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Identification of restriction fragment length polymorphisms linked to genes controlling soluble solids content in tomato fruit

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Summary. Gene(s) conferring high soluble solids (SS) in tomato fruit had been backcrossed previously from a wild tomato species, *Lycopersicon chmielewskii* LA1028 (~ 10% SS), into a *L. esculentum* cultivar, VF36 (~ 5% SS), to derive a BC_5S_5 line, LA1563, similar to 'VF36' but with 7–8% SS. DNAs from these lines and a tomato breeding line, H2038, were screened for restriction fragment length polymorphisms (RFLPs) using four restriction endonucleases and sixty clones chosen at random from a tomato cDNA library. Most of the cDNA clones (56) identified the same RFLP in 'VF36' and LA1563 and a different RFLP in LA1028. However, two cDNA clones identified the same RFLP in LA1563 and LA1028 and a different RFLP in 'VF36'. To determine whether RFLPs identified by these two cDNA clones were linked to SS genes, a H2038 × LA1563 F_2 population was screened for segregation of the RFLPs and for SS content. The segregation ratios of these RFLPs were consistent with ratios expected for codominant alleles at unlinked loci. Analysis of variance of SS content for different RFLP genotypic classes indicated that RFLP alleles at one of the loci were linked to genes controlling SS content. The RFLP allele from the high SS tomato line, LA1563, was associated with significantly higher SS content and, therefore, could be useful in selecting for high SS gene(s) in a tomato breeding program.

Key words: Restriction fragment length polymorphisms (RFLPs) – Quantitative trait loci – Linkage – Tomato – Soluble solids

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Introduction

Homologous fragments of DNA that vary in length after being cleaved with restriction endonucleases have been termed restriction fragment length polymorphisms (RFLPs). The detection and potential uses of RFLPs as genetic markers in plants have been considered (Beckmann and Soller 1983; Burr et al. 1983; Soller and Beckmann 1983). RFLPs can be used to establish linkage maps, to identify cultivars and to study genes controlling quantitative traits. They have potential uses in crop improvement as aids to plant breeders in the selection of desired genotypes. Although much attention has been given to their potential uses; very little work has been done with RFLPs in plants. The level of RFLP variation has been reported for maize and tomatoes (Helentjaris et al. 1985; Rivin et al. 1983) and RFLPs have been genetically mapped in these crops (Helentjaris et al. 1986; Vallejos et al. 1986).

RFLPs have potential applications as markers linked to genes controlling quantitative traits. They can be used in basic research on the genetic control of quantitative traits and in crop improvement by providing a means for marker-based selection of desired polygenes. In tomatoes, a quantitative trait of great interest to the tomato processing industry is soluble solids (SS) content of the tomato fruit, which includes primarily fructose, glucose and other sugars. Tomatoes used in formulating solids-based products are often priced according to their SS content and because of the large amount of tomatoes grown worldwide for processing, small increases in the SS content of the raw tomatoes can have an enormous economic impact. Although the cultivated tomato (*L. esculentum*) is relatively low in SS (~ 5%), some wild relatives of the tomato have much higher proportions (Rick 1974). An

accession, LA1028, of a wild relative, *L. chmielewskii* (formerly *L. minutum*), was used as a high SS (~ 10%) donor parent to backcross one or more genes for high SS into the tomato cultivars, VF36 and VF145-22-8 (Rick 1974). The derived BC_5S_5 lines had high SS (7–8%) and were phenotypically similar to 'VF36'.

In attempts at using one of the derived backcross lines, LA1563, to transfer the high SS trait to superior processing tomato lines, high environmental variation and a low heritability were observed making it difficult to identify high SS genotypes (Steve Schroeder, personal communication). Therefore, the identification of genetic markers linked to gene(s) conferring high SS could be very useful in a tomato breeding program. In this study, we identified RFLPs with potential linkage to genes controlling SS content and then tested for linkage relationships between these RFLPs and SS genes.

