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Genes from *Lycopersicon chmielewskii* affecting tomato quality during fruit ripening

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Abstract Three chromosomal segments from the wild tomato, *L. chmielewskii*, introgressed into the *L. esculentum* genome have been previously mapped to the middle and terminal regions of chromosome 7 (7M, 7T respectively), and to the terminal region of chromosome 10 (10T). The present study was designed to investigate the physiological mechanisms controlled by the 7M and 7T segments on tomato soluble solids (SS) and pH, and their genetic regulation during fruit development. The effects of 7M and 7T were studied in 64 BC_2F_5 backcross inbred lines (BILs) developed from a cross between LA1501 (an *L. esculentum* line containing the 7M and 7T fragments from *L. chmielewskii*), and VF145B-7879 (a processing cultivar). BILs were classified into four homozygous genotypes with respect to the introgressed segments based on RFLP analysis, and evaluated for fruit chemical characteristics at different harvest stages. Gene(s) in the 7M fragment reduce fruit water uptake during ripening increasing pH, sugars, and SS concentration. Gene(s) in the 7T fragment were found to be associated with higher mature green fruit starch concentration and red ripe fruit weight. Comparisons between tomatoes ripened on or off the vine suggest that the physiological mechanisms influenced by the *L. chmielewskii* alleles are dependent on the translocation of photosynthates and water during fruit ripening.

Key words BILs · *Lycopersicon esculentum* (Mill) · RFLPs · Soluble solids · Tomato quality

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Introduction

A major goal of many tomato breeding programs is higher soluble solids (SS), since this character is positively correlated with processed product yield and negatively correlated with the energy costs for dehydration (Rick 1974). Soluble solids are also of prime concern in fresh market tomato production for the contribution that sugars and acids make to the overall flavor of the fruit (Stevens et al. 1977; Jones and Scott 1983). SS account for approximately 75% of the total dry matter (total solids), with the insoluble fraction consisting of proteins, pectic substances, cellulose and hemicellulose. SS are composed primarily of the reducing sugars, fructose and glucose (accounting for 70% of the SS), and the organic acids, citrate and malate (accounting for 15% of the SS) (Davies and Hobson 1981). In processed tomatoes, organic-acid concentration is important because it enhances fruit flavor, and is associated with fruit pH (Jones and Scott 1983). A pH of less than 4.5 is required in processed and canned tomato products to control the growth of thermophilic microorganisms (Thompson et al. 1964).

In order to develop tomato lines with increased SS concentration it is important to understand how the main components of SS accumulate during fruit ripening. Total sugar concentration dramatically increases during the initial stages of cell enlargement in immature fruits and keeps rising during ripening from the mature green to the breaker stage. Many environmental factors, such as day length, shading, leaf to fruit ratio and mineral nutrition, affect sugar concentration in tomato fruits (Davies and Hobson 1981).

Dinar and Stevens (1981) found a high correlation between starch concentration in green fruits and the total solids (TS) of red ripe fruits. Starch concentration increases during fruit development rising to a peak (20% of the dry matter) at 25 to 30 days after anthesis (Ho et al. 1983). After this, starch breaks down into sugars reaching a final concentration of about 1% dry matter at the inception of fruit ripening.

In immature fruit, the predominant acid is malate, with citrate accounting for only 25%. As the fruit matures, citric-acid concentration increases and malic-acid concentration declines (Davies 1964). Fruit acidity reaches its maximum at the breaker stage, followed by a progressive decrease as ripening continues (Winsor et al. 1962). Variation in fruit acidity is partially explained by the concentration of citric acid in the fruit, although other acids as well as certain minerals, particularly phosphorus, may also affect fruit pH (Davies 1964).

Genetic variation for SS concentration is limited among the cultivated forms of *L. esculentum* (Lower and Thompson 1966). However, some wild relatives of the tomato have much higher concentrations. In 1974, C.M. Rick introgressed chromosomal segments from *L. chmielewskii* into the *L. esculentum* genome by repeated backcrosses. As a result, several lines with high SS concentration were released, including LA1501 and LA1563. Using molecular markers the introgressed chromosomal segments have been mapped in LA1563 to the middle and terminal regions of chromosome 7 (7M, 7T respectively), and to the terminal region of chromosome 10 (10T) (Tanksley and Hewitt 1988). In a previous study, using a backcross inbred population generated from a cross between LA1501 and VF145B-7879, Azanza et al. (1994) characterized the effect of two of these *L. chmielewskii* fragments (7M and 7T) on SS and yield in red ripe tomato fruits. The 7M fragment, when homozygous, was associated with greater SS (26%) and higher pH (0.10) than the control group without reducing fruit yield. The 7T fragment did not influence either SS or pH, but was observed to significantly increase fruit yield (12%).

The physiological mechanisms responsible for the enhanced SS concentration and yield observed in these lines are as yet unknown. For this study we use a tomato backcross inbred population (Azanza et al. 1994) to investigate the genetic regulation during fruit ripening and the possible physiological roles of the 7M and 7T chromosome segments introgressed from *L. chmielewskii* into *L. esculentum*.

Materials and methods

Plant materials

LA1500, LA1501, LA1502, LA1503 and LA1563 are several of the high-solids breeding lines developed by C. M. Rick (1974) from repeated backcrossing of the wild green-fruited species *L. chmielewskii* to *L. esculentum* cv 'VF36' (BC_{1-2}) and cv 'VF145B-22-8' (BC_{3-5}). These lines were graciously provided by Professor C.M. Rick, University of California at Davis.

The sixty-four BILs were developed using LA1501 as the donor and VF145B-7879 as the recurrent parent as previously described by Azanza et al. (1994). These 64 backcross inbred lines, the two parents, LA1500, LA1502, LA1503 and LA1563 were all evaluated in the field at the University of Illionis, Champaign, in the summer of 1990 in a randomized complete block design with four replications. One experimental unit consisted of a single row containing nine tomato plants of each BIL or parental line.

