>
<td>Gain and loss of mtDNA common to maize inbred lines</td>
<td>Lough et al. (2008)</td>
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<td>Gain and loss of cpDNA common to maize inbred lines</td>
<td>Roark et al. (2010)</td>
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<td>Expression of several bacterial genes in sweet potatoes</td>
<td>Kyndt et al. (2015)</td>
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<td>Bacterial genes</td>
<td>&gt;60 wild relatives have been used for &gt;100 characteristics (80% involve pest or disease resistance) in 13 crops</td>
<td>Hajjar and Hodgkin (2007)</td>
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<td></td>
<td>Dozens of alien genes used in wheat breeding</td>
<td>Jones et al. (1995)</td>
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<td>Crossing with wild relatives</td>
<td>Stable viral DNA in rice genome</td>
<td>Liu et al. (2012)</td>
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<td>Stable viral DNA in tomato (previously also seen in potato)</td>
<td>Staginnus et al. (2007)</td>
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<tr>
<td>Pararetroviruses</td>
<td>Stable integrations in all plants</td>
<td>Geering et al. (2014)</td>
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<td>Submergence-tolerant rice</td>
<td>Xu et al. (2006)</td>
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<td></td>
<td>Dwarf sorghum</td>
<td>Multani et al. (2003)</td>
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<td>Yellow soybean seeds</td>
<td>Tuteja et al. (2004)</td>
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<tr>
<td>Indels</td>
<td>Maize proteins (300-400 aa long) from 2 alleles differ by 3-4 aa</td>
<td>Tenaillon et al. (2001)</td>
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<td>Maize genome has 55 million SNPs</td>
<td>Gore et al. (2009)</td>
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<td>Green Revolution gene has 2 SNPs for dwarf wheat</td>
<td>Peng et al. (1999)</td>
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<td>One SNP caused loss of shattering in domestic rice</td>
<td>Konishi et al. (2006)</td>
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<td>Tall or short pea plants (Mendel)</td>
<td>Ellis et al. (2011)</td>
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<td>7 new SNPs created per meiosis per billion base pairs</td>
<td>Ossowski et al. (2010)</td>
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<td>Single nucleotide polymorphisms (SNPs)</td>
<td>856 wild-type soybean genes absent in cultivated varieties (and &gt;186K DNA insertions/deletions)</td>
<td>Lam et al. (2010)</td>
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<td>&gt;10^6 SNPs, 30K insertion/ deletions and a few large chromosomal deletions (&gt;18 genes) in 6 elite maize varieties</td>
<td>Lai et al. (2010)</td>
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<td>Copy number variation relates to soybean cyst nematode resistance</td>
<td>Cook et al. (2012)</td>
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<td>Pinot Noir, Corvina &amp; Tannat wine grapes have 1873 genes not found in other wine grapes</td>
<td>Da Silva et al. (2013)</td>
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<td>Only 81% of Brassica genes are always present in the same number</td>
<td>Golicz et al. (2016)</td>
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<td>2500 genes found only in either B73 or PH207</td>
<td>Hirsch et al. (2016)</td>
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<td>G. soja genotypes can vary by 1000-3000 gene families from each other</td>
<td>Li et al. (2014)</td>
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<td>Glenn, K. et al. (2017)</td>
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</table>
Some Genetic Differences Can be Visible e.g. Brassica family

Nine vegetable species are all derived from a common ancestor by selecting for traits or combining traits

Swarup et al, submitted
Phenotypic Differences Does Not Always Reflect Genotypic Differences in Plants

Corn is 14 times more diverse than humans

Genomic tools can differentiate a plant with desired characteristics in a mix of plants

Chen and Li, 2001
Genetic Change (Mutations) is the Source of Genetic Diversity

**Natural mutations**
- 70 *denovo* mutations per generation
- 17 bp per generation
- 1 every 2000 bp mutations per generation
- 1 every 540 bp mutations per generation

**Induced mutations**
- 2-10 mutations every Mb
How is Genetic Diversity Incorporated Into a Crop Variety?

Create

Natural mutations

Induced mutations

Utilize

Incorporate
Genetic Diversity Is Strategically Added into Varieties: Keep Out Unwanted Characteristics and Retain Wanted Characteristics

- **Task (scientific term)**
  - **Check point**
  - **Breeding Method**

- **Create combinations (inter cross)**
  - inter cross elite parents
  - cross pollination

