Towards Understanding the Genetic Basis of Apple Acidity

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Fruit acidity greatly affects overall eating quality and flavor. The major organic acid in mature apple fruit is malic acid although citric, quinic and other acids are also detectable. Because of its importance in determining fruit flavor and quality, fruit acidity has been a subject of genetic investigations. An early inheritance study conducted on fruit acidity (Nybom 1959) based on pH measurements, reported several important findings: 1) Apple varieties can be categorized into two groups: an acid/sub-acid group with a fruit pH<3.8 and a sweet group of pH about or over 4.0. 2) The acid/sub-acid group is much more prevalent than the sweet group in cultivated apples. 3) The sweet flavor is determined by one recessive gene present in 80-90% of apple varieties studied. These findings were independently confirmed in later studies based on fruit pH (Visser and Verhaegh 1978) as well as malic acid concentration (Brown and Harvey 1971). The major gene governing fruit acidity was designated Ma with the Ma allele (an variant of the Ma gene) representing the dominant high and medium acid, and the ma allele for low acid (Visser and Verhaegh 1978).

The Ma gene has been mapped to the distal end of linkage group (syn. chromosome) 16 in a presumably Mama x Mama cross (‘Prima’ × ‘Fiesta’) (Maliepaard et al. 1998). In this study, fruit acidity was evaluated with pH indicator paper and the progeny were classified into two categories of three genotypes based on fruit color and aroma) over a six week period estimated based on fruit color and aroma) over a six week period.

Materials and Methods

Plant materials. An interspecific mapping population derived from a cross of ‘Royal Gala’ (M. × domestica) × PI 613988 (M. sieversii) was created at the USDA National Apple Germplasm Collection in 2002, and the seedlings were planted on their own roots in 2004 in an orchard in Geneva, New York, and were used in this study. This population, named GMAL 4595, has a total of 222 individuals, and 188 of them were previously used to construct the genetic maps of ‘Royal Gala’ and PI 613988 (Wang et al. 2011). The maternal parent ‘Royal Gala’ is a widely grown commercial variety, whereas the paternal parent PI 613988 was one of the elite M. sieversii clones collected from Kazakhstan (Forsline et al. 2003). PI 613988 bear fruits of size close to cultivated apples (http://www.ars-grin.gov/cgi-bin/npgs/acc/display.pl?1531529). Fruiting in the population was first recorded on a few trees in 2006 and most of the trees have fruited since 2008.

Evaluation of fruit pH and titratable acidity (TA). Ten fruits were randomly harvested for each genotype at maturity (initially estimated based on fruit color and aroma) over a six week period from August 9 through September 20 in 2010. Fruits were stored overnight at 4°C and processed for fruit juice extraction on the day following harvest. To obtain juice from fruits with relatively uniform ripening stages, fruits were cross sliced into two halves. One half was used for maturity evaluation and the other was processed for fruit juice extraction. The evaluation of fruit maturity was conducted by dipping the cut side of apples in an iodine solution (2.2 gm of I₂ plus 8.8 gm of KI per liter) for 1 min and then rating from 1 (most immature) to 8 (over mature) according to the Cornell Starch Index (Blanpied and Silsby 1992). Fruit at stages 4 through 6, a common indicator for mature apples, were selected correspondingly in the second set of halves, resulting in 5-10 fruits (in halves) per genotype for fruit juice extraction. In cases where most fruits in a sample were harvested prematurely, the samples were re-taken at a later time when there were at least five or more mature fruits. The selected fruit halves were pooled and blended using a household food proces-
suggesting a bimodal distribution for fruit pH. The boundary between
the lower pH range for the sweet genotypes, and the other in the higher pH range representing most of the population of high and medium acid
variation in both pH and TA in the GMAL 4595 population (Figure 1).

Results

Segregation of fruit pH and titratable acidity. 190 of the 222 trees in the GMAL 4595 population bore fruits and were evaluated for fruit pH and TA. The population mean of fruit pH was $3.49\pm0.46$, ranging from 2.76 to 4.65. The average TA showed $7.14\pm3.74$ mg/ml, varying between 1.08 and 18.68. This is a considerable range of variation in both pH and TA in the GMAL 4595 population (Figure 1).

There were two peaks in the pH distribution: one in the lower pH range and the other in the higher pH range for the sweet genotypes, suggesting a bimodal distribution for fruit pH. The boundary between the low and high/medium pH seemed to be around pH 3.80 as there were no fruits in the pH 3.81-3.90 range (Figure 1). Among the 190 fruiting trees, there are 144 and 46 trees of low and high/medium fruit pH, respectively, a segregation pattern fitting the ratio of 3:1 ($P_{(df=1, X^2=0.344)} = 0.56$). This suggested that there is a complete dominance gene, presumably $Ma$ designated previously for fruit pH (Maliepaard et al. 1998; Nybom 1959), in controlling fruit pH.

