Mapping quantitative trait loci in inbred backcross lines of *Lycopersicon pimpinellifolium* (LA1589)

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Abstract: Although tomato has been the subject of extensive quantitative trait loci (QTLs) mapping experiments, most of this work has been conducted on transient populations (e.g., F_2 or backcross) and few homozygous, permanent mapping populations are available. To help remedy this situation, we have developed a set of inbred backcross lines (IBLs) from the interspecific cross between *Lycopersicon esculentum* cv. E6203 and *L. pimpinellifolium* (LA1589). A total of 170 BC_2F_1 plants were selfed for five generations to create a set of homozygous BC_2F_6 lines by single-seed descent. These lines were then genotyped for 127 marker loci covering the entire tomato genome. These IBLs were evaluated for 22 quantitative traits. In all, 71 significant QTLs were identified, 15% (11/71) of which mapped to the same chromosomal positions as QTLs identified in earlier studies using the same cross. For 48% (34/71) of the detected QTLs, the wild allele was associated with improved agronomic performance. A number of new QTLs were identified including several of significant agronomic importance for tomato production: fruit shape, firmness, fruit color, scar size, seed and flower number, leaf curliness, plant growth, fertility, and flowering time. To improve the utility of the IBL population, a subset of 100 lines giving the most uniform genome coverage and map resolution was selected using a randomized greedy algorithm as implemented in the software package MapPop (http://www.bio.unc.edu/faculty/vision/lab/mappop/). The map, phenotypic data, and seeds for the IBL population are publicly available (http://soldb.cit.cornell.edu) and will provide tomato geneticists and breeders with a genetic resource for mapping, gene discovery, and breeding.

Key words: tomato, *Lycopersicon esculentum*, IBLs, QTL, mapping.

Résumé : Bien que la tomate ait été l'objet de nombreuses études de cartographie des loci de caratère quantitatif (QTL), la plupart de ces travaux ont été réalisés sur des populations non-fixées (F_2 ou rétrocroisement) et peu de populations homozygotes, permanentes sont disponibles. Afin de remédier à cette situation, les auteurs ont développé une collection de lignées rétrocroisées fixées (« inbred backcross lines », IBL) à partir d'un croisement interspécifique entre le *Lycopersicon esculentum* cv. E6203 et le *L. pimpinellifolium* (LA1589). Cent soixante-dix plants BC_2F_1 ont été reproduits par descendance monosporale pendant cinq générations pour produire des lignées BC_2F_6 homozygotes. Ces lignées ont ensuite été génotypées à l'aide de 127 locus marqueurs couvrant l'ensemble du génome de la tomate. Ces IBL ont été évaluées pour 22 caractères quantitatifs. Au total, 71 QTL significatifs ont été identifiés, dont 15 % (11/71) étaient situés à la même position chromosomique que lors d'études antérieures faisant appel à ce même croisement. Pour 48 % (34/71) des locus détectés, l'allèle sauvage était associé à une performance agronomique améliorée. Plusieurs nouveaux QTL ont été identifiés dont plusieurs de grande importance agronomique : la forme des fruits, la fermeté, la couleur des fruits, la taille de la cicatrice, le nombre de graines et de fleurs, l'enroulement des feuilles, la croissance des plantes, la fécondité et le nombre de jours à la floraison. Afin d'accroître l'utilité de la population de lignées IBL, un jeu de 100 lignées offrant la couverture génomique la plus uniforme et la meilleure résolution de la carte génétique a été constitué à l'aide d'un algorithme de randomisation disponible dans la suite logicielle MapPop (http://www.bio.unc.edu/faculty/vision/lab/mappop). La carte, les données phénotypiques et les graines de la population de lignées IBL sont du domaine publique (http://soldb.cit.cornell.edu) et procureront aux généticiens et aux sélectionneurs de la tomate une ressource génétique pour la cartographie, la découverte de gènes et la sélection.

Mots clés : tomate, *Lycopersicon esculentum*, IBL, QTL, cartographie.

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Introduction

To date, many QTL identification and mapping studies have been conducted on balanced populations in which both parental alleles are in high frequency, such as backcrosses, F_2s, recombinant inbred lines, or doubled haploids (e.g., Stuber et al. 1992; deVicente and Tanksley 1993; Azanza et al. 1994; Xiao et al. 1996; Doganlar et al. 1999). Using balanced populations allows efficient determination of the chromosomal positions of each QTL, estimation of the minimal number of QTL affecting a trait of interest, as well as definition of the relative contribution of each QTL to the expression of the trait of interest. Alternatives to balanced populations are unbalanced populations, like advanced backcross generations, in which alleles from one parent are at a much lower frequency (Tanksley and Nelson 1996). Unbalanced populations can also be used to construct genetic maps and identify QTLs, but often at the loss of resolution and efficiency as compared with balanced populations (Butruille et al. 1999; Tanksley and Nelson 1996). The advantage of unbalanced populations is that the population is much more genetically and phenotypically similar to the recurrent parent. This characteristic is useful in identifying and introgressing QTLs from unadapted germplasm into elite germplasm (Tanksley and Nelson 1996).

One type of unbalanced population that has been widely used is inbred backcross lines (IBLs). Wehrhan and Allard (1965) first proposed the use of IBLs for estimating both gene number and magnitude of effect of each locus for a trait of interest. A set of IBLs can be generated by crossing two parents to produce an F_1 generation, which is then backcrossed at least once to the recurrent parent. Multiple backcross lines are then advanced by single-seed descent to fix any segregating loci and reach the desired level of homozygosity. Thus, the production of IBLs combines the desirable features of both the backcross and single-seed descent breeding methods. Recovery of the recurrent parent genotype is facilitated by the backcross procedure and single-seed descent allows fixation of the recurrent parent alleles (Chetelat and Meglic 2000).

Many researchers have used the inbred-backcross method as a means of estimating the minimum number of genes that contribute to the expression of quantitatively inherited traits (Baker 1978; Thurling and Vijendra 1979; Rau et al. 1994). Additionally, the IBL method has been successfully used as a breeding technique to improve quantitative traits by introgressing genes from wild germplasm into elite breeding lines while maintaining the favorable horticultural characteristics of the elite materials (Bliss 1983). Examples of the use of IBLs in breeding programs include improvements in seed protein and nitrogen-fixing ability in common bean (Sullivan and Bliss 1983; St. Clair and Bliss 1991), fruit mass in cucumber (Owens et al. 1985), seed yield in spring rape (Thurling 1982), several fruit characteristics (Rau et al. 1994; Triano and St. Clair 1995), insect resistance (Hartman and St. Clair 1998) and soluble solids content (Triano and St. Clair 1995) in tomato, seed dormancy and heading date in rice (Lin et al. 1998), and several agronomic traits including yield and oil characteristics in *Brassica* (Butruille et al. 1999).

Although tomato has been the subject of extensive QTL mapping and cloning work, most of this work has used transient populations. Very few homozygous, permanent mapping populations exist. A notable exception is a set of introgression lines developed from the cross *L. esculentum* × *L. pennellii* (Eshed and Zamir 1995); however, the mapping resolution provided by this population is somewhat limited because each line contains a relatively large introgression. The goals of this research were to develop and characterize a permanent inbred population, to use this population to identify QTL of agronomic interest, and to compare this QTL mapping with mapping that was done using earlier generations of the same cross.

In this report, we describe the development of an inbred backcross population in tomato comprising 196 IBLs (BC_2F_6) and a selected subset of 100 lines giving the most uniform genome coverage and map resolution (Vision et al. 2000). The donor was the wild species *L. pimpinellifolium* (LA1589) and the recurrent parent was the elite processing line, *L. esculentum* cv. E6203. These parents were chosen for several reasons as follows: (*i*) the two species differ dramatically for many phenotypes; (*ii*) the cross is very fertile, which facilitates the development of advanced generations; and (*iii*) the interspecific nature of the cross provides ample DNA polymorphism for mapping. Each IBL was evaluated in the field for a variety of morphological and agronomic traits. In addition, the IBL population was characterized with a set of molecular markers covering the entire tomato genetic map and QTL analysis was performed for all traits. We compare the efficiency of QTL mapping in the IBL population with two alternative, unbalanced population structures (BC_1F_1 and BC_2F_1) using the same donor and recurrent parents. The results of this study shed light on the detection and introgression of QTLs using unbalanced populations. Moreover, the set of IBLs described herein represents a new and valuable resource for tomato genetics.

