Quantitative trait locus analysis of a recombinant inbred line population derived from a *Lycopersicon esculentum* × *Lycopersicon cheesmanii* cross

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Abstract  Quantitative trait loci influencing fruit traits were identified by restriction fragment length polymorphism (RFLP) analysis in a population of recombinant inbred lines (RIL) derived from a cross of the cultivated tomato, *Lycopersicon esculentum* with a related wild species *Lycopersicon cheesmanii*. One hundred thirty-two polymorphic RFLP loci spaced throughout the tomato genome were scored for 97 F₂ RIL families. Fruit weight and soluble solids were measured in replicated trials during 1991 and 1992. Seed weight was measured in 1992. Significant (P<0.01 level) quantitative trait locus (QTL) associations of marker loci were identified for each trait. A total of 73 significant marker locus-trait associations were detected for the three traits measured. Fifty-three of these associations were for fruit weight and soluble solids, many of which involved marker loci significantly associated with both traits. QTL with large effects on all three traits were detected on chromosome 6. Greater homozygosity at many loci in the RIL population as compared to F₂ populations and greater genomic coverage resulted in increased precision in the estimation of QTL effects, and large proportions of the total phenotypic variance were explained by marker class variation at significant marker loci for many traits. The RIL population was effective in detecting and discriminating among QTL for these traits previously identified in other investigations despite skewed segregation ratios at many marker loci. Large additive effects were measured at significant marker loci. Lower fruit weight, higher soluble solids, and lower seed weight were generally associated with RFLP alleles from the *L. cheesmanii* parent.

Key words  Recombinant inbred line – Quantitative trait locus – Restriction fragment length polymorphism – Tomato

Introduction

Efforts to localize and characterize associations between segregating molecular marker loci and quantitative traits have expanded in recent years. Linkage between DNA markers and quantitative trait loci (QTL) controlling important agronomic traits has been reported (Beavis et al. 1991; Edwards et al. 1987; Lander and Botstein 1989; Patterson et al. 1988; Edwards et al. 1992). Much of the published QTL information in crop plants has been obtained from experiments conducted in early segregating generations, such as F₂ or F₃. Generally, mean phenotypic estimates have been regressed on genotypic marker classes at molecular marker loci in order to estimate QTL effects. Given the difficulty of obtaining replicated phenotypic data from individual F₂ plants in obligate sexually-propagated species, genetically advanced progeny (e.g., beyond the F₂ generation) should be better suited for QTL analysis. Populations advanced beyond the F₂ have only recently been utilized in QTL mapping efforts (Mansur et al. 1993; Eshed and Zamir 1994) Recombinant inbred lines (RIL), which are produced by inbreeding the progeny of an F₂ plant derived from two inbred lines, offer certain specific advantages in QTL analysis. Since each RIL family representing a segregate from the original F₂ population is in essence an inbred line, multi-environment trials can be conducted to obtain increased precision of genetic variance estimates for a particular trait (Burr et al. 1988). In addition, RIL constitute a permanent mapping population in which near-homozygosity is often obtained (Burr et al. 1988); thus multiple workers can contribute to genetic mapping and subsequent QTL analysis efforts.
A major objective in tomato breeding programs throughout the world is to improve the soluble solids (SS) content in fruits of high-yielding varieties. The amount of processed product which can be obtained from processing tomatoes is directly related to the SS content, and in fresh market tomatoes high SS is associated with superior taste. The cultivated tomato, *Lycopersicon esculentum* is relatively low in SS (approximately 5%). A source for increased SS content (approximately 15% SS) has been identified in two related wild species: *L. chmielewskii* and *L. cheesmanii*, both of which also possess much smaller fruit than *L. esculentum* (Rick 1974; Osborn et al. 1987, Tanksley and Hewitt 1988). Despite the economic importance of SS in tomatoes and the availability of donor germ plasm with high solids levels, efforts to improve this trait have generally been unsuccessful because of a negative correlation between yield and SS content. A number of recent investigations have identified QTL for SS in crosses of *Lycopersicon* species (Paterson et al. 1988, 1990, 1991; Tanksley and Hewitt 1988; Osborn et al. 1987), however these studies have been carried out on F2 and F3 populations, which render the estimation of quantitative effects more difficult than for inbred lines.