Materials and methods

Plant materials, plant growth and SS determination

Seeds of the tomato lines 'VF36', LA1028 and LA1563 were obtained from Charles M. Rick, University of California, Davis, CA. Seeds of the tomato breeding line H2038 were obtained from Steve Schroeder, Heinz USA, Stockton, CA. These tomato lines were grown in the greenhouse and expanding axillary shoots were harvested for DNA isolation. Seeds of H2038, LA1563 and the F_1 and F_2 of H2038 × LA1563 were planted in double rows spaced 36 cm apart on 107 cm wide beds with beds spaced 168 cm apart at Stockton, CA in the spring of 1984. Plants were thinned to 20 cm apart within rows and watered by furrow irrigation. Expanding axillary shoots were harvested just prior to the onset of flowering for DNA isolation. At the end of the growing season, three to five fruit per plant were harvested for SS determinations. The fruit were ground in a blender and the percentage SS in the juice measured as °Brix on a refractometer.

DNA isolation and blot preparation

Harvested leaves were frozen immediately in a −80 °C freezer and then lyophilized. Lyophilized leaves were pulverized by rolling a cylinder over a bag containing the leaves. Total cellular DNA was isolated from the powdered, lyophilized leaves according to the method of Saghi-Maroot et al. (1984) except that DNA was precipitated with 2–3 volumes of cold ethanol instead of isopropanol. The DNA was dissolved in 10 mM Tris-HCl pH 7.5, 1 mM EDTA, 10 mM NaCl. Approximately 10 µg of DNA/RNA was incubated overnight at 37 °C with 20 units of one of four different restriction endonucleases, *Bam*HI, *Eco*RI, *Hind*III or *Sst*I (BRL) and 5 µg of RNase A. The digested DNAs were separated by electrophoresis in 0.8% agarose gels and 40 mM Tris-acetate, 1 mM EDTA and transferred onto either Gene Screen Plus (NEN) or Magna Nylon 66 (MSI) nylon membranes as described by Southern (1975). Blots were prehybridized at 65 °C for 4–24 h in sealed bags containing a solution (45 µl/cm² of membrane) of 5 × SSPE (0.74 M NaCl, 0.05 M $NaH_2PO_4H_2O$, 6 mM EDTA, pH 7.4), 5 × Denhardts (0.1% Ficoll, 0.1% polyvinyl pyrrolidone, 1% BSA), 0.5% SDS, 10% dextran sulfate and 10 µg/ml denaturated calf thymus DNA.

Probe selection

Probes for detecting RFLPs were selected from a tomato cDNA library prepared as described previously (Alexander et al. 1984) using the pARC7 cDNA cloning vector and mRNA extracted from leaves of heat shocked tomato seedings of 'VFNT Cherry'. The cDNA library was screened by *in situ* colony hybridization (Taub and Thompson 1982) using [^{32}P]cDNA of the mRNA used for cloning. After exposing to film, the colony lift filters were boiled in 1 mM EDTA to remove the probe and then reprobed with cDNA of control leaf mRNA. In general, clones which hybridized with the same intensity to both sources of cDNA were selected for plasmid isolation. Plasmids were isolated essentially as described by Birnboim and Doly (1979). The insert size of cDNAs were determined by digesting plasmids with *Sma*I restriction endonuclease and separating on 1.5% agarose gels. Plasmid preparations containing cDNAs ≥ 400 bp were nick translated for use in screening parental lines for RFLPs. The two plasmids used for screening the F_2 population were isolated by banding twice in CsCl gradients (Kahn et al. 1979).

Probe labeling and hybridization to blots

Selected plasmids were nick translated using a Nick Translation Reagent Kit (BRL) and [^{32}P]dCTP (600 Ci/mM, NEN). Labeled probes were isolated by low speed centrifugation of the nick translation mixture through two beds of Biogel P4 (BioRad). The labeled probes (0.5×10^6 cpm/ml hybridization solution) were mixed with calf thymus DNA (100 µg/ml hybridization solution), denatured by boiling and added to bags containing the blots and prehybridization solution. Hybridization was carried out at 65 °C for 20–40 h. Blots were washed two times in 2 × SSC (0.30 M NaCl, 30 mM sodium citrate, pH 7.0) at room temperature for 5 min, two times in 2 × SSC, 1% SDS at 65 °C for 30 min and 2 times in 0.25 × SSC at 65 °C for 30 min. They were blotted dry, wrapped in plastic wrap and exposed to XAR-5 film with one intensifying screen for 4–7 days. Blots were prepared for reuse by washing in 0.1 M NaOH at room temperature for 15 min followed by a wash in 0.1 × SSC, 0.1% SDS, 0.2 M Tris-HCl, pH 7.5 at room temperature for 15 min.