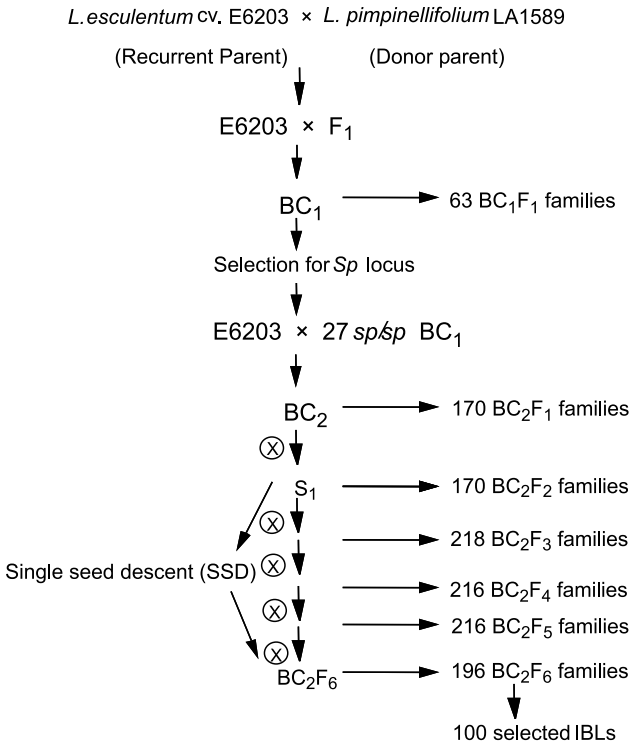
Materials and methods

Population development

The inbred-backcross lines (IBL) were derived from a population that had been developed as part of an advanced backcross (AB) QTL strategy to identify useful alleles from a small red-fruited wild relative of tomato, *L. pimpinellifolium* (LA1589) (hereafter referred to as PM) (Grandillo and Tanksley 1996; Tanksley et al. 1996). Briefly, a single plant of the wild species was used as the donor parent in crosses to an open pollinated cultivar, *L. esculentum* cv. E6203 (hereafter referred to as LE). One individual F_1 hybrid was subsequently backcrossed to LE. The resulting 63 BC_1 plants were first genotyped with the RFLP marker TG279 to select for homozygous LE alleles at the *sp* locus on chromosome 6. This initial selection ensured that the plants would have a determinate growth habit, an essential requirement for field evaluations. The 27 selected BC_1 plants were then individually backcrossed to LE to produce the BC_2 population. Each of these BC_2 plants was advanced by single-seed descent for five generations of selfing, resulting in a population of 196 IBLs (BC_2F_6) (Fig. 1).

Single plants of the 196 individual IBLs along with 3 of each of the LE and PM controls were transplanted to the field in Ithaca, N.Y. at the end of May 1997 at a row and

Fig. 1. Diagram depicting development of inbred backcross lines using *L. pimpinellifolium* LA1589 as the donor parent and *L. esculentum* cv. E6203 as the recurrent parent.



plant spacing of 1 m^2. All plants were harvested at the beginning of October for trait evaluations.

RFLP analysis

Genomic DNA isolation, Southern hybridization, washing, and autoradiography were performed as described in Bernatzky and Tanksley (1986). RFLP markers used in this study were selected from the previously published *L. pimpinellifolium* map (Grandillo and Tanksley 1996). A total of 126 RFLP markers were used to genotype 196 IBLs, along with the morphological marker *u* (uniform ripening).

Phenotypic analysis

Twenty-two agronomically important traits were scored for each IBL and the controls. Details of the evaluation of each trait follow. Plant growth (GRO) was visually measured as the amount of vegetative growth of each line on a scale of 1 to 5 (1 = minimal growth, 5 = excessive growth). Leaf curliness (CURLY) was determined as the extent of adaxial rolling or curling up of the plant's leaves using a scale from 1 to 5 (1, no rolling; 5, severe rolling of leaves). Flowering time (DFL) and fruit ripening time (DFR) were measured as number of days from sowing to the appearance of the first opened flowers and the complete change of color (green to red) of the first fruit, respectively. Number of flowers per inflorescence (NOF) was measured by taking an average of five randomly chosen inflorescences per plant. Maturity (MAT) was evaluated by a visual assessment of the percentage of mature fruit on harvest day using a scale from 1 to 5 (1, many ripe fruit; 5, very few ripe fruit). Fertility (FER) was determined visually as the degree to which flowers set fruit per plant using a scale from 1 to 5 (1, reduced fruit set; 5, heavy fruit set). Radial fruit cracking (RFC) was determined as the degree of the splitting of the epidermis radiating from the calyx end to the blossom end of the fruit using a scale from 1 to 5 (1, no cracked fruit; 5, majority of fruit cracked on a plant). Concentric fruit cracking (CFC) was determined as the degree of splitting of the epidermis in circular patterns around the calyx end of the fruit using a scale from 1 to 5 (1, no cracked fruit; 5, majority of fruit cracked on a plant). Fruit rot (ROT) was evaluated as the percentage of healthy ripe fruit with a scale from 1 to 5 (1, no rotten fruit; 5, majority of fruit with rot). Fruit weight (FW) was measured as the average weight of 20 ripe fruits per plant. Internal (IC) and external fruit color (EC) were visually determined by a scale from 1 to 5 (1, low color; 5, more intense red color) by observing at least 10 transversely cut fruits. Fruit firmness (FIRM) was subjectively determined by hand squeezing at least 10 ripe fruits using a scale from 1 to 5 (1, soft; 5, very firm). Soluble solid content (SSC) was measured on puree derived from five randomly selected fruits per plant in degrees Brix using a refractometer. Puffiness (PUF), a measurement of free air space in the locules, was evaluated on a scale of 1 to 5 (1, not puffy; 5, very puffy) by examining transversely cut fruits. For fruit shape analysis, at least 10 fruits of each line were sliced longitudinally and scanned using Adobe Photoshop version 3.0 software for the Macintosh. Fruit length (FL) was determined by measuring the average polar length (stem to blossom ends) (cm) of 10 fruits. Fruit diameter (FD) was determined by measurement of the equatorial diameter of the fruit (taken midway between the stem and blossom end). Fruit shape (FS) was determined as the ratio of fruit length to fruit diameter (FL:FD) for a minimum of 10 ripe fruit. Fruit stem scar size (SCAR) was measured as the approximate mean diameter (in mm) of the stem scar on the 20 fruit. Seed weight (SW) was determined by weighing 100 seeds (g) from each IBL. Total seed weight for all of the seed extracted from 20 fruits per line was measured and, with the 100 seed weight, was used to determine seed number per fruit (SN).

Statistical analysis

Marker segregation was tested for significant deviation from the expected ratio of 223 LE/LE : 31 PM/PM: 2 LE/PM for a BC_2F_6 by the χ^2 goodness-of-fit analysis. For each chromosome, the MAPMAKER "group" and "ripple" commands at LOD 3.0 were used to test the most likely marker order (Lander et al. 1987). Using this information and the previously published *L. pimpinellifolium* maps (Grandillo and Tanksley 1996; Tanksley et al. 1996), a consensus map containing 151 markers was constructed. The genetic distances between markers were based on the map of Grandillo and Tanksley (1996).

Pearson's correlation coefficients were calculated for each trait combination using the QGENE computer program (Nelson 1997). Single-point regression analysis was used to determine the effect of each molecular marker on each trait using the QGENE program (Nelson 1997). Because one of the goals of this work was to determine if the IBLs could be used to detect the same QTL identified in the AB-QTL populations, a relatively lenient significant threshold, $P < 0.01$, was used to declare linkage of a trait to a marker locus.

Fig. 2. A consensus genetic linkage map for the inbred backcross lines (IBLs). Distances between markers are from Grandillo and Tanksley (1996). When more than one marker in a chromosomal region showed significant association with a trait, the QTL was positioned adjacent to the most significant marker. Underlined QTL were also identified in at least one previous study using the same donor parent.

