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Fgr, a major locus that modulates the fructose to glucose ratio in mature tomato fruits

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Abstract A genetic trait determining the ratio of fructose to glucose in mature tomato fruits is described. A backcross breeding program based on the interspecific cross of *Lycopersicon hirsutum* and *L. esculentum* yielded stable genotypes with a high ratio of fructose to glucose (>1.5:1) compared with the approximately equimolar ratios found in *L. esculentum*. Two inter-simple-sequence repeat (ISSR) DNA sequences, highly associated (20 <LOD score <21) with the trait, were identified. The markers were found to be less associated with either glucose or fructose levels individually (2 <LOD score <3) and were statistically unlinked to total sugars and total soluble solids (TSS). These two ISSR bands segregated in a dominant fashion and were found to be allelic to each other, one associated in coupling and the other in repulsion with the trait of high fructose to glucose ratio. Both ISSR markers were mapped to the centromeric region of tomato chromosome 4. Quantitative analysis of the identified locus, based on data from segregating F_2, BC and F_3 populations from the cross between genotypes having high and low fructose to glucose ratios, suggested that the *L. hirsutum*-derived allele (*Fgr*^{H}), which increases the fructose to glucose ratio, is partially dominant. *Fgr*^{H} leads to an increase in fructose levels and a subsequent decrease in glucose levels, with no effect on total hexose levels. Accordingly, we conclude that the *Fgr* locus modulates the partitioning of hexose sugars between fructose and glucose, with no effect on total sugars or TSS.

Key words *Lycopersicon hirsutum* · *Lycopersicon esculentum* · PCR · Fructose · Glucose

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Introduction

The soluble solids content of tomato fruit is primarily comprised of sugars, organic acids and salts (Davies and Hobson 1981) and is a major determinant of fruit quality, both for industrial use (Hankin 1986) and for fresh market consumption (Stevens et al. 1977). Approximately half of the soluble solids content is contributed by the sugar fraction which, in all standard cultivars of *Lycopersicon esculentum*, consists of the monosaccharide reducing sugars glucose and fructose in approximately equimolar concentrations (Davies and Hobson 1981). There is, however, an obvious advantage in fructose accumulation with respect to fruit quality, given that fructose is approximately twice as sweet as its isomer, glucose (Biester 1925).

L. hirsutum fruit has a distinct carbohydrate metabolism, and this species, as well as other green-fruited *Lycopersicon* species, has been utilized as a gene source for modifying sugar accumulation patterns in tomatoes. For example, the trait of sucrose accumulation has been transferred from *L. chmielewskii* (Chetelat et al. 1995) and from *L. hirsutum* (Hadas et al. 1995; Schaffer et al. 1998). The sucrose-accumulating *L. hirsutum* fruit is characterized by concomitant low hexose levels but has a relatively high ratio of fructose to glucose (Davies 1966). Stommell and Haynes (1993) presented results, based on an interspecific F_2 population, that suggested that the trait of high fructose to glucose ratio is polygenically inherited.

The trait of high fructose to glucose ratio can be combined with standard *L. esculentum* hexose levels (Stommell and Haynes 1993; Schaffer et al. 1999). Tomato genotypes with a high fructose to glucose ratio in

the mature fruit have been developed in a backcross breeding program based on an initial interspecific cross between *L. esculentum* and *L. hirsutum*, with selection for individual sugar levels (Schaffer et al. 1999). However, precision breeding towards higher fructose levels in the mature tomato fruit involves the assessment of individual reducing sugars via time-consuming chemical, enzymatic or chromatographic analysis. DNA markers could potentially alleviate this problem, enabling the identification and selection of genetic material at the seedling stage, thus significantly reducing effort and time.

During recent years, international efforts have been invested in the genome mapping of several plant species, such as tomato, potato and maize, by means of DNA markers (Helentjaris et al. 1986; Tanksley et al. 1992). Apart from being an efficient tool for many breeding and genetic analyses (reviewed by Hillel et al. 1992), DNA markers also provide initial map-based cloning sites for genes of interest. Recently, there have been several reports of successful gene isolations in higher plants by positional cloning (reviewed by Tanksley et al. 1995)

We report here the discovery of a major locus that modulates the fructose to glucose ratio in the mature tomato fruit. Two inter-simple-sequence repeat (ISSR) DNA markers linked to the locus were identified by means of the polymerase chain reaction (PCR) (Saiki et al. 1985). These DNA markers made the chromosomal localization of the trait possible, and they will be useful in the early marker-assisted selection of plants with this potentially important trait as well as for the map-based cloning of the gene.