Statistical analysis

The goodness of fit of observed segregation ratios to expected ratios was determined using analysis of chi squares by orthogonal function (Mather 1951). Linkage relationships between RFLPs and genes controlling SS content and analysis of types of gene action were determined by analysis of variance (SAS computer program) using RFLP genotypic groups as class variables. Significant differences in the SS content of RFLP genotypes were interpreted as an indication of linkge between RFLPs and SS gene(s).

Results

Parental screening

Southern blots of DNAs from four tomato lines, 'VF36' (low SS recurrent parent), H2038 (low SS breeding line), LA1563 (high SS backcross-derived line) and LA1028 (high SS donor parent), digested with four restriction endonucleases, were hybridized with ap-

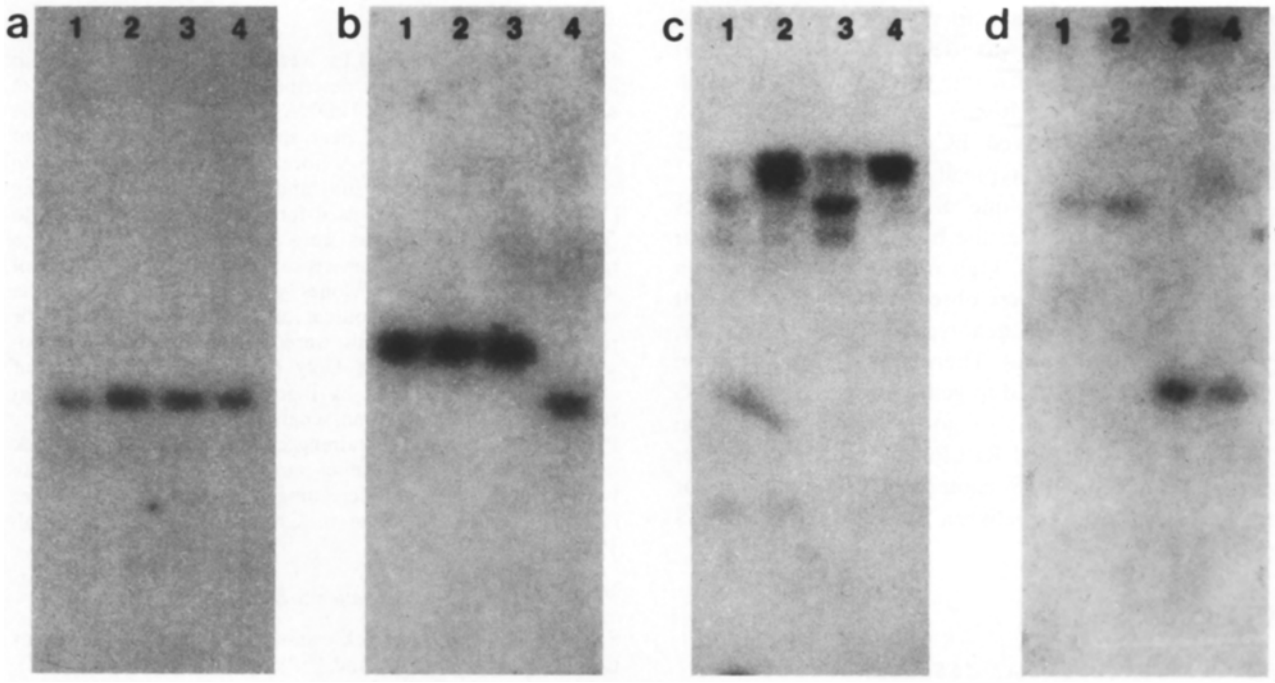

Fig. 1. Examples of the four different classes of RFLP patterns observed on autoradiographs of restriction enzyme digested DNAs from four tomato lines probed with different tomato cDNA clones. Tomato lines: *1* 'VF36'; *2* H2038; *3* LA1563; *4* LA1028. *a* *Hind*III digest, pTHL83 probe; *b* *Hind*III digest, pTHL82 probe; *c* *Bam*HI digest, pTHL32 probe; *d* *Hind*III digest pTHL75 probe