Physiological analysis

Ten randomly sampled tomato fruits were harvested from each experimental unit at three different stages of fruit development (mature green, breaker and red ripe). Fruits at the mature green stage were harvested from previously tagged flowers at 28 days after anthesis, since around this time starch concentration rises to a peak (Ho et al. 1983). The second harvest was conducted at the breaker stage, which is described as the first appearance of external pink, red, or tannish-yellow color. At the breaker stage two sets of fruits from each experimental unit were harvested. One was stored at −20 °C and the other was allowed to ripen on trays at 25 °C until red ripe. Off-the-vine ripe red fruit were included as a harvest treatment since fresh market tomatoes are harvested at the breaker stage and ripened during shipment. Vine-ripened fruits were harvested 10 days after the breaker stage. Tomato fruits from each experimental unit at each stage, were weighed after harvesting to obtain an averaged fruit weight and then stored in freezer bags at −20 °C for subsequent analysis.

Total and soluble solids, serum, pH, color, and the concentration of citrate, malate, fructose and glucose were analyzed from each of the samples following procedures described by Azanza et al. (1994). Starch was first extracted from fruit tissue and then hydrolyzed with ten units of amylase and 0.4 units of amyloglucosidase. The resultant glucose concentrations were determined by coupling the oxidation of glucose to the reduction of $NADP^+$ with three units of hexokinase and 0.5 units of glucose 6-P dehydrogenase (Sigma Chemical Company), using a technique modified from Mao and Craker (1990). The number of locules per fruit, fruit diameter, and fruit wall thickness were measured from 12 fresh red ripe fruits cut in half perpendicular to the core.

RFLP analysis

Leaves of all the lines were harvested and oven dried. Total DNA was isolated from the powdered leaf sample, using a procedure described by Bernatzky and Tanksley (1986a). DNA was digested with four restriction enzymes (*Eco*RI, *Eco*RV, *BSTN1*, and *Dra*I), and subjected to Southern analysis as described by Bernatzky and Tanksley (1986b). Filters were probed with nine genomic clones in the following probe/enzyme combinations: TG183, TG143 and TG149 with *Eco*RI; TG174 and CD56 with *Eco*RV; TG13A and TG202 with *Dra*I; and TG313 and TG303 with *BSTN*1. These probes were known to map within the three regions introgressed from *L. chmielewskii* (Tanksley et al. 1992) (see Fig. 1). Six probes, known to be polymorphic between the high SS lines and VF145B-7879, were used to assay the BIL population. Based on probe polymorphisms, the 64 BILs were classified into four groups segregating for two of the segments.

Statistical analysis

Analysis of variance using SAS (SAS 1985) was conducted for all the variables to assay the variation among replicates, BILs, parental lines, LA1500, LA1502, LA1503 and LA1563 at mature green, breaker, red on-the-vine and red off-the-vine tomato fruit. Each genotype mean for all the physiological parameters is the average of four replicates. Starch concentration was measured at mature green and breaker stages and fruit diameter, wall thickness, and number of locules from red on-the-vine fruit.

The 64 BILs were classified into four genotypes (with respect to 7M, 7T). Linkage relationships between RFLPs and genes controlling the traits under study at each of the harvest treatments were determined by one-way analysis of variance, using RFLP genotypic groups as class variables. Multiple comparisons using LSD tests were conducted to compare genotype means and to ascertain the significance of the effect of the individual segments on each of the variables. Only one backcross inbred was found to contain both 7M and 7T chromosomal segments from *L. chmielewskii*, which was insufficient replication to make inferences about the effect of both fragments in association. Therefore, this line was excluded from the analysis and comparisons were only conducted among the three remaining geno-