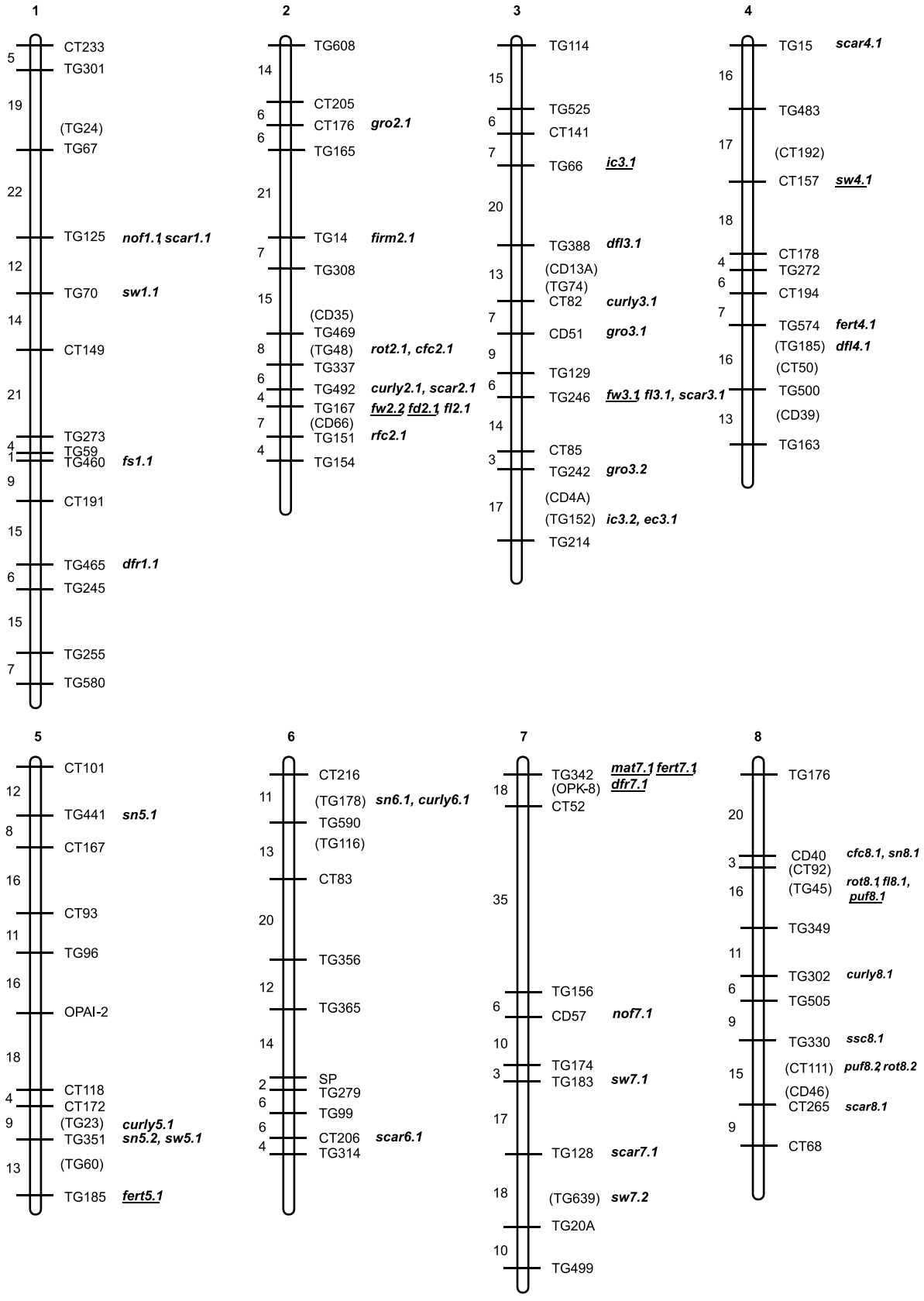
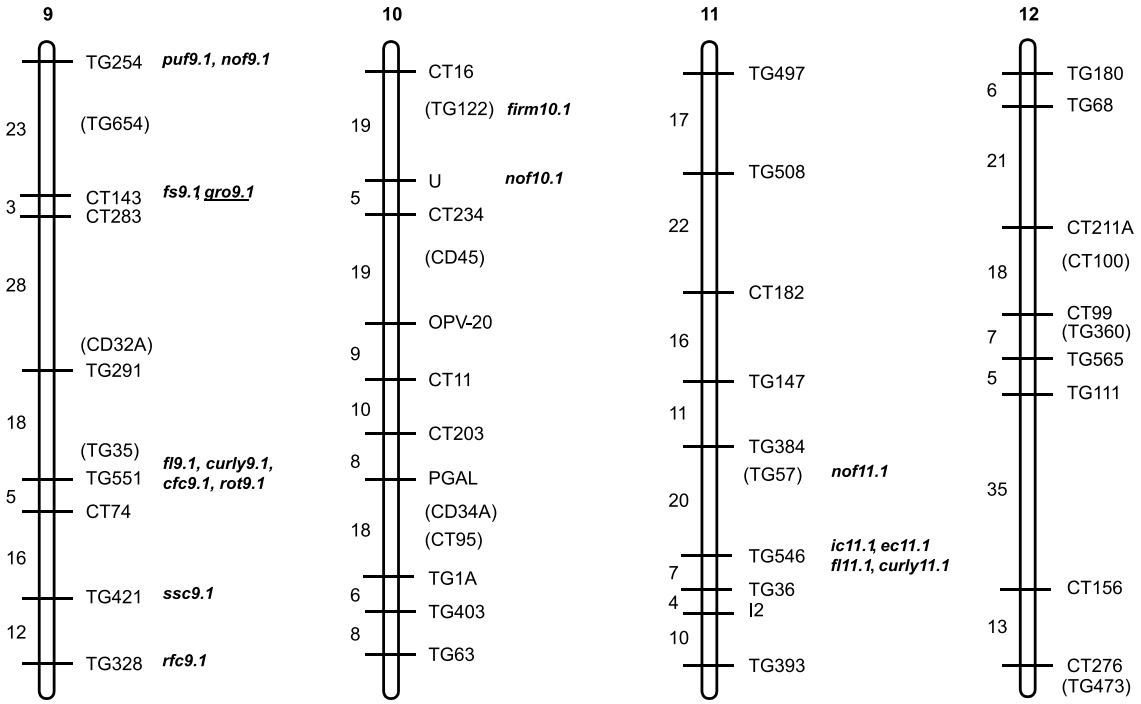


Fig. 2 (*concluded*).



When more than one marker in a chromosomal region showed significant association with a trait, the QTL was positioned adjacent to the most significant marker. The observed percentage of phenotypic variation explained by each marker was calculated by QGENE from the regression of each marker–phenotype combination. Based on the mapping data generated for the entire IBL population, a subset of 100 IBL was selected using a randomized greedy algorithm as implemented in the software package MapPop (Vision et al. 2000) (http://www.bio.unc.edu/faculty/vision/lab/mappop/). This subset provides uniform genome coverage and map resolution with a minimum number of plants.

Results and discussion

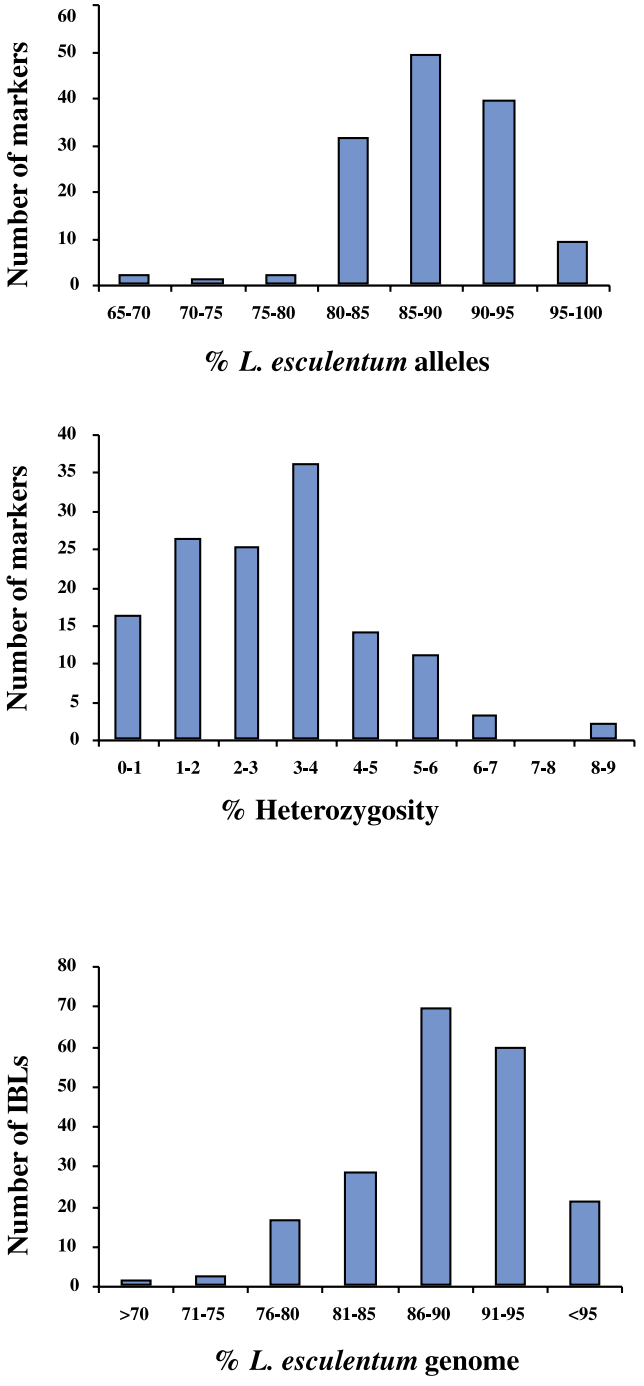
Marker segregation and genome composition of the IBL population