Materials and methods

Plant material and growing conditions

Three parental lines, 1406, 1408 and 1415, were used in this study. Lines 1406 and 1408 are BC_3F_4 lines derived from the interspecific cross of *L. hirsutum* (accession LA1777) and an *L. esculentum* male-sterile breeding line developed at the Volcani Center (1630), with successive selection at each generation for fructose and glucose levels in the mature fruit. Line 1415 is another Volcani Center *L. esculentum* breeding line. Lines 1406 and 1408 were each crossed to line 1415 to yield F_1 seeds, and F_1 plants were selfed to yield two F_2 segregating populations. F_1 plants (1406×1415) were also backcrossed to line 1415 to yield a BC population. The parental lines, F_1 and segregating BC and F_2 plants were grown in an environmentally controlled greenhouse at the Volcani Center, Bet Dagan, Israel during the winter of 1996/1997. The number of plants grown from each of the segregating populations is presented in Table 1.

The genotypes of all F_2 plants were determined by means of the markers developed during the present study. F_2 plants solely carrying the *L. hirsutum* allele, which is associated with a high fructose to glucose ratio, were scored as HH, heterozygous F_2 plants were scored as HE. F_2 plants solely carrying the *L. esculentum* allele, which is associated with a lower fructose to glucose ratio, were scored as EE. F_3 populations were generated from selected selfed F_2 plants. A total of nine F_3 populations were derived from three F_2 plants scored as HH, four heterozygous HE and two homozygous EE plants. The genotype of the F_3 plants was also determined with the markers developed, and a total of 25 EE, 37 HE and 43 HH plants were grown in a screen-house at the Volcani Center, Bet Dagan, Israel during the summer of 1997 for the purpose of progeny testing.

Table 1 Average fructose to glucose ratio in P_1, P_2, F_1, F_2, BC and F_3 populations used in this study (*n*=number of plants. *SE*= standard error)

Line	Generation	*n*	Mean±SE
1406	P_1	4	1.99±0.11
1408	P_1,	4	2.33±0.17
1415	P_2	3	1.12±0.01
1471	F_1 (1406×1415)	3	1.15±0.1
1419	F_1 (1408×1415)	5	1.35±0.16
1420	F_2 (1406×1415)	99	1.44±0.04
1424	F_2 (1408×1415)	63	1.34±0.04
1421	BC (1471×1415)	36	1.22±0.01
1500	F_3 families	105	1.68±0.08

DNA extraction

Genomic DNA was extracted from individual plants of the three parental lines and the F_1, F_2, BC and F_3 populations. The extraction procedure was according to Fulton et al. 1995.

PCR primers

All DNA primers used during the course of this study were purchased from Pharmacia Biotech, Austria. Sixteen primers containing di-, quadri- and penta-nucleotide SSR sequences were used to screen for polymorphic loci. These primers were applied to the PCR reaction as single primers or in two-primer combinations (a total of 120 combinations). Two of the primers that produced bands linked to the fructose to glucose ratio, MS6 (TCTCTCTCTCTCTCTCCC) and MS8 (TCTCTCTCTCTC TCTCCG), were extended by one of the four possible nucleotides at their 3' end and a single base (T) was deleted from their 5' end (primers MS61=CTCTCTCTCTCTCTCCCA, MS62=CTCTCTC TCTCTCTCCCT, MS63=CTCTCTCTCTCTCTCCCC, MS64= CTCTCTCTCTCTCTCCCG, MS81=CTCTCTCTCTCTCTCCGA, MS82=CTCTCTCTCTCTCTCCGT, MS83=CTCTCTCTCTCTCT CCGC and MS84=CTCTCTCTCTCTCTCCGG). Single-locus PCR primers that were designed following cloning and sequencing of the PCR product, originally generated using the MS6 primer, were: SC65=CGGCGGAAAGATGTGAACTT, SC6M5=ACGG TCATGTGAATGGGACT, SC6M3=AGTCCCATTCACATGAC CGT and SC63=GCTTTGTCTCATTGCAGGAC (Fig. 2).