proximately 80 tomato cDNA clones. Sixty of these clones gave distinguishable RFLP patterns on autoradiographs of Southern blots. RFLP patterns for clones which had shown strong hybridization to total cDNA by *in situ* colony hybridization (i.e., clones of abundant mRNA) were compared to each other and all were unique. Therefore, all cDNA clones probably were hybridizing to unique genomic sites.

For individual clones, RFLP patterns could be divided into four classes (Fig. 1, Table 1). Class one included two cDNA clones which detected the same RFLP pattern in the four lines for all four restriction enzymes used to digest the DNAs. In class two (54 cDNA clones), the same RFLP pattern was observed in 'VF36', H2038 and LA1563 DNAs and a different RFLP pattern in LA1028 DNA for at least one of the restriction enzymes. This indicated that the restriction fragments in LA1563 identified by these clones had been introduced from 'VF36' by backcrossing. This was also true of the fragments identified by two clones in class three; however, these clones also detected a different RFLP pattern in H2038. Class four included two cDNA clones which identified one RFLP pattern in H2038 and 'VF36' and a second, different RFLP pattern in both LA1563 and LA1028. The restriction fragments in LA1563 identified by these two clones, pTHL75 and pTHL104, apparently had been introduced from the high SS donor parent, LA1028, by backcrossing and, therefore, represented RFLPs that potentially were linked to gene(s) conferring high SS.

Table 1. Number of cDNA clones detecting the four polymorphism classes shown in Fig. 1

Polymorphism class	No. of cDNA clones
1. One RFLP pattern in all four lines for all enzymes tested (Fig. 1 a)	2
2. One RFLP pattern in 'VF36', H2038 and LA1563, second pattern in LA1028 for at least one enzyme tested (Fig. 1 b)	54
3. One RFLP pattern in 'VF36' and LA1563, second pattern in LA1028, third pattern in H2038 for at least one enzyme tested (Fig. 1 c)	2
4. One RFLP pattern in 'VF36' and H2038, second pattern in LA1028 and LA1563 for at least one enzyme tested (Fig. 1 d)	2

F_2 segregation analysis

F_2 plants from a cross between H2038 and LA1563 were analyzed for segregation of RFLPs detected by the cDNA clones, pTHL75 and pTHL104. Examples of