- **Move trait (introgress)**
  - >99% known genetic background
  - >95% accurate marker
  - MAS-MABC; zygosity assay

- **Find trait (mapping)**
  - Defined DNA size
  - QTL mapping

- **Maintain germplasm (germplasm)**
  - Elite germplasm
  - Fingerprinted
  - Yrs of safe use
  - Traits collection
  - Monitored and maintained

- **New Parents (inbreds)**
  - 6-7 rounds of selfing
  - Self pollination /DH

- **Off types (Field testing)**
  - > 60 agronomic parameters
  - ~99.9% plants eliminated
  - Selection & elimination

- **Predict (GWS and Chipping)**
  - >95% accurate markers
  - Protect embryo
  - Predictive analytics

- **Variety**

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**Time**

>10 years
Conventional Breeding Uses **Backcrossing, Meiosis and Selection** to Incorporate Diversity

- **Backcrossing**
  - Find trait (introgress)

- **Meiosis**
  - Create combinations (inter cross)
  - Maintain germplasm (germplasm)

- **Selection**
  - Off types (Field testing)
  - Predict (GWS and Chipping)

- **New Parents** (inbreds)

- **Variety**

**Create combinations**

**Off types** (Field testing)

**Predict** (GWS and Chipping)
Conventional Breeding: A Multi-Stage Long-Term Strategic Process

- Generation 1
  - 50% dad background

- X
  - 6 generations
  - >99% dad background

- Yield
- Kernel Quality
- Disease resistance

- F1
- F2
- F6

Backcrossing Relies on Cell Division To Eliminate Unwanted Effects

- Generation 1
  - 75% dad background

- F1
  - X
  - Disease susceptible

- F2

- X

- F6
  - >6 generations
  - >99% dad background
  - Yield
  - Kernel Quality
  - Disease resistance
It Can Take Years of Backcrossing to Eliminate *Genetic Background Effects* and *Linkage Drag*

Desired gene (fusarium resistance)  

\[ \text{wild tomato} \times \text{commercial tomato} \]

*Linkage drag* (bacterial spot genes genetically linked to fusarium resistance region)

Genetic background (other genes)
Conventional Breeding Uses **Backcrossing, Meiosis and Selection** to Incorporate Diversity

- **Find trait (mapping)**
- **Move trait (introgress)**
- **Create combinations (inter cross)**
- **Maintain germplasm (germplasm)**
- **New Parents (inbreds)**
- **Predict (GWS and Chipping)**
- **Off types (Field testing)**
- **Variety**
Germplasm Acts As a Reservoir for Genetic Diversity for Different Traits

Bayer global maize germplasm uses a well characterized germplasm
Diversity is Created to Develop Parents With Beneficial Genetic Combinations

Plants suited for specific environments are cross pollinated

New genetic combinations in progenies

Use progenies that are better performers as new parents

New characteristic added

Germplasm Collection is Routinely Monitored and Recycled with New Genetic Combinations and Traits
Conventional Breeding Uses **Backcrossing, Meiosis and Selection** to Incorporate Diversity

- **Find trait** (mapping)
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- **Maintain germplasm** (germplasm)
- **Create combinations** (inter cross)
- **New Parents** (inbreds)
- **Predict** (GWS and Chipping)
- **Off types** (Field testing)
- **Variety**
- **Selection**
Tools Such as Predictive Analytics Has Shrunk Our Footprint

Field testing

Select the seeds with desirable features

- Replaces 900,000 plants
- One chip replaces 14 acres
- 6-8 weeks vs months

Chipping & Genome wide selection

Conventional Breeding: A Multi-Stage Long-Term Strategic Process

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Conventional Breeding: A Multi-Stage Long-Term Strategic Process

Select the seeds with desirable features

- Replaces 900,000 plants
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Chipping & Genome wide selection
All Crops - Conventional, Biotech and Genome Edits - Go Through Intense Selection Criteria

0.1% plants selected; 99.9% discarded over 6-8 years of testing across 100 locations

Young Plant
- Percent emergence
- First leaf shape
- Plant vigor
- Seedling height
- Seedling color

During Pollination
- Pre-anthesis brittle snap
- Pre-anthesis root lodging
- Density of spikelets
- Glume color
- Anther color
- Leaf color
- Silk color
- Days to pollen shed
- Days to silking