PCR reaction

The amplification reaction of ISSR sequences (25-ml final volume) was performed with 10 ng template DNA, 25 m*M* TAPS (pH=9.3 at 25 C), 50 m*M* KCl, 2 m*M* MgCl2, 1 m*M* β-mercaptoethanol, 0.2 m*M* of each of the four deoxyribonucleotide triphosphates (dATP, dCTP, dGTP and dTTP), 20 ng of a single primer (or 10 ng of each of 2 primers when combinations of 2 primers were used), and 1 U of thermostable *Taq* DNA polymerase (SuperNova *Taq* polymerase, Madi Ltd, Rishon Le Zion, Israel). Reaction mixtures were overlaid by 15 ml of light mineral oil, and reactions were carried out in an automated thermocycler (MJ Research, Watertown, Mass., USA). Initial incubation was at 94°C for 1.5 min, followed by 34 cycles of denaturation at 94°C for 1 min, annealing at 45°C for 1 min and polymerization at 72°C for 1 min. A final polymerization cycle at 72°C was carried out after the above cycles were been completed. The amplification products were visualized by electrophoresis in 1.5% agarose gels and were detected by staining with ethidum bromide. Single-locus PCR amplification reactions were carried out at an annealing temperature of 55°C.

Soluble sugar determination

Fruit pericarp portions of about 500 mg fresh weight were placed in 80% ethanol, and soluble sugars were extracted from the tissue by heating to 70°C as previously described (Miron and Schaffer 1991). Sugars were separated chromatographically by HPLC in a Biorad Fast Carbohydrate column according to the manufacturer's directions (Bio-Rad Laboratories, Hercules, Calif., USA). Sucrose, glucose and fructose were identified refractometrically by their retention time and quantified by comparison with sugar standards. The total soluble solids (TSS) content of expressed fruit juice was determined using an Atago digital refractometer.

Cloning of the PCR fragments generated by the MS6 and MS8 primers

The polymorphic bands generated by the MS6 and MS8 primers were excised from an agarose gel and purified with the GENECLEAN II kit (BIO 101, La Jolla, Calif., USA). The PCR bands were cloned into an pGEM-T Easy vector using the pGEM-T and pGEM-T Easy Vector System according to the manufacturer's recommendations (Promega Corp, Madison, Wis., USA).

Sequencing of the PCR fragments generated by the MS6 and MS8 primers

The DNA clones generated by the MS6 and MS8 primers were sequenced with an automated sequencer (Applied Biosystems, Foster City, Calif., USA). Sequence analysis and locus-specific primer design for the clone produced by primer MS6 were carried out with CLONEWORKS, clone design system version 1.94 (Anteater Software Corp, Los Angeles, Calif., USA). Sequence analysis and locus-specific primer design for the DNA clone generated by primer MS8 are yet to be carried out.

Mapping the markers linked to the fructose to glucose ratio

The markers linked to the fructose to glucose ratio were mapped by means of *L. pennellii* introgression lines (Eshed et al. 1992; Eshed and Zamir 1994a, b). DNA extracted from individual plants of each of the introgression lines, including their original parental lines M82 and *L. pennellii*, were used as templates in PCR reactions. The DNA marker generated by the MS6 primer was mapped by means of single-locus PCR primers that were designed on the basis of their nucleotide sequence (SC6M5 and SC63). The other ISSR DNA marker, initially identified with the MS8 primer, was mapped with the modified MS81 and MS82 primers.

Statistical analyses

Statistical analyses were carried out with the JMP Statistical Discovery software (SAS Institute, Cary, N.C., USA) and the MAPMAKER computer package (Lander et al. 1987). In order to evaluate the linkage between the markers and the trait of fructose to glucose ratio, we applied a cross-classified analysis of variance to data obtained from the segregating populations. The presentation of the results was simplified by combining data obtained from the two F_2 and BC populations that were used to identify the markers. The results obtained from the F_3 population that had been used for progeny testing are presented separately.

