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R. T. Chetelat · J. W. DeVerna · A. B. Bennett

Introgression into tomato (*Lycopersicon esculentum*) of the *L. chmielewskii* sucrose accumulator gene (*sucr*) controlling fruit sugar composition

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Abstract High sucrose concentration in fruit of *Lycopersicon chmielewskii* is governed by the recessive sucrose accumulator gene (*sucr*) that is situated in the pericentromeric region of chromosome 3. The *sucr* gene was introgressed into the genetic background of the hexose-accumulating cultivated tomato (*L. esculentum* cv 'Hunt 100') by marker-assisted selection using tightly linked RFLP markers and a tomato acid invertase cDNA as probes for *sucr*. RFLP mapping indicated that the segment containing *sucr* comprised over 43.2 cM in the BC_1F_2 generation, representing over one-third of the total length of chromosome 3. By selecting for crossovers between *sucr* and the flanking visual marker *r* (yellow fruit flesh) and RFLP marker TG288, we were able to reduce the size of the *sucr* introgression fragment to 0.8–7.1 cM by the BC_5 generation. Smaller recombinant fragments were not obtained despite screening a large BC_6F_2 population. The smallest *sucr* introgression reduced recombination between the flanking visual markers *sy* (sunny) and *bls* (baby lea syndrome) by 38%. To facilitate future introgression and recombination experiments, a PCR-based test for the *sucr* gene was developed using primers specific to the tomato invertase gene. This assay takes advantage of a small deletion that maps to the second intron of the *L. chmielewskii* invertase gene. The assay detected significant allelic variation both within and between hexose- and sucrose-accumulating *Lycopersicon* spp.

Key words Marker-assisted selection · RFLPs · Breeding · PCR · Invertase

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R. T. Chetelat (✉) · A. B. Bennett
Department of Vegetable Crops, University of California, Davis, CA 95616, USA

J. W. DeVerna
Campbell's Research and Development, 28605 County Road 104, Davis, CA 95616, USA

Introduction

Cultivated varieties of tomato (*Lycopersicon esculentum* Mill.) are, as a whole, extremely depleted in genetic variation, whereas the related wild species are by all measures highly diverse (Miller and Tanksley 1990; Rick and Fobes 1975; Williams and St. Clair 1993). All eight of the wild *Lycopersicon* spp. can be hybridized to tomato, with varying degrees of difficulty, and therefore represent an important resource for enhancing economic traits. For example, the vast majority of disease resistances in tomato represent single dominant genes introgressed from wild *Lycopersicon* spp. into tomato cultivars (Rick 1986). Among the difficulties encountered in introgression experiments of this type are associations with hybrid sterility or inviability, segregation distortion, and novel variation (for examples, see: Chetelat et al. 1989; Rick 1963, 1969; Zamir and Tadmor 1986). The generally undesirable horticultural qualities of wild tomatoes (e.g. small fruit, poor color, self-incompatibility, etc.) are in most cases caused by genes other than those responsible for economic traits. However, pleiotropic effects are possible and would be very difficult to distinguish from the action of tightly linked genes. For example, the *Pto* locus confers both resistance to *Pseudomonas syringae* pv. *tomato* and sensitivity to the herbicide Fenthion, traits encoded by two tightly linked genes (Loh and Martin 1995). Elimination of such negative traits is a major objective of introgression experiments.

Soluble solids content (SSC) is a major fruit quality determinant and breeding objective for processing varieties and is controlled largely by the concentration of soluble sugars. Fruit of the wild tomato species *L. chmielewskii* possess SSC levels roughly twice that of modern cultivars (Rick 1974). While fruit of *L. esculentum* accumulate primarily reducing sugars (glucose and fructose) and very little sucrose, fruit of *L. chmielewskii* accumulate high amounts of sucrose (Yelle et al. 1988). Yelle et al. (1991) reported that sucrose accumulation is associated with higher soluble sugar concentrations in an early generation backcross to *L. esculentum*.

Genetic experiments have demonstrated that the trait of sucrose accumulation in *L. chmielewskii* is controlled by a single recessive gene, *sucr*, located in the pericentromeric region of chromosome 3 (Chetelat et al. 1993). All other green-fruited *Lycopersicon* spp. examined also accumulate sucrose (Davies 1966; Miron and Schaffer 1991; Stommel 1992). In *L. hirsutum* the trait is also monogenic and recessive in crosses to *L. esculentum* (Stommel and Haynes 1993) and is allelic to *sucr* from *L. chmielewskii* (Chetelat et al. 1993).

