DNA INFORMATION FACILITATES UTILIZATION OF TREE FRUIT GENE BANKS

Instant Utilization

(DNA information not needed this time)

Cameron Peace
To Facilitate Utilization of Tree Fruit Genebanks:

1. Think like a curator
   ➞ Think like a breeder

2. Static collections
   ➞ Segregating descendant populations

3. Descriptors
   ➞ Comprehensive and standardized performance information

4. Phenotypes
   ➞ Performance-predictive DNA information
Asst Prof
Tree Fruit Genetics

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Member, Apple CGC

Co-Project Director
MAB Pipeline Team Leader

WHO IS THIS GUY?
ACKNOWLEDGEMENTS

Breeders: Tom Gradziel, Amy Iezzoni, Jim Olmstead, Kate Evans, Nnadozie Oraguzie, Jim Luby

Germplasm curators: Phil Forsline, Gennaro Fazio, Ed Stover, John Preece

Germplasm DNA evaluators: Gayle Volk, Nahla Bassil, Malli Aradhya

Co-PIs and participants of NRI project “Candidate genes for fruit softening in Prunus”

Co-PIs and participants of NRI project “Functional gene markers for Rosaceae tree fruit texture”

Co-PIs and participants of SCRI project “RosBREED: Enabling marker-assisted breeding in Rosaceae”
Outline of Presentation

- Value of Genebanks to Crop Improvement
- Think Like a Breeder
- Segregating Descendant Germplasm
- Comprehensive & Standardized Performance Information
- Performance-Predictive DNA Information
- Genebanks as Conduits to Utilization
Value of Genebanks to Crop Improvement
Value of Genebanks to Crop Improvement

Security of crop production

Utilization is rare for tree fruit

Output is basis of crop production

Ultimate source of useful alleles, less protected

WILD POPULATIONS

GENEBANK

BREEDING
Value of Genebanks to Crop Improvement

- Allelic diversity is not useful if not used
- Primary users for crop improvement = BREEDERS
- Encourage utilization by breeders!
Think Like a Breeder
Think Like a Breeder

Select parents

Cross

Select seedlings

Release commercially
Think Like a Breeder

Genebank accessions

Select parents

Cultivars and selections
Think Like a Breeder

I can’t see any value in that!
Think Like a Breeder

Do I need that trait?

Even if I want it, it’s a lot of work to chip it out and polish it up!
Think Like a Breeder

Much better! I can incorporate that!
Think Like a Breeder

*Select parents*

Genebank accessions

Cultivars and selections
Think Like a Breeder

Select parents

Genebank accessions

Cultivars and selections
Think Like a Breeder

Select parents

Genebank accessions

USE

Cultivars and selections

IGNORE
Think Like a Breeder

• To support “Use as parent/Ignore” decisions (and encourage “Use”), need to get into breeders’ heads!

Those genebank accessions *could* be useful, but…

What are the actual allele effects?

Is it dominant?

Recessive? Additive?

Is there pleiotropy with other traits? Linkage?
Think Like a Breeder

- Phenotype is an incomplete predictor of underlying genetics

**Phenotype is an incomplete predictor of underlying genetics**

- Sweet sometimes, v. high yield
- Mid sweetness, large size
- Poor taste, small

**Think Like a Breeder**

- Phenotype is an incomplete predictor of underlying genetics

- Sweet sometimes, v. high yield
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- Poor taste, small
Phenotype is an incomplete predictor of underlying genetics
Think Like a Breeder

Normal view

- MID SWEETNESS, LARGE SIZE
- POOR TASTE, SMALL
- SWEET SOMETIMES, V. HIGH YIELD

Breeders’ optimal view

- Genetic Block 2C
  - Sweetness: 90% linkage
  - Weight

- Recessiveness
- Epistasis
- Pleiotropy

+2  -1
+10  +5
+2  -1
-18  +5
+3
Think Like a Breeder

Breeders’ optimal view

(+6) 0 -5

+2 -1

+10 +5

-18 +5

+3
Think Like a Breeder

Putting the jewels together…

Breeders’ optimal view
Think Like a Breeder

Breeders’ optimal view

Putting the jewels together…
1. Marker-trait association approaches require enough contrasting individuals for statistical power.

- E.g. Red flesh and the *MYB10* gene in apple
  - F<sub>1</sub> mapping population: 516 seedlings
  - NPGS genebank: >50 accessions (>30 red)
Provide Segregating Descendant Germplasm

2. Cryptic alleles – not detected by phenotype

If favorable allele is recessive or epistatic, then
- not detected, or
- mistaken for dominance or additive effect

Typical cultivar  Wild forest cherry  e.g. Fruit size in sweet cherry

QTL study found a tiny-fruited wild cherry accession contains common large-fruit allele at major locus

(large-fruit allele masked by another locus)
Provide Segregating Descendant Germplasm

3. Wild germplasm, even if carrying wonderful alleles, is far from being cultivar-producing parents. How can breeders rapidly respond? 