Results

Identification of DNA markers linked to the fructose to glucose ratio

Sixteen single ISSR primers and 120 ISSR primer combinations were screened in PCR reactions with DNA extracted from the parental lines differing in the fructose to glucose ratio in their mature fruit. Fifteen polymorphic bands were identified: 8 of these appeared solely in those parental lines characterized by a high fructose to glucose ratio, whereas 7 appeared solely in those parental lines characterized by a low fructose to glucose ratio. To evaluate the linkage between each of these dominantly segregating bands and the trait of fructose to glucose ratio, we carried out a PCR tail analysis (Plotsky et al. 1990; Michelmore et al. 1991) on plants from the 1420 segregating F_2 population. According to this analysis, 2 of the markers generated by the single primers MS6 and MS8 were associated with the fructose to glucose ratio. The PCR fragment produced by primer MS6 was coupled to high fructose to glucose ratios (Fig. 1). The PCR fragment obtained by primer MS8, on the other hand, was coupled to low fructose to glucose ratios (data not presented). Primers MS6 and MS8 were modified by extending their 3' end by one of the four possible nucleotides and reducing their 5' end, as outlined in the Materials and methods, to increase their locus specificity. Primers MS62 and MS63 regenerated the original band coupled to high fructose to glucose ratios, whereas primers MS81 and MS82 regenerated the original DNA band coupled to low fructose to glucose ratios. These primers were used in PCR reactions with DNA templates individually extracted from each of the plants of the two F_2 and the BC populations. The presence or absence of each of the marker bands was scored for each of these individual plants.

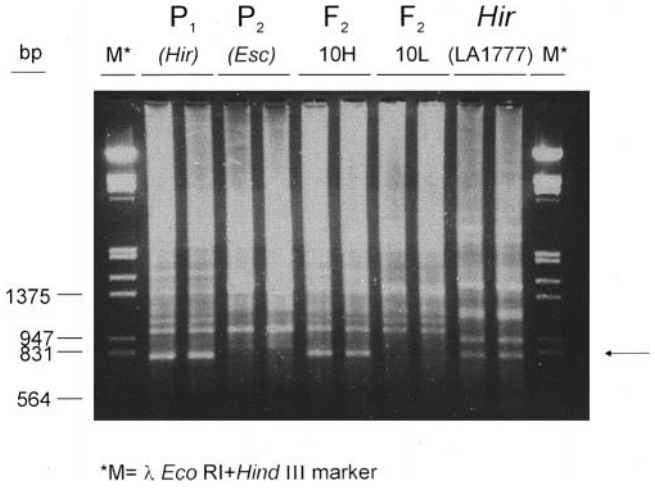

Fig. 1 Tail analysis of an inter-SSR marker (primer MS6) closely associated with the fructose to glucose ratio in tomatoes. Included in the analyses are (in duplicate): a high fructose to glucose parental line originating from *L. hirsutum* (P_1), a low fructose to glucose parental line originating from *L. esculentum* (P_2), a DNA mix of 10 F_2 individuals of the highest fructose to glucose ratio originating from the two parental lines presented (*10H*), a DNA mix of 10 F_2 individuals of the lowest fructose to glucose ratio originating from the two parental lines presented (*10L*) and DNA extracted from the original *L. hirsutum* accession used in this study (*Hir*)

Table 2 Association between the DNA markers generated by the MS6 and MS8 primers and the fructose to glucose ratio, fructose levels and glucose levels

Fructose to glucose ratio

Source of variation[a]	Population	LOD score[b]	Percentage of total variation (R^2)
MS6 allele	F_2+BC	11 <LOD <12	22.6
MS8 allele	F_2+BC	16 <LOD <17	31.5
Zygosity	F_2+BC	20 <LOD <21	40.8
Zygosity	F_3	11 <LOD <12	40.9

Fructose levels

Source of variation[a]	Population	LOD score[b]	Percentage of total variation (R^2)
MS6 allele	F_2+BC	3 <LOD <4	6.2
MS8 allele	F_2+BC	0 <LOD <1	0.4
Zygosity	F_2+BC	2 <LOD <3	6.2
Zygosity	F_3	3 <LOD <4	14.7

Glucose levels

Source of variation[a]	Population	LOD score[b]	Percentage of total variation (R^2)
MS6 allele	F_2+BC	0 <LOD <1	1.0
MS8 allele	F_2+BC	3 <LOD <4	6.5
Zygosity	F_2+BC	2 <LOD <3	6.5
Zygosity	F_3	6 <LOD <7	24.0