A total of 127 markers including one morphological (*u*, uniform ripening) and 126 RFLP markers were scored in 196 IBLs and were assigned to their positions in 12 linkage groups. The linear order of markers used in this study agreed with the previously published map (Grandillo and Tanksley 1996) and a consensus map is presented in Fig. 2. Averaged over all loci, the frequency of LE alleles in the IBL population was 88% (ranging from 69 to 100%), which is quite close to the expected value of 87% (Fig. 3*a*). However, of the 127 markers scored in the IBLs, 105 (83%) deviated significantly ($P < 0.05$) from the expected ratio for a BC_2F_6 generation. The percentage of skewed markers in IBLs was 10 times greater than that observed in a BC_1 population using the same parents (Grandillo and Tanksley 1996) in which only 8% of the markers were skewed. The skewed markers were observed throughout the genome. The most common type of skewing was an excess of heterozygotes (86% of the skewed markers). As a result, the average heterozygosity for the IBL population was 3%, significantly higher than the expected value of <1% (Fig. 3*b*). Six markers on chromosome 6 were skewed towards the homozygous LE allele. All 6 were located on the bottom of chromosome 6 near the self-pruning gene, *sp* (Fig. 2). This part of the genome was subjected to marker assisted selection (for the LE allele) to develop determinate type BC_2 lines (Grandillo and Tanksley 1996). No IBL was entirely homozygous over all loci sampled (Fig. 3*c*).

Trait correlations

Significant ($P < 0.05$) phenotypic correlations were observed between many physiologically related traits (Fig. 4). For the fruit characteristics, the strongest positive correlations were observed between fruit weight (FW) and its component traits fruit diameter (FD) ($r = 0.85$) and fruit length (FL) ($r = 0.81$). This result was expected because as FL and FD increase, there will be a concomitant increase in FW. Thus these correlations were most likely due to pleiotropic effects of the same genes. FW and stem scar (SCAR) were positively correlated with each other ($r = 0.65$) as larger fruits tended to have larger stem scars. There was a small positive correlation between FW and number of seeds per fruit (SN) ($r = 0.42$). This result is consistent with a study that indicated that final fruit size and weight were, in part, determined by the number of developing seeds (Nitsch 1970). In agreement with several previous reports (Tanksley et al. 1996; Fulton et al. 1997; Bernacchi et al. 1998; Chen et al. 1999) that indicated that FW and soluble solid content (SSC) are negatively correlated traits, a negative correlation ($r = –0.16$) was observed between these two traits in this study. Additional correlations were found between FW and days to first flower (DFL) ($r = 0.22$), days to first ripe fruit (DFR) ($r = 0.28$), and number of flowers per inflorescence (NOF) ($r = –0.22$) (Fig. 4). For fruit color traits, a significant

Fig. 3. Distributions of (*a*) the percent *L. esculentum* alleles, (*b*) the percent heterozygosity for the molecular markers, and (*c*) the percent *L. esculentum* alleles for the inbred-backcross lines (IBLs).



positive correlation was found between internal color (IC) and external color (EC) (r = 0.52). Similar results were reported in the previous studies that used *Lycopersicon peruvianum* and *Lycopersicon parviflorum* as donor parents for the development of mapping populations (Fulton et al. 1997; Fulton et al. 2000). The two types of fruit cracking showed a strong positive correlation (r = 0.51) suggesting that radial (RFC) and concentric (CFC) cracking may be the result of pleiotropic effects of some of the same loci. Furthermore, cracking was positively correlated with fruit rot (r = 0.38 and 0.53 for RFC and CFC, respectively). This was not unexpected because the cracks could provide an entry into the fruit for the fungus and bacteria that cause rot. Earliness traits (DFL, DFR, and MAT) all showed strong positive correlations (r = 0.43 to 0.63). Thus, lines that were late to flower were also likely to take longer to produce ripe fruit.

QTL analysis

A total of 71 significant ($P < 0.01$) QTL were identified for 22 characters of agronomic importance on the basis of single-point linear regression analysis. Table 1 summarizes the QTL identified with the P value and the percent phenotypic variance explained by the most significant marker for each QTL. Figure 2 depicts the most likely positions of each QTL on the genetic map. The number of QTL identified for each trait ranged from 1 (for fruit diameter and maturity) to 7 (for stem scar size and curly leaf); the magnitude of effect of each individual QTL (% phenotypic variation = R^2) ranged between 4 and 17%. The number of QTL detected per chromosome ranged from 2 (chromosomes 10) to 10 (chromosomes 2, 3, 8, and 9). The only chromosome that did not have any QTL associated with it was chromosome 12. Overall, 11 (15%) of the QTLs identified in this population were also identified in the other QTL studies that examined the earlier generations of the IBL population (Grandillo and Tanksley 1996; Tanksley et al. 1996). QTLs with overlapping map positions in the different studies were considered to be identical. The correspondence between the results of this and previous studies using the same donor parent was relatively low because the current study examined many traits that were not examined in either of the other studies. For many of the QTLs, (34 (48%)), the wild alleles showed agronomically favorable effects on the traits. Of the novel QTLs identified in this study, approximately 23% (14 of 60 new QTLs) have potential agronomic value because they had the appropriate direction of effect and were not associated with any negative traits. These QTLs controlled various traits including FS, FIRM, IC, EC, NOF, SCAR, SN, DFL, FERT, GRO, and CURLY. The QTLs identified for each trait are summarized in the following sections.

Fruit weight (FW)

Two QTLs for FW were identified on chromosomes 2 (*fw2.2*) and 3 (*fw3.1*), which accounted for 15 and 7%, respectively, of phenotypic variance for this trait (Table 1; Fig. 2). For both QTLs, the wild alleles were associated with a reduction in FW. This result is consistent with the phenotype of PM, which has an average fruit size of 1–2 g as compared with 80–90 g for the LE parent. The map positions of the two QTL identified in this study were also found to be associated with FW in several previous studies (reviewed in Grandillo et al. 1999; Chen et al. 1999). Of these, *fw2.2* has been reported to be conserved among the wild tomato species and is considered to be a major fruit weight QTL (Alpert et al. 1995). *fw2.2* was recently isolated via map-based cloning (Frary et al. 2000).

Fruit diameter, length, and shape (FD, FL, and FS)

One QTL was identified for FD on chromosome 2 (*fd2.1*), accounting for 15% of the phenotypic variance for this trait

Fig. 4. Significant ($p < 0.01$) correlations among traits in the inbred backcross lines.