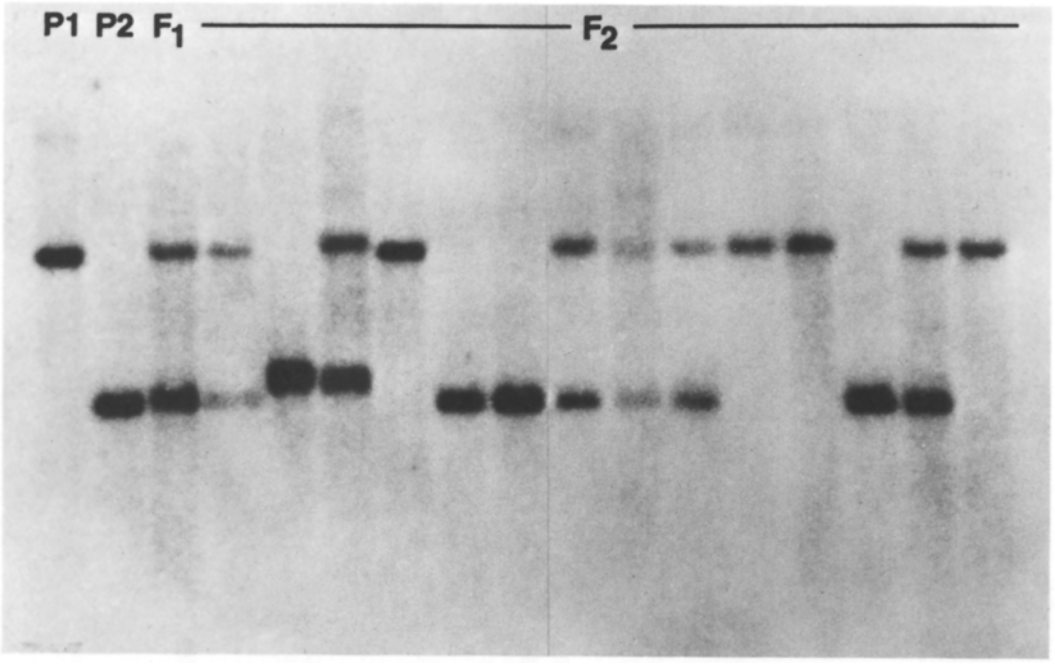

Fig. 2. RFLP patterns observed on autoradiograph of *Hind*III digested DNAs from H2038 (P1), LA1563 (P2), H2038×LA1563 (F_1) and the F_2 progeny probed with pTHL75

the RFLP patterns observed in the parental, F_1 and F_2 progeny DNAs when cut with restriction enzyme *Hind*III and probed with pTHL75 are shown in Fig. 2. For H2038, one 5.6 kb fragment was observed, whereas LA1563 had one 1.5 kb fragment. Both fragments were observed in the F_1, and the F_2 segregated for the parental and F_1 patterns. Similar RFLP patterns of fragments having all together different sizes were observed for the parental, F_1 and F_2 progeny DNAs when digested with *Hind*III and probed with pTHL104. Therefore, F_2 plants could be assigned RFLP genotypes at the two loci identified by these cDNA clones. We have assigned the loci identified by pTHL75 and pTHL104 as *A1* and *A2*, respectively, and the alleles from H2038 and LA1563 as *e* (for *L. esculentum*) and *c* (for *L. chmielewskii*), respectively.

The observed and expected segregation ratios of H2038 × LA1563 F_2 plants are shown for the individual RFLP loci, *A1* and *A2*, in Table 2 and for the loci combined in Table 3. The total number of F_2 plants used in the combined analysis (Table 3) was less than in the analysis for locus *A1* or *A2* individually because of missing data for some plants at one or the other locus. The chi square values of observed segregation ratios based on ratios expected for single gene inheritance at loci *A1* and *A2* individually and the probabilities of greater chi square values also are shown in Table 2. The total chi square value for data at loci *A1* and *A2* combined (Table 3) was partitioned into chi square

Table 2. Observed and expected segregation ratios of H2038 × LA1563 F_2 plants for RFLP genotypes at loci *A1* and *A2* individually

Locus[a]	Allelic constitution of F_2 plants[b]							
	e/e		*e/c*		*c/c*			
	obs	exp	obs	exp	obs	exp	X^2	*P*
A1	45	41.25	76	82.5	44	41.25	1.04	0.59
A2	43	36.75	71	73.5	33	36.75	1.53	0.47

[a] RFLPs at loci *A1* and *A2* are detected by cDNA clones pTHL75 and pTHL104, respectively
[b] *e* is the RFLP allele from H2038 and *c* is the RFLP allele from LA1563

Table 3. Observed and expected cosegregation ratios of H2038×LA1563 F_2 plants for RFLP genotypes at loci *A1* and *A2* combined[a]

	$A1^e/A1^e$		$A1^e/A1^c$		$A1^c/A1^c$	
	obs	exp	obs	exp	obs	exp
$A2^e/A2^e$	15	8.88	19	17.75	9	8.88
$A2^e/A2^c$	15	17.75	30	35.5	23	17.75
$A2^c/A2^c$	13	8.88	14	17.75	4	8.88

[a] x^2 for segregation at locus *A1* = 2.49 (2 df), $P = 0.29$
x^2 for segregation at locus *A2* = 2.28 (2 df), $P = 0.32$
x^2 for linkage between loci *A1* and *A2* = 7.76 (4 df), $P = 0.10$

