Quantitative Trait Loci for Total Soluble Solids in Different Melon Crosses under Greenhouse and Field Environments

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ABSTRACT

Our purpose was to identify randomly amplified polymorphic DNA (RAPD) markers associated with quantitative trait loci (QTL) for total soluble solids (TSS) using bulked segregant analysis in an F₂ population from the melon (Cucumis melo L.) cross of ‘TAM Dulce’ (high TSS) x TGR1551 (low TSS) in a greenhouse experiment. Additionally, we attempted to confirm the associations of RAPD markers with QTL for the fruit sweetness trait in an F₂ population from the different cross of ‘Deltex’ (high TSS) x TGR1551 in a field experiment. Five RAPD markers were detected to be significantly associated with QTL for TSS in the F₂ population of the ‘TAM Dulce’ x TGR1551 cross in the greenhouse based on simple linear regression. Three unlinked markers associated with QTL were significant in a stepwise multiple regression analysis where the full model explained 19% of the total phenotypic variation for TSS. Two RAPD markers were confirmed in the F₂ population of the ‘Deltex’ x TGR1551 cross in the field to be consistently associated with QTL for the sweetness trait. A significant association of andromonoecious (a) with TSS was consistently expressed in our genetic populations under greenhouse and field environments. These RAPD and a markers associated with QTL for the fruit sweetness trait identified in the greenhouse and confirmed in the field here could be useful in melon breeding for improving the mature fruit sweetness.

Additional Index Words: Cucumis melo, brix, randomly amplified polymorphic DNA markers, andromonoecious

Due to consumer preference for sweet fruit (Lester and Shellie, 1992), sugar content is a highly important quality trait of different market melon classes (United States Federal Government, 1990). The improvement of sugar content is one of the most significant goals in breeding programs of most melon types worldwide. Total soluble solids are regarded as a major factor in determining mature melon fruit sweetness, and quantitatively inherited (Park et al., 2004b). Total soluble solids were found to be positively correlated with several melon traits such as sucrose (Park et al., 2004b), overall consumer preference, sweetness, flavor, texture (Lester and Shellie, 1992), and maturation period (Welles and Buitelaar, 1988). Kalb and Davis (1984) calculated a moderately high narrow sense heritability for TSS in a melon population.

Bulked segregant analysis (Michelmore et al., 1991) is an efficient method to rapidly identify molecular markers linked to a specific gene using DNA bulks from F₂ plants. This technique, along with RAPDs, has been used in melons to tag the single recessive ms-3 gene controlling male sterility (Park et al., 2004c) as well as single genes for disease and pest resistance such as the dominant Fom 2 gene for resistance to fusarium wilt (Wechter et al., 1995), the dominant Vat gene for resistance to melon aphid (Klingler et al., 2001), and the recessive nsv gene for resistance to the Carmovirus melon necrotic spot virus (Morales et al., 2002). Also, it has been applied in identifying RAPD markers associated with quantitative genes for mature melon fruit sweetness, size, and shape traits (Park and Crosby, 2004; Park et al., 2004b).

Molecular mapping of QTL for TSS has been reported in several horticultural crops such as tomato (Causse et al., 2002), peach (Etienne et al., 2002), and watermelon (Hashizume et al., 2003). Those markers linked to QTL for the sweetness trait may improve the breeder’s ability to recover high sugar genotypes and aid in the development of high sugar elite cultivars. However, markers associated with QTL affecting TSS present in ‘TAM Dulce’, a western shipper muskmelon type, have not been reported.

Our objective in the current study was to identify RAPD and andromonoecious (a) markers associated with QTL controlling TSS using bulked segregant analysis in an F₂ population derived from the melon cross of ‘TAM Dulce’ (high TSS) x TGR1551 (low TSS) in a greenhouse experiment. Park et al. (1999) emphasized the importance of confirming the marker-QTL associations in different populations and environments before using molecular markers for marker-assisted selection in breeding programs. Thus, our additional goal was to confirm the associations of RAPD and a markers with QTL for the fruit sweetness trait in an F₂ population from the different cross of ‘Deltex’ (high TSS) x TGR1551 in a field experiment.

MATERIALS AND METHODS

Plant Material. For identification of QTL for TSS, 105 F₂ plants derived from the melon cross of ‘TAM Dulce’ x TGR1551 were planted in a greenhouse at the Texas Agricultural Experiment Station-Weslaco on 15 Oct. 2002. The ‘TAM Dulce’ parent is a western shipper muskmelon type with high fruit quality, while the TGR1551 parent, originally obtained from Zimbabwe, is a wild agrestis type with low fruit quality. One plant was grown per
11 L pot containing soil-less media, Sunshine Mix #4 (Sun Gro Horticulture Inc., Bellevue, Wash.). Peters 20N-8.7P-16.6K water soluble fertilizer (Scotts, Marysville, Ohio) was applied weekly. Pesticides were applied as needed to control diseases. Approximate day/night greenhouse temperatures were 25±3/22±2 °C. Day lengths ranged from 12 to 10 h light in this experiment.