	FW	FL	FD	FS	FIRM	EC	IC	PUF	SSC	SCAR	RFC	CFC	ROT	SW	SN	DFL	DFR	NOF	FERT	MAT	GRO
FL	0.81																				
FD	0.85	0.66																			
FS	-	-0.45	-0.28																		
FIRM	-	-	-	-																	
EC	-0.22	-0.23	-0.15	-0.19	-																
IC	-0.29	-0.25	-0.28	-	-	0.52															
PUF	0.42	0.32	0.42	-	-	-0.20	-0.45														
SSC	-0.16	-	-0.21	-	-	-	0.19	-													
SCAR	0.65	0.50	0.54	-	-	-	-	-	-												
RFC	-	-	-	-	-	-	-	-	-	-											
CFC	-0.20	-0.19	-0.20	-	-	-	-	-	-	-	0.51										
ROT	-0.21	-0.20	-0.26	-	-	-	-	-	-	-	0.38	0.53									
SW	0.23	0.27	0.19	-	-	-	-	-	-	0.28	-	-	-								
SN	0.42	0.22	0.30	-	-	-	-	-	-	0.25	0.16	0.16	0.18	-							
DFL	0.22	0.22	-	-	-	-	-0.21	-	-	0.17	-	-	-	0.23	-						
DFR	0.28	0.34	0.24	0.22	0.27	-0.19	-0.19	-	-	-	-	-0.23	-0.34	0.24	-	0.63					
NOF	-0.21	-0.30		-0.16	-	-	0.14	-	-	-0.22	-	-	-	-0.21	-	-	-				
FERT	-	-	0.17	-0.22	-	0.15	-	-	-0.37	-	-	-	-	-0.18	0.15	-	-0.22	-			
MAT	-	-	-	-	0.26	-	-	-	-	-	-0.19	-0.21	-0.24	-	-	0.43	0.46	-	-0.17		
GRO	-	-	-	-	-	-	-	-	-	-	-0.18	-	-0.19	-	-	0.20	0.27	-	0.26	0.37	
CURLY	0.21	0.25	0.24	-	-	-0.15	-	-	-0.18	-	0.18	-	-	0.18	-	-	-	-	-	-0.27	-0.25

and coinciding with *fw2.2* (Fig. 2). Five QTLs were identified for FL (Table 1; Fig. 2). Again, the most significant QTL for FL was detected on chromosome 2 in the vicinity of *fw2.2* and reflects the pleiotropic effects of the FW QTL. In addition to the chromosome 2 QTL, the other four FL QTLs (on chromosomes 3, 8, 9, and 11) were detected both in this work and in a study using a population derived from a cross between *L. pimpinellifolium* (LA1589) and an extremely large-fruited *L. esculentum* cultivar (Lippman and Tanksley 2001).

Two QTLs were identified for FS (FL/FD) on chromosomes 9 and 1 accounting for 5 and 9% of the phenotypic variance for this trait, respectively (Table 1; Fig. 2). For both *fs1.1* and *fs9.1*, the PM alleles made the fruit more elongated, a desirable shape for processing tomatoes. Fulton et al. (1997) also detected *fs9.1* in a population derived from a cross with *L. peruvianum* (LA1708). Interestingly, *fs8.1*, a major QTL that has been detected in many previous studies including five AB-QTL populations (Grandillo et al. 1999) was not identified in the IBLs. However, a FL QTL (*fl8.1*) that made fruit shorter was identified in the same genomic location as *fs8.1*. This agrees with the known phenotypic effect of *fs8.1* as developmental analysis of the locus has shown that it exerts its effect on shape by affecting fruit elongation (Ku et al. 2000).

Firmness (FIRM)

Fruit firmness is a way of assessing ripening and a significant trait for reducing fruit damage during harvesting and shipping. This trait was controlled by two QTLs that each accounted for 6% of the total phenotypic variance for the trait (Table 1; Fig. 2). For one QTL (*firm2.1*), the PM allele was associated with an increase in firmness, whereas for the other QTL (*firm10.1*), the wild allele was associated with a decrease in the trait. Neither were identified in the previous PM study that examined this trait (Tanksley et al. 1996); however, *firm10.1* was identified in an AB-QTL study with *L. parviflorum* (LA1223) (Fulton et al. 2000). The QTL on chromosome 2 did not coincide with any other QTL with unfavorable effects; therefore, this locus might be useful in breeding programs for improving fruit firmness.

Internal and external fruit color (IC and EC)

Two QTLs were identified for EC on chromosomes 3 and 11 (Table 1; Fig. 2). For these QTL, the PM alleles had a favorable effect on the trait, increasing the intensity of external red fruit color. Three QTLs were detected for IC on chromosomes 3 and 11 accounting for 5–9% of the total phenotypic variance for the trait (Table 1; Fig. 2). For two of the QTLs, *ic3.2* and *ic11.1*, the PM alleles were associated with an increase in IC, whereas for *ic3.1*, the PM alleles were associated with a decrease in IC. *ic3.1* was also detected by Tanksley et al. (1996). In addition, the QTLs on chromosomes 3 and 11 were identified in the same map positions in two previous studies using populations derived from *L. hirsutum* (LA1777) and *L. parviflorum* (Bernacchi et al. 1998; Fulton et al. 2000). Because *ec3.1* and *ic3.2* are located in the same region and did not coincide with any other QTL with unfavorable effects, these QTLs may be useful for the improvement of fruit appearance by introgression of the PM alleles to increase red color.

Puffiness (PUF)

Three QTLs were identified for puffiness accounting for 7–13% of the total phenotypic variance for the trait (Table 1; Fig. 2). For *puf8.1*, the PM allele improved the trait and resulted in less intralocular air space. *puf8.1* was also identified at the same chromosomal position in a previous study (Tanksley et al. 1996). Moreover, this QTL coincides with *fs8.1*, suggesting that rounder fruit are, by nature, less puffy. For the other two QTLs, the PM alleles were associated with an increase in PUF, an undesirable trait for processing tomatoes. Interestingly, the fact that for the majority of the QTLs the wild alleles caused an increase in fruit puffiness agrees with the results of other studies that used *L. peruvianum* and *L. hirsutum* as the donor parents for QTL analysis (Fulton et al. 1997; Bernacchi et al. 1998).

Soluble solid content (SSC)

Two genomic regions were associated with SSC (Table 1; Fig. 2). For the QTL on chromosome 9, two doses of the wild allele increased SSC by 0.8°Brix; however, the wild allele reduced soluble solids for the QTL on chromosome 8. The QTL on chromosome 9 was also identified in the same

Table 1. QTL for horticultural traits identified in a population of 195 inbred backcross lines derived from a cross between *Lycopersicon esculentum* cv. E6203 × *L. pimpinellifolium* (LA1589).

Trait	QTL designation	Marker	Enzyme[a]	Chromosome	R^2 [b]	*P* value	EE[c]	*N*	PP[d]	*N*	EP[e]	*N*	*d*/*a*[f]
Fruit weight (FW)	*fw2.2*	TG167	*Xba*I	2	15	0	57	171	34	18	47	7	0.1
	fw3.1	TG246	*Hin*dIII	3	7	0.001	56	172	43	17	42	7	–1.1
Fruit diameter (FD)	*fd2.1*	TG167	*Xba*I	2	15	0	4.6	169	3.9	18	4.2	7	–0.1
Fruit length (FL)	*fl2.1*	TG492	*Dra*I	2	17	0	4.8	169	3.8	18	4.5	7	0.5
	fl11.1	TG546	*Sca*I	11	9	0.0002	4.7	163	4.0	13	4.4	9	0.2
	fl8.1	TG45	*Xba*I	8	9	0.0001	4.8	155	4.2	21	4.3	11	–0.5
	fl3.1	TG246	*Hin*dIII	3	7	0.0008	4.7	170	4.2	17	4.2	7	–1.0
	fl9.1	TG551	*Hin*dIII	9	5	0.006	4.7	180	4.2	13	3.4	1	–4.6
Fruit shape (FS)	*fs9.1**	CT143	*Sca*I	9	9	0.0002	1.0	155	1.1	30	1.2	6	–7.0
	*fs1.1**	TG460	*Hin*dIII	1	5	0.01	1.0	181	1.1	12	1.3	1	–4.3
Fruit firmness (FIRM)	*firm10.1*	TG122	*Eco*RI	10	6	0.004	2.7	174	1.7	16	3.5	2	2.5
	*firm2.1**	TG14	*Hin*dIII	2	6	0.004	2.5	178	3.3	15	4.3	3	–3.6
External fruit color (EC)	*ec3.1**	TG152	*Eco*RI	3	7	0.002	3.0	155	3.7	21	3.0	5	0.9
	ec11.1	TG546	*Sca*I	11	6	0.005	3.1	165	3.7	13	2.4	9	2.9
Internal fruit color (IC)	*ic3.2**	TG152	*Eco*RI	3	9	0.0002	2.9	155	4.0	21	3.4	5	0.0
	ic11.1	TG546	*Sca*I	11	5	0.007	3.1	165	3.9	13	2.3	9	2.7
	*ic3.1**	TG66	*Eco*RV	3	5	0.008	3.2	169	2.4	20	2.4	7	–0.9
Fruit puffiness (PUF)	*puf8.2*	CT111	*Hin*dIII	8	13	0	1.6	146	2.1	20	3.5	4	–5.8
	puf8.1	TG45	*Xba*I	8	7	0.001	1.8	156	1.2	21	1.1	11	–1.5
	puf9.1	TG254	*Hin*dIII	9	7	0.001	1.7	181	4.0	1	2.8	5	0
Soluble solids content (SSC)	*ssc9.1**	TG421	*Xba*I	9	7	0.003	5.2	162	6.0	11	5.5	1	0.3
	ssc8.1	TG330	*Eco*RV	8	6	0.003	5.4	166	4.9	23	4.9	5	–0.9
Fruit stem scar size (SCAR)	*scar2.1*	TG492	*Dra*I	2	14	0	73	169	53	20	62	6	–0.1
	*scar8.1**	CT265	*Dra*I	8	10	0	73	159	58	28	66	8	0.1
	scar3.1	TG246	*Hin*dIII	3	11	0	73	172	57	17	53	6	–1.6
	scar1.1	TG125	*Hin*dIII	1	8	0.0003	72	181	52	11	62	3	0
	*scar7.1**	TG128	*Dra*I	7	8	0.0005	73	159	61	31	65	3	–0.3
	scar6.1	CT206	*Dra*I	6	4	0.007	70	191	93	4	—	0	0
	*scar4.1**	TG15	*Dra*I	4	7	0.001	72	173	59	17	58	5	–1.1
Radial fruit cracking (RFC)	*rfc2.1*	TG151	*Xba*I	2	13	0	1.7	174	2.7	15	3.3	6	–2.5
	rfc9.1	TG328	*Bst*NI	9	5	0.007	1.8	175	2.0	11	3.7	3	–18
Concentric fruit cracking (CFC)	*cfc8.1*	CD40	*Xba*I	8	10	0.0001	1.8	162	2.7	23	1.9	7	0.8
	cfc2.1	TG48	*Hin*dIII	2	8	0.0006	1.9	162	1.8	19	3.6	5	59
	cfc9.1	TG551	*Hin*dIII	9	7	0.0006	1.8	182	2.9	13	2.0	1	0.7
Fruit rot (ROT)	*rot2.1*	TG48	*Hin*dIII	2	9	0.0001	2.1	162	2.2	19	4.0	5	–46
	rot9.1	TG551	*Hin*dIII	9	6	0.002	2.1	182	3.1	13	3.0	1	–0.8
	rot8.1	TG45	*Xba*I	8	5	0.006	2.1	157	2.8	21	2.4	11	0.2
	rot8.2	CT111	*Hin*dIII	8	5	0.002	2.1	146	2.4	20	3.6	5	–8.9
Seed weight (SW)	*sw4.1*	CT157	*Hin*dIII	4	17	0	0.29	163	0.24	21	0.26	11	0

