BRIDGING THE CHASM BETWEEN GENOMICS & BREEDING: ENABLING MARKER-ASSISTED BREEDING

Cameron Peace, MAB Pipeline Team Leader, WSU Pullman
The RosBREED Approach

Socio-economics information

Objectively define

SELECTION TARGETS

Optimize

SELECTION STRATEGIES

DNA information

IMPROVED CULTIVARS
Writing the Instruction Manual

Instruction Manual

Socio-Economically & DNA-Informed Breeding

RosBREED
Enabling marker-assisted breeding in Rosaceae
Outline of Presentation

- The MAB Pipeline
- A High-Impact Target for Cherry Breeding
- Example of MAB Pipelining in Action: Large Fruit for Sweet Cherry
The MAB Pipeline
The MAB Pipeline

Socio-economics information
DNA information

Routine Breeding Operations

RosBREED
Enabling marker-assisted breeding in Rosaceae
www.rosbreed.org
The Pipeline’s Eight Stages

1. Choose best targets
2. Choose efficient genetic screening technologies/services
3. Adapt genetic tests
4. Validate genetic tests
5. Assess utility of genetic tests in breeding germplasm
6. Inform parent selection and crossing choices
7. Identify efficient seedling selection schemes
8. Trial seedling selection schemes

Routines Breeding Operations

RosBREED
Enabling marker-assisted breeding in Rosaceae
www.rosbreed.org
Fast-Tracked Pipelining in 2010

1. Chose best M-L-T associs from those already available
2. Using RosBREED’s Genotyping Center: lab of Nahla Bassil (USDA-ARS Corvallis)
3. Using existing SSR and SCAR markers
4. Genotyping and Phenotyping on Crop Reference Sets, PBA
5. Genotyping and Phenotyping on Breeding Pedigree Sets, PBA, describe parent alleles
6. Inform parent selection and crossing choices of 2011
Fast-Tracker Pipelining in 2010 for Fruit Quality

**Peach**
1. Texture: *endoPG F-M* locus
2. Flavor: sweetness & acidity QTL

**Cherry**
1. Fruit quality: fruit size and firmness QTL
2. Flavor: acidity QTL

**Apple**
1. Flavor and Texture: acidity, crispness, juiciness QTL
2. Texture: Firmness QTL
A High-Impact Target for Cherry Breeding
A High-Impact Target

- Fruit size for sweet cherry

Elite sweet cherry cultivars
12 grams

Domestication and breeding

2 grams
Wild forest cherries

Breeding for resistance

Disease resistant
Disease susceptible

RosBREED
Enabling marker-assisted breeding in Rosaceae
Mapping & QTL Studies

- Sweet cherry genetic map created
- Nice QTL for fruit size identified


QTL Study

• Functional alleles reported (thanks Amy!)

CPSCT038  BPPCT034

QTL peak
(location of gene influencing cell number)

Fruit size

EF

NY

A  190  204
B  255  235
A  190  192
N  255  225
Reported M-L-T Association

- QTL paper published

**Fruit size QTL analysis of an F₁ population derived from a cross between a domesticated sweet cherry cultivar and a wild forest sweet cherry**

Guorong Zhang · Audrey M. Sebolt · Suneth S. Sooriyapathirana · Dechun Wang · Marco CAM Bink · James W. Olmstead · Amy F. Iezzoni

Received: 15 January 2009 / Revised: 18 June 2009 / Accepted: 24 June 2009
© Springer-Verlag 2009

**Abstract** Maximizing fruit size is critical for profitable sweet cherry (*Prunus avium* L.) production. Yet, despite its importance, little is known about the genetic control of fruit size. The objective of this study was to identify quantitative trait loci (QTLs) controlling fruit size, and one QTL was identified for mesocarp cell number. Fruit size QTLs were found on linkage group 2 on the EF map (EF 2) and linkage groups 2 and 6 on the NY map (NY 2 and NY 6). On EF 2, the cell number QTL clustered...
Plenty of Reported M-L-T Associations!