Low acid invertase activity in fruit is the principle cause of sucrose accumulation in *L. chmielewskii* (Yelle et al. 1991). The properties of this enzyme have been well studied, and its subcellular localization assigned to the vacuole (Konno et al. 1993; Yelle et al. 1991). The low invertase activity of *L. chmielewskii* fruit is due to low protein levels (Yelle et al. 1991) and apparently not to higher levels of an invertase inhibitor, as has been found in *L. esculentum* (Pressey 1994). Tomato fruit acid invertase cDNAs have been cloned from *L. esculentum* (Elliott et al. 1993; Klann et al. 1992; Ohyama et al. 1992; Sato et al. 1993) and *L. pimpinellifolium* (Elliott et al. 1993). Invertase mRNA was not detected in *L. chmielewskii* or sucrose-accumulating introgression lines derived thereof (Klann et al. 1993), and genetic mapping of the invertase gene placed it at the same locus as *sucr* (Chetelat et al. 1993). Recent experiments demonstrate that down regulation of invertase by antisense or sense inhibition leads to sucrose accumulation (Klann and Bennett submitted; Wilde et al. 1994). Therefore, in all likelihood, the *sucr* gene represents an invertase allele with little or no expression in fruit.

The objective of the work reported herein was to develop suitable germ plasm to enable an evaluation of the effects of sucrose accumulation on SSC and other quality traits in the genetic background of a processing tomato cultivar with acceptable horticultural traits. Accordingly, we have introgressed, by repeated backcrosses, the *sucr* gene from *L. chmielewskii* into *L. esculentum* and reduced the size of flanking foreign DNA by marker-assisted selection. An analysis of sugar accumulation and fruit quality parameters of these introgression lines is presented in an accompanying paper (Chetelat et al. 1995). Following elucidation of the function of *sucr* as an inactive invertase allele, a rapid, codominant polymerase chain reaction (PCR)-based test for the invertase gene was also developed to facilitate future selection experiments and to examine allelism within *Lycopersicon*.

Materials and methods

Plant material

L. chmielewskii accession LA1028 was used as the donor parent for the sucrose accumulation trait, *sucr*. The tomato cultivars used at various stages in the backcrossing program were 'UC82B', 'UC204C', and 'Hunt 100'. The F_1 *L. esculentum*×*L. chmielewskii* hybrid was synthesized using cv. 'UC82B' as the female parent. A sucrose-accumulating F_2 individual derived thereof was crossed as male parent to cv. 'UC204C' to obtain the first backcross (BC_1) generation. The recurrent parent for further backcrosses was cv 'Hunt 100'. The chromosome 3 linkage tester stock containing the visual markers *sy, bls* and *sf* (LA1430) was used to monitor recombination in the vicinity of *sucr*. Other wild *Lycopersicon* spp. examined for allelic variation at *sucr* included *L. esculentum* var *cerasiforme* accession LA1673, *L. pimpinellifolium* LA722 and LA2184, *L. cheesmanii* LA166, *L. chmielewskii* LA1306 and LA2663, *L. parviflorum* LA1326, *L. hirsutum* LA1777 and LA1223, *L. pennellii* LA716 and LA1926 and *L. peruvianum* LA111, LA2151 and LA1292. Seed samples of all genotypes used in this study were obtained from the C. M. Rick Tomato Genetics Resource Center (TGRC), Department of Vegetable Crops, University of California at Davis.

Linkage analysis

To determine the gene order of and map distances between markers flanking *sucr*, recombination was monitored in the backcross populations. For the *sucr* – TG66 interval, results are based on a BC_2F_2 population of 103 plants; for the *sucr* – TG42 interval, on a BC_5F_1 population of 218 plants. Estimates of recombination rates were obtained with the LINKAGE-1 program, version 3.50 (Suiter et al. 1983).

Restriction fragment length polymorphism (RFLP) analysis

RFLP markers were used to map *sucr* and to select for crossovers in the introgressed segment containing *sucr*. RFLP analysis was performed essentially as previously described (Chetelat and DeVerna 1991). Probes consisted mainly of tomato genomic ("TG") clones of known map location (Tanksley et al. 1992), provided by Dr. S. Tanksley at Cornell University. Other probes included a tomato fruit acid invertase cDNA, TIV1 (Klann et al. 1992), used in later backcross generations as a direct probe for *sucr* (Chetelat et al. 1993), and a proton ATPase cDNA, LHA1, which maps to chromosome 3 (Ewing et al. 1990).