For confirmation of the marker-QTL associations, 64 F2 plants from the cross of ‘Deltex’ x TGR1551 were planted on black plastic mulch with drip irrigation on sandy clay loam soil at Weslaco, Texas on 10 Mar. 2002. The high sugar ‘Deltex’ parent is a commercial ananas cultivar (Nunhems, Parma, Idaho). Single bed length and width were 31.7 m and 1.0 m, respectively, with 30.5 cm spacing between plants and 1.0 m between beds. A fertilizer containing 5N-11.3P-27.4K (Wilbur Ellis, Edinburg, Texas) was applied weekly. Irrigation was supplied as needed. Approximate day/night field temperatures were 30±3/23±3 °C. Day lengths ranged from 12 to 14 h light.

Data Collection. Data for TSS were obtained from the two parental pairs as well as the 105 and 64 F2 plants using a temperature corrected refractometer with digital readout (Reichert Scientific Instruments, Buffalo, N.Y.). Stamen presence and absence in female flowers were checked thrice on all F2 plants and parents at different times during flowering.

RAPD. Fully expanded leaves of the 105 and 64 F2 plants along with their parental pairs were collected at 21 days after planting. Total genomic DNA was extracted from the leaf tissue using the method of Skroch and Nienhuis (1995). A total of 500 random 10-mer primers (Operon Technologies, Alameda, Calif.) were used for the RAPD analysis (Williams et al., 1990). Polymerase chain reactions (PCR) were performed on 96-well plates in a MJ Research thermalcycler (model PTC-0100; MJ Research, Waltham, Mass.). Protocols for PCR and the composition of the final volume of reactants were the same as those described by Skroch and Nienhuis (1995). A 100-base pair (bp) DNA ladder (Life Technologies, Grand Island, N.Y.) was used to estimate the length of RAPD markers. The name of each RAPD marker is derived from an “O” prefix for Operon primers, the letters identifying the Operon kit, Operon primer number, and the approximate length (bp) of the marker (Park et al., 2004a).

Bulked Segregant Analysis. Two low and high bulks were prepared from equal volumes of standardized DNA (10 ng μL-1) from eight selected F2 plants of the ‘TAM Dulce’ x TGR1551 cross with the highest and lowest TSS values, respectively. The 500 primers were used to simultaneously screen between the low and high DNA bulks, and between the parents ‘TAM Dulce’ and TGR1551. Primers that generated marker polymorphisms between the low and high DNA bulks were tested in the F2 population from the cross between ‘TAM Dulce’ and TGR1551 for identifying QTL. Four primers were tested in the F2 population of the ‘Deltex’ x TGR1551 cross for confirming the RAPD marker-QTL associations.

Linkage Analysis. To detect segregation distortion of markers and andromonoecious, F2 population marker data were tested for goodness-of-fit to a 3:1 ratio using the chi-square test. Due to the dominant nature of RAPD markers, the linkage analyses of two markers obtained from ‘TAM Dulce’ and four markers including a obtained from TGR1551 were separately performed on the data for 105 F2 plants of the ‘TAM Dulce’ x TGR1551 cross using MAPMAKER version 3.0 (Lander et al., 1987). We also executed separately the linkage analyses of two markers from ‘Deltex’ and two markers from TGR1551 on the data for 64 F2 plants of the ‘Deltex’ x TGR1551 cross.

Detection of QTL. Simple linear regression, for each pairwise combination of quantitative traits and marker loci, was used to analyze the greenhouse and field data for detection and confirmation of QTL for TSS. Significant differences in trait associations were based on F-tests (P<0.05) (Edwards et al., 1987). Loci with the lowest P value per QTL were chosen and added in a stepwise multiple regression to select the best set of markers (P<0.05) for prediction of the total trait phenotypic variation.

Figure 1. Frequency distributions for total soluble solids (%) of F2 plants derived from two melon crosses of ‘TAM Dulce’ (high total soluble solids) x TGR1551 (low total soluble solids) and ‘Deltex’ (high total soluble solids) x TGR1551 in greenhouse and field experiments.
Table 1. Pearson correlations of total soluble solids (TSS) with other fruit sweetness traits in two F2 populations derived from melon crosses of ‘TAM Dulce’ x TGR1551 (TT) and ‘Deltex’ x TGR1551 (DT) in greenhouse and field experiments.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Cross</th>
<th>Sucrose</th>
<th>Glucose</th>
<th>Fructose</th>
<th>SPTS*</th>
<th>GPTS</th>
<th>FPTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>TSS</td>
<td>TT</td>
<td>0.66**</td>
<td>0.90**</td>
<td>0.96**</td>
<td>0.33**</td>
<td>-0.16NS</td>
<td>-0.37**</td>
</tr>
<tr>
<td></td>
<td>DT</td>
<td>0.57**</td>
<td>-0.20NS</td>
<td>-0.08NS</td>
<td>0.53**</td>
<td>-0.52**</td>
<td>-0.48**</td>
</tr>
</tbody>
</table>

*SPTS=sucrose percentage of total sugars, GPTS=glucose percentage of total sugars, FPTS=fructose percentage of total sugars.