<table>
<thead>
<tr>
<th>Crop</th>
<th>Number of Traits</th>
<th>Number of QTLs(^a)</th>
<th>Number of MTLs(^b)</th>
<th>Number of Genes(^c)</th>
<th>Number used in MAPS(^d)</th>
<th>Number used in MASS(^e)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apple</td>
<td>55</td>
<td>180</td>
<td>36</td>
<td>5</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Pear</td>
<td>5</td>
<td>7</td>
<td>3</td>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Peach</td>
<td>21</td>
<td>36</td>
<td>4</td>
<td>2</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Almond</td>
<td>10</td>
<td>8</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Cherry (sweet)</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Cherry (tart)</td>
<td>3</td>
<td>14</td>
<td>-</td>
<td>1</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Strawberry</td>
<td>11</td>
<td>31</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>Raspberry</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Blackberry</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Rose</td>
<td>11</td>
<td>41</td>
<td>9</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>117</strong></td>
<td><strong>317</strong></td>
<td><strong>58</strong></td>
<td><strong>11</strong></td>
<td><strong>6</strong></td>
<td><strong>3</strong></td>
</tr>
</tbody>
</table>

\(^a\) QTLs = quantitative trait loci with linked markers  
\(^b\) MTLs = major/ trait loci with linked markers  
\(^c\) Genes = known genes controlling a trait  
\(^d\) MAPS = marker-assisted parent selection  
\(^e\) MASS = marker-assisted seedling selection
“Available M-L-T Associations Warehouse”

<table>
<thead>
<tr>
<th>Crop</th>
<th>QTLs</th>
<th>MTLs</th>
<th>Genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cherry (tart)</td>
<td>7</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Raspberry</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rose</td>
<td>11</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>117</strong></td>
<td><strong>31</strong></td>
<td><strong>58</strong></td>
</tr>
</tbody>
</table>
Example of MAB Pipelining in Action: Large Fruit for Sweet Cherry
The MAB Pipeline

Socio-economics information

DNA information

Routine Breeding Operations

RosBREED: Enabling marker-assisted breeding in Rosaceae
The MAB Pipeline for Fruit Size

- Which crosses to make for efficient genetic gain in fruit size
- Efficient wild introgression
- Identify new genetic sources for large fruit
- Efficient seedling selection scheme to avoid wasting resources on small-fruited seedlings

Consumer & industry value of large fruit

QTLs for fruit size

RosBREED: Enabling marker-assisted breeding in Rosaceae
Warehouse

1. “Selection Target Identifier”

2. Technology Portfolio”

3. “Genome Database Resources”

4. “Crop Reference & Breeding Pedigree Germplasm Databases”

5. “Cross Planning Tool”

6. “Seedling Selection Efficiency Tool”

7. “Seedling Selection Efficiency Tool”
1. Prioritization

Trait groups

- **DNA info available?**
  - Usually Yes

**MAB approach**

- **Market-defining Harvest season,** Self-fertility, Fruit color, PM resistance
  - Usually Yes

**Parent selection**

- **Primary**
  - Fruit size, Firmness
  - Usually Yes

- **Secondary**
  - Sweetness, Acidity, Taste, Low astringency and bitterness
  - Usually Yes

**Parent & Seedling selection**

- **Secondary**
  - Sweetness, Acidity, Taste, Low astringency and bitterness
  - Usually Yes
1. Prioritization

"Selection Target Identifier"

$$PI = \$ \times G \times H$$
# 1. Prioritization

"Selection Target Identifier"

<table>
<thead>
<tr>
<th>PI</th>
<th>Marker-Locus- Trait Association</th>
</tr>
</thead>
<tbody>
<tr>
<td>9.7</td>
<td>Fruit size 8-12 g</td>
</tr>
<tr>
<td>3.1</td>
<td>Fruit size 12-14 g</td>
</tr>
<tr>
<td>1.2</td>
<td>Sweetness 16-22° Brix</td>
</tr>
<tr>
<td>1.1</td>
<td>Fruit size 14+ g</td>
</tr>
<tr>
<td>1.0</td>
<td>Self-fertility</td>
</tr>
<tr>
<td>0.9</td>
<td>Powdery mildew resistance</td>
</tr>
<tr>
<td>0.8</td>
<td>Acidity - in balance with sweetness</td>
</tr>
</tbody>
</table>
2. Genetic Screening Efficiency