DNA isolations from large populations were performed as described by Bernatzky and Tanksley (1986), with modifications as follows. Several young leaflets were placed in a small plastic weigh boat to which 800 µl extraction buffer and a dash of sterilized sand were added, then crushed with a plastic rod. The homogenate was rinsed into a 2-ml microcentrifuge tube with a second 800 µl aliquot of extraction buffer. Nuclei were pelleted at 10,000 rpm for 10 min, then resuspended in 300 µl of extraction buffer and lysed with 200 µl nuclei lysis buffer and 120 µl 5% sarkosyl. Following a 1-h incubation at 60°C, samples were extracted with 1 ml chloroform-isoamyl alcohol (24:1). DNA was precipitated from the aqueous phase with 0.6 ml cold isopropanol and pelleted 2 min at 10,000 rpm. The DNA pellet was washed with 70% ethanol, vacuum dried, resuspended in 50 µl TE buffer pH 8.0 and incubated for 1 h at 60°C. The extraction buffer consisted of 63.7 g/l sorbitol, 12.1 g/l TRIS, 1.68 g/l EDTA (disodium), at pH 7.5, to which 3.8 g/l sodium bisulfite was added just prior to use. The nuclei lysis buffer consisted of 0.2 M TRIS, 0.05 *M* EDTA, 2 *M* NaCl, 20 g/l CTAB, at pH 7.5.

PCR detection of *sucr*

On the basis of the known sequences of the invertase cDNA (Klann et al. 1992; Genbank Accession no. M81081) and genomic clones (Elliott et al. 1993; Genbank Accession no. Z12027), primers were chosen to permit PCR amplification of a fragment of the invertase gene. The sequence of the upstream and downstream primers were CTATCTTCTATTATGGCCACTCAG and CCCAAATAGCTGAATCTGGATTGT, respectively. Each PCR reaction contained approximately 500 ng of total DNA template, 0.2 m*M* each dNTP, 2.5 m*M* $MgCl_2$, 50 m*M* KCl, 10 m*M* TRIS-HCl pH 8.3, 10 pmol of each primer and 5 units Ampli*Taq* DNA polymerase (Applied Biosystems) in a total reaction volume of 100 µl. PCR was carried out for 40 cycles under the following conditions: 1 min at 94°C, 1 min at 60°C

and 2 min. at 72°C. PCR products were digested with the appropriate restriction enzyme according to manufacturer's recommendations, then electrophoresed on 2% agarose/1×TBE gels, stained with ethidium bromide and visualized under UV light.

Results

Size of the *sucr* introgression in BC_1F_2

Several plants accumulating sucrose were identified in the segregating BC_1F_2 generation by an analysis of fruit sugars (Yelle et al. 1991). One of these plants (GH4077-13) was used for further backcrossing to cv 'Hunt 100'. RFLP mapping indicated that this plant carried a large chromosome 3 segment from *L. chmielewskii*, that spanned markers TG66 and TG242 but not markers TG56, TG152 or TG244 (Fig. 1). On the basis of the RFLP map of tomato (Tanksley et al. 1992), the estimated fragment size is at least 43.2 cM, representing over one-third of the total genetic length of chromosome 3.

Fragment size reduction

Analysis of the BC_1F_2 population with the TG102 probe indicated that some plants homozygous for the *L. chmielewskii* marker (i.e. putatively *sucr/sucr*) did not produce fruit under greenhouse conditions (Chetelat et al. 1993). Sucrose accumulators also had yellow fruit, caused by an allele of *r* (Chetelat et al. 1993). Additional backcrosses accompanied by selection for *sucr* were therefore carried out in order to break the putative linkage between sucrose accumulation and these associated traits and to simultaneously recover a greater proportion of the recurrent parent genome.