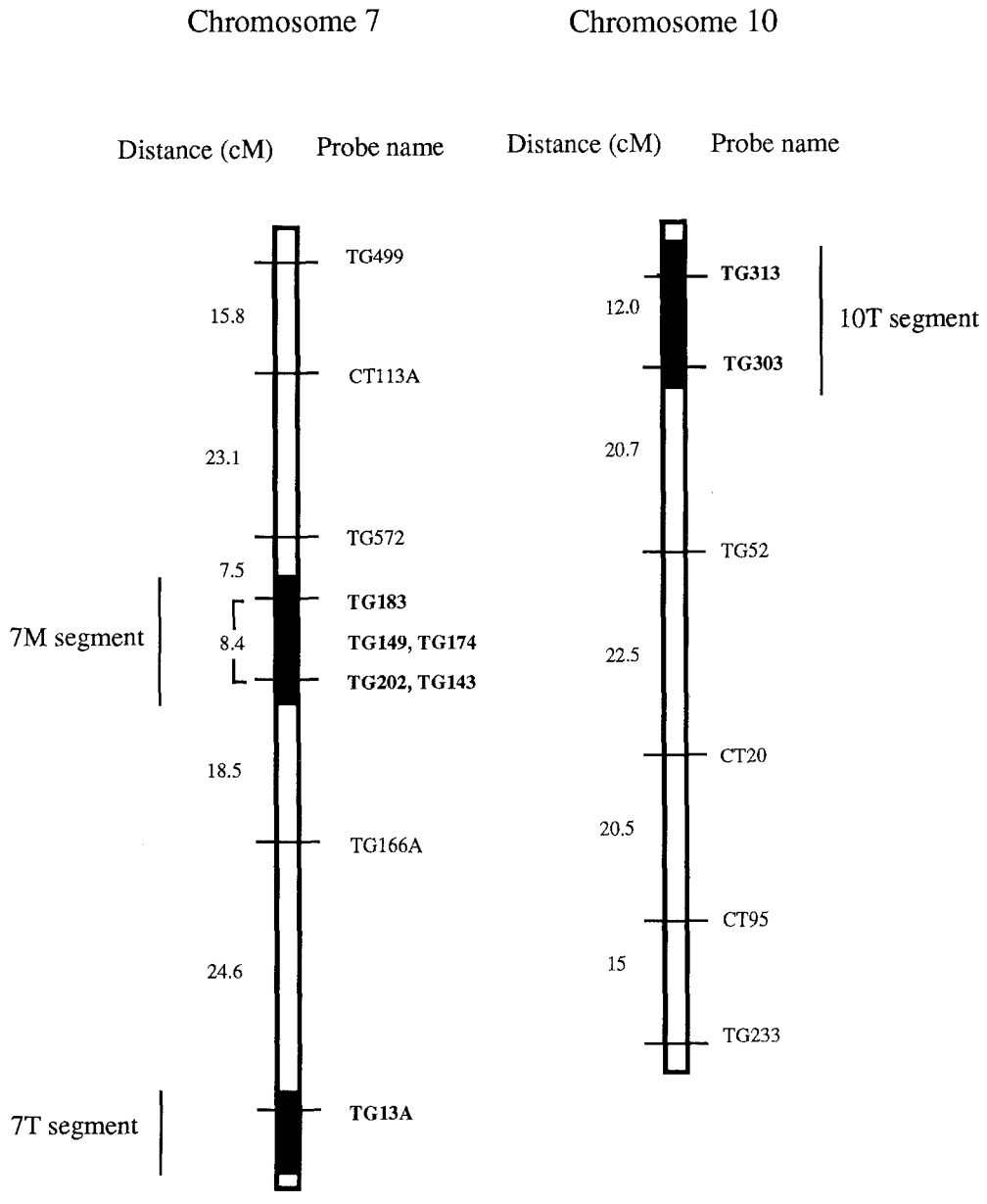

Fig. 1 Tomato RFLP map for chromosome 7 and 10 (Tanksley et al. 1992). *Probes in bold* were used to assay high SS lines, VF145B-7879, and BILs

types. Means for the fourth genotype (7M/7T) were, however, included in the tables for comparsion. Correlations among different variables at different maturity stages were also calculated to study physiological associations.

Results

The high SS lines derived from C.M. Rick's breeding program have been previously assayed for the presence of three introgressed segments from *L. chmielewskii* (Tanksley and Hewitt 1988; Azanza et al. 1994). These segments were designated: 7M for the segment in the middle region of chromosome 7; 7T, a segment at the terminal position on the long arm of chromosome 7; and 10T, a segment at the terminal position on chromosome 10 (Fig. 1) in the tomato map (Tanksley et al. 1992).

Although derived from the same breeding program, these lines showed significant differences for most of the fruit parameters under study at different harvest stages (Table 1). This may be explained by differences among C.M. Rick's original lines in the number of *L. chmielewskii* introgressed segments each contains, and/or in variability in the alleles contributed from their cultivated parents (VF145B-7879 or VF36). The interaction of the *L. chmielewskii* genes with allelic variation between the cultivated parents may also contribute to the observed variation among the lines.

High SS lines were classified into two groups. The 7M/7T + class consists of LA1501 and LA1503, both having the *L. chmielewskii* segments on chromosome 7 but not the terminal segment on 10. The 7M/7T/10T class have all three introgressed segments, and included

Table 1 Fruit-parameter means for the parental and the high SS lines at the four different harvest treatments

Variable		VF145B	LA1501	LA1500	LA1502	LA1503	LA1563	LSD (0.05)[a]
	10T	–[b]	–	+	+	–	+	
	7M, 7T	–	+	+	+	+	+	
Fruit weight (g/fruit)	Mature green	64 ab	60 abc	46 bcd	42 cd	66 a	40 d	19
	Breaker	86 ab	83 ab	94 a	82 ab	86 ab	71 b	15
	Ripe on vine	104 bc	133 a	125 ab	66 c	106 bc	103 c	21
	Ripe off vine	80 a	85 a	93 a	76 a	75 a	75 a	18
pH	Mature green	4.65 b	4.97 a	5.03 a	5.12 a	4.99 a	5.07 a	0.15
	Breaker	4.21 a	4.21 a	4.12 a	4.21 a	4.13 a	4.13 a	0.09
	Ripe on vine	4.35 c	4.51 b	4.58 ab	4.62 a	4.39 c	4.62 a	0.10
	Ripe off vine	4.32 c	4.42 bc	4.52 ab	4.64 a	4.47 b	4.52 ab	0.13
Total solids (g/100 g)	Mature green	6.11 b	7.46 a	7.77 a	7.33 a	7.58 a	7.07 a	0.71
	Breaker	5.27 a	5.71 a	5.63 a	6.20 a	5.72 a	6.01 a	1.16
	Ripe on vine	5.34 b	5.50 b	5.65 b	6.35 a	5.29 b	5.51 b	0.68
	Ripe off vine	4.76 b	5.31 ab	5.16 b	5.77 a	5.24 ab	5.30 ab	0.56
Soluble solids (g/100 g)	Mature green	4.38 a	4.52 a	4.81 a	4.66 a	4.77 a	4.72 a	0.62
	Breaker	3.95 b	4.23 ab	4.25 ab	4.64 a	4.32 ab	4.43 a	0.43
	Ripe on vine	3.32 d	4.39 ab	3.86 c	4.70 a	4.40 ab	4.23 bc	0.39
	Ripe off vine	3.73 b	4.09 ab	4.29 ab	4.71 a	4.51 a	4.13 ab	0.63
SS/TS (%)	Mature green	71.7 a	60.7 b	67.4 ab	63.6 ab	63.3 ab	66.6 ab	10.2
	Breaker	75.0 a	74.1 a	75.5 a	75.0 a	75.4 a	73.7 a	8.7
	Ripe on vine	62.3 d	80.3 ab	68.5 cd	73.9 bc	83.6 a	77.0 abc	9.0
	Ripe off vine	78.3 ab	76.9 b	83.3 ab	79.2 ab	86.1 a	78.0 ab	8.5
# Locules	Ripe on vine	5.3 b	7.0 a	7.0 a	7.2 a	—	6.5 a	1.0
Wall thickness (mm)	Ripe on vine	5.36 ab	5.55 a	4.00 d	4.40 cd	—	4.85 b	5.67
Fruit diameter (mm)	Ripe on vine	60.4 c	67.9 a	61.3 bc	58.7 c	—	65.8 ab	4.7