- Sampling
- DNA extraction
- Genotyping
- Information delivery

Silica Bead Method

ABI (or PAGE)

Electronic database
2. Genetic Screening Efficiency

"Technology Portfolio"

Welcome to the Technology Portfolio

Please indicate your location

RosBREED
Enabling marker-assisted breeding in Rosaceae

www.rosbreed.org
3. Improved Markers

CPSCT038  SSR, 4 alleles

BPPCT034  SSR, 7 alleles

…and fine mapping underway: SNPs being developed, controlling gene being sought
3. Improved Markers

“Genome Database Resources”

Candidate genes to test (SCAR, SSR, SNP, or seq)
4. Validation

Validation on “Pedigree Set”

41 cultivars and 3 populations (417 seedlings)
4. Validation

"Crop Reference Germplasm Databases"

Sweet Cherry Crop Reference Set

57 cultivars and 23 populations (183 seedlings)
5. Utility

Utility assessment on fruiting seedlings

22 populations (219 seedlings)
5. Utility

<table>
<thead>
<tr>
<th>Size (g)</th>
<th>Firmness (kgf)</th>
<th>Sweetness (*Brix)</th>
<th>Flavor (1-5 score)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A 8.6</td>
<td>E 300</td>
<td>C 22</td>
<td>E 2.7</td>
</tr>
<tr>
<td>B 6.8</td>
<td>D 240</td>
<td>A 20</td>
<td>F 1.7</td>
</tr>
</tbody>
</table>

- **E** = large, firm
- **A** = large, soft
- **B, C, D, F** = small

- **“Good” genetic group**
- **“Okay” genetic group**
- **“Bad” genetic group**

- “good” group averages higher than this value
- “bad” group averages lower than this value

[Diagram with genetic groups and trait values]
5. Utility

“Breeding Pedigree Germplasm Databases”

PNWSCBP Breeding Pedigree Set

66 cultivars and 59 populations (414 seedlings)

RosBREED
Enabling marker-assisted breeding in Rosaceae

www.rosbreed.org
6. Parent Selection Decisions

Pedigree of sweet cherry fruit size genotypes
6. Parent Selection Decisions

“Cross Planning Tool”

Glacier

large (DE)
self-fert.
(S4’S9)

DD

med (AF)
self-incomp.
(S1’S4)
7. Seedling Selection Cost Efficiency & Logistics

Spreadsheet tool: Predicted savings for 1 marker, 50% cull

TRADITIONAL PHENOTYPIC SELECTION
- Fruit evaluation & further maintenance: $14
- Maintenance until fruiting: $12
- Orchard planting: $10
- Early rearing costs: $8
- Later rearing & evaluation costs: $6
- Genotyping costs: $4
- Early rearing costs: $2
- Early rearing costs: $0

Savings: $14

MASS ESTIMATED
7. Seedling Selection Cost Efficiency & Logistics

“Seedling Selection Efficiency Tool”

Welcome to your Seedling Selection Efficiency Tool

Set-up  Calculate

RosBREED
Enabling marker-assisted breeding in Rosaceae
www.rosbreed.org
8. Trial Use

Fall 2010: Genotype ~1000 seedlings for fruit size, cull predicted small-fruited
The MAB Pipeline for Fruit Size

- QTL for fruit size
- Which crosses to make for efficient genetic gain in fruit size
- Efficient wild introgression
- Identify new genetic sources for large fruit
- Efficient seedling selection scheme to avoid wasting resources on small-fruited seedlings
- Consumer & industry value of large fruit
- The most efficient use of SE and DNA information!
Further Benefits

• Describe genetic potential of new cultivars to enhance cultivar adoption by growers

Dr. Nnadozie Oraguzie, sweet cherry breeder

Breeding orchard

Fruits of our labor
Acknowledgements

This project is supported by the Specialty Crops Research Initiative of USDA’s National Institute of Food and Agriculture.
Questions?