The *sucr/sucr* BC_1F_2 individual GH4077-13 was backcrossed for two additional generations, using cv 'Hunt 100' as the *L. esculentum* recurrent parent, followed by one generation of selfing. The resulting BC_3F_2 progeny were genotyped at *sucr* using the tightly linked RFLP marker, TG102. Out of a total population of 300 plants, 40 *sucr/sucr* individuals were identified, of which 11 had red fruit, indicating recombination events between *sucr* and *r*. Progeny resulting from the selfing of 1 red-fruited recombinant (GH5180-184) were scored for the RFLP marker TG66, which is closely linked to *r* (Fig. 1). This permitted the identification of plants homozygous for the recombinant chromosome (i.e. of genotype $TG66^+/TG66^+$, r^+/r^+, *sucr/sucr*, where "+" indicates the reference allele, in this case from *L. esculentum*), from which subsequent backcross generations were derived.

In the BC_5F_1 generation, 218 plants were genotyped at *sucr* using TIV1 and several markers along the chromosome on the side opposite *r*. The resulting gene order (Fig. 1) is in good agreement with the existing RFLP map, except for the position of TG102, which was placed between TG222 and TG246 (Tanksley et al. 1992). One

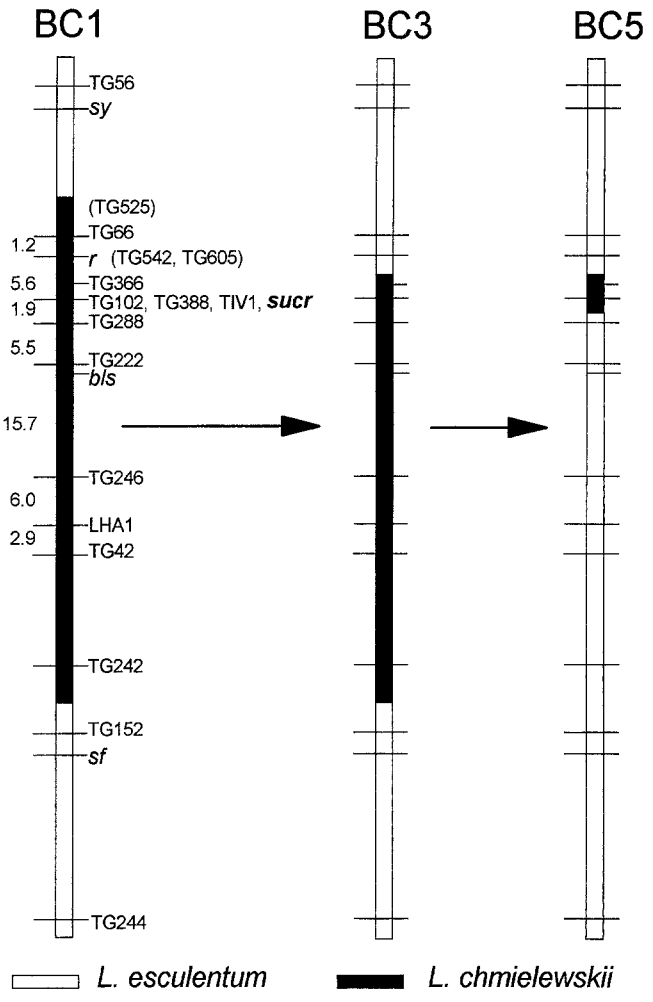

Fig. 1 Reduction in size of the chromosome 3 introgression fragment containing the sucrose accumulator gene, *sucr*, from *L. chmielewskii* during backcrossing to *L. esculentum*. Solid sections represent *L. chmielewskii* DNA, open sections *L. esculentum* DNA. Markers used include tomato genomic clones (*TG*), cDNA clones of the invertase gene (TIV1) and a proton ATPase gene (LHA1) and the visual markers *sy*, *r*, *bls* and *sf*. *BC*n indicates the number of backcross generations. Map distances are given in centiMorgans and are based on recombination rates obtained during the introgression process. The approximate positions of other markers are based on the maps of Tanksley et al. (1992) and van der Biezen et al. (1994)

BC_5F_1 individual (GH6086-182) carried a recombination between TIV1 and the next closest marker, TG288; this plant, which was heterozygous for TIV1 and TG102 but homozygous (+/+) for TG288 and all other markers tested on chromosome 3, carried the smallest introgression fragment detected. Another plant (GH6086-218) contained a slightly larger introgression fragment containing a crossover between TG288 and TG222. The BC_5F_2 progeny from these 2 plants (families 92L6770 and 92L6773, respectively) were utilized to study the effects of sucrose accumulation on yield and fruit quality traits, the results of which are reported in an accompanying paper (Chetelat et al. 1995).

