The Effects of Lycopene β-Cyclase (CYC-B) Allele Introgression on Cherry Tomato Fruit Quality

Eka Sari

Ohio Agricultural Research and Development Center (OARDC)
The Ohio State University
46th Tomato Breeders Roundtable
2016
SPARKLING, TAP, OR TOILET?
Research Background

- Consumer dissatisfaction
- Increased health concerns

Sensory and nutritional quality traits

Question:
Do different $B$ allele introgressions affect tomato fruit quality?

Research objectives
1. Integrate marker assisted backcrossing (MAB) into the breeding program
2. Assess the fruit quality through instrumental and sensory evaluations
Carotenoid Biosynthesis Pathway in Plants

The image was modified from: Causse et al., 2003
Lycopene β-Cyclase

1600 bp promoter sequence
Plant Materials

Jaune Flamme
*S. habrochaites*

97L97
*S. galapagense*

Purdue 89-28-1
*S. habrochaites*

Tainan (PI 647556)
<table>
<thead>
<tr>
<th>Promoters</th>
<th>Primers</th>
<th>Markers</th>
</tr>
</thead>
</table>
| **Jaune Flammee** | **Forward:** 5’ CGTCTTAGGCTTGAGGTTAGTTG 3’  
                      **Reverse:** 5’ TGAGAAAGTTGCGAAGCTCA 3’ | **Indel (23 bp)**  
                      Jaune Flammee allele: 5’ TTCTATCA------------------------AATA 3’  
                      Tainan allele: 5’ TTCTATCAACCTGTTGAGTTCTCTTTA TAAAATA 3’ |
| **Purdue 89-28-1** | **Forward:** 5’ AATATACCTGGCGCCTCCA 3’  
                      **Reverse:** 5’ TGTGCCCAACTGCAAATA 3’ | **Indel (8 bp)**  
                      Purdue 89-28-1 allele: 5’ ATAACCTAGATCAAGTATAGTGC 3’  
                      Tainan allele: 5’ ATAACCTAG--------ATATAGTGC 3’ |
| **97L97** | **Forward:** 5’ GCAGTTGGGCACATGAACAA 3’  
                      **Reverse:** 5’ TCTTGCAGCTCATTCGGTTT 3’ | **SNP (Rsal restriction site)**  
                      97L97 allele: 5’ TTGTAGAAAGTATTTTTCATT 3’  
                      Tainan allele: 5’ TTGTAGAAAGTATTTTTCATT 3’ |

100-bp ladder
Available marker resources for tomato:
- 7,720 SNPs - SolCap array
  (http://solcap.msu.edu/tomato_genotype_data.shtml)
- 384 FM tomato SNPs– 0.2 Mbp windows
  (http://www.extension.org/pages/61007)
Recurrence Parent Genome Proportions

Histogram of BC$_2$ Tainan genome proportion (%) within Purdue 89-28-1 allele source population
**BC₁S₁ Field Evaluation**

RCBD
Promoter sources: JF; Purdue 89-28-1; 97L97; Tainan
Allele states: BB; Bb; bb
Locations: Wooster, OH; Fremont, OH.
Blocks: 2

**BC₂S₁ GH Evaluation**

RCBD
Promoter sources: JF; Purdue 89-28-1; 97L97; Tainan
Allele states: BB; bb
Locations: Wooster, OH
Blocks: 2
Measured Traits

Instrumental measurements:
- $\beta$-carotene
- Total lycopene
  - All-trans-lycopene
  - All-cis-lycopene
- Total soluble solids
- Titretable acidity
- Fruit size and shape
  - Fruit shape index 1
  - Circular
  - Height mid width

Sensory evaluations:
- Aroma
- Flavor
- Mouthfeel
Y = FAMILY:ALLELE_STATE + LOC + LOC:BLOCK + FAMILY:ALLELE_STATE:LOC

Variance Table:

<table>
<thead>
<tr>
<th>Trait</th>
<th>FAMILY:ALLELE_STATE</th>
<th>LOC</th>
<th>LOC:BLOCK</th>
<th>FAMILY:ALLELE_STATE:LOC</th>
<th>Residual</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-carotene</td>
<td>26.23</td>
<td>17.64</td>
<td>22.01</td>
<td>12.21</td>
<td>20.00</td>
</tr>
<tr>
<td>Total lycopene</td>
<td>34.34</td>
<td>45.43</td>
<td>47.50</td>
<td>24.47</td>
<td>40.00</td>
</tr>
<tr>
<td>All-trans-lycopene</td>
<td>30.49</td>
<td>47.50</td>
<td>75.18</td>
<td>18.84</td>
<td>60.00</td>
</tr>
<tr>
<td>All-cis-lycopene</td>
<td>24.82</td>
<td>16.55</td>
<td>16.84</td>
<td>16.32</td>
<td>80.00</td>
</tr>
<tr>
<td>Total soluble solids</td>
<td>73.77</td>
<td>70.11</td>
<td>45.59</td>
<td>43.59</td>
<td>100.00</td>
</tr>
<tr>
<td>Titratable acidity</td>
<td>12.21</td>
<td>24.47</td>
<td>16.89</td>
<td>39.55</td>
<td>43.21</td>
</tr>
<tr>
<td>Fruit Shape Index</td>
<td>45.43</td>
<td>43.59</td>
<td>39.52</td>
<td>34.53</td>
<td>120.00</td>
</tr>
<tr>
<td>External 1</td>
<td>26.23</td>
<td>70.11</td>
<td>16.55</td>
<td>18.84</td>
<td>16.32</td>
</tr>
<tr>
<td>Height mid width</td>
<td>34.34</td>
<td>45.59</td>
<td>45.59</td>
<td>39.58</td>
<td>43.21</td>
</tr>
<tr>
<td>Circular</td>
<td>30.49</td>
<td>47.50</td>
<td>75.18</td>
<td>16.32</td>
<td>80.00</td>
</tr>
</tbody>
</table>

% of variances: 73.77, 45.43, 47.50, 75.18, 12.21, 45.59, 43.59, 23.04, 43.21, 43.58
Do promoter sources affect fruit quality traits?

\[ Y = PROMOTER\_SOURCE + LOC + LOC\_BLOCK + PROMOTER\_SOURCE:LOC \]

<table>
<thead>
<tr>
<th>Traits</th>
<th>Significance</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\beta)-carotene</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Total lycopene</td>
<td>S</td>
<td>0.00004539***</td>
</tr>
<tr>
<td>All-cis-lycopene</td>
<td>S</td>
<td>0.002838**</td>
</tr>
<tr>
<td>All-trans-lycopene</td>
<td>S</td>
<td>0.00004446***</td>
</tr>
<tr>
<td>Total soluble solids</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Titratable acidity</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Fruit Shape Index External_1</td>
<td>S</td>
<td>0.013747*</td>
</tr>
<tr>
<td>Circular</td>
<td>S</td>
<td>0.014996*</td>
</tr>
<tr>
<td>Height mid width</td>
<td>S</td>
<td>0.000204***</td>
</tr>
</tbody>
</table>

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Total lycopene (moles/100 g)

- **Jaune Flamme**
- **Purdue 89-28-1**
- **97L97**

Promoters

- **a**
- **b**

Fruit Shape Index External_1

- **Purdue 89-28-1**
- **Jaune Flamme**
- **97L97**

Promoters

- **a**
- **b**

Circular

- **97L97**
- **Jaune Flamme**
- **Purdue 89-28-1**

Promoters

- **a**
- **b**

Height mid width

- **Jaune Flamme**
- **97L97**
- **Purdue 89-28-1**

Promoters

- **a**
- **b**
Hypothesis:

- Genes controlling sugar production are linked to the \( B \) gene.
- Lycopene \( \beta \)-cyclase might somehow affect the regulation of sugars in tomato.
- The carotenoid cleavage products affect the production of sugars.
Quantitative Descriptive Analysis® (QDA®) (Stone and Sidel, 1993)

Selection
Training:
- Language generation
- Reference standards
- Refinement of descriptors
- Ballot generation
- Reproducibility

Evaluation

(Lawless and Heymann, 2010)

<table>
<thead>
<tr>
<th>AROMA</th>
<th>Tomato-like intensity</th>
<th>647</th>
<th>489</th>
<th>285</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anemic</td>
<td>Intense</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Sensory Evaluation

- Tomato-like aroma*
- Explosiveness*
- Skin toughness
- Sourness
- Sweetness**
- Tomato-like flavor**
- Meltiness
- Chewiness
- Crunchiness*
- Bursting energy*
- Juiciness

Legend:
- Jaune Flamme
- Purdue 89-28-1
- 97L97
Conclusions

• Different $B$ allele introgressions have impacts on the several fruit quality parameters.
• Need a replication to confirm whether the total soluble solid production is correlated to the $B$ allele.
Acknowledgements

Advisor:
Dr. David Francis

Student Committee:
Dr. Joseph Scheerens
Dr. Leah McHale

Francis’ Lab:
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Scheerens Lab:
Lisa Robbins

Panelists
Thank you. Questions?
High Performance Liquid Chromatography (HPLC) Analysis Process
$B$ allele is a semi dominant gene

![Bar chart showing $\beta$-carotene concentration (mg/100 g fresh weight) for different alleles: BB (2.79), Bb (2.16), and bb (0.54).]

Data source: Orchard, 2014
<table>
<thead>
<tr>
<th>Forward</th>
<th>Reverse</th>
</tr>
</thead>
<tbody>
<tr>
<td>5’ CGTCTTAGGCTGGGTTAGTG 3’</td>
<td>5’AACAGGCACGGAAGACCTTA 3’</td>
</tr>
<tr>
<td>5’ CAAAGCCTGTGCCITCTC 3’</td>
<td>5’ TGTGCCAACTGCAAAATTA 3’</td>
</tr>
<tr>
<td>5’ AATATACCTGCCTCCATGC 3’</td>
<td>5’ GATGAAAAGGCTTGAGAAGA 3’</td>
</tr>
</tbody>
</table>

2. Sequence alignment
   Clustal Omega ([http://www.ebi.ac.uk/Tools/msa/clustalo/](http://www.ebi.ac.uk/Tools/msa/clustalo/))

3. Primer development ([http://bioinfo.ut.ee/primer3-0.4.0/](http://bioinfo.ut.ee/primer3-0.4.0/))
Methods of Sensory Evaluation

- Discriminative analysis
- Descriptive analysis
  - Flavor Profile®/Profile Attribute Analysis:
    Flavor intensities, order of perception, aftertaste, overall impression.
  - Texture Profile®
  - Sensory Spectrum®
  - Quantitative Descriptive Analysis®
### Mean separation of Brix

<table>
<thead>
<tr>
<th>trt</th>
<th>means</th>
<th>M</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 97L97BB</td>
<td>9.133333</td>
<td>a</td>
</tr>
<tr>
<td>2 puBB</td>
<td>8.666667</td>
<td>ab</td>
</tr>
<tr>
<td>3 jfBB</td>
<td>8.650000</td>
<td>ab</td>
</tr>
<tr>
<td>4 jfhhet</td>
<td>8.600000</td>
<td>ab</td>
</tr>
<tr>
<td>5 puhet</td>
<td>8.241667</td>
<td>b</td>
</tr>
<tr>
<td>6 97L97het</td>
<td>8.191667</td>
<td>b</td>
</tr>
<tr>
<td>7 Tainanbb</td>
<td>8.177273</td>
<td>b</td>
</tr>
</tbody>
</table>
(Fix the data!!!) Results from BC1S1 evaluation

Tomato Aroma Score Means

Explosiveness Score Means

Sweetness Score Means

Tomato Flavor Intensity Score Means

Fruit Bursting Energy Score Means

Crunchiness Score Means

Promoter sources

Promoter sources

Promoter sources

Promoter sources

Promoter sources

Promoter sources