[a] Analyses were carried out on average values for three fruits per plant. MS6 designates the allele originating from *L. hirsutum* using primer MS6 (H). MS8 designates the allele originating from *L. esculentum* (E). Zygosity indicates allelic combinations (HH, HE and EE)
[b] LOD score >3 is highly significant

Table 3 Effect of the genotypes identified at the *Fgr* locus on fructose to glucose ratio and on fructose, glucose, sucrose and total sugar levels (mg/g fresh weight) in the combined F_2 and BC populations and in the F_3 population (values presented are means±SE)

Genotype	Population	Fructose/glucose ratio[a]	Fructose levels[a]	Glucose levels[a]	Sucrose levels[a]	Total sugars
Fgr^H/Fgr^H	F_2+BC	1.83^A±0.05	19.0^A±0.9	11.4^B±0.8	1.3^A±0.2	31.7^A±2.2
Fgr^H/Fgr^E	F_2+BC	1.39^B±0.03	19.2^A±0.5	14.6^A±0.5	1.5^A±0.1	35.3^A±1.2
Fgr^E/Fgr^E	F_2+BC	1.15^C±0.03	16.4^B±0.6	14.8^A±0.5	1.4^A±0.1	32.6^A±1.9
Fgr^H/Fgr^H	F_3	2.25^A±0.10	18.7^A±0.9	8.8^C±0.4	1.4^A±0.1	28.9^A±1.3
Fgr^H/Fgr^E	F_3	1.52^B±0.10	15.5^B±1.0	10.7^B±0.6	1.5^A±0.1	27.7^A±1.6
Fgr^E/Fgr^E	F_3	0.93^C±0.13	12.5^B±0.2	13.5^A±0.7	1.4^A±0.1	27.4^A±1.7

Different superscripts indicate statistically significant differences between means (P<0.05) based on the Tukey-Kramer HSD test (Kramer 1956)

Analysis of the association between the DNA markers and the fructose to glucose ratio

Analyses of variance applied to data obtained from the two F2 and the BC populations indicated that the marker bands originally generated by MS6 and MS8 primers were strongly associated with the average fructose to glucose ratio in the mature fruit (11 <LOD score <12 and 16 <LOD score <17, respectively; Table 2). Because the polymorphic DNA bands generated by MS6 and MS8 primers were allelic to each other, the dose effect (zygosity) of the gene could be analyzed. The zygosity of the gene was significantly higher than that of each of the single alleles generated by the MS6 or MS8 primers (20 <LOD score <21, Table 2). Similar analyses carried out after the transformation of the glucose to fructose ratios to their arc sine values (arc sine transformation of the fructose to glucose ratios was mathematically impossible for most of the data cases), as required when analyzing ratios, substantially increased the association between the markers and the trait (30 <LOD score <31, data not presented). The statistical association of the markers with either fructose or glucose levels individually was less significant (2 <LOD score <3; Table 2). The markers were not associated with either total sugar levels (Table 3) or TSS values (data not presented).

Progeny testing

The confirmation of linkage between the PCR markers and the fructose to glucose ratio was carried out for F_3 plants originating from selected F_2 plants, as outlined above in the description of plant material and growing conditions. Analysis of variance revealed a statistically significant association between the markers and the trait and, therefore, confirmed the linkage (Tables 2 and 3).