Table 1 (Continued)

Variable		VF145B		LA1501		LA1500		LA1502		LA1503		LA1563		LSD (0.05)[a]	
	10T	–[b]		–		+		+		–		+			
	7M, 7T	–		+		+		+		+		+			
		Fresh	Dry	Fresh	Dry	Fresh	Dry	Fresh	Dry	Fresh	Dry	Fresh	Dry	Fresh	Dry
Citric acid (g/100g)	Mature green	0.37 a	8.5 a	0.51 a	11.2 a	0.42 ab	8.6 a	0.45 ab	9.6 a	0.41 ab	8.8a	0.39 ab	8.5 a	0.12	3.1
	Breaker	0.47 c	11.8 b	0.59 b	14.0 ab	0.67 ab	15.8 a	0.67 ab	14.5 ab	0.63 ab	14.6 ab	0.73 a	16.5 a	0.10	3.1
	Ripe on vine	0.39 a	11.8 a	0.34 ab	7.7 b	0.36 ab	9.6 ab	0.30 b	6.4 b	0.39 a	9.1 ab	0.29 b	6.9 b	0.07	2.7
	Ripe off vine	0.40 a	10.6 a	0.43 a	10.8 a	0.36 a	8.3 a	0.37 a	7.7 a	0.46 a	10.3 a	0.34 a	8.1 a	0.14	3.4
Glucose (g/100g)	Mature green	1.17 a	26.9 a	1.26 a	27.9 a	1.17 a	24.1 a	1.11 a	24.2 a	1.24 a	26.0 a	0.92 a	19.9 a	0.34	8.6
	Breaker	1.08 a	27.3 a	1.08 a	25.4 ab	1.00 a	23.5 b	1.16 a	24.9 ab	1.05 a	24.2 b	1.01 a	22.7 b	0.17	2.9
	Ripe on vine	0.71 c	21.5 a	1.07 ab	24.2 a	1.00 ab	25.9 a	1.15 a	24.3 a	0.87 bc	21.9 a	0.97 ab	22.9	0.24	4.4
	Ripe off vine	0.80 a	21.4 a	1.00 a	24.5 a	0.98 a	22.6 a	0.99 a	20.8 a	1.06 a	23.7 a	0.97 a	23.5 a	0.26	5.4
Fructose (g/100g)	Mature green	1.13 ab	26.0 a	1.37 a	30.3 a	1.40 a	29.1 a	1.27 ab	27.8 a	1.17 ab	23.4 a	1.00 b	21.7 a	0.36	9.1
	Breaker	1.25 a	31.7 a	1.20 a	28.1 b	1.16 a	27.3 b	1.29 a	27.9 b	1.18 a	27.3 b	1.20 a	27.1 b	0.17	2.8
	Ripe on vine	1.02 c	30.6 ab	1.33 ab	30.4 ab	1.30 ab	34.0 a	1.49 a	31.9 ab	1.14 bc	27.6 b	1.28 ab	30.1 ab	0.25	5.3
	Ripe off vine	1.15 a	30.7 a	1.28 a	31.7 a	1.26 a	29.3 a	1.35 a	28.6 a	1.32 a	29.5 a	1.20 a	29.1 a	0.30	6.9
Total sugars (g/100g)	Mature green	2.31 ab	52.9 a	2.63 a	58.3 a	2.57 a	53.2 a	2.38 ab	52.0 a	2.41 ab	49.4 a	1.92 b	41.6 a	0.64	17.0
	Breaker	2.33 ab	59.0 a	2.28 ab	53.5 b	2.16 b	50.8 b	2.46 a	52.8 b	2.23 b	51.5 b	2.21 b	49.8 b	0.19	5.3
	Ripe on vine	1.73 d	52.2 ab	2.40 ab	54.7 ab	2.31 b	60.0 a	2.64 a	56.2 a	2.01 c	49.5 b	2.25 bc	53.0 ab	0.28	9.5
	Ripe off vine	1.95 a	52.1 a	2.27 a	56.2 a	2.24 a	51.9 a	2.34 a	49.4 a	2.38 a	53.2 a	2.17 a	52.6 a	0.51	12.0
Starch (g/100g)	Mature green	1.52 a	24.9 a	1.70 a	22.8 a	1.54 a	19.8 a	1.36 a	18.6 a	1.48 a	19.5 a	1.78 a	25.1 a	0.42	10.2
	Breaker	0.29 a	5.5 a	0.24 a	4.2 a	0.27 a	4.8 a	0.19 a	3.1 a	0.21 a	3.7 a	0.23 a	3.8 a	0.10	3.5

[a] Least significant difference at $P = 0.05$. Values followed by the same letter (a–d) are not significantly different

[b] +, indicates presence and –, absence of the different chromosomal fragments

lines LA1500, LA1502, and LA1563. Using T-test comparisons between both groups (data not shown), the 10T segment was found to significantly increase fruit pH in mature green (0.09) and in red ripe tomatoes (0.16), but not at the breaker harvest stage. This suggests that genes in the 10T segment are expressed prior to ripening, and either undergo some kind of repression during the breaker stage or else the action of other genes and physiological events during this stage of ripening overcome these differences. The association between the 10T fragment and red fruit pH has been previously reported by both Tanksley and Hewitt (1988) and by Azanza et al. (1994).