In an attempt to identify further recombinants within the smallest fragment, 854 BC_6F_2 plants (derived from

BC_5F_1 plant GH6086-182) were genotyped at *sucr* using the TIV1 and TG102 probes as well as the linked RFLP markers TG366 and TG388; TG388 is situated within the same cluster of genes to which *sucr* maps, whilst TG366 is between *sucr* and TG66 (Fig. 1; Chetelat et al. 1993; Tanksley et al. 1992). No recombinants were found among plants carrying at least one copy of *sucr*. A BC_6F_3 family was also genotyped for several more distal markers located between TG66 and *sucr* (TG525, TG542 and TG605), of which all were homozygous +/+, indicating the introgression fragment did not span these loci (Fig. 1). Using these data, we estimate the size of the smallest introgression fragment to be between 0.8 cM (min) and 7.1 cM (max), based on the RFLP map (Tanksley et al. 1992), or less than 3 cM, based on an integrated map (van der Biezen et al. 1994).

Recombination between flanking visual markers

To determine if recombination on chromosome 3 is affected by the presence of the *sucr* introgression fragment, the recessive seedling markers *sy, bls* and *sf* were used. A *sucr/sucr* plant from BC_5F_2 (derived from GH6086-182) was crossed to a chromosome 3 tester stock homozygous for all three visual markers. The F_1 was selfed, or backcrossed (as female parent) to the linkage tester, to produce F_2 and BC progeny, respectively, in which the seedling markers were scored (Table 1). Single locus segregations fit the expected Mendelian ratios, except for *bls* in the F_2 population, which showed a significant (χ^2=6.13, P<0.05) deviation in the direction of the *L. esculentum* (mutant) allele (Table 1). The observed recombination rate for the *sy–bls* interval ranged from 17.5% (F_2) to 18.9% (BC), while for the *bls–sf* interval the values ranged from 41.4% (F_2) to 42.6% (BC) (Table 1). The BC data were compared to a control cross free of introgressed segments and made in the same direction (i.e. heterozygous female) by C. M. Rick (personal communication, 1971). Recombination between *sy* and *bls* was reduced by 38.4% in the presence of the *sucr* introgression (χ^2 heterogeneity=18.0, significant at P<0.001), while recombination between *bls* and *sf* was not significantly affected. The unexpectedly high frequency of double crossovers indicates a slightly negative interference in the presence of the *sucr* introgression (Table 1).

Table 1 Segregation and recombination in self (F_2) and backcross (BC) progeny of the linkage cross *sy–bls–sf* × +–+–+, where the nonmutant chromosome carried an introgression from *L. chmielewskii* conferring sucrose accumulation (*sucr*). Backcross data are compared to a control cross from C. M. Rick (personal communication) in which the nonmutant chromosome carried no introgressions

Crossovers	Phenotypic classes			Number of plants		
				F_2 (*sucr*)	BC (*sucr*)	BC (control)
Parental	+	+	+	244	156	120
	sy	*bls*	*sf*	29	159	134
Single	+	*bls*	*sf*	19	29	55
	sy	+	+	22	33	67
	+	+	*sf*	63	102	85
	sy	*bls*	+	64	116	77
Double	+	*bls*	+	32	28	31
	sy	+	*sf*	7	34	31
Total no.:				480	657	600
% Coincidence:				113	117	90
% Recombination (± SE):						
	sy – bls			17.5 ± 4.4	18.9 ± 1.5	30.7 ± 1.9
	bls – sf			41.4 ± 3.7	42.6 ± 1.9	37.3 ± 2.0