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TCTCTCTCTC TCTCTCCCTA AATATCTTAT CAATTGCTGA AGAACTCTAT

                SC65 ⇨⇨⇨⇨⇨⇨⇨⇨⇨⇨⇨⇨⇨⇨⇨⇨⇨⇨
ATATGGCCGA TCCACCACCG GCGGAAAGAT GTGAACTTCA TCACGTAATC

GATGGTCTTG CAGCTATACC TCTTGACCGT ACTTTTTACG CCCTACACAT

CATCACAACA TACCAAGAAA ATTTTGAAGA TTTCCTATGG AATTTCAAAA

TGTTAATTTG ACAACCGATG CCATCATTCT TGATAAATCA TTTAATGATA

ATTTTTCAAA CCCTATCAAT GATGATGCAT TTCAAAACCC ATAATCTACC

AGACTGTCGC GGGTCGGAGT AAGGTTTTGA CTCAAATCAA ATGAACCCAA

CTAGCTTTTT TTTATTGAAT TTTATGAAAA ACATCTTGAA AGAGTTTTGA

SC6M5 ⇨⇨⇨⇨⇨⇨⇨⇨⇨⇨⇨⇨⇨⇨⇨⇨⇨⇨
CTATTGACGG TCATGTGAAT GGGACTTATT GTTCTGGGAA ATACGGTGGT
      ⇦⇦⇦⇦⇦⇦⇦⇦⇦⇦⇦⇦⇦⇦⇦⇦ SC6M3

AAAGATCATA GCTCCCATTG TTGGTGGGGA GATCAGGGTG AAAGCAATAT

GAAAAATACC AATTAGTTTG CAGATGAGTT TACTCATATG GGCAATGGCA

CTGATCCTCT GGAAGCCTCT CTGTATGTCC CAGGGAATGA TAAACTTGTT

CAGATTGATG GGAAGTTGAT AATTCAATCC GTATTGGCAA GTGAGAAAGC

CATGGTATTT CATGGAAGTG CTCATAAGAA AAATAGAGAG TTAGGCCTCA

CAGGTGATTT AGCCCCTACT ATCCCAGGAA TCCATCCTCA CCTTTATCAA

 ⇦⇦⇦⇦⇦⇦⇦⇦⇦⇦⇦⇦⇦⇦⇦⇦⇦⇦ SC63
AGTCCTGCAA TGAGACAAAG CATTTCTTGC ATATGAGGGG AGAGAGAGAG

AGAGA
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Fig. 2 Sequence of the DNA fragment obtained by the MS6 ISSR primer. The annealing sites of the original MS6 primer are *underlined*. The 20 bp primers designed to generate single-locus PCR products are indicated by *arrows*

Inheritance of the markers

Each of the markers segregated in an expected Mendelian fashion in the F_2 and different F_3 populations (data not presented).

Effects of *Fgr* on sugar levels

The effects of the identified locus on the ratio of fructose to glucose and total sugar levels as well as on the individual fructose, glucose and sucrose levels in the F_2, BC and F_3 populations are presented in Table 3. In both the combined F_2 and BC populations as well as in the F_3 population the characteristic of fructose to glucose ratio shows a dosage effect, with the values for the HE genotype intermediate between those for the HH and EE genotypes. This is due to a general trend of increasing fructose levels and decreasing glucose levels in response to the *L. hirsutum*-derived allele (Fgr^H). However, the fructose and glucose levels individually do not show a clear intermediate response. Although the results from the segregating F_3 population do suggest a dosage response, the data from the F_2+BC populations show similar fructose levels for the HH and HE genotypes and similar glucose levels for the HE and EE genotypes. Sucrose as well as total sugar levels were not affected by the allelic status of the locus (Table 3).

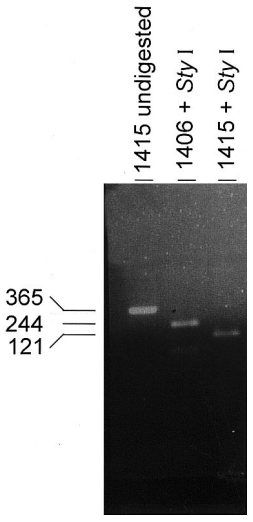

Fig. 3 Codominant single-locus PCR marker obtained by sequence analysis of the DNA fragment produced by the MS6 ISSR primer. PCR amplification products were obtained using DNA templates extracted from line 1406 (originating from *L. hirsutum)* and line 1415 (originating from *L. esculentum)* after digestion the with *StyI* endonuclease

Sequence analysis of the PCR band generated by the MS6 primer and generation of a locus-specific PCR probe