Differences between the means of VF145B-7879 and LA1501 and the rest of high SS lines were tested for significance for all the physiological characteristics at different harvest treatments (mature green, breaker and red ripe on-and-off the vine) (Table 1). LA1501 was found to display a higher pH, TS, and a lower SS to TS ratio than VF145B-7879 at the mature green fruit stage. During the breaker, differences in pH, TS and SS disappear (Table 1). When tomatoes are ripened on the vine, LA1501 was found to display higher SS concentration and higher pH, as well as larger fruit, and more locules per fruit, than VF145B-7879 (Table 1). Glucose and fructose concentrations were higher and citrate concentration lower, but only when expressed as a proportion of fresh weight. In contrast, when tomatoes are ripened off the vine differences between both lines disappeared or were greatly reduced.

No significant differences based on *F* values were detected for fruit color and malate concentration; consequently these variables were not included in the discussion of the results. Fruit color was used as a test for physiological maturity, and therefore the absence of significant color differences between BILs at a given harvest stage suggests that the variation due to differences in maturity is not significant (Young et al. 1993).

Since LA1501 is the high SS parent of the BILs, only two fragments (7M and 7T) were segregating in the population of BILs. The sixty-four BILs were classified into four genotypes, based on probe polymorphisms at the 7M and 7T regions. The ++ group (51 BILs) consisted of all the lines which were genetically identical to the recurrent parent (VF145B-7879) at 7M and 7T. The 7M/+ group was made up of six BILs that have the *L. chmielewskii* introgressed segment in the medial region of chromosome 7, but not the terminal segment. The +/7T group consisted of six BILs that are lacking the medial segment but are homozygous for the 7T *L. chmielewskii* segment. A single 7M/7T genotype had both *L. chmielewskii* segments. None of the BILs were found to be heterozygous for either 7M or 7T. The observed genotypic segregation conforms to the expected ratio, suggesting that no selection during the backcrossing or selfing generations took place and that the loci segregated independently (Azanza et al. 1994).

Effect of the 7M fragment

The *L. chmielewskii* segment in the middle of chromosome 7 (7M) was associated with a significant increase in TS and pH at both mature green and red ripe stages (Table 2). The increase in SS was significant throughout all the harvest stages. In contrast to what was observed by Winsor et al. (1962), TS (data not shown) and SS (Fig. 2) significantly decreased during fruit ripening in the control group (+/+) from mature green to breaker and from breaker to red pipe. These decreases in TS and SS may be partially due to respiration (Walker et al. 1978) and through dilution from fruit water uptake during ripening (Young et al. 1993). The decrease in the SS fraction (1.06 g/100 g fresh matter) explained most of the decrease in the TS (1.24 g/100 g fresh matter). The decrease in the insoluble fraction can be explained by the degradation of pectic compounds associated with a loss of firmness during ripening (Hobson and Davies 1971), and also by the catabolism of starch and cellulose. In contrast, in the 7M/+ group, TS and SS significantly decreased from mature green to breaker, but increased from breaker to red ripe on-the-vine (Fig. 2). Fruits produced by inbreds with the 7M fragment ripened normally and losses due to respiration are presumed similar to those in the control group. The increase in TS and SS during the latest ripening stages suggests that genes in this region either reduced the fruit water uptake or else participated in the accumulation of more water soluble compounds (by increasing the rate of sucrose uptake or by increasing the period in which the fruit is actively taking up sucrose).

Hewitt et al. (1982), using a high SS line genetically similar to LA1501, proposed that sucrose hydrolysis occurred at a faster rate in fruits of LA1563 than in VF145B-7879. This higher hydrolysis rate would increase the accumulation of sugars (glucose and fructose) in these lines. To further investigate whether the effect of the 7M fragment was associated with an increase in the accumulation of sugars and acids as previously suggested (Hewitt et al. 1982), or with a reduction in water uptake, the amounts of these chemicals in the fruit were calculated on a fresh and dry weight basis. Sugar concentration on a fresh-weight basis decreased from mature green to red ripe in the control group (+/+), but increased in the 7M/+ group (Fig. 2). The significant increase in sugar concentration from mature green to breaker in the 7M/+ group (Fig. 2) may be caused by differences in maturity or by differences in the accumulation pattern of sugars in the fruit. By the beginning of ripening, the amounts of sugars on a fresh and dry weight basis are equivalent to those found in the control group (+/+) (Table 2). At the red ripe stage, inbreds homozygous for the 7M fragment had significantly less sugar on a dry-weight basis than the control group but more sugars on a fresh weight basis (Table 2), indicating that fruits from these lines had lower concentrations of water. This suggests that the effect of the 7M segment is associated with a physiological

Table 2 Mean values and LSDs for the fruit variables for the four different genotypic classes based on segregation of both fragments on chromosome 7 (7M and 7T) at all harvest stages

Variable		+/+	7M/+	+/7T	7M/7T	LSD (0.05)[a]
Fruit weight (g/fruit)	Mature green	55 a	54 a	67 b	78	10
	Breaker	75 a	74 a	81 a	83	8
	Ripe on vine	104 ab	98 b	114 a	122	13
	Ripe off vine	75 a	78 a	84 a	80	10
pH	Mature green	4.70 b	4.80 a	4.67 b	4.94	0.09
	Breaker	4.11 a	4.16 a	4.13 a	4.15	0.05
	Ripe on vine	4.37 b	4.47 a	4.37 b	4.52	0.05
	Ripe off vine	4.27 b	4.42 a	4.28 b	4.38	0.04
Total solids (g/100g)	Mature green	6.39 b	6.97 a	6.31 b	7.43	0.27
	Breaker	5.40 a	5.62 a	5.35 a	6.05	0.38
	Ripe on vine	5.15 b	5.83 a	4.94 b	6.33	0.39
	Ripe off vine	4.94 b	5.28 a	5.02 ab	5.56	0.32
Soluble solids (g/100 g)	Mature green	4.58 b	4.90 a	4.66 b	4.64	0.25
	Breaker	3.83 b	4.07 a	3.91 ab	4.59	0.23
	Ripe on vine	3.52 b	4.45 a	3.68 b	5.17	0.36
	Ripe off vine	3.73 b	4.17 a	3.87 b	4.75	0.29
SS/TS (%)	Mature green	71.9 a	71.1 a	73.9 a	62.3	3.2
	Breaker	71.6 a	72.8 a	73.0 a	75.9	3.4
	Ripe on vine	68.6 b	76.1 a	74.2 a	81.9	4.3
	Ripe off vine	75.6 b	79.4 a	77.3 ab	84.3	3.6
# Locules	Ripe on vine	4.3 b	5.9 a	5.6 a	6.2	1.1
Wall thickness (mm)	Ripe on vine	5.5 a	5.0 a	5.4 a	4.9	0.7
Fruit diameter (mm)	Ripe on vine	57.6 a	56.8 a	56.8 a	59.6	3.2