	sw7.1	TG183	*Xba*I	7	14	0	0.29	150	0.26	24	0.24	6	–3.0
	sw7.2	TG639	*Hin*dIII	7	9	0.0003	0.29	152	0.26	21	0.25	12	–2.0
	sw5.1	TG351	*Eco*RV	5	5	0.008	0.29	176	0.31	9	0.25	10	5.0
	sw1.1	TG70	*Xba*I	1	5	0.01	0.29	185	0.23	6	0.27	4	0.5
Seed No. (SN)	*sn5.1**	TG441	*Eco*RV	5	9	0.0002	56	169	71	18	67	7	–0.5
	sn6.1	TG178	*Sca*I	6	6	0.003	61	139	52	42	53	6	–0.6
	sn8.1	CD40	*Xba*I	8	5	0.006	57	160	64	23	73	7	–3.3
	sn5.2	TG351	*Eco*RV	5	5	0.007	59	175	52	9	43	10	–3.2
Days to first flower (DFL)	*dfl3.1**	TG388	*Xba*I	3	7	0.001	34	163	38	23	33	6	1.3
	dfl4.1	CT185	*Hin*dIII	4	6	0.003	35	155	34	12	30	14	–6.6
Days to first ripe fruit (DFR)	*dfr7.1*	TG342	*Sca*I	7	14	0	77	176	90	10	85	5	–0.5
	dfr1.1	TG465	*Sca*I	1	8	0.0003	77	175	81	13	88	7	–4.4
No. of flowers (NOF)	*nof10.1*	u	—	10	5	0.002	5.1	172	—	0	—	0	0
	nof7.1	CD62	*Eco*RV	7	7	0.0006	4.8	167	5.8	23	5.3	6	0
	nof9.1	TG254	*Hin*dIII	9	8	0.0007	4.9	182	5.0	1	6.9	5	–48
	nof1.1	TG125	*Hin*dIII	1	7	0.001	4.9	182	6.1	11	5.9	3	–0.6
	nof11.1	TG57	*Eco*RI	11	5	0.008	4.9	169	5.0	10	6.5	6	–75
Fertility (FERT)	*fert7.1*	TG342	*Sca*I	7	11	0	3.6	176	2.6	9	1.8	6	–2.8
	fert5.1	TG185	*Eco*RV	5	6	0.002	3.6	173	3.2	13	2.3	9	–7.0
	*fert4.1**	TG574	*Bst*NI	4	6	0.004	3.4	167	4.0	26	4.5	2	–2.4
Maturity (MAT)	*mat7.1*	TG342	*Sca*I	7	9	0.0001	3.3	176	4.6	9	4.7	6	–1.5
Plant growth habit (GRO)	*gro3.1*	CD51	*Eco*RV	3	6	0.004	3.7	173	4.4	16	4.7	7	–1.7
	gro9.1	CT143	*Sca*I	9	6	0.004	3.9	157	3.2	30	3.2	6	–1.1
	*gro3.2**	TG242	*Dra*I	3	5	0.006	3.7	177	4.6	16	4.7	3	–1.3
	gro2.1	CT176	*Sca*I	2	5	0.008	3.8	174	3.2	18	5.0	4	4.7
Curly leaf (CURLY)	*curly11.1*	TG546	*Sca*I	11	13	0	3.4	165	1.5	13	2.0	9	–0.4
	*curly3.1**	CT82	*Hin*dIII	3	8	0.0004	3.4	160	2.4	27	2.0	9	–1.8
	curly5.1	TG23	*Hin*dIII	5	6	0.002	3.3	171	2.1	13	2.2	10	–0.8
	curly2.1	TG492	*Dra*I	2	6	0.004	3.3	169	2.9	20	1.4	7	–7.8
	curly6.1	TG178	*Sca*I	6	6	0.004	3.4	141	2.8	42	1.7	6	–5.0
	curly9.1	TG551	*Hin*dIII	9	5	0.006	3.3	182	2.0	13	1.0	1	–2.6
	curly8.1	TG302	*Eco*RI	8	5	0.008	3.0	162	4.0	21	3.9	9	–0.8

[a]Polymorphic enzyme used for each marker.
[b]R^2 indicates the percent phemotypic variation explained.
[c]EE indicates homozygous *L. esculentum* alleles;
[d]PP indicates homozygous *L. pimpinellifolium* alleles;
[e]EP indicates heterozygous alleles.
[f]d/a indicates degree of dominance.
*QTL with agronomically favorable effect and no association with any negative trait.

genomic region in several previous studies (Eshed and Zamir 1995; Bernacchi et al. 1998; Chen et al. 1999; Fulton et al. 2000; Saliba-Colombani et al. 2001). This QTL, *ssc9.2*, has recently been cloned (Fridman et al. 2000).

Stem scar (SCAR)

Seven QTLs were identified for SCAR (Table 1; Fig. 2). The QTL on chromosome 2 had the most significant effect on the trait and explained 14% of the total phenotypic variance for the trait (Table 1). For six of the QTLs, the PM alleles had a favorable effect, reducing the size of the fruit stem scar. Only one QTL (*scar6.1*) had been previously identified (Fulton et al. 2000). Stem scar size is an important trait for processing tomatoes because fruit with small stem scars usually release better during harvest, yield attractive whole tomato products and peel more easily resulting in less waste during processing. In addition, large stem scars may penetrate into the fruit and be visible as a "yellow eye" if the processed peeled fruit is whole or diced. However, varieties with very small stem scars are undesirable because the fruit may fall off the plant prematurely during mechanical harvesting. The most significant QTL (*scar2.1*) for stem scar was located in the same genomic region where the most significant fruit weight QTL (*fw2.2*) was located (Fig. 2). Because the PM allele for *fw2.2* is associated with a decrease in fruit weight, a change that is unacceptable for any breeding program, *scar2.1* is not an appropriate target for reducing stem scar. However, the QTL on chromosome 8 that accounted for 10% of the phenotypic variance for the trait (Fig. 2) did not coincide with any reduced fruit weight QTL. Therefore, *scar8.1* might be useful in breeding tomatoes with smaller stem scars without sacrificing fruit weight.