The PCR markers generated by primers MS6 (Fig. 1) and MS8 (not presented) were extracted from a gel and cloned, and 3 independent clones were sequenced. Analysis of the nucleotide sequence of the PCR marker coupled to a high fructose to glucose ratio (MS6) revealed that all 3 inserts were 805 bp in length and showed the same nucleotide sequence (Fig. 2). To generate a locus-specific PCR marker for the fructose to glucose ratio, we designed 4 primers complementary to the 805 bp insert and designated them SC65, SC6M5, SC6M3 and SC63 respectively (Fig. 2). With these primers, 3 locus-specific PCR DNA bands homologous to the product generated by MS6 primer could be amplified and visualized. Analysis of DNA extracted from individual plants of the parental lines, 1406 and 1415, indicated that primer combinations SC65+SC63 and SC65+SC6M3 generated a PCR product only in the parental line originating from *L. hirsutum*, which is characterized by a high fructose to glucose ratio. These results indicate a dominant segregation and sequence divergence at the 5' end of the marker produced by the MS6 primer (data not shown). The primer combination SC6M5+SC63, on the other hand, produced a single PCR product, 365 bp in length, when DNA extracted from lines 1406 and 1415 was used as templates. In order to generate a codominant marker, a set of different restriction enzymes was used to digest that PCR product. The restriction enzyme *Sty*I generated a restriction fragment length polymorphism (RFLP) between the lines and could, therefore, be suitable for the production of a codominantly segregating, locus-specific PCR marker for the fructose to glucose ratio in the ma-

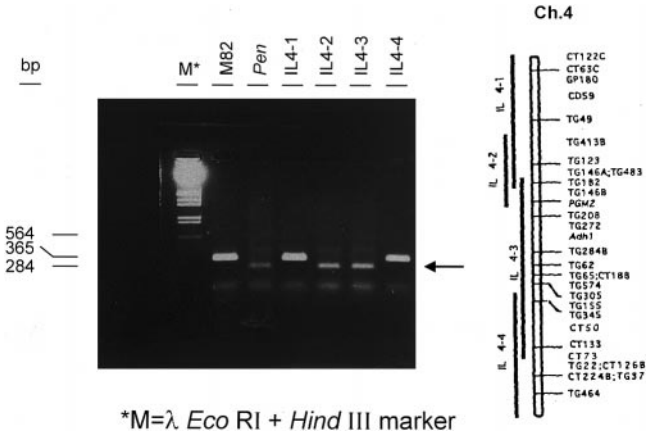

Fig. 4 Mapping of the DNA fragment obtained by the MS6 ISSR primer. PCR analysis of a partial set of introgression lines spanning tomato chromosome 4, showing the map location of the DNA marker originally produced by the MS6 primer (*arrow*). Included in the analysis are DNA samples extracted from M82, *L. pennellii* (*Pen*) and introgression lines IL 4–1 through IL 4–4. Primers used are SC6M5 and SC63

ture tomato fruit (Fig. 3). This locus-specific marker was used on a subset of 100 F_2 plants, and cosegregation between that codominant marker and the PCR products separately obtained by means of MS6 and MS8 primers was confirmed (data not shown).

Mapping of the PCR bands associated with the fructose to glucose ratio

The single-locus PCR primers SC6M5 and SC63 were used to map the DNA marker initially generated by the MS6 primer. DNA extracted from *L. pennellii*, M82 and the 55 *L. pennellii* introgression lines (Eshed et al. 1992; Eshed and Zamir 1994a, b) were used as templates in the PCR reactions. In each line, a PCR product, 365 bp in length, was visualized, and a set of different restriction enzymes was used to digest that product. The restriction endonuclease *Hae*III generated a RFLP between *L. pennellii* and M82. That polymorphism was mapped to *L. pennellii* introgression fragments selected for in the introgression lines IL 4–2 (LA-3492) and IL 4–3 (LA-3493) that are presented in Fig. 4. The PCR product, originally visualized by the MS8 primer, was mapped to the same introgression lines with the modified, more specific, primers, MS81 and MS82 (data not presented).

Discussion

Our goal was to identify DNA markers linked to a putative gene or genes that modulate the fructose to glucose ratio in the mature tomato fruit. The identification of such markers would serve to generate a locus-specific probe for use in a marker-assisted breeding program directed towards modulating that ratio as well as to determine the inheritance of the trait. The ISSR random PCR technique was successful in identifying 2 DNA markers linked to a major locus which modulates the fructose to glucose ratio in tomatoes. Each of the 2 identified markers segregated in a dominant fashion and appeared on an agarose gel together with a multi-band pattern, which is a characteristic of ISSR PCR analysis (Gupata et al. 1994). Such a multi-band pattern could be dependent upon the genetic background and could potentially confound polymorphism in genetic backgrounds other than those used in the present study. Therefore, the 2 PCR markers identified were cloned and 1 of them, based on MS6, was shown to produce a single-locus PCR probe which segregated in a codominant fashion. The sequence analysis of the other PCR product, originally produced by primer MS8, was not further pursued.