		Fresh	Dry	Fresh	Dry	Fresh	Dry	Fresh	Dry	Fresh	Dry
Citric acid (g/100 g)	Mature green	0.37 a	8.2 a	0.35 a	7.3 a	0.36 a	7.9 a	0.43	9.3	0.05	1.2
	Breaker	0.54 a	14.1 a	0.57 a	14.2 a	0.53 a	13.8 a	0.67	14.6	0.04	0.9
	Ripe on vine	0.36 a	10.3 a	0.34 a	8.0 b	0.34 a	9.6 a	0.37	7.2	0.03	1.3
	Ripe off vine	0.42 a	11.4 a	0.38 a	9.2 b	0.41 a	10.4 a	0.48	10.4	0.05	1.6
Glucose (g/100 g)	Mature green	1.09 a	24.1 a	0.96 b	20.0 b	1.03 a	22.5 ab	1.10	24.1	0.12	3.3
	Breaker	0.96 a	25.1 a	1.01 a	24.8 a	0.98 a	24.8 a	1.16	25.1	0.08	1.2
	Ripe on vine	0.77 b	21.9 a	0.93 a	20.3 a	0.74 b	20.3 a	1.28	24.5	0.12	2.2
	Ripe off vine	0.82 a	22.1 a	0.89 a	20.8 a	0.81 a	20.9 a	1.06	24.1	0.08	2.0
Fructose (g/100 g)	Mature green	1.25 a	27.5 a	1.01 b	21.0 b	1.17 a	25.5 a	1.13	24.8	0.13	3.3
	Breaker	1.16 a	30.4 a	1.20 a	29.3 b	1.19 a	30.4 a	1.25	27.3	0.07	1.1
	Ripe on vine	1.09 b	31.3 a	1.23 a	27.5 b	1.05 b	29.2 ab	1.49	31.4	0.12	2.4
	Ripe off vine	1.13 ab	30.7 a	1.19 a	28.1 a	1.08 b	28.6 a	1.27	28.8	0.10	2.9
Total sugars (g/100 g)	Mature green	2.34 a	51.7 a	1.96 b	41.1 b	2.20 ab	47.9 a	2.23	48.9	0.25	6.5
	Breaker	2.13 a	55.4 a	2.21 a	54.1 a	2.17 a	55.2 a	2.29	52.3	0.15	2.2
	Ripe on vine	1.86 b	53.3 a	2.15 a	47.8 b	1.79 b	49.9 b	2.77	53.1	0.24	2.8
	Ripe on vine	1.95 ab	52.7 a	2.08 a	48.9 a	1.89 b	49.5 a	2.33	52.9	0.19	4.8
Starch (g/100 g)	Mature green	1.29 b	20.2 b	1.17 b	16.8 b	1.83 a	29.0 a	1.95	26.2	0.20	4.2
	Breaker	0.24 a	4.5 a	0.25 a	4.4 a	0.27 a	4.8 a	0.26	4.2	0.05	0.8
	Ripe on vine	–	–	–	–	–	–	–	–	–	–
	Ripe off vine	–	–	–	–	–	–	–	–	–	–

[a] Least significant difference at $P = 0.05$. Values followed by the same letter are not significantly different. Values for the 7M/7T genotypic group were not included in the analysis, but can be used as a reference

mechanism that reduces fruit water uptake during ripening and not with an increase in the amount of sugar in the fruit.

Although the concentration of fructose was always larger than the concentration of glucose, the amounts of these sugars were highly correlated during ripening. This implies that both sugars are under the same form of regulation and are affected similarly by genes in 7M, as would be expected if genes in this fragment affect fruit water uptake. The 7M fragment did not affect starch concentration at the mature green stage (Table 2) in this experiment. Dinar and Stevens (1981) reported that a higher concentration of starch in mature green fruit was correlated with high SS concentration at the red ripe stage. No association between these two variables was found in this study.