NS,**Nonsignificant or significant at P < 0.01.

explained by the identified QTL (Paterson et al., 1991). Pearson correlations of mature fruit sweetness traits were also determined in our populations. All statistical analyses were conducted using the Statistical Analysis System (SAS Inst., Cary, N.C.).

RESULTS AND DISCUSSION

Frequency Distribution. A clear separation was observed for TSS content between ‘TAM Dulce’ and TGR1551 in the greenhouse experiment (Fig. 1). Mature fruits of ‘TAM Dulce’ had high TSS (9.1-12.5%) content. In contrast, those of TGR1551 possessed low TSS (3.8-5.8%) content. Fruits of all F1 plants derived from the ‘TAM Dulce’ x TGR1551 cross had intermediate TSS content (Fig. 1). A continuous frequency distribution for TSS content was noted for F2 plants of the ‘TAM Dulce’ x TGR1551 cross in the greenhouse (Fig. 1), indicating that the fruit sweetness trait was quantitatively inherited. In addition, a continuous distribution for the sweetness trait was observed for F2 plants of the ‘Deltex’ x TGR1551 cross in the field experiment (Fig. 1) consistent with the quantitative inheritance for the trait. The quantitative inheritance pattern of TSS concentration in this study was similar to that reported previously in other horticultural crops (Causse et al., 2002; Etienne et al., 2002; Georgelis et al., 2004; Hashizume et al., 2003; Paterson et al., 1991) as well as melon (Burger et al., 2002). Low (0.23), intermediate (0.63-0.66), and high (0.75) estimates of narrow sense heritability for TSS were reported in several genetically different melon populations (Burger et al., 2002; Kalb and Davis, 1984).

Correlation. Total soluble solids were significantly and positively correlated with sucrose and sucrose percentage of total sugars in the two F2 populations in the greenhouse and field experiments (Table 1), indicating that selection of the three fruit sweetness traits is feasible. The positive correlation between TSS and sucrose was nearly the same as that reported previously in peach by Etienne et al. (2002). A significant negative correlation of TSS with fructose percentage of total sugars was found in the two genetic populations.

Identification of QTL. A total of 500 primers were used for the RAPD analysis of two different bulks for TSS developed from selected greenhouse F2 plants with low and high values along with their parents ‘TAM Dulce’ and TGR1551. Five RAPD markers were polymorphic between the two different DNA bulks. Two displayed an amplified DNA fragment in the high bulk that was absent in the low bulk. Three showed an amplified DNA fragment in the low bulk that was absent in the high bulk. An example of marker OAS03.450 is shown in Fig. 2. These five marker fragments segregated in the F2 population of the cross ‘TAM Dulce’ x TGR1551. A goodness-of-fit to a 3:1 ratio for band presence to band absence for each of the five markers was observed in 105 F2 plants (Table 2). These markers were unlinked based on linkage analysis. A total of six genetic markers including five RAPDs and andromonoecious were identified to be significantly associated with QTL affecting TSS content in the F2 population of the ‘TAM Dulce’ x TGR1551 cross in the greenhouse experiment based on simple linear regression (Table 3). Two markers were amplified from ‘TAM Dulce’, while four markers were generated from TGR1551. The high TSS parent ‘TAM Dulce’ contributed high TSS alleles for these six markers. Markers OAW06.1250 from ‘TAM Dulce’ and OAS03.450 from TGR1551 explained 9% and 8% of the variation for the TSS content, respectively. Also, OAT03.250, OAP03.800, OAW06.600, and the a locus accounted...
Table 2. Chi-square analyses for segregation of five RAPD markers and andromonoecious (a) associated with total soluble solids in two F2 populations derived from melon crosses of ‘TAM Dulce’ x TGR1551 (TT) and ‘Deltex’ x TGR1551 (DT).