The 2 markers identified were found to be allelic, in *trans*-orientation to one another, according to the segregation patterns observed in the two F_2 populations analyzed in the present study. This observation was confirmed by the mapping results obtained using the *L. pennellii* introgression lines. Both markers were mapped to two contiguous *L. pennellii* introgression fragments appearing in lines IL 4–2 (LA-3492) and IL 4–3 (LA-3493). The *L. pennellii* introgression fragment contained in line IL 4–2 (LA-3492) spans approximately 15 cM near the centromeric region of chromosome 4 and overlaps introgression fragment IL 4–3 (LA 3493), which spans approximately 46 cM on the same chromosome (Eshed et al. 1992; Eshed and Zamir 1994a, b). The overlapping DNA region between the 2 introgression lines spans approximately 7 cM on chromosome 4 that contains the phosphoglucomutase 2 (*PGM-2*) gene and the RFLP marker TG182 (Eshed et al. 1992; Eshed and Zamir 1994a, b). Further examination indicated that the locus for the trait is very closely linked to the *Adh-1* locus, which is also located near the centromere of chromosome 4 (data not presented).

Based on the segregation and mapping results, the zygosity status of each individual plant used in our study could be identified by analyzing the absence or presence of each of the PCR bands visualized by the MS6 and MS8 primers. Such an analysis could also be carried out with the single-locus PCR probe generated by the sequence analysis of the PCR band produced by the MS6 primer. The analysis of our data, with attention being given to these considerations, revealed that the markers identified were highly associated with the fructose to glucose ratio and accounted for more than 40% of the variation in this trait (20 <LOD score <21). Interestingly, both markers were found to be only marginally associated with absolute glucose or fructose levels and were not linked to either total sugars or total soluble solids (TSS) in the F_2 and BC populations. Similar results were obtained with data obtained from the F_3 population used for progeny testing, with only a single exception: the linkage between the PCR markers and glucose levels was found to be significantly greater than that obtained from the analysis carried out on the F_2 and BC populations. Nevertheless, the association between the markers and the fructose to glucose ratio remained significantly stronger than their association with glucose levels in the F_3 population. The results presented

in Tables 2 and 3 indicate that although the locus identified shows lower linkage effects on the glucose and fructose levels separately, nevertheless the locus does modulate the fructose to glucose ratio by reciprocally modulating the levels of the individual sugars. Most importantly, the locus showed no effect on total sugars (Table 3) or TSS (data not shown). No effect was observed on sucrose levels, as was expected in the absence of the *L. hirsutum*-derived *sucr* allele (Chetelat et al. 1995).

Our data suggest that the *L. hirsutum*-derived allele (*Fgr*^{H}) that increases the fructose to glucose ratio is incompletely dominant. This is clearly seen in the effect on the ratio of the two sugars in both the combined F_2+BC and the F_3 populations. In the F_3 populations there was also an intermediate effect on the levels of each of the individual sugars which was not observed in the F_2 and BC populations. Therefore, selection should be made for plants homozygous for *Fgr*^{H} if the fixation of genetic material with maximal fructose to glucose ratios is desired.

In summary, our results suggest that a major locus, which we term *Fgr,* can modulate the fructose to glucose ratio in the mature tomato fruit without affecting total sugar levels. Although other loci with similar effects may be present in the tomato genome, we have established the significant effect of the identified locus by progeny testing. The PCR markers produced may, therefore, be used efficiently in marker-assisted selection directed at modulating the fructose to glucose ratio in the ripe tomato fruit and in map-based cloning of the locus or loci involved in such modulation. With the development of genetically defined near-isogenic lines for the *Fgr* locus we can study the gene function at the biochemical and physiological levels. The characterization of hexose metabolism in these tomato fruits is presently being carried out.

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