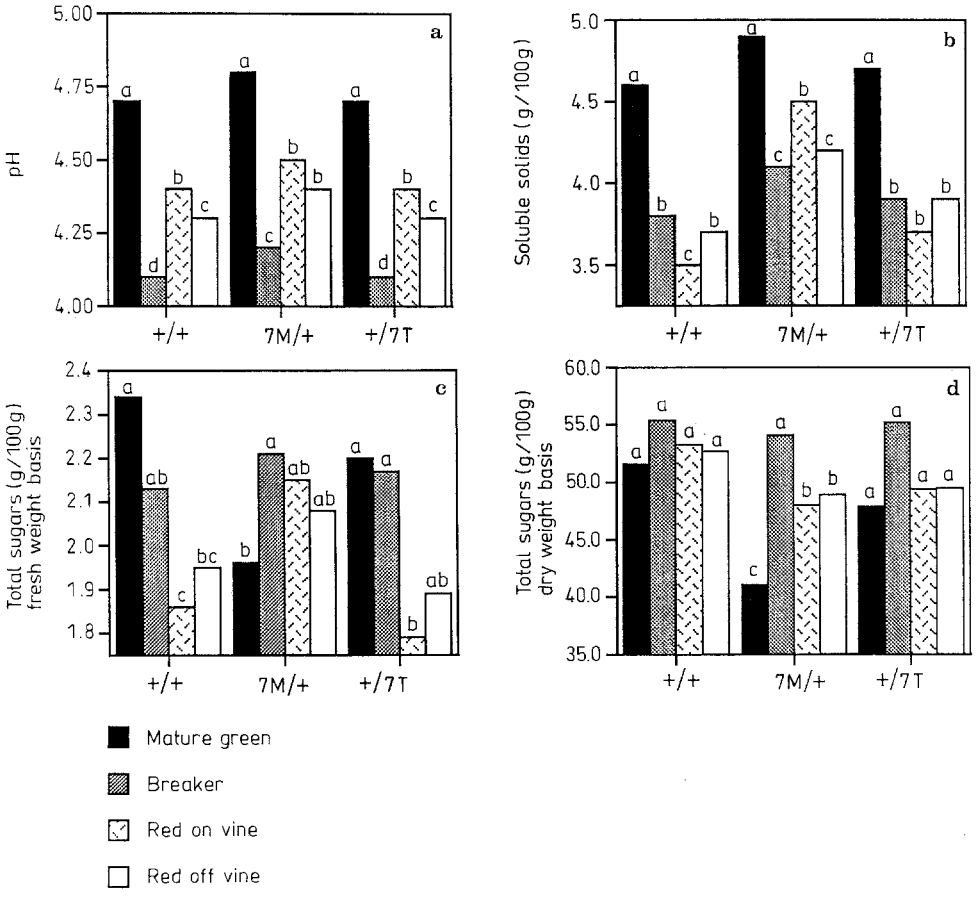

Fig. 2 Changes in pH (**a**), soluble solids, (**b**) and total sugar concentrations on a fresh (**c**) and dry weight (**d**) basis at different fruit harvest stages. *Columns with different letters* represent means significantly different for comparisons within the same RFLP group using LSDs

Inbreds homozygous for the 7M fragment also have a higher pH than the control group (Table 2) at both mature green and red ripe stages. The increase in pH ranged from 0.05 to 0.15 units. Our results agree with previous studies (Winsor et al. 1962) which have shown that pH decreases during the breaker stage and increases with the appearance of lycopene pigmentation in the fruit (Fig. 2). The 7M fragment did not affect citric-acid concentration on a fresh weight basis, suggesting that the differences in pH between the control (+/+) and the 7M/+ groups were not associated with differences in citric-acid concentration between both genotypic groups. This lack of association between fruit pH and citric-acid concentration was also observed among the high SS lines. The effect of the 10T fragment on fruit pH was not associated with a reduction in citric-acid concentration. Other fruit chemical constituents are most likely interacting with citrate in the regulation of pH during ripening. The 7M fragment did not affect either fruit weight during ripening or fruit diameter at the red ripe stage, but was associated with an increase in the number of locules per fruit (Table 2).

Effect of the 7T fragment

The terminal fragment on chromosome 7 did not affect TS, SS, pH or the concentrations of sugars and citric-acid. However, the 7T fragment increased the number of locules in red fruits and fruit weight during all the developmental stages (from 8 to 22%), although this increase was only significant at the mature green stage. This fragment was also associated with a higher concentration of starch at the mature green stage (Table 2). In a previous study, in which starch concentration was measured in tomato fruits at different stages of development, a linear relationship ($r = 0.68$ $P < 0.01$) was observed between starch and the specific growth rate of the fruit (Walker and Thornley 1977). Fruits with a higher starch concentration at the mature green stage would grow either faster or for longer time periods, achieving greater fruit weight. Assuming plants have an equivalent number of fruits, an increase in fruit weight would result in greater yields. Inbreds homozygous for the 7T fragment had higher starch concentrations at the mature green stage and larger fruits at the red ripe stage (Table 2) and were found to have significantly greater yields (Azanza et al. 1994) than the control group (+/+).

Water and photosynthate translocation during ripening

Tomatoes ripened on and off the vine were compared for all the fruit characteristics to determine if the physiological processes affected by the 7M and 7T fragments are

dependent or independent of the mother plant. When compared to tomatoes at the breaker stage, vine-ripened tomatoes of all genotypes showed a greater increase in pH than those ripened off the vine (Fig. 2). In the 7M/+ group, SS only increased from the breaker to the red ripe stage when tomatoes were ripened on the vine (Fig. 2). Differences in SS and sugar concentration between the control group and the 7M/+ group were reduced or disappeared when tomatoes are ripened off the vine (Table 2), suggesting that the effect of this fragment is dependent on the translocation of photosynthates and/or water during ripening. The control (+/+), vine-ripened tomatoes showed a greater decrease in sugar concentration on a fresh weight basis during ripening than those ripened off the vine (0.27 against 0.18 g/100 g) (Fig. 2). In contrast, in the 7M/+ group, sugar concentration did not vary significantly when tomatoes were ripened on or off the vine (Fig. 2). These data agree with the previously suggested hypothesis that the 7M fragment reduces water uptake during the latter stages of vine ripening.

Discussion

The 7M and 7T chromosomal fragments from *L. chmielewskii* differentially influenced fruit physiological parameters during fruit development and appear to be activated at different times. The 7T fragment may be acting early in fruit development affecting cell division and cell expansion, resulting in larger fruits with more locules. Gene(s) in the 7T fragment increase starch concentration at the mature green stage and result in fruit that grow more rapidly or for longer time periods achieving greater fruit weight and yield. This segment is possibly influencing the import of assimilates early during fruit development, but not during fruit ripening.

The 7M fragment also increased the number of locules of the fruit, indicating an early activation during development. However, the expression of gene(s) located in this fragment appeared to exert larger physiological effects at the end of fruit development and during fruit ripening. Walker and Ho (1977) observed no correlation between changes in the levels of soluble components and the import rate by the fruit, proposing that this was due to the very dynamic role that glucose and fructose have as intermediates in fruit synthetic reactions. Sucrose synthase activity, was observed to be ten-times larger than needed to hydrolyze the sucrose imported by the fruit (Sun et al. 1992), suggesting that enzyme activity is not the limiting factor for higher sugar concentration in the fruit. These two observations imply that differences in fruit enzyme activities among backcross inbred lines in the population under study may not be responsible for the observed differences in SS concentration from the breaker to the ripe red stage. In contrast, higher fresh weight SS concentration appears to be associated with a reduced rate of fruit water uptake during ripening, which could operate independently of fruit enzyme activity and sucrose assimilation.