Development of a PCR-based assay for the invertase gene

To facilitate future introgression experiments, a method for the rapid, non-radioactive detection of *sucr* was needed. Accordingly, a test based on the polymerase chain reaction was developed. Using the published sequences of the tomato fruit acid invertase cDNA (Klann et al. 1992) and genomic (Elliott et al. 1993) clones, we chose primers to permit amplification from the 5′ end of the coding sequence (nucleotide no. 3521 of the *L. esculentum* genomic clone) to the beginning of the third exon (nucleotide no. 5490). A single band of approximately the expected size (ca. 1.98 kb for *L. esculentum*) was obtained from genomic DNA of both *L. esculentum* and *L. chmielewskii*, with no difference in amplification efficiency noted (data not shown). A Southern blot of the gel probed with TIV1 confirmed that the PCR products were bona-fide invertase sequences (not shown). On certain gels the *L. chmielewskii* invertase fragment was detectably smaller, however digestion with either *Xba*I or *Hinf*I revealed consistently detected, codominant polymorphism (Fig. 2). In the case of *Xba*I, which cuts the *L. esculentum* genomic clone at nucleotide 4597, a small deletion (<40 bp) is detectable in the *L. chmielewskii* allele (Fig. 2). In the case of *Hinf*I, the *L. chmielewskii* invertase gene lacks one of the seven restriction sites found within this amplified region of the *L. esculentum* gene, generating a distinct restriction pattern (Fig. 2). The approximate position of the *L. chmielewskii* deletion was determined by digesting the same PCR products with additional restriction enzymes (*Alu*I, *Bgl*II, *Hin*dIII, *Hpa*II, *Nde*I and *Pst*I) that cut the genomic clone at known sites within the amplified region. The *Alu*I digest indicated a location of the deletion between nucleotides 4661 and 5481, while the *Bgl*II digest indicated a position between nucleotide 4694 and 5490; hence the deletion is somewhere between 4694 and 5481, a position corresponding to the large second intron (Elliott et al. 1993).

Using this PCR assay, we analyzed a sample of several wild *Lycopersicon* spp. for polymorphisms in the invertase gene. On the basis of the *Xba*I digest, *L. esculentum* cv 'Hunt 100' could be distinguished from another hexose accumulator, a cherry tomato (*L. esculentum* var *cerasiforme*) by the slight size difference of the larger fragment (Fig. 3). The two additional *L. chmielewskii* accessions analyzed were indistinguishable from LA1028 (do-

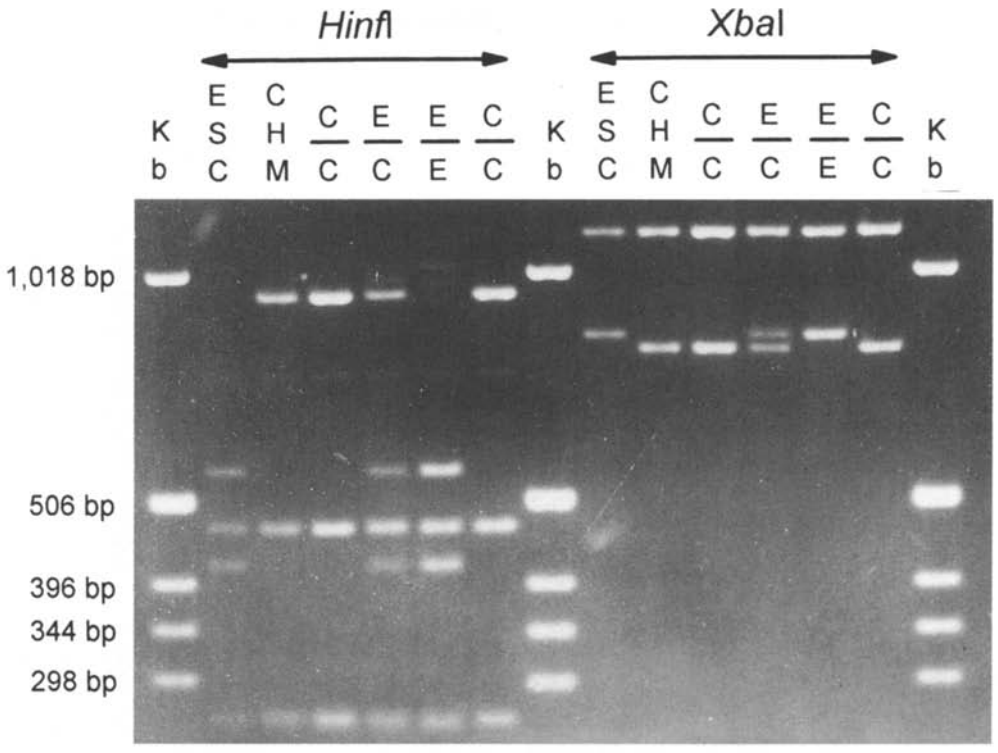

Fig. 2 Detection of the *L. chmielewskii* invertase gene by PCR. Lanes are: *Kb* DNA size ladder, *ESC L. esculentum* cv 'Hunt 100', *CHM L. chmielewskii* LA1028, *C/C* BC_6F_2 homozygous for *L. chmielewskii* allele; *E/C* BC_6F_2 heterozygous, *E/E* BC_6F_2 homozygous for *L. esculentum* allele. Amplification products representing approximately 1.9 kb starting at the 5′ end of the gene were digested with the restriction enzymes *Hinf*I or *Xba*I and are visualized with ethidium bromide following electrophoresis

