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Recombinant inbred lines for genetic mapping in tomato

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Abstract A cross between the cultivated tomato *Lycopersicon esculentum* and a related wild species *L. cheesmanii* yielded 97 recombinant inbred lines (RILs) which were used to construct a genetic map consisting of 132 molecular markers. Significant deviation from the expected 1:1 ratio between the two homozygous classes was found in 73% of the markers. In 98% of the deviating markers, *L. esculentum* alleles were present in greater frequency than the *L. cheesmanii* alleles. For most of the markers with skewed segregation, the direction of the deviation was maintained from F_2 to F_7 generations. The average heterozygosity in the population was 15%. This value is significantly greater than the 1.5% heterozygosity expected for RILs in the F_7 generation. On average, recombination between linked markers was twice as high in the RILs than in the F_2 population used to derive them. The utility of RILs for the mapping of qualitative and quantitative traits is discussed.

Key words Tomato · Recombinant inbred lines · Genetic mapping · Molecular markers

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Introduction

Genetic maps consisting of molecular markers have been developed in recent years for many crop plants (Paterson and Wing 1993). Most of the mapping studies with molecular markers have used single-meiosis populations such as F_2s or backcrosses. The use of dihaploid populations has been restricted to a few crop species, such as pepper and barley, in which the construction of such populations is feasible (Heun et al. 1991; Lefebvre et al. 1992). In contrast to single-meiosis populations, recombinant inbred lines (RILs) are derived by selfing or sib-mating the progeny of F_2 or backcross generations until the lines reach homozygosity.

RILs have several advantages over other populations used for genetic mapping. Becuase the lines are genetically homozygous, they can be propagated without further segregation. The lines can, therefore, be distributed and replicated for experiments in different laboratories and environments. This characteristic is particularly useful for the analysis of quantitative traits because it allows for a reduction of the environmental component of the total phenotypic variance. Many studies of quantitative trait locus (QTL)-mapping have used one or limited environments for evaluation of the phenotype (e.g., Nienhuis et al. 1987; Paterson et al. 1988). These studies have, therefore, ignored the genotype-by-environment interaction that exists for quantitative traits. The importance of using multiple environments for QTL-mapping was demonstrated by Paterson et al. (1991) who showed that only 4 out of 29 QTLs identified for fruit characters in tomato were detected in all three environments tested. These results point to the need for a mapping population that can be replicated and evaluated in multiple environments. QTL-mapping with RILs is more efficient than with an F_2 population because fewer individuals are needed to detect linkage of the same magnitude between a marker and QTL (Simpson 1989).

Because RILs undergo several rounds of meiosis, the chance that a recombination event will occur between linked loci is greater than in a single-meiosis population of the same size. For RILs obtained by selfing or by sib-mating, there is a twofold or fourfold increase in recombination frequency, respectively, between two closely linked markers (Taylor 1978). Higher resolution maps can therefore be constructed with RILs than with single-meiosis populations and more accurate map distances are obtained with RILs than with an F_2 population of similar size (Burr and Burr 1991). The use of RILs for mapping different types of markers, e.g, co-dominant markers such as restriction fragment length polymorphisms (RFLPs) and dominant markers such as random amplified polymorphic DNAs (RAPDs), is equally efficient (Reiter et al. 1992). In an F_2, which is the most commonly used population for genetic mapping, the mapping of dominant markers is less efficient than the mapping of co-dominant ones (Allard 1956).

RILs have been used extensively for genetic mapping in mice (Bailey 1981). RIL populations have also been used for genetic mapping in several plant species, such as pea, maize, soybean and *Arabidopsis* (Burr et al. 1988; Ellis et al. 1992; Reiter et al. 1992; Lister and Dean, 1993; Mansur et al. 1993). In tomato, a highly saturated genetic map was constructed using an F_2 population derived from a *Lycopersicon esculentum* × *L. pennellii* cross (Tanksley et al. 1992). Additional F_2 and backcross populations were used for the mapping of qualitative and quantitative traits (e.g., Nienhuis et al. 1987; Behare et al. 1991). However, to-date no RIL population has been used for genetic mapping in tomato.

In this report, we describe the construction of a RIL population in tomato. This population was derived from an F_2 of a cross between *L. esculentum* and *L. cheesmanii*. The latter is a wild red-fruited species, closely related to the cultivated tomato, which is characterized by small fruits and high total soluble solids. High total soluble solids is of interest to tomato breeders because it is an important fruit quality parameter for both fresh-market sales and the processing of tomatoes. The original F_2 population from which the RILs were derived was used by Paterson et al. (1991) to map QTLs for fruit characters. This F_2 population was advanced to the F_8 generation by selfing to obtain the RILs. The availability of F_2 and F_8 populations that were mapped with identical RFLP markers enabled us to follow changes in allele and recombination frequencies between the two generations. Use of the RIL population for the mapping of quantitative trait loci is reported in a companion paper (Goldman et al. 1994).

Materials and methods

Plant material

The RILs were constructed from an interspecific cross between *L. esculentum*, cv UC204B (hereafter E) and *L. cheesmanii*, LA483 (hereafter C) as described by Paterson et al. (1991). A total of 350 F_2 plants were self pollinated and advanced to the F_5 generation using single-seed descent.

Because of sterility problems in many of the lines at F_5, only 120 lines remained. From the F_5 generation, five individuals from each line were planted and the most fertile ones were used as seed parents for the next generation until the F_8 generation. The final population consisted of 97 lines each originating from a different F_2 individual.

DNA extraction and Southern hybridization

DNA was extracted from a bulk of 30 individuals from each F_8 line. This bulk reconstituted the genotype of the F_7 plant from which seeds were obtained for the next generation. Procedures for DNA extraction, Southern blotting, and hybridization were as described by Bernatzky and Tanksley (1986). Five restriction enzymes were used for most of the markers: *Bst*NI, *Dra*I, *Eco*RI, *Eco*RV and *Hae*III. Monomorphic markers for these enzymes were screened with four additional enzymes: *Hin*dIII, *Msp*I, *Sca*I and *Xba*I. A total of 132 RFLP markers were mapped in the RIL population. The markers were selected to allow a coverage of the tomato genome at approximately 10 cM intervals between adjacent markers, based on the F_2 map constructed by Tanksley et al. (1992). Markers that were used to construct the F_2 map from the cross between *L. esculentum* × *L. cheesmanii* were also included (Paterson et al. 1991). For a few chromosomes, intervals between markers were greater than 20 cM because of a lack of polymorphism with the above enzymes. In addition to the RFLP markers, we scored one isozyme, *Adh1*, and two morphological markers, *B* and *sp* (Stevens and Rick 1986).

Linkage analysis

The markers used in this study have already been mapped in other populations and no change in gene order was observed between the *L. esculentum* × *L. pennellii* and *L. esculentum* × *L. cheesmanii* maps (Paterson et al. 1991; Tanksley et al. 1992). Markers were therefore assigned to linkage groups based on their known chromosome location, and their order was then verified by the use of the MAPMAKER program (Lander et al. 1987). For each chromosome, loci were ordered at a minimum LOD score of 2.0. Only markers that were consistent with the previously published order were retained for further analysis. Recombinant frequencies and map distances were calcualted with the aid of the RI Plant Manager program (version 2.3 of the program RI Manager; Manly 1993). The relationship between map distance in a single-meiosis population (r) and the percent of recombinants in the RILs (R) was calculated by the formula $r = R/(2 - 2R)$. This formula assumes complete homozygosity, a situation that was not maintained in the RIL population. Therefore, the estimates of map distances should be considered as an approximation.

Statistical analysis

Tests of significance for the monogenic segregation in the F_2 and F_7 generations were performed by a chi-square goodness of fit. Significance tests for differences in recombinant frequencies between the F_2 and F_7 generations, and between the expected and observed recombinant frequencies in the F_7 generation, were computed by a 2×2 contingency table.

Results

Marker segregation

A strong selection against C alleles was detected in the RILs at most of the marker loci (Fig. 1A). Seventy-three percent of the loci deviated significantly ($P < 0.05$) from

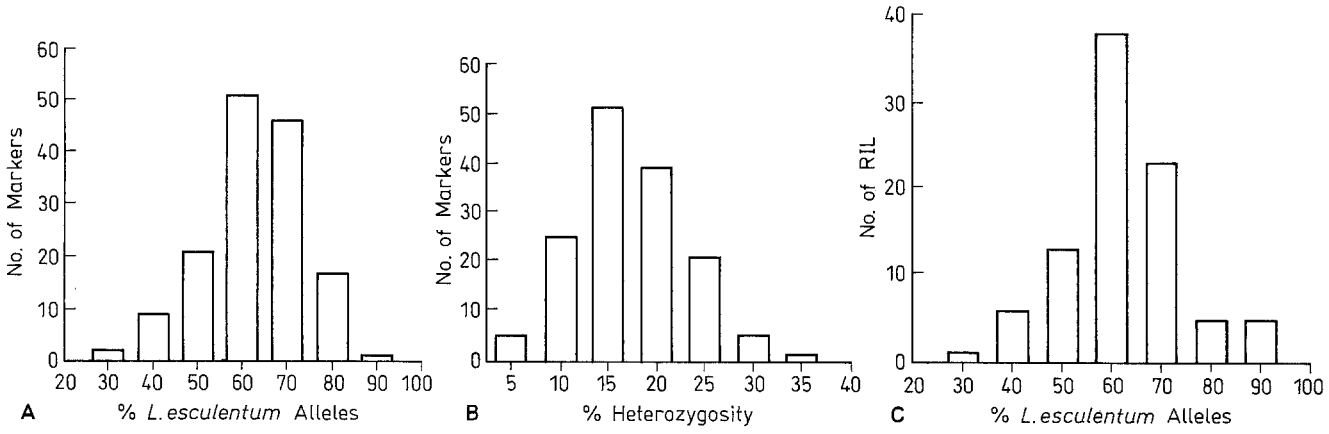

Fig. 1A–C Distribution of the percent *L. esculentum* alleles and the percent heterozygosity in tomato recombinant inbreds. **A** Distribution of the percent *L. esculentum* alleles for a number of molecular markers. **B** Distribution of the percent heterozygosity of the RILs for a number of molecular markers. **C** Distribution of the percent *L. esculentum* alleles for a number of recombinant inbred lines

the expected 1:1 ratio between the homozygous genotypes, favoring E alleles. The skewed loci were scattered throughout the genome. In most genomic regions, the degree of deviation was similar for linked markers. However, for few regions, one marker deviated strongly while its nearest neighbor did not, e.g., *TG140* and *TG188* in chromosome 3 (E = 0.63, E = 0.47 respectively). Average frequencies of E and C homozygous classes at the examined markers were 0.59 and 0.24 respectively. The average frequency of the heterozygous class was 0.15 (Fig. 1B). The strongest deviation was observed on chromosome 11 in the vicinity of the gametophytic factor × (Rick and Butler 1956), which causes selective abortion of gametes containing C alleles. For *TG523* in this region, the frequency of E was 0.93. Additional regions that deviated strongly were: *CT255* on chromosome 2 (E = 0.88), *TG75* on chromosome 4 (E = 0.82), and *TG280* on chromosome 10 (E = 0.81). For a few markers, however, C alleles were favored, namely *TG42* (E = 0.35) on chromosome 3, *B* and *TG178* on chromosome 6 (E = 0.47 for both loci); and *CD54* and *CD65* on chromosome 7 (E = 0.37, E = 0.46 respectively).

Genome composition of the RIL population

As expected from the skewed segregation of the markers, the average RIL was comprised mostly of the E genome (Fig. 1C). The average RIL was homozygous E and homozygous C at 59% and 25% of the scored markers, respectively. The respective distribution ranges for the homozygous classes were 27–91% and 0–67%. The average RIL was heterozygous for 16% of the loci with a range of 0–50%. The observed percent heterozygosity in the RIL population after seven generations of selfing was higher than the expected 1.5%.

Comparison of segregation of markers between F_2 and F_7 generations

The monogenic segregation of markers that were genotyped in both F_2 (Paterson et al. 1991) and F_7 is presented in Table 1. Segregation distortion ($P < 0.05$) for an expected 1:1 ratio between the two homozygous classes was observed for 50% of the markers genotyped in the F_2 generation. Out of the same markers genotyped in the F_7 generation, 79% deviated significantly from the expected 1:1 ratio for the two homozygous classes. The average ratio between the homozygous E and homozygous C classes was increased from 1.59 in the F_2 to 2.83 in the F_7 (Table 1). For most of the markers with skewed segregation, the direction of the deviation was maintained from the F_2 to the F_7 generations. For loci that deviated significantly towards E in the F_2, the deviation became more significant in the RILs. For loci that had non-significant deviation towards E in the F_2, the deviation became significant in the RILs. For loci such as *TG42* on chromosome 3 and *CD65* and *CD54* on chromosome 7, that deviated significantly towards C in the F_2, the direction of the deviation was maintained in the RILs. However, for loci, such as *CD4* on chromosomes 3 and *TG20* on chromosome 7, which deviated towards C in the F_2, a significant deviation towards E was observed in the RILs.

Recombination in the RILs

In an ideal RIL (where alleles are fixed and no segregation distortion is present), recombination frequency between closely linked markers is expected to be twofold higher than in an F_2 population (Taylor 1978). A twofold increase in recombination results in difficulties in the establishment of linkage between markers that are more than 20 cM apart (Burr et al. 1988). Using the RIL population, we constructed a genetic map consisting of 132 markers (Fig. 2). Due to the map expansion, we failed to detect linkage for markers in six linkage groups. The map spans 1209 cM within the linked markers at an average spacing of 10 cM between markers.

Table 1 Comparison of the deviation from a 1:1 ratio between the homozygous classes and of the level of heterozygosity in F_2 and F_7 generations of the cross *L. esculentum* × *L. cheesmanii*[a]

Chrom.	Marker	F_2					F_7				
		E	H	C	E/C	Freq. of H	E	H	C	E/C	Freq. of H
1	*CD15*	91	168	64	1.4*	0.52	34	18	22	1.5*	0.24
	TG125	67	168	70	0.9	0.55	34	22	21	1.6**	0.29
	TG59	81	166	76	1.0	0.51	49	8	15	3.3**	0.11
	TG71	82	165	71	1.1	0.52	48	11	13	3.7**	0.15
	TG83	90	160	76	1.1	0.49	51	13	11	4.6**	0.17
	TG465	50	168	56	0.9	0.61	42	18	11	3.8**	0.25
2	*TG165*	126	177	20	6.3**	0.55	64	16	14	4.5**	0.17
	TG48	126	144	27	4.6**	0.48	48	22	26	1.8**	0.23
	CD66	112	163	34	3.3**	0.53	35	13	16	2.2**	0.20
3	*TG114*	87	153	84	1.0	0.47	52	7	14	3.7**	0.10
	TG130	69	140	60	1.1	0.52	26	5	12	2.1*	0.12
	TG74	51	174	89	0.6**	0.55	34	15	27	1.2	0.20
	TG42	22	159	124	0.2**	0.52	20	21	47	0.4**	0.24
	CD4A	39	166	109	0.3**	0.53	39	17	27	1.4*	0.20
4	*TG15*	91	160	71	1.3	0.50	32	10	21	1.5	0.16
	TG182	84	163	71	1.1	0.51	55	10	11	5.0**	0.13
	TG2	90	161	51	1.7**	0.53	58	9	12	4.8**	0.11
	TG75A	97	145	46	2.1**	0.50	58	6	9	6.4**	0.08
	CD39	86	169	59	1.4*	0.54	35	14	33	1.4	0.28
	TG37	84	160	71	1.1	0.51	32	21	23	1.4*	0.28
5	*TG23*	82	165	74	1.1	0.51	57	18	15	3.8**	0.20
	TG185	72	165	69	1.0	0.54	51	10	21	2.4**	0.12
6	*TG178*	85	122	57	1.5**	0.46	36	5	40	0.9	0.06
	TG118	85	162	64	1.3	0.52	49	15	30	1.6*	0.16
	TG54	88	135	63	1.4*	0.47	46	12	23	2.0**	0.15
	TG253	79	141	69	1.1	0.49	37	12	31	1.2	0.15
	TG314	39	187	68	0.6**	0.64	47	19	16	2.9**	0.23
7	*CD65*	65	159	81	0.8	0.52	31	20	38	0.8*	0.22
	TG20	53	181	91	0.6**	0.56	44	17	33	1.3*	0.18
	TG170	78	151	87	0.9	0.48	46	14	29	1.6*	0.16
	CAB4	78	178	59	1.3*	0.57	44	12	26	1.7*	0.54
	CD54	51	169	71	0.7*	0.58	19	6	35	0.5*	0.10
8	*TG181*	27	45	20	1.3	0.49	47	11	13	3.6**	0.15
9	*CD32A*	58	136	83	0.7*	0.49	36	11	28	1.3	0.15
	TG35	69	177	44	1.6**	0.61	58	14	18	3.2**	0.16
10	*TG52*	67	140	33	2.1**	0.58	54	14	7	7.7**	0.19
	CD34	70	145	44	1.6*	0.56	56	8	9	6.2**	0.11
	TG63	62	182	63	0.9	0.59	69	10	17	4.1**	0.10
11	*TG36*	109	166	43	2.5**	0.52	47	19	21	2.2**	0.22
	TG30	114	166	43	2.6**	0.51	51	18	20	2.5**	0.20
	TG147	146	154	18	8.1**	0.48	70	10	12	5.8**	0.11
12	*TG111*	77	179	71	1.1	0.55	65	12	14	4.6**	0.13
	TG50A	46	183	58	0.8	0.64	53	9	12	4.4**	0.12
	TG68	85	167	73	1.1	0.51	39	15	36	1.1	0.17
Average					1.6	0.53				2.9	0.17

* $0.01 < P < 0.05$
** $P < 0.01$
[a] E: homozygotes for *L.esculentum*, C: homozygotes for *L.cheesmanii*, H; heterozygotes.
Expected frequency of each of the homozygous classes was 0.25 and 0.5 in the F_2 and F_7 respectively

To determine whether recombinant frequencies were higher in the RILs than in the F_2, we compared the percent of recombinants between pairs of markers that were genotyped in both the F_2 (Paterson et al. 1991) and the F_7 generations (Table 2). For all pairs of markers except for *TG111-TG50* on chromosome 12, the recombinant frequency was higher in the RILs than in the F_2 generation, as was expected. Pairs of markers that were at least 20–25 cM apart in the F_2 were unlinked in the RIL population. The average recombinant frequency for the compared markers increased from 0.13 in the F_2 generation to 0.25 in the RILs (excluding markers that were unliked in the F_7). We also compared the expected and observed recombinant frequencies in the RILs (Table 2). For most pairs of markers there was no significant difference between the expected and observed values.