Post Pollination
- Brace root color
- Stalk anthocyanin
- Internode direction
- Internode length
- Leaf sheath pubescence
- Husk color fresh
- Plant height
- Ear height
- Stalk diameter
- Nodes with brace roots
- Leaf angle
- Upper leaf number
- Ear leaf length
- Ear leaf width
- Tassel length
- Tassel spike length
- Tassel peduncle length
- Tassel branch number
- Ear position
- Ears per stalk
- Days to husk opening
- Husk color dry
- Husk cover
- Husk length

At Harvest
- Ear length
- Ear shape
- Ear weight
- # kernel rows
- # kernels per row longitudinally
- Ear diameter
- Cob diameter
- Cob color
- Kernel length
- Kernel width
- Kernel thickness
- Kernel row direction
- Kernel type
- Kernel cap color
- Kernel side color
- Endosperm color
- Endosperm type
- 100 kernel weight

Glenn et al, 2017
Breeding Methods - Backcrossing, Selection, Cross Pollination - Used To Incorporate Biotechnology

- Find transgene
  - Insertion (transformation)
  - Move germplasm (biotech donor pool)
- Move transgene (introgress)
- Cross/Hybrid (breeding stack)
- Create combinations (inter cross)
- New Parents (inbreds)
  - Predict (GWS and Chipping)
- Cross/Hybrid (breeding stack)
- Variety
  - Off types (Field testing)
  - Maintain germplasm (biotech donor pool)
  - Find trait (mapping)
  - Move trait (introgress)
  - Maintain germplasm (biotech donor pool)
  - Create combinations (inter cross)
  - Insertion (transformation)
  - Find transgene
Selection is used to identify a parent plant to use for trait introgression at the next stage.

- Insertion
- Efficacy
- Genetic and environmental stability in plants
Has modern plant breeding exhausted genetic diversity?
Genetic Diversity is Actively Maintained While Improving Performance (Wheat)
Genetic Diversity is Actively Maintained in Commercial Breeding for Desirable Consumer Traits

Commercial Breeding Utilize Genetic Diversity for Different Environments
How Can Genome Editing Be Used in Plant Breeding?
Genome Editing Sits at the Interface of Breeding and Genetic Modification

Genome Editing is the Latest Tool in the Breeder’s Toolkit

**Plant Breeding**
- Desired gene
- Target Plant Genome
- Only selected gene transferred
- Modified Plant Genome

**Gene Editing**
- Deactivate an unfavorable characteristic
- Enable a beneficial characteristic

**Genetic Modification**
- Desired gene
- Target Plant Genome
- Only selected gene transferred
- Modified Plant Genome
Genome Editing Can Make Precise Changes Mimicking Natural Changes

**Comparison of cDNAs**

- **Nature**
- **SNP**
- **Insertion (4736 bp)**
- **Stop**
- **Gene Edit**
- **Insertion (1 bp)**
- **Stop**

**Brachytic gene edit allele**
- elite inbred

**Brachytic transposon allele**
- inbred

**Wild-type allele**
- elite inbred
Genome Editing Can Overcome Limitations of Conventional Breeding Approach: Soybean Cyst Nematode (SCN) Disease

race 1 resistant + race 2 resistant = race 1 and 2 resistant

Targeted recombination using genome editing

$1B loss per year
Genome Edits, Just Like Biotech and Conventionally Bred Crops, Go Through Rigorous Field Testing

![Diagram showing the process of genome editing and field testing with stages labeled as follows:

1. Native trait introgression to selected elite germplasm
2. New Line Development
3. Pre-Commercial (PCM) Field Testing
4. Traitd PCM Testing
5. Commercial

Key points:

- More than 99% of plants are discarded by breeding selection and testing.
- Create Diversity
- Make edits in the elite plants directly replacing backcrossing
- Make edits in after biotech trait introgression

Swarup et al, submitted]
Gene Editing Screening Assays Ensures Clean Edit For Field Testing

Before Field Testing

- Unintended effects: develop edit-specific assay
- Potential human error: add selection step between assay points
- Prevent cross pollination: perform selection before flowering
Contribution of Technologies to Genomic Stability and Variation is Negligible

Breeding

Mutagenesis

Transgenic

Genome Editing

Safe

Safe

Safe

Safe

Robert Stupar (University of Minnesota)
Key Take home Messages

- Plants are not animals: plant breeders can throw away plants.
- Plant genomes are labile in nature.
- Three concepts used to incorporate genetic diversity into a variety: backcrossing, meiosis and selection.
- Breeding methods are used to introduce traits from technologies.
Thank You!