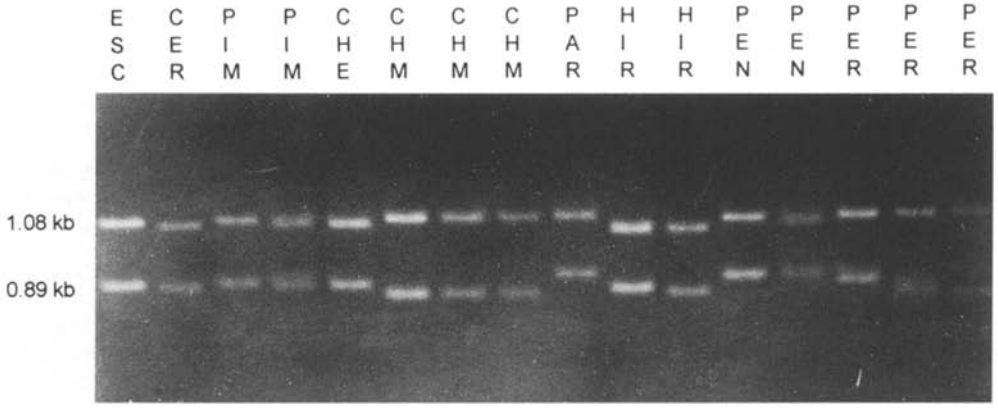

Fig. 3 Comparison of several *Lycopersicon* spp. using PCR detection of the invertase gene followed by digestion with *Xba*I. Lanes are, from left to right: *ESC L. esculentum* cv 'Hunt 100'; *CER L. esculentum* var *cerasiforme* LA1673, *PIM L. pimpinellifolium,* LA722 and LA2184, *CHE L. cheesmanii* LA166, *CHM L. chmielewskii* LA1028, LA1306 and LA2663, *PAR L. parviflorum* LA1326, *HIR L. hirsutum* LA1777 and LA1223, *PEN L. pennellii* LA716 and LA1926, *PER L. peruvianum* LA111, LA2151 and LA1292. Template DNAs were bulk samples of genomic DNA from 5 plants within each accession

nor of *sucr*), while the sibling species *L. parviflorum* had a banding pattern similar to that of *L. pennellii* (Fig. 3). Additional polymorphisms were seen between *L. hirsutum*, *L. pennellii* and *L. peruvianum*, and among the three *L. peruvianum* accessions (Fig. 3). Digestion of PCR products with *Hinf*I yielded similar results (data not shown).

Discussion

The use of marker-assisted selection can facilitate genetic introgression by (1) accelerating recovery of the recurrent parent genome, (2) identifying recombinants in the target region and (3) providing codominant markers for phenotypically recessive traits (for review see: Tanksley et al. 1989). Without the use of markers, identification of recombinants near the target gene may be ineffective, and the introgressed fragment can remain surprisingly large, despite many backcross generations, due to linkage drag (Young and Tanksley 1989). Application of whole genome selection can significantly accelerate recovery of the recurrent parent genome (Tanksley et al. 1989). Computer simulations by Hospital et al. (1992) suggest an optimal strategy to achieve both goals with minimum population sizes: in early backcross generations, perform whole genome selection accompanied by selection for recombinants in the target region using distal markers, then, in later generations, select only for recombinants using tightly linked markers. Although recombination involving alien chromosome seg-

ments may be progressively reduced during backcrossing (Rick 1969), crossovers can nonetheless be identified in highly advanced generations using marker-assisted selection (Young et al. 1988).