<table>
<thead>
<tr>
<th>Marker</th>
<th>Source</th>
<th>TT plants (no.)</th>
<th>DT plants (no.)</th>
<th>Presence</th>
<th>Absence</th>
<th>Ratio</th>
<th>$\chi^2$</th>
<th>P</th>
<th>Presence</th>
<th>Absence</th>
<th>Ratio</th>
<th>$\chi^2$</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>OAW06.1250</td>
<td>TAM Dulce &amp; Deltex</td>
<td>80</td>
<td>25</td>
<td>3:1</td>
<td>0.02</td>
<td>0.87</td>
<td></td>
<td></td>
<td>55</td>
<td>9</td>
<td>3:1</td>
<td>3.52</td>
<td>0.06</td>
</tr>
<tr>
<td>OAT03.250</td>
<td>TAM Dulce &amp; Deltex</td>
<td>75</td>
<td>30</td>
<td>3:1</td>
<td>0.53</td>
<td>0.46</td>
<td></td>
<td></td>
<td>44</td>
<td>20</td>
<td>3:1</td>
<td>1.01</td>
<td>0.31</td>
</tr>
<tr>
<td>OAP03.800</td>
<td>TGR1551 &amp; Deltex</td>
<td>81</td>
<td>24</td>
<td>3:1</td>
<td>0.15</td>
<td>0.69</td>
<td></td>
<td></td>
<td>44</td>
<td>20</td>
<td>3:1</td>
<td>1.01</td>
<td>0.31</td>
</tr>
<tr>
<td>OAS03.450</td>
<td>TGR1551</td>
<td>70</td>
<td>35</td>
<td>3:1</td>
<td>3.45</td>
<td>0.06</td>
<td></td>
<td></td>
<td>44</td>
<td>20</td>
<td>3:1</td>
<td>3.52</td>
<td>0.06</td>
</tr>
<tr>
<td>OAW06.600</td>
<td>TGR1551 &amp; Deltex</td>
<td>80</td>
<td>25</td>
<td>3:1</td>
<td>0.02</td>
<td>0.87</td>
<td></td>
<td></td>
<td>44</td>
<td>20</td>
<td>3:1</td>
<td>1.01</td>
<td>0.31</td>
</tr>
<tr>
<td>a</td>
<td>TGR1551</td>
<td>74</td>
<td>31</td>
<td>3:1</td>
<td>0.91</td>
<td>0.34</td>
<td></td>
<td></td>
<td>44</td>
<td>20</td>
<td>3:1</td>
<td>3.52</td>
<td>0.06</td>
</tr>
</tbody>
</table>

No segregation of OAP03.800 and OAW06.600 in the DT F2 population.

Table 3. Simple linear regression (SLR) and stepwise multiple regression (SMR) analyses of marker and data for detection of QTL for total soluble solids in an F2 population derived from the melon cross of ‘TAM Dulce’ (high total soluble solids) x TGR1551 (low total soluble solids) in the greenhouse experiment.

<table>
<thead>
<tr>
<th>RAPD marker</th>
<th>Source</th>
<th>SLR Average value</th>
<th>SMR Average value</th>
</tr>
</thead>
<tbody>
<tr>
<td>OAW06.1250</td>
<td>TAM Dulce</td>
<td>0.001  9 6.8</td>
<td>0.001  9</td>
</tr>
<tr>
<td>OAT03.250</td>
<td>TAM Dulce</td>
<td>0.029  5 6.7</td>
<td>0.014  5</td>
</tr>
<tr>
<td>OAS03.450</td>
<td>TGR1551</td>
<td>0.004  8 6.1</td>
<td>0.004  8</td>
</tr>
<tr>
<td>OAP03.800</td>
<td>TGR1551</td>
<td>0.005  7 6.1</td>
<td>0.011  6</td>
</tr>
<tr>
<td>OAW06.600</td>
<td>TGR1551</td>
<td>0.010  6 6.1</td>
<td>0.011  5</td>
</tr>
<tr>
<td>a</td>
<td>TGR1551</td>
<td>0.044  4 6.2</td>
<td>Cumulative $R^2 = 19$</td>
</tr>
</tbody>
</table>

An average value of F2 plants with band presence for the marker.

An average value of F2 plants with band absence for the marker.

Andromonoecious.
on the basis of simple linear regression (Table 4). Marker OAS03.450 obtained from TGR1551, associated with QTL for the fruit sweetness trait in the greenhouse population, was also found to be significantly associated with QTL for the sweetness trait in the field population, and accounted for 17% of the phenotypic variation for the trait. A significant association of OAW06.1250 from ‘Deltex’ with QTL for the fruit sweetness trait was consistently expressed in our populations derived from two different crosses under greenhouse and field environments. The andromonoecious locus on linkage group 4 of the classical melon map regulating stamen absence or stamen presence in female flowers was also significantly associated with the sweetness trait in the field, and explained 18% of the TSS variation. However, marker OAT03.250 from ‘Deltex’, slightly associated with the TSS content identified in the greenhouse F2 population, was not confirmed in this field F2 population. The RAPD and a markers linked to the TSS QTL identified and confirmed in two populations and environments in this study should be more reliable for marker-assisted selection than those evaluated in a single population and environment.

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**LITERATURE CITED**


