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Use of Molecular Markers to Locate Quantitative Trait Loci Linked to High Soluble Solids Content in a Hybrid of *Lycopersicon cheesmanii*

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Abstract. An **interspecific hybrid was made between an accession of *Lycopersicon cheesmanii* f. *minor Riley (LA 1508)* from the Galapagos Islands, Ecuador, and *L. pennellii* (Corr.) D'Arcy (LA 716). LA 1508 was used because of its high soluble solids content (SSC). It was crossed with LA 716 to test for linkage between isozymes and morphological markers and loci conditioning high SSC. For both accessions, chromosome numbers are equal and there are large differences between SSC and no barriers to crossing. Modified BC_1 populations derived from the hybridization were assayed for isozyme markers using starch gel electrophoresis. Associations between marker loci and quantitative-trait loci (QTL) conditioning high SSC were determined using analysis of variance. Six isozymes located on five chromosomes and one morphological marker had significant associations with SSC, indicating linkage to QTL. Digenic epistatic interactions between pairs of independent markers did not appear to play an important role in the interactions between QTL that condition SSC.**

Many important genetic traits in the tomato *(Lycopersicon* sp.) are quantitatively inherited. The continuously varying characteristic of SSC in processing tomatoes is of particular interest to geneticists, plant breeders, and processors (Rick, 1974). Increasing SSC in the tomato fruit would decrease the energy inputs required to produce tomato products. Knowledge of the number and location of genes or clusters of genes would be invaluable to plant breeders, since it would permit investigators to choose efficient breeding strategies for introgressing high SSC genes.

Tomato has many mapped genetic markers that can be used to locate QTL (Ibarbia and Lambeth, 1969; Lander and Botstein, 1989; Tanksley and Hewitt, 1988; Tanksley and Rick, 1980; Tanksley et al., 1981). *Lycopersicon cheesmanii* f. *minor (minor)* is a useful source of variation for high SSC (Garvey and Hewitt, 1984). Furthermore, a survey by Rick and Fobes (1975a) indicated the *minor* is polymorphic for isozyme loci when compared to the isozyme marker stock *L. pennellii LA* 716 'Atico'.

The present study was undertaken to detect associations between QTL conditioning SSC and segregating isozyme loci and to identify higher-order interactions between QTL linked to isozyme markers.

Materials and Methods

Interspecific hybridizations. A cross was made between *L. cheesmanii* LA 1508 (female parent SSC X = 13.16) and *L. pennellii* 'Atico' LA 716 (SSC X= 6.84). F_1 plants were crossed to *L. esculentum* Mill (SSC X = 5.86) VF 36 LA 490 (used as a female parent) to create modified backcross populations (MBC_1). Seeds were provided by C.M. Rick. The introduction of LA 490 as the recurrent parent increases the progeny vigor while maintaining both the differences in SSC and the high level of polymorphism seen between LA 1508 and LA 716 (Rick and Fobes, 1975b).

Plant culture. MBC_1 seeds were stratified (2.5% sodium hypochlorite), sown in modified UC mix (Matkin and Chandler, 1957) with controls, and germinated in a greenhouse kept at 24/18C (day/night). One hundred ninety-eight 4-cm MBC_1 and 16 control plants were transplanted into a 1 sand : 1 modified UC mix medium and fertilizer (16N–20P–16K) and grown under fluorescent lighting (350 $\mu mol \cdot s^{-1} \cdot m^{-2}$) on a 12-h day/night regime for 10 days. Leaf tissue from the second true leaf was ground in a 3% reduced glutathione (Sigma, St. Louis) buffer solution. Samples were drawn onto 8 × 3-mm Whatman (Maidstone, England) No. 3 paper wicks for electrophoresis. Root samples were ground using no buffer, and plants were repotted to 3.8-liter (180 mm top diameter) containers. Plants were watered, fertilized, and spaced in such a way as to prevent mutual shading.

Reestablished plants were pollinated using LA 716 pollen. Five fruit samples per plant were evaluated for SSC on a B&L model 3100 refractometer (Bausch and Lomb, Rochester, N.Y.).

Electrophoretic analysis. Leaf and root samples from 198 MBC_1 plants and 16 controls were genotyped using Tris·citrate and histidine-free base horizontal starch gel electrophoresis (Tanksley and Rick, 1980; Tanksley et al., 1982). Genotypes of plants were determined for 12 isozymes and two morphological markers *(pts,* chromosome 6, and *sym,* chromosome location unknown, Table 1). Four linkage groups had no markers and could not be tested.

Statistical treatments. Chi-square analysis detected deviations from expected Mendelian ratios for the MBC_1. One-way analysis of variance using Anova-1 of SPSS (SPSSX, Chicago) tested the hypothesis that the means of the genotype of the dependent variable, SSC, were equal. Significant F ($P \leq 0.01$ or 0.05) values were interpreted to indicate segregation of genotypes at a QTL that was linked to an isozyme locus. Univariate analysis and Levene's test of equal variances using the procedures of SAS (SAS, 1985) were performed to validate the assumptions of the analysis of variance. Multiple classification analysis using

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Abbreviations: SSC, soluble solids content; QTL, quantitative-trait loci.

Table 1. Chromosome location, genotype means, and analysis of variation for effects of introgressed segments on tomato fruit SSC in the MBC_1 population.

Chromosome	Isozyme locus	Mean soluble solids values +/c[z]	Mean soluble solids values +/p	Significance of F values
1	*PRX-1*	9.80	9.84	0.835
1	*SKDH-1*	9.81	9.81	0.990
2	*PRX-2*	10.26	9.28	0.000**
2	*EST-157*	10.10	9.56	0.007**
3	*PRX-7*	9.46	10.11	0.000**
6	*GOT-2*	9.58	10.02	0.030*
6	*PTS*	9.73	9.93	0.295
7	*APS-1*	9.77	9.85	0.675
8	*APS-2*	9.66	10.22	0.049*
10	*PRX-4*	9.78	9.82	0.822
12	*ACO-1*	9.05	9.21	0.766
12	*6PGDH-2*	9.75	9.82	0.739
12	*PGI-1*	9.6	9.98	0.037*
?	*SYM*	10.10	9.47	0.001**

[z]Originating from *Lycopersicon esculentum* (+), *L. cheesmanii (c),* or *L. pennellii* (p).
*,**Significance at $P = 0.05$ and 0.01, respectively.

Table 2. Detected deviations from expected Mendelain ratios for isozyme markers of tomato in the MBC_1 population (Yates adjusted chi square).

Isozyrne marker locus	Adjusted x^2
ACO-I	0.00
GOT-2	16.66*
PGI-1	3.72
APS-1	0.00
APS-2	3.68
PRY-1	0.66
PRX-2	0.75
PRX-4	11.25*
PRX-7	4.9*
EST-157	9.97*
6PGDH-2	3.92*
SKDH-1	*2.8*
PTS	1.31
SYM	*2.73*

*Significant at $P = 0.05$.

MCA and SPSSX (SPSSX, 1983) determined the multiple correlation coefficient *R,* which, when squared (MR^2), indicated the partial variance contributed by the dependent variable (SSC) (Steele and Torrie, 1960). A two-way analysis of variance using Anova-2 of SPSSX (SPSSX, 1983) tested for significant interactions between pair-wise combinations of genotypes. Linkage disequilibrium was measured according to the methods of Allard (1956).

Results and Discussion

Eleven isozymes of *L. cheesmanii* differ from those in LA 716, and there was a large difference in the SSC observed between LA 1508 and LA 716.

Segregation ratios. Segregation ratios of isozymes and morphological loci showed that the modified backcross population (MBC_1) had two forms: *L. cheesmanii +/c* homozygote and the +/p heterozygote. Of the loci examined, 38% had aberrant Mendelian segregations (Table 2). Three of the isozyme markers deviated toward *L. pennellii* parent and two deviated toward the *L. cheesmanii* parent. The observed deviations were consistent with results of other researchers (Edwards et al., 1987; Zamir and Tadmor, 1986). In the present interspecific hybrid, many loci are in the heterozygous condition, which could lead to an unbalanced state for various factors, including selection against linkage blocks that would result in reduced variation or unbalanced transmission of genes affecting SSC (Zamir and Tadmor, 1986).

Detected QTL. Significant linkages between QTL conditioning SSC and isozymes loci were determined to be PRX-2, *EST-157,* and SX14 (positive QTL) and PRX-7, GOT-2, APS-2, and *PG1-1* (negative QTL), as described by Tanksley et al. (1982) (Table 1).

The pattern exhibited by PRX-2 and EST-157 is interesting in that both reside in close proximity on chromosome 2 of the tomato and are separated by 12 map units (Tanksley and Rick, 1980). It is not clear if each marker was linked to a separate QTL or if a single QTL was exhibiting an association with both of these markers.

Similar work has been done on *Lycopersicon* by Tanksley et al. (1982) using isozymes and by Osborne et al. (1987) using RFLPs. In the former study, it was possible to show associations of isozyme markers and several quantitatively inherited morphological characters. In the latter work, it was possible to show associations of molecular markers with genes controlling high SSC.

Variance. The proportion of the partial phenotypic variances (MR^2) for high SSC described in terms of isozyme markers was calculated (Table 3). Values for seven loci account for 38% of the observed variance.

Two-way interactions between QTL. ANOVA-2 revealed interactions between loci linked to genes influencing SSC. Significant interactions ($P \leq 0.01$ or 0.05) could be due to either epistasis (digenic interaction) or linkage disequilibrium. In the MBC_1 (Table 4), five of 169 pair-wise interactions were significant at $P \leq 0.05$. This result could be due to chance alone; therefore, there is no strong evidence for epistasis. Possible reduced recombination resulting from an interspecific cross can maintain populations in disequilibrium ($D \neq 0$). Estimates of gametic phase disequilibrium for two codominant loci for each of the marker variables were calculated. Disequilibrium solutions obtained by iteration failed to converge. In contrast, Tank-

Table 3. Proportion of total variance in SSC in tomato for QTL from the MBC_1 population.

Isozyme marker locus	Multiple R^2
ACO-1	0.004
GOT-2	0.027*
PGI-1	0.022*
APS-1	0.001
APS-2	0.020*
PRX-1	0.000
PRX-2	0.147**
PRX-4	0.000
PRX-7	0.065**
EST-157	0.041**
6PGDH-2	0.001
SKDH-1	0.000
PTS	0.006
SYM	*0.059***

, *Significant at $P = 0.05$ and 0.01, respectively.

Table 4. Significance of *F* values for two-way interactions for the MBC_1 population.

Isozyme locus	Isozyme locus												
	ACO-1	*GOT-2*	*PGI-1*	*APS-2*	*APS-1*	*PRX-1*	*PRX-2*	*PRX-4*	*PRX-7*	*EST-157*	*6PGDH-2*	*SKDH*	*PTS*
SYM													
GOT-2													
PGI-1									*				
APS-2													
APS-1											*		
PRX-1									*				
PRX-2									*				
PRX-4													
PRX-7											*	*	
EST-157													
6PGDH-2													
SKDH													
PTS													
SYM													

*Significant at $P = 0.05$.

sley et al. (1982) reported interactions between isozyme pairs in the analysis of quantitative metric characters in tomatoes. Isozyme analysis of maize *(Zea mays* L.) found that 15 of the 82 traits examined showed digenic epistatic interactions (Edwards et al., 1987).

Conclusions. Segregating isozyme loci in a backcross population appeared to be an efficient method of identifying genome regions that condition high SSC in the genus *Lycopersicon.* These regions are distributed throughout the tomato genome, except on four linkage groups not tested, with some affecting SSC more than others. Digenic epistasis does not appear to be an important factor contributing to interactions between regions. Inferences regarding the identity and location of regions in the genome that condition SSC are limited to the populations examined and the single environment in which they were measured.

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