The distribution of TA revealed a group of low acidity with TA <3.00 mg/ml that correspond to the high pH genotypes (Figure 1, Figure 2). However, there appeared to have two overlapping groups in high/medium acid range as there were significantly less genotypes with TA 8.01-9.00 mg/ml. Regression analysis demonstrated that fruit TA and fruit pH were highly correlated and are predictable with a polynomial function of order 3 ($r^2=0.8827$, Figure 2). Together, these data suggested that fruit pH and TA contents were under a control of the same major gene $Ma$; but for TA, the dominance of $Ma$ over $ma$ is incomplete and both additive and dominance effects of the $Ma$ allele appeared to be strong.

QTL analyses of fruit pH and TA. A major QTL, presumably the $Ma$ locus, was detected for both fruit pH and TA on linkage group 16 (Figure 3 and Table 1). Interval mapping in 'Royal Gala' found the $Ma$ QTL peaked at or around marker CH05c06 and was supported with LOD scores of 18.34 and 19.82 and explained 41.7% and 42.3% for fruit pH and TA variation, respectively (Figure 3a). In the Kruskal-Wallis analyses, the $Ma$ QTL is supported with highly significant ($P<0.0001$) values of the K statistic, 69.2 for pH and 68.6 for TA (Table 1). In $M. sieversii$ PI 613988, the $Ma$ QTL was detected near marker CH05c06 on linkage group 16. However, the peak of the QTL was located in the 8.2 cM (centi-Morgans) interval between markers CH02a03 and CH05c06 (Figure 3b), and the $Ma$ QTL was associated with lower LOD scores (10.35 for pH, 6.13 for TA), a lower percentage of variance explained (28.3% for pH, 17.0% for TA) and explained 41.7% and 42.3% for fruit pH and TA variation, respectively (Figure 3a). In the Kruskal-Wallis analyses, the $Ma$ QTL is supported with highly significant ($P<0.0001$) values of the K statistic, 69.2 for pH and 68.6 for TA (Table 1).

In addition to the $Ma$ locus, two minor QTLs, tentatively designated $M2$ and $M3$, were detected for fruit pH and TA based on the Kruskal-Wallis analyses (Table 1). $M2$ was represented by marker C12360 ($K=14.1$ and 10.1, $P<0.0005$ and 0.001, LOD=1.59 and 2.32, percentage variance explained=4.3% and 6.2% for pH and TA, respectively) on linkage group 6 of 'Royal Gala'. $M3$ was represented
Table 1. QTLs and their associated parameters detected for apple juice pH and TA (titratable acidity) in the GMAL 4595 population

<table>
<thead>
<tr>
<th>Trait</th>
<th>QTL</th>
<th>Parental Map</th>
<th>LG</th>
<th>Marker</th>
<th>Position (cM)</th>
<th>Ka</th>
<th>Significance levels</th>
<th>QTL Peak position (cM)</th>
<th>QTL most closely linked/ flanking markers</th>
<th>LOD</th>
<th>Significant LOD thresholds (Chr/Genome)</th>
<th>Variance explained (%)</th>
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<tr>
<td>pH</td>
<td>Ma (pH)</td>
<td>Royal Gala</td>
<td>16</td>
<td>CH05c06</td>
<td>0.0</td>
<td>69.2</td>
<td>0.0001</td>
<td>2.0</td>
<td>CH05c06-C13393 (0.0-16.8cM)</td>
<td>18.34</td>
<td>2.3/3.4</td>
<td>41.7</td>
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<tr>
<td></td>
<td>Ma (pH)</td>
<td>PI 613988</td>
<td>16</td>
<td>CH05c06</td>
<td>12.9</td>
<td>20.8</td>
<td>0.0001</td>
<td>9.7</td>
<td>CH02a03-CH05c06 (4.7-12.9 cM)</td>
<td>10.35</td>
<td>2.5/3.8</td>
<td>28.3</td>
</tr>
<tr>
<td></td>
<td>M2 (pH)</td>
<td>Royal Gala</td>
<td>6</td>
<td>C12360</td>
<td>2.8</td>
<td>14.1</td>
<td>0.0005</td>
<td>2.8</td>
<td>C12360</td>
<td>1.59</td>
<td>2.4/3.4</td>
<td>4.3</td>
</tr>
<tr>
<td></td>
<td>M3 (pH)</td>
<td>PI 613988</td>
<td>1</td>
<td>C12063</td>
<td>13.6</td>
<td>11.1</td>
<td>0.001</td>
<td>13.6</td>
<td>C12063</td>
<td>0.80</td>
<td>2.3/3.8</td>
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<tr>
<td>TA</td>
<td>Ma (TA)</td>
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<td>CH05c06</td>
<td>0.0</td>
<td>68.6</td>
<td>0.0001</td>
<td>0.0</td>
<td>CH05c06</td>
<td>19.82</td>
<td>2.2/3.4</td>
<td>42.3</td>
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<td></td>
<td>Ma (TA)</td>
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<td>CH05c06</td>
<td>12.9</td>
<td>18.8</td>
<td>0.0001</td>
<td>10.7</td>
<td>CH02a03-CH05c06 (4.7-12.9 cM)</td>
<td>6.13</td>
<td>2.6/3.5</td>
<td>17.0</td>
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<td>Royal Gala</td>
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<td>8.0</td>
<td>0.005</td>
<td>13.6</td>
<td>C12063</td>
<td>1.59</td>
<td>2.4/3.5</td>
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</table>