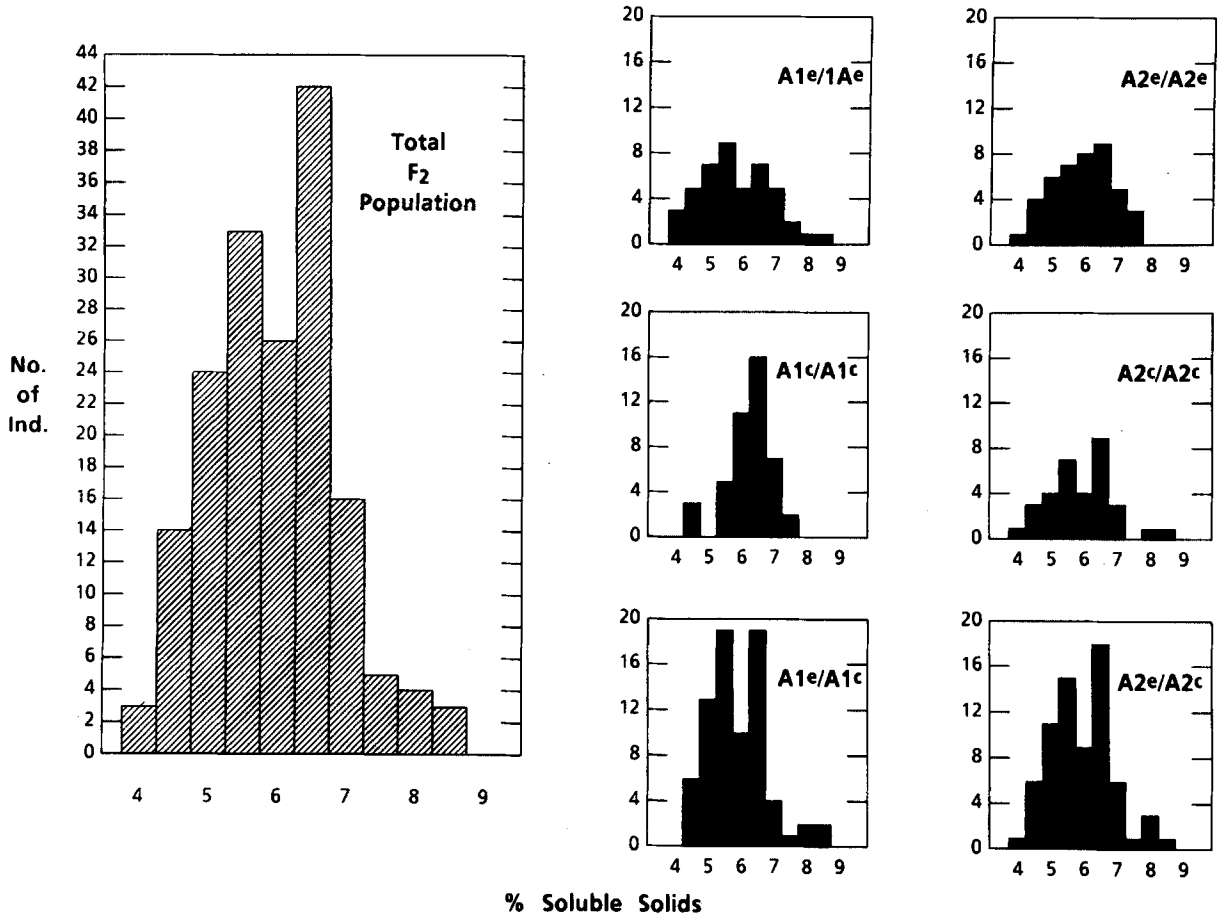

Fig. 3. Histograms showing the distributions of H2038 × LA1563 F_2 plants from the total F_2 population analyzed and the F_2 plants having different RFLP genotypes

values for segregation at each locus individually and a chi square value for linkage between loci. These values and the probabilities of greater chi square values also are presented in Table 3.