Fruit cracking (RFC and CFC)

Two QTLs were identified for RFC on chromosomes 2 (*rfc2*.1) and 9 (*rfc9.1*) (Table 1; Fig. 2). The QTL on chromosome 2 accounted for 13% of the total phenotypic variance for the trait. For both QTLs, the PM alleles were associated with an increase in RFC (Table 1). Three QTLs were identified for CFC and accounted for 7 to 10% of the total phenotypic variance for the trait (Table 1; Fig. 2). In general for CFC, the PM allele was associated with an increase in cracking; however, for *cfc2.1*, an appreciable increase in cracking was only detected in the heterozygous individuals. Two of the cracking loci (*rfc2.1* and *cfc2.1*) were colocalized to the bottom of chromosome 2 suggesting that the two types of cracking may, in some cases, be the pleiotropic effect of a single locus. Saliba-Colombani et al. (2001) observed that smaller fruit had more cracks, which they attributed to reduced elasticity. Results from this study agree with their findings as two different cracking loci mapped to the vicinity of *fw2.2* and the PM alleles at these loci were associated with reduced fruit size and increased cracking.

Fruit rot (ROT)

Four QTLs were identified for ROT on chromosomes 2, 8, and 9 (Table 1; Fig. 2). The QTL on chromosome 2 had the most significant effect on the trait and accounted for 9% of the total phenotypic variance for ROT. For all QTLs, the PM allele was associated with more ROT. Interestingly, three of the four ROT QTLs were mapped to regions that also contained loci controlling concentric fruit cracks (CFC). This is not surprising, because increased cracking may make fruit more susceptible to rot.

Seed weight (SW)

Five QTLs were identified for SW (Table 1; Fig. 2). The QTL on chromosome 4 (*sw4.1*) had the most significant effect on SW and explained 17% of the total phenotypic variance for this trait (Table 1). For the three QTLs on chromosomes 1 (*sw1.1*) and 7 (*sw7.1* and *sw7.2*), the PM alleles were associated with a decrease in SW. This result is consistent with the phenotype of PM, which has an average seed size of approximately 1 mg as compared with 3 mg for the LE parent. Some of the seed weight QTLs detected in the IBLs have also been reported in several other studies that examined different wild species (reviewed in Doganlar et al. 2000). Among these QTLs, *sw4.1* was shown to be a major locus controlling seed size and the most conserved seed weight gene in tomato, suggesting that *sw4.1* is an orthologous seed weight gene found in all wild tomato species (Doganlar et al. 2000).

Seed number (SN)

Four QTLs were identified for SN (Table 1; Fig. 2). One of the QTLs on chromosome 5 (*sn5.1*) had the strongest effect on SN and explained 9% of the total phenotypic variation for this trait (Table 1). For this QTL, one dose of the PM allele increased seed number by as many as 11 more seeds per fruit. Increased seed number per fruit might be an important issue, especially for hybrid seed production, which is costly and often yields only a very limited number of seed per fruit. Therefore, *sn5.1* might be used in breeding programs for improving SN.

Days to first flowers (DFL)

Two QTLs were identified for DFL, each explaining 6–7% of the total phenotypic variance for the trait (Table 1; Fig. 2). For the QTL on chromosome 3 (*dfl3.1*), two doses of the PM allele increased number of days to first flower by approximately 4 days as compared with homozygosity for the LE allele. *dfl4.1* corresponds to the same region of chromosome 4 to which a QTL was mapped for early fruit ripening in a BC_1F_1 population using the same donor and recurrent parents (Tanksley et al. 1996). In that study, the heterozygotes (LE/PM) were also associated with earliness. This QTL may also correspond to a fast fruit set locus mapped to approximately the same location in an intraspecific F_2 population derived from an breeding line for which *L. pimpinellifolium* was a progenitor (Lindhout et al. 1994). *dfl3.1* maps to the same region of chromosome 3 to which a major flowering time QTL was previously mapped using the interspecific cross *L. pennellii* (LA716) × *L. esculentum* (de Vicente and Tanksley 1993). It seems likely that *dfl3.1* and the locus reported in that earlier study are allelic. The QTL *dfl4.1* could be useful for improving DFL in hybrid cultivars because it reduces flowering time by 5 days when it is in the heterozygous condition.

Days to first ripe fruit (DFR)

Two QTLs were identified for DFR (Table 1; Fig. 2). For

both, the PM alleles increased the length of time from sowing to first ripe fruit by as much as 13 days (*dfr7.1*). This more significant QTL mapped to the short arm of chromosome 7 in a position coincident with a QTL mapped for delayed maturity and reduced fertility in a BC_2F_1 population involving the same parents as the current study (Tanksley et al. 1996). The other QTL, *dfr1.1*, did not coincide with maturity or fertility QTLs.

Number of flowers per inflorescence (NOF)

PM has significantly more flowers per inflorescence, an average of 18, than the LE parent, which has an average of 5. Five QTL were identified for the number of flowers per inflorescence with phenotypic effects ranging from 5 to 8%. The QTL on chromosome 1 (*nof1.1*) mapped to the same location as a major QTL affecting flower number reported for the wild species *L. hirsutum* (Bernacchi and Tanksley 1997). We propose that the *hirsutum* and *pimpinellifolium* QTL are allelic and, in both cases, the wild species alleles specify production of a higher number of flowers compared with the corresponding *L. esculentum* allele. The QTL *nof11.1* could be employed to increase NOF in hybrid cultivars because individuals that are heterozygous at this locus produce nearly two more flowers per truss than either parental type.

Fertility (FERT)

Three QTLs were detected for fertility (increased fruit set) with effects ranging between 6 and 11% of the total phenotypic variance (Table 1; Fig. 2). For the QTLs on chromosomes 5 and 7, the PM alleles were associated with a reduction in fruit set and mapped to the same position as a FERT QTL mapped in the BC_2F_1 population (Tanksley et al. 1996). For the QTL on chromosome 4, the PM allele was associated with an increase in fruit set and did not coincide with any other QTL with unfavorable effects. Therefore, this QTL might be used to improve the trait.

Maturity (MAT)

One QTL was detected for maturity (Table 1; Fig. 2). For this QTL, the PM allele was associated with a delay in fruit ripening. *mat7.1* was also identified in the *L. pimpinellifolium* BC_2F_1 population (Tanksley et al. 1996). *mat7.1* colocalized with *fert7.1*, suggesting that their effects may be due to pleiotropic effects of a single locus.

Plant growth (GRO)

Four QTLs were detected for GRO, the amount of vegetative plant growth, with phenotypic effects ranging from 5 to 6% (Table 1; Fig. 2). Two separate QTLs mapped to chromosome 3 and in both instances the PM allele was associated with an increase in plant growth. For the QTLs on chromosomes 2 and 9, the PM alleles were associated with reduced plant growth. *gro9.1* was also identified in a *L. pimpinellifolium* derived population by Tanksley et al. (1996). In addition, *gro9.1* mapped to the same region as a GRO QTL in a *L. peruvianum* derived population (Fulton et al. 1997).

Leaf curliness (CURLY)

Leaf curliness describes the adaxial rolling or curling up of the plant's leaves. This normally occurs late in the season and can result in fruit sunscald because fruit are less protected from the sun when leaves curl. Seven QTLs were identified for CURLY (Table 1; Fig. 2). The QTL on chromosome 11 had the most significant effect on leaf curliness and explained 13% of the phenotypic variance for the trait with the PM allele being associated with reduced leaf curl (Table 1). The other CURLY QTLs were of lesser effect and, in all but one case (*curly8.1*), the PM allele was associated with an increase in leaf curliness.

QTL clustering and possible pleiotropic effects

Sixteen genomic regions were identified with significant effects on more than one trait and 31 regions were found to be associated with single traits (Fig. 2). The regions characterized by the largest clusters of QTLs were the bottom of chromosomes 2 (6) and the top of chromosome 8 (5). For example, the major QTLs for FW, FL, FD, and SCAR were all localized in clusters on chromosome 2 and 3. Some of these QTL colocalizations could be due to physiological relationships among traits. For example, FL, FD, and SCAR are components of FW, thus it is expected that QTLs controlling these traits would map to the same regions of chromosome 2 and 3. In addition, significant QTLs for cracking and rot also mapped to the vicinity of the chromosome 2 cluster. Many of the aforementioned traits (FL, CURLY, CFC, and ROT) were also found in clusters on chromosomes 8 and 9. Although we assume that pleiotropy played a significant role in the expression of morphologically and physiologically related traits, it is in fact unknown if the colocalization of related QTL was due to linkage or pleiotropic effects of the same gene. Additional genetic studies including high resolution mapping and perhaps gene cloning are required to distinguish between linkage and pleiotropy. To date, two of the QTLs identified in this study have been isolated: *fw2.2* and *ssc9.1* (Frary et al. 2000; Fridman et al. 2000).

Comparison of the IBLs with AB-QTL analysis

Tanksley and Nelson (1996) proposed the AB-QTL strategy as a breeding method for combining QTL analysis (the detection of favorable alleles for quantitative traits from exotic germplasm) with variety development (the introgression of these alleles into elite breeding lines). AB-QTL analysis differs from other methods of QTL mapping because the marker-trait analysis is delayed until the BC_2 or BC_3 generation when the investigated population resembles the recurrent parent of the cross. AB-QTL analysis has been demonstrated in tomato (Grandillo and Tanksley 1996; Tanksley et al. 1996; Bernacchi et al. 1998; Fulton et al. 1997, 2000), rice (Xiao et al. 1998), and barley (Pillen et al. 1998). The inbred backcross breeding method uses populations that have a similar structure to those used for AB-QTL analysis because the recurrent parent alleles occur at a high frequency. In this study, the IBLs were developed from a BC_2 AB-QTL population by selfing individual plants for an additional five generations.

Table 2 shows how BC_1, BC_2 families, and IBL-QTL populations derived from a single F_1 individual differ in terms of the number of QTLs detected, percent variation (R^2) explained by each QTL and gene action. For all three experiments, single plant evaluations were done. Only five traits, fruit ripening time, fruit color, soluble solid content, fruit

Table 2. Comparison of QTL detected in the BC_1, BC_2, and IBL (inbred backcross lines) populations derived from a single *L. esculentum* cv. E6203 × *L. pimpinellifolium* (LA1589) F_1 individual.

	Population used in QTL analysis								
	L. esculentum 'E6203' × *L. pimpinellifolium* (LA1589)[a] (257 BC_1)			L. esculentum 'E6203' × *L. pimpinellifolium* (LA1589)[b] (170 BC_2)			*L. esculentum* 'E6203' × *L. pimpinellifolium* (LA1589)[c] (196 BC_2)		
Traits	No. of QTLs	% PVE (R^2)	No. of favorable QTLs	No. of QTLs	% PVE (R^2)	No. of favorable QTLs	No. of QTLs	% PVE (R^2)	No. of favorable QTLs
Fruit ripening time	3	11	3	4	20	3	2	14	0
Fruit color	2	28	2	5	10	3	3	9	2
Soluble solid content	3	47	3	12	18	11	2	7	1
Fruit weight	7	32	0	8	20	1	2	15	0
Fruit shape	2	27	0	4	37	2	2	9	2

[a]Grandillo and Tanksley 1996.
[b]Tanksley et al. 1996.
[c]This work.

weight, and fruit shape, were examined in all three populations. In the BC_1 generation, a total of 17 QTLs were detected for the aforementioned five traits (Grandillo and Tanksley 1996). In the study that used BC_2 families, 33 QTLs were identifed for these five traits (Tanksley et al. 1996). However, only 11 QTLs were detected in the IBL population. Five of these QTLs were also detected in at least one of the previous studies. It was expected that fewer QTL would be detected in the IBL population because 88% of the loci were fixed for LE alleles and the population was segregating for fewer loci than the AB-QTL populations. Thus, some of the QTL that were detected in the previous studies were probably not identified in the current work because they were not segregating in the IBLs.

For each trait, the percent variation explained by each QTL detected in the BC_1 and BC_2 populations was generally much higher than that detected in the IBLs. In addition, a greater number of QTLs with favorable effects from the PM parent were identified in the AB-QTL population than in the IBLs. These results suggest that, compared with the IBLs, the AB-QTL populations may be more efficient for mapping and precisely estimating QTL effects (Tanksley and Nelson 1996). A major objective of AB-QTL analyses has been the creation of near-isogenic lines (NILs) that will carry a specific targeted region of the donor parent. This allows one to attribute any phenotypic differences between the recurrent parent and the NIL to the existence of a specific QTL from the donor parent within the specific introgression. On the contrary, each IBL contains at least several segments from the donor parent (Tanksley and Nelson 1996). In this study, none of the IBLs contained single introgressions from the donor parent. Thus, there will always be the possibility of problems associated with linkage drag in the IBLs (Tanksley and Nelson 1996).

Despite the drawbacks of IBLs, the population structure has a major advantage when compared with other advanced backcross population: IBLs are permanent mapping resources. As a result, new markers can be added to the map as they become available. Because of the genetic relatedness of the two parents, the *L. esculentum* × *L. pimpinellifolium* population described in this paper displayed good genetic recombination and, therefore, can be used for enriching the molecular genetic linkage map of tomato. The permanent nature of the lines also makes it possible to extend the QTL analysis of the population to additional traits. Thus, data for the population can accumulate over time from many different studies.

Selection of optimized subset of IBL population

To facilitate use of the IBL population, a subset of 100 of the 196 lines was selected using a randomized greedy algorithm in the software package MapPop, which attempts to minimize the expected distance between flanking recombination breakpoints for a marker placed uniformly at random along the genetic map (Vision et al. 2000). The expected distance between flanking recombination breakpoints (i.e., bin size) for the optimized subset of 100 lines is 1.7 cM (ranging from 0.5 to 1.9 cM), which is only slightly less than that of the whole population, 1.2 cM. In comparison, a randomly selected set of 100 plants had a bin size of 2.3 cM. In addition, the optimized subset has less segregation distortion

than both the entire population and the randomly selected subset. Genotypic and phenotypic data for the optimized subset of the IBL population are available through the Solanaceae Genome Network (http://soldb.cit.cornell.edu).

Conclusions

Inbred-backcross lines (IBLs) were originally developed for determining the minimum number of genes controlling a quantitative trait (Wehrhahn and Allard 1965). This technique has also been used as a breeding method for transferring desirable genes from exotic germplasm into cultivated backgrounds (Bliss 1983). Furthermore, such populations have been used for detecting QTLs for many agronomically important traits in various crop species using molecular markers (Hartman and St. Clair 1998; Lin et al. 1998; Butruille et al. 1999). These researchers reported that such populations can be used to perform molecular analyses of quantitative traits despite their unbalanced population structure.

In this work, we studied 22 quantitatively inherited traits in a population of 196 BC_2F_6 inbred backcross lines of tomato. Based on the marker analysis, we found that the genome of each IBL was composed, on average, of 88% *L. esculentum* alleles, a value quite comparable with the expected value of 87%. This result demonstrated that the recurrent parent's genome was progressively recovered through two backcrosses and five generations of selfing.

We were able to identify QTLs for all of the traits examined. A total of 47 different genomic regions in the IBLs were found to be associated with at least one trait. For 16 of these regions, QTLs that had significant effects on more than one trait were identified. In most cases, traits that were colocalized showed significant correlations between each other. For example, fruit cracking and fruit rotting, which are correlated attributes, usually mapped in close proximity to one other. This is consistent with previous QTL studies conducted in other populations derived from various wild species (Paterson et al. 1988, 1990; de Vicente and Tanskley 1993; Grandillo and Tanksley 1996; Tanksley et al. 1996; Fulton et al. 1997; Chen et al. 1999). Thus, the colocalization of QTLs and correlations among traits may be due to pleiotropic effects of the same genes rather than linkages of independent genes. However, it is almost impossible to separate linkages and pleiotropy unless QTLs have been cloned. Currently, only two of the aforementioned QTL for FW (*fw2.2*, Frary et al. 2000) and SSC (*ssc9.2*, Fridman et al. 2000) have been cloned.

Several novel QTLs for agronomically important traits were identified in this study, including loci for fruit shape, firmness, color, stem scar size, seed and flower number, leaf curliness, plant growth, fertility, and flowering time. Because each IBL contains relatively small introgressions from the wild parent, the lines are valuable resources for introducing these new, useful wild alleles into the elite background of cultivated tomato. Thus, the lines can serve as an important breeding tool. We have also shown that a population of inbred-backcross lines can be a valuable permanent genetic resource. To facilitate such use of this population, seed for a selected subset of 100 IBLs will be available through the Tomato Genetics Resource Center (http://tgrc.ucdavis.edu; Davis, Calif.).

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