Due to its abundance of well-characterized molecular marker loci (Tanksley et al. 1993) the tomato has played an important role in the development of QTL analysis strategies (Paterson et al. 1988, 1990; Tanksley and Hewitt 1988). These contributions have also provided a wealth of information on QTL positions in tomato (DeVicente and Tanksley 1993; Osborn et al. 1987, Tanksley and Hewitt 1988, Paterson et al. 1988, 1991). Paterson et al. (1988) identified QTL for mass per fruit, soluble solids, and pH in F2 and BC1 populations derived from *L. esculentum* and *L. chmielewskii*. Collectively these QTL accounted for 44–58% of the total phenotypic variation for these traits. Subsequent fine-mapping of these QTL (Paterson et al. 1990) has revealed close marker-QTL associations. Recent work by Paterson et al. (1991) detailed the identification of 29 putative QTL for soluble solids, mass per fruit, and pH in a *L. esculentum* × *L. cheesmanii* cross. *Lycopersicon cheesmanii* is a wild red-fruited species related to *L. esculentum* and native to the Galapagos Islands. Many of these QTL mapped to similar chromosomal locations as those identified in crosses with *L. chmielewskii*, suggesting an overall positional consistency for QTL in *Lycopersicon* species. Since phenotypic estimates were based upon F2 and F3 family data, experimental error associated with these measurements would be expected to be larger than for replicated progenies. More precise estimates of quantitative traits should be available with the use of RIL. The objective of this investigation was to utilize a RIL population in examining associations between polymorphic molecular marker loci and quantitative traits in tomato.

**Materials and methods**

RIL families were developed from a cross of the inbred cultivar ‘UC204C’ with the *L. cheesmanii* accession LA483 (Paterson et al. 1991). Three hundred and fifty individual F3 plants derived from this cross were grown in a completely randomized design in Davis, California, in 1987. Single-seed descent was practiced for six generations on all plants descended from the original F2 population however, due to lethality and inbreeding depression, 97 F3 RIL families remained following selfing. These RIL families formed the segregating population used in QTL analyses. Quantitative trait measurements were assessed with each RIL family represented by six replications of single plants. Measurements were based on mean values for all mature fruit harvested from each family. Plots containing families exhibiting the determinate and indeterminate growth habit were 0.5 m² and 1 m², respectively. The experiment was planted in a randomized complete block design and grown in 1991 and 1992 at the Acre Experiment Station in Acre, Israel. Fruit weight in grams per fruit (FW) and soluble solids (SS, °Brix) were determined as described by Tanksley and Hewitt (1988). RIL families were visually scored for fruit color (yellow/orange/red) and growth habit (determinate/indeterminate). These morphological traits correspond to the B and SP loci, respectively. Seed weight (SDWT) was measured on seed from the F3 generation as the mass per 100 seeds.

DNA isolation, restriction digestion, Southern blotting, and hybridization were performed as described in Paterson et al. (1991). DNA was extracted from a bulk of 30 F3 individuals from each RIL. Marker-restriction enzyme combinations were chosen for their ability to identify restriction fragment length polymorphism (RFLP) between *L. esculentum* and *L. cheesmanii*. Nine restriction enzymes were used for digesting total genomic DNA: BstI, DraI, EcoRI, EcoRV, HaeIII, HindIII, MspI, ScaI, and XbaI. A linkage map consisting of 132 RFLP markers was constructed in the population of RIL (see companion paper, Paran et al. 1995). General similarity and collinearity between this genetic map and published linkage maps derived from interspecific crosses (Tanksley et al. 1992) was observed. The probes used were a subset of those described in Tanksley et al. (1992) selected for thorough coverage of the tomato genome and polymorphism between the *L. esculentum* and *L. cheesmanii* species. Forty-five of the 73 markers used to construct the genetic map described in Paterson et al. (1991) were also included in this investigation. A single isozyme marker, Adh1, was scored in the RIL population.

Means and standard errors were determined for each trait for the RIL population (Table 1). Skewness and kurtosis of the phenotypic distribution for each of these traits was tested, and normality was improved by log10 transformation of all three traits (Fig. 1). Phenotypic correlations were calculated for all traits. One-way analysis of variance (ANOVA) was performed on all loci for each trait separately (Edwards et al. 1987) using an F-test (SAS Institute 1988). The heterozygous marker class was included in the ANOVA. Associations of marker loci with QTL linkage were considered to be significant when the F-test exceeded a value necessary for a probability value less than 0.01. The explained variance (R²) value was calculated for each significant marker locus. The additive effects (a) were obtained for each locus by substituting one homozygous marker class from the other homozygous marker class and dividing by 2.

**Results**

Phenotypic evaluation of RIL families

Significant differences among RIL families were detected for each trait measured. No significant family × year interaction was measured for FW and SS; thus these traits were combined over years for analyses. SDWT was measured only once on F8 seed; thus no environmental component existed for this trait.

The mean FW in the RIL population was 8.03 g (Table 1), which is close to values reported for F2 and F3 generations from which this RIL population was derived (Pa-