Analysis of linkage between RFLPs and SS genes

The SS content was determined in fruit from single parental, F_1 and F_2 plants. The SS frequency distributions of the entire F_2 population and subsets of the population having different RFLP genotypes are shown in Fig. 3. The population as a whole appears normally distributed for SS content and based on statistical analysis, it did not deviate significantly ($P > 0.05$) from a normal distribution. The frequency distributions of plants having homozygous genotypes at the *A1* locus ($A1^e/A1^e$ and $A1^c/A1^c$) appear to be different: the distribution of the $A1^e/A1^e$ plants is shifted to the lower end of the SS scale whereas the distribution of the $A1^c/A1^c$ plants is shifted to the upper end of the scale. The distribution of the heterozygous $A1^e/A1^c$ plants is simlar to the total F_2 population. For the *A2* locus, all genotypic subsets of plants have similar distributions.

Table 4. Analysis of variance among RFLP genotypes at locus *A1* for SS content

Source	df	MS	F	*P*
Genotype	2	2.341	2.94	0.056
e/e vs *c/c*	(1)	3.942	4.95	0.028
1/2(*e/e*+*c/c*) vs *e/c*	(1)	0.765	0.96	0.329
Error	162	0.797		

Linkage relationships between RFLPs and genes controlling SS content and information on the types of gene action were determined by analysis of variance. Variation in SS content was analyzed among RFLP genotypic classes at loci *A1* and *A2* separately. In testing for variation due to the three RFLP genotypes at locus *A1* (Table 4), the F statistic was found to have a probability just greater than 0.05. This variation was partitioned into single degree freedom contrasts to

Table 5. Soluble solids content associated with RFLP genotypes in parental, F_1 and F_2 generations

Line or cross	Marker genotype	Statistic	Total	% Soluble solids[a]					
				Locus *A1*			Locus *A2*		
				e/e	*c/c*	*c/e*	*e/e*	*c/c*	*c/e*
H2038	*e/e*	mean	5.41	–	–	–	–	–	–
LA1563	*c/c*	mean	6.73	–	–	–	–	–	–
F_1	*e/c*	mean	5.68	–	–	–	–	–	–
F_2	*e/e, e/c, c/c*	mean	5.96	5.82 –*–	6.24	5.90	5.93 –ns–	5.96	5.93
		median	6.00	5.60	6.35	5.75	6.00	6.00	5.90

[a] ns = nonsignificant; $P > 0.05$ and * = significant; $P < 0.05$ as determined by single degree freedom contrasts from analyses of variance

compare 1) the two homozygous RFLP classes to each other and 2) the mean of these classes to the heterozygous class. The first contrast was significant ($P < 0.05$) whereas the second contrast was non-significant ($P > 0.05$) (Table 4). This analysis indicated linkage between the *A1* locus and one locus or a group of linked loci affecting SS content with gene(s) conferring relatively high SS content linked to the $A1^c$ allele and gene(s) conferring relatively low SS content linked to the $A1^e$ allele. Variation in SS content due to the three RFLP genotypes at locus *A2* and the contrasts comparing specific genotypic classes at this locus were all non-significant (P values > 0.90) indicating no linkage relationship between locus *A2* and loci affecting SS content (data not shown). The magnitudes of interlocus interactions (epistatis) were determined as contrasts from analysis of variance using data for genotypic classes of loci *A1* and *A2* combined and all were non-significant (P values > 0.20, data not shown). The mean percentage SS in fruit of parental and F_1 plants and the mean and median percentage SS in fruit of the total F_2 population and the different F_2 RFLP genotypic classes is shown in Table 5.

Discussion

In this study, we utilized previously developed genetic materials (Rick 1974) to identify RFLP loci that potentially were linked to loci affecting SS content in tomato fruit. By screening the low SS recurrent parent, the high SS donor parent and the high SS derived backcross line for variation in RFLPs detected by 60 random cDNA clones, we were able to identify two cDNA clones that hybridized to RFLPs which had been introduced into the backcross line from the high SS donor parent. The proportion of homozygous RFLP loci introduced by backcrossing (2 out of 58 informative loci or 3.4%, assuming single locus detection by cDNA clones) is only slightly greater than the average proportion of homozygous donor parent genome expected in a BC_5S_5 line developed without selection (1.6%). The larger observed proportion can be explained by selection for donor parent linkage group(s) in conjunction with backcrossing. By using this procedure for identifying a few RFLP loci with potential linkage to SS loci, we were able to avoid analyzing a large population for segregation of RFLPs at many loci scattered randomly throughout the genome.