* e.g. *Malus floribunda*-derived disease resistance in apple
Provide Segregating Descendant Germplasm

SOLUTION

• Genebanks need to have, or else energetically facilitate, availability of descendant segregating germplasm

→ Enough contrasting individuals

→ Reveal cryptic alleles

→ Closer to elite

Useful alleles easier to find

Useful alleles easier to extract
Provide Segregating Descendant Germplasm

- RosBREED’s reference germplasm set of apple merges breeding & NPGS

- **85** NPGS accessions
- **135** cultivars (breeding progs)
- **260** seedlings (breeding progs)

- NPGS accessions = ancestors. Allow joining of many populations in stats
Provide Comprehensive & Standardized Performance Information
Provide Comprehensive & Standardized Performance Information

- Phenotypic evaluation: performance, not descriptors

  - Acute apex petiole length = 24 mm
  - Performance at harvest:
    - Storage 10w+7d
    - Storage 20w+7d

  - Firmness:
    - Instr, Sens
  - Crispness:
    - Instr, Sens
  - Juiciness:
    - Sens
  - Harvest date
  - Maturity
  - Fruit size
  - Sweetness
  - Acidity
  - Internal ethylene conc

→ Relevant to breeding
Provide Comprehensive & Standardized Performance Information

• Excellent quality phenotypic information (and available in GRIN) is critical
  → Reduces unpredictable components of performance

Contributors to variation among trees

...needed by researchers to develop excellent quality genetic tests for performance
Provide Comprehensive & Standardized Performance Information

- Standardized phenotyping for Rosaceae (led by Gayle Volk, USDA-ARS Fort Collins)

<table>
<thead>
<tr>
<th>Eight Guiding Principles for Phenotyping</th>
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<tbody>
<tr>
<td>1. Commercial relevance</td>
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<td>2. Heritability &amp; Precision (Genetic relevance)</td>
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<td>3. Redundancy avoidance</td>
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<td>4. Availability of expertise &amp; instruments</td>
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<td>5. Throughput</td>
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<td>6. Personnel fatigue</td>
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<td>7. Cost &amp; Cost-efficiency</td>
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<td>8. Standardization</td>
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For:
- Genebanks
- Breeding programs
- Research programs

First presented at ASHS July 2009
Provide Comprehensive & Standardized Performance Information

• Protocols
  www.rosbreed.org/resources/fruit-evaluation/

Fruit Maturity and Harvest

- Check trees weekly for fruit color changes that indicate onset of fruit maturation – changes in background color (from green to cream or light yellow) and brightening in red blush color development.

- When the tree looks like it may be ready for the first harvest sampling, select 1-2 fruit with the most advanced maturity appearance and conduct a starch-iodine test. Aim for a maturity indicator of SPI 3-5 on the 1-5 Cornell (Blanched) Chart below (available at: http://commons.library.cornell.edu/bitstream/1813/32892/Predicting%20Harvest%20Date%20Window%20for%20Grapes.pdf) or 1.5-2.5 on a 0-6 starch pattern chart as a generic harvesting ‘go date’. This is equivalent to commercial harvest, and is a good intermediate range where it is unlikely for any trees to be too immature or over-mature.

- If the SPI indicates onset of maturation for a tree, visually evaluate the remaining fruit on the tree for their range in maturity. If ~25% of the fruit have a ‘harvest-mature’ appearance (roughly similar to the SPI-tested fruit), take the sample of 15-45 fruit selectively picked to be at the correct harvest maturity. 15 fruit is minimum to cover all evaluation stages (5 fruit x 3 evaluation times), and extra fruit provides some backup in case of fruit rot in storage.

Crop Germplasm Committees: trait priorities -> + protocols?
Provide
Performance-Predictive DNA Information
Provide Performance-Predictive DNA Information

- Research required to develop genetic tests that provide functional genotypes for valuable traits
Provide Performance-Predictive DNA Information

• Target appropriate traits
Provide Performance-Predictive DNA Information

• Need excellent quality phenotypic data

At harvest
Storage 10w+7d
Storage 20w+7d
5 fruit (reps) per evaluation

Firmness
– Instr, Sens
Crispness
– Instr, Sens
Juiciness
– Sens
Harvest date
Maturity
Fruit size
Sweetness
Acidity
Internal ethylene conc

{ Multiple harvests
  Multiple locations
  Multiple years
  Multiple tree reps }
Provide Performance-Predictive DNA Information

- Need efficient DNA technologies
Provide Performance-Predictive DNA Information

• Need appropriate germplasm

→ Segregating descendant germplasm

○ Associations are discovered and characterized in training set(s)

○ Then extrapolated to the rest of the crop – breeding material, repositories, wild populations
Provide Performance-Predictive DNA Information

• A jewel for sweet cherry

Some functional genotypes (haplotypes) in cultivars

<table>
<thead>
<tr>
<th>Marker 1 allele</th>
<th>Marker 2 allele</th>
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<tr>
<td><strong>Effect on fruit</strong></td>
<td><strong>Single source</strong></td>
</tr>
<tr>
<td>237 Large Firm</td>
<td>190</td>
</tr>
<tr>
<td>255 Large Soft</td>
<td>190</td>
</tr>
<tr>
<td>235 Small Med-firm</td>
<td>204</td>
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→ Using already in breeding
→ Screening Davis Repository cherries
  o further sources of the good haplotype?
  o new useful haplotypes?
  o tag bad haplotypes (avoid in introgression)
Provide Performance-Predictive DNA Information

→ Replace this … with this

MID SWEETNESS (11.5-13.4 °Brix)
LARGE SIZE (140-180 g)

LOW SWEETNESS (6.7-9.0 °Brix)
SMALL (45-68 g)

SWEET SOMETIMES (8.4-14 °Brix)
V. HIGH YIELD but TINY (19-31 g)
The Current System

WILD POPULATIONS

Genebank

Security of crop production

Ultimate source of useful alleles, less protected

Output is basis of crop production
1. Think like a breeder

2. Provide segregating descendant populations

3. Provide comprehensive & standardized performance information

4. Provide performance-predictive DNA information
Genebanks as Conduits to Utilization

Ultimate source of useful alleles
Safeguarded source of useful alleles
Training sets for genetic test development
Allele providers for breeding
Output is basis of crop production
Genebanks as Conduits to Utilization

REPOSITORY

WILD POPULATIONS

BREEDING

SEGREGATING DESCENDANT GERMPLASM