In order to study the effects of sucrose accumulation on soluble solids concentration, yield and other parameters, the recessive gene *sucr* was introgressed from *L. chmielewskii* into tomato. The primary objective of this introgression program was to overcome the negative traits (unfruitfulness, color defects) associated with *sucr/sucr* genotypes. These associated traits were tentatively attributed to the action of linked genes rather than pleiotropic effects of *sucr* since: (1) the original *sucr* introgression segment comprised over one-third of the total length of chromosome 3, ample size to include numerous genes and (2) the yellow fruit character was shown to be allelic to *r* (Chetelat et al. 1993). The size of the *sucr* introgression fragment was reduced in two steps by selecting for recombinants between *sucr* and *r* on one side and between *sucr* and TG288 on the other. This process was greatly facilitated by the use of the tightly linked RFLP marker TG102, and later an invertase cDNA clone, TIV1, both of which provided codominant markers for the recessive *sucr* trait. Emphasis was placed on breaking the linkage between *sucr* and the negative traits, hence genomic selection with unlinked markers was not applied. However, even in the absence of selection, the proportion of the *L. esculentum* genome recovered should exceed 98.5% by BC_5, assuming Mendelian inheritance. Though complicated somewhat by changes in the recurrent parent cultivar in early generations, this process should be accelerated by segregation distortions in the direction of *L. esculentum* often seen in backcrosses from interspecific hybrids (Paterson et al. 1990; Rick 1969, 1971).

On the basis of the existing RFLP map (Tanksley et al. 1992), the smallest introgression segment containing *sucr* is between 0.8 and 7.1 cM in length. The failure to obtain recombinants closer to *sucr* in a large BC_6F_2 population could be due to recombination suppression in this region. This is consistent with the map location of *sucr* in the pericentromeric region of chromosome 3 (Chetelat et al. 1993). This region shows the most pronounced clustering of genes observed anywhere in the genome, a phenomenon attributed to recombination suppression around the centromere (Tanksley et al. 1992). Furthermore, crosses to the chromosome 3 linkage tester stock indicated that the *sucr* introgression significantly reduces recombination between the flanking markers *sy* and *bls* relative to that observed within *L. esculentum*. Recombination suppression near *sucr* could be even more pronounced since the introgression fragment comprises less than one-fifth of the *sy–bls* interval based on the integrated map of Koornneef et al. (1993). Similarly, Rick (1971) observed an approximately tenfold recombination suppression in the *sy–bls* interval during introgression of an entire *L. pennellii* chromosome 3. Also consistent with our results, this effect did not extend to the long arm, marked by *bls – sf* (Rick 1971b). The observed segregation distortion towards *bls* in the F_2 population is consistent with previous data showing the reduced transmission of *sucr* in certain F_2 but not BC populations (Chetelat et al. 1993). On the basis of available maps for chromosome 3 (Chetelat et al. 1993; Tanksley et al. 1992; van der Biezen et al. 1994), both *bls* and *sucr* are near the centromere, a fact which probably explains why segregation for the distal markers *sy* and *sf* was normal.

By using primers recognizing the tomato invertase cDNA, we were able to amplify by PCR an approximately 1.9–2-kb fragment from the genomic DNA of either *L. esculentum* or *L. chmielewskii*. Under the stringent annealing temperature used, this result suggests considerable sequence homology between invertase alleles in the two species, consistent with the near identity of the *L. esculentum* and *L. pimpinellifolium* sequences (Elliott et al. 1993). Following restriction enzyme mapping, a small deletion was identified in the large second intron of the *L. chmielewskii* allele, which provided a clear size polymorphism that can be used to score *sucr* in future introgression experiments. The use of this non-radioactive PCR test should expedite screening of large populations since the quantity and purity of DNA template needed are lower than for Southern analysis. Performing PCR directly on plant tissues (Klimyuk et al. 1993) and the converting of flanking RFLP markers to PCR-based markers provides an efficient strategy to search for rare recombinants (Balint-Kurti et al. 1994). To enrich recombinant individuals, future molecular genotyping will be restricted to plants with crossovers between the visual markers *sy* and *bls,* thereby providing roughly a fivefold greater efficiency.

The observation that all red or orange-fruited tomato species accumulate hexoses whilst the green-fruited species accumulate sucrose has prompted the suggestion of a common evolutionary origin for sucrose (or hexose) accumulation (Chetelat et al. 1993). Therefore, the PCR-based assay was used to compare the invertase alleles in a broad sample of *Lycopersicon* germ plasm. With just one restriction enzyme, more than one invertase allele could be resolved within each of the subgeneric groups. Significant similarities between accessions within a species were also noted. The data therefore indicate that mutations have accumulated within this region of the invertase gene since the divergence between green and red/orange-fruited species. This is not unexpected since primarily non-coding (intronic) sequences were amplified.

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