In order to determine if the RFLPs identified by the two selected cDNA clones were linked to genes controlling SS, we analyzed a F_2 population derived from a cross between a low SS processing tomato line and the high SS backcross line for segregation of RFLPs identified by the two cDNA clones and for SS content. Segregation ratios of RFLPs identified by both cDNA clones were consistent with ratios expected for co-dominant alleles at single loci. Cosegregation ratios of the loci combined also were consistent with single gene inheritance at each locus individually and the chi square values for linkage had a probability value of $P > 0.05$, indicating independent assortment of alleles at loci *A1* and *A2*. However, one of the single degree of freedom contrasts from the linkage analysis was significant ($P < 0.05$) and, therefore, caution must be taken in asserting non-linkage between these loci.

In analyzing the relationship between the RFLP genotypes and SS content of F_2 plants, differences in the SS distributions between RFLP genotypic subsets of the F_2 population suggested linkage between SS genes and alleles at locus *A1* but not locus *A2*. Statistical analysis of the data confirmed this observation. The two homozygous RFLP classes at locus *A1* had significantly different SS contents, whereas those at locus *A2* did not. The difference in mean values between homozygous classes at the *A1* locus was 0.42% SS with the $A1^c/A1^c$ genotypic class having a higher percentage SS. The difference in median value was even greater (0.75% SS). This would be expected if there was a low frequency of recombination between the *A1* locus and the linked SS loci. Although these

differences are not large, we reemphasize that even small increases in the SS content of tomato cultivars may have great economic importance. Also, the difference between the high and low SS parents observed in this growing season (1.32% SS) was less than the difference reported previously (2.3% SS, Rick 1974). In a more typical growing season, a larger difference in the two RFLP genotypic classes might be observed.

The data presented in this study provide some insight into the types of gene action involved in controlling SS content in the cross we analyzed. The mean SS value of the H2038 × LA1563 F_1 plants was intermediate to the two parents, but closer to the low SS parent. The heterozygous $A1^e/A1^c$ F_2 class was also intermediate to the two homozygous classes, but closer to the $A1^e/A1^e$ F_2 class. These data suggest some dominance of genes controlling low SS. However, in the analysis of variance of the *A1* locus, the contrast representing the test for dominance gene action ($\frac{1}{2}(e/e + c/c)$ vs e/c) was non-significant whereas the contrast representing additive gene action (e/e vs c/c) was significant. Rick (1974) found dominance of genes controlling high SS in the F_1 of VF36 × LA1028; however, he did not report results of crosses between the high SS backcross-derived lines and low SS lines. In other studies involving different genetic materials and different types of mating designs, various results were obtained. Additive gene action for SS content was the most important component of genetic variance in studies by Lower and Thompson (1967) and Ibarbia and Lambeth (1969), whereas dominance and epistatic gene action were the most important in a study by Stoner and Thompson (1966).

In this study, we identified a RFLP locus linked to one or a group of linked loci affecting SS content in tomato fruit. For a second RFLP locus which apparently was unlinked to the first RFLP locus, no linkage to SS loci could be detected. Other loci that significantly effect SS content probably exist in the lines we used and these could have been detected if more donor parent RFLP marker loci had been identified in the backcross line and/or if more F_2 plants had been analyzed. Also, the ability to detect SS loci is dependent on what alleles are present at loci affecting SS content and, therefore, may be dependent on what genetic materials are used. In the F_2 progeny we analyzed, the RFLP allele, $A1^c$, from LA1563 was linked to gene(s) which significantly increased SS content in tomato fruit. This linkage relationship could be used in a tomato breeding program to select for lines with increased SS content by selecting for the linked RFLP allele.

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