Differences in water concentration in fruits with and without the 7M fragment could be based on variation in fruit water loss during ripening. This, however, is unlikely since when fruits with and without the 7M fragment were ripened off the vine they displayed no significant differences in sugar concentration on a wet-weight basis. Translocation of water operates via diffusion, and is rate dependent on concentration gradients between the source and the sink. If we assume that fruits exert a controlling influence on the rate of import of assimilates, fruits with higher sugar concentration on a fresh, but not dry, weight basis do not necessarily have to be more efficient in maintaining a large gradient to increase the translocation rate as was suggested by Hewitt et al. (1982), but could be more efficient in compartmentalizing fructose and glucose inside the cell so that less water is needed to maintain the appropriate cell osmotic potential for translocation. Perhaps the simplest hypothesis to explain the effect of the 7M fragment on fruit soluble solids does not even involve source-sink relationships. A gene(s) in the 7M fragment could reduce the rate of water uptake during ripening from the xylem without influencing assimilate translocation in the phloem. This would be consistent with our data from on- and off-the-vine ripened fruit.

An experiment to study the effect of these fragments in different genetic backgrounds will ascertain the utility of these segments for the commercial tomato industry. Future research to study the effect of the 7M fragment on the translocation of water and assimilates to the fruit will provide new information to more clearly understand the effect of the 7M fragment on SS concentration and pH during fruit ripening.

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References

Azanza F, Young TE, Kim D, Tanksley SD, Juvik JA (1994) Characterization of the effects of introgressed segments of chromosome 7 and 10 from *Lycopersicon chmielewskii* on tomato soluble solids, pH, and yield. Theor Appl Genet 87:965–972

Bernatzky R, Tanksley SD (1986a) Genetics of actin-related sequences in tomato. Theor Appl Genet 72:314–321

Bernatzky R, Tanksley SD (1986b) Methods for detection of single or low-copy sequences in tomato on Southern blots. Plant Mol Biol Rep 4:37–41

Davies JN (1964) The effect of variety on the malic and citric acid content of tomato fruit. Annu Rep Glasshouse Crops Res Inst 139

Davies JN, Hobson GE (1981) The constituents of tomato fruit—the influence of environment, nutrition and genotype. CRC Crit Rev Food Sci Nutrit 15:205–278

Dinar M, Stevens MA (1981) The realtionship between starch accumulation and soluble-solids content of tomato fruits. J Am Soc Hor Sci 106:415–418

Hewitt JD, Dinar M, Stevens MA (1982) Sink strength of fruits of two tomato genotypes differing in total fruit-solids content. J Am Soc Hor Sci 107:896–900

Ho LC, Sjut V, Hoad GV (1983) The effect of assimilate supply in fruit growth and hormone level in tomato plants. Plant Growth Reg 1:155–171

Hobson GE, Davies JN (1971) The biochemistry of fruits and their products. In: Hulme AC (ed) The tomato. Academic Press, New York, pp 437–472

Jones RA, Scott SJS (1983) Improvement of tomato flavor by genetically increasing sugar and acid contents. Euphytica 32:845–855

Lower RL, Thompson AE (1966) Sampling variation of acidity and solids in tomatoes. Proc Am Soc Hort Sci 89:512–522

Mao Z, Craker LE (1990) Abscission: the carbohydrate factor. Plant Sci 71:167–172

Rick CM (1974) High soluble-solids content in large-fruited tomato lines derived from a wild green-fruited species. Hilgradia 42: 493–510

SAS Institute Inc. (1985) SAS user's guide, 5th ed. North Carolina Cary, SAS Institute Inc.

Stevens MA, Kader AA, Albright-Houlton M, Algazi M (1977) Genotypic variation for flavor and composition in fresh market tomatoes. J Am Soc Hort Sci 102:680–689

Sun J, Loboda T, Sung SJS (1992) Sucrose synthase in the wild tomato, *Lycopersicon chmielewskii*, and tomato fruit sink strength. Plant Physiol 98:1163–1169

Tanksley SD, Hewitt J (1988) Use of molecular markers in breeding for soluble-solids content in tomato – a re-examination. Theor Appl Genet 75:811–823

Tanksley SD, Ganal MW, Prince JP, de Vicente MC, Bonierbale MW, Broun P, Fulton TM, Giovannoni JJ, Grandillo S, Martin GB, Messeguer R, Miller JC, Miller L, Paterson AH, Pineda O, Roder MS, Wing RA, Wu W, Young ND (1992) High-density molecular linkage maps of the tomato and potato genomes. Genetics 132:1141–1160

Thompson AE, Lower RL, Helper RW (1964) Increasing acidity content of tomatoes by breeding and selection. Proc Am Appl Genet 75:811–815

Walker AJ, Ho LC (1977) Carbon translocation in the tomato: carbon import and fruit growth. Ann Bot 41:813–823

Walker AJ, Thornley JHN (1977) The tomato fruit: import, growth, respiration and carbon metabolism at different fruit sizes and temperatures. Ann Bot 41:977–985

Walker AJ, Ho LC, Baker DA (1978) Carbon translocation in the tomato: pathways of carbon metabolism in the fruit. Ann Bot 42:901–909

Winsor GW, Davies JN, Massey DM (1962) Composition of tomato fruit. III. Juices from whole fruit and locules at different stages of ripeness. J Sci Food Agric 13:108–112

Young TE, Juvik JA, Sullivan JG (1993) Accumulation of the components of total solids in ripening fruits of tomato. J Am Soc Hort Sci 118:286–292