K: the highest value of the K statistic obtained in the Kruskal–Wallis (KW) rank-sum test. bP=0.01

Discussion

Segregation of fruit acidity and the genetic effect of Ma. There was not a single genotype with fruit pH 3.81-3.90 in the interspecific GMAL 4595 population while there were two peaks of distribution of pH ranges 3.21-3.30 and 4.21-4.30, suggesting that classification of fruits with pH values higher than 3.80 into low acid is rational. Apples with pH>3.8 are commonly considered to be low acid or sweet of the Mama genotype (Mallepaard et al. 1998; Nybom 1959; Visser and Verhaegh 1978). Consistent with these findings, the segregation ratio of the high/medium acid (pH<3.80) to the low acid groups fits the 3:1 ratio in the GMAL 4595 population, indicating that the parents are all of the Mama genotype (Figure 1).

Within the dominance of the high/medium acid class (TA>3.0 mg/ml), there appeared to be two overlapping classes for TA (Figure 1), suggesting that both additive and dominance genetic effects of Ma strongly influenced the variation of fruit acidity in the GMAL 4595 population.
population. It is known that the *Mana* genotype has selection advantage over *MaMa* and *mama* as most apple varieties are heterozygous at the *Ma* locus. The two markers CH02a03 and CH05c06 flanking the *Ma* QTL may be useful for selection of seedlings of the *Mana* genotype.

**QTL analyses of fruit acidity.** QTL analyses of fruit acidity measured with both pH and TA in this study identified a major QTL, i.e. the *Ma* locus on linkage group 16, and two minor QTLs on linkage groups 6 (*Royal Gala*) and 1 (PI 613988). Detection of the major QTL of *Ma* appears consistent with previous studies (Kenis et al. 2008; Liebhard et al. 2003). The peak of the major QTL of *Ma* was located between markers CH02a03 and CH05c06 in PI 613988. The CH02a03-CH05c06 interval was best compared with the interval between markers CH05e04z and CH05c06 in 'Telamon', where a major fruit acidity QTL was detected although the same interval was inverted in 'Braeburn' (Kenis et al. 2008). Except for the *Ma* locus, there were no common QTLs detected for fruit acidity among the crosses studied to date. Notably, another major QTL for fruit acidity on linkage group 8 (Liebhard et al. 2003), was neither detected in 'Telamon' × 'Braeburn' (Kenis et al. 2008), nor in our GMAL 4595 population.

This QTL study was conducted with only one-year of fruit pH and TA data. Although a similar previous study was conducted for fruit acidity (Liebhard et al. 2003), the variability of fruit acid between years and between individual fruits of the same genotype could still be a concern. Nevertheless, a study addressing such variations between years concluded that the relative acidity trend in 17 cultivars evaluated remained “much the same” between years while fruit malic acid contents varied slightly (Brown and Harvey 1971). The same study also found that the variation in individual fruits harvested from different positions for a given cultivar was not comparable with that observed between different cultivars, and suggested “samples from the mixed juice from a few fruits can be relied upon to give a reasonably accurate figure for the cultivar.” This method of bulkling of several juice samples was also used in Kenis et al. (2008) for fruit acidity QTL study.

**Conclusion**

The *Ma* locus has been shown to be the primary genetic factor in determining fruit pH and TA in both ‘Royal Gala’ and the *M. sieversii* PI 613988. In addition, there are two minor QTLs detected for fruit TA and pH with one on linkage group 6 specific to ‘Royal Gala’ and the other on linkage group 1 to PI 613988. The variations of fruit acidity in the GMAL 4595 population are better explained by the additive-dominance gene action of allele *Ma* as it has a strong additive effect in increasing fruit acidity and is incompletely dominant over *ma*. The SSR markers CH02a03 and CH05c06 flanking the *Ma* QTL would be useful in marker assisted breeding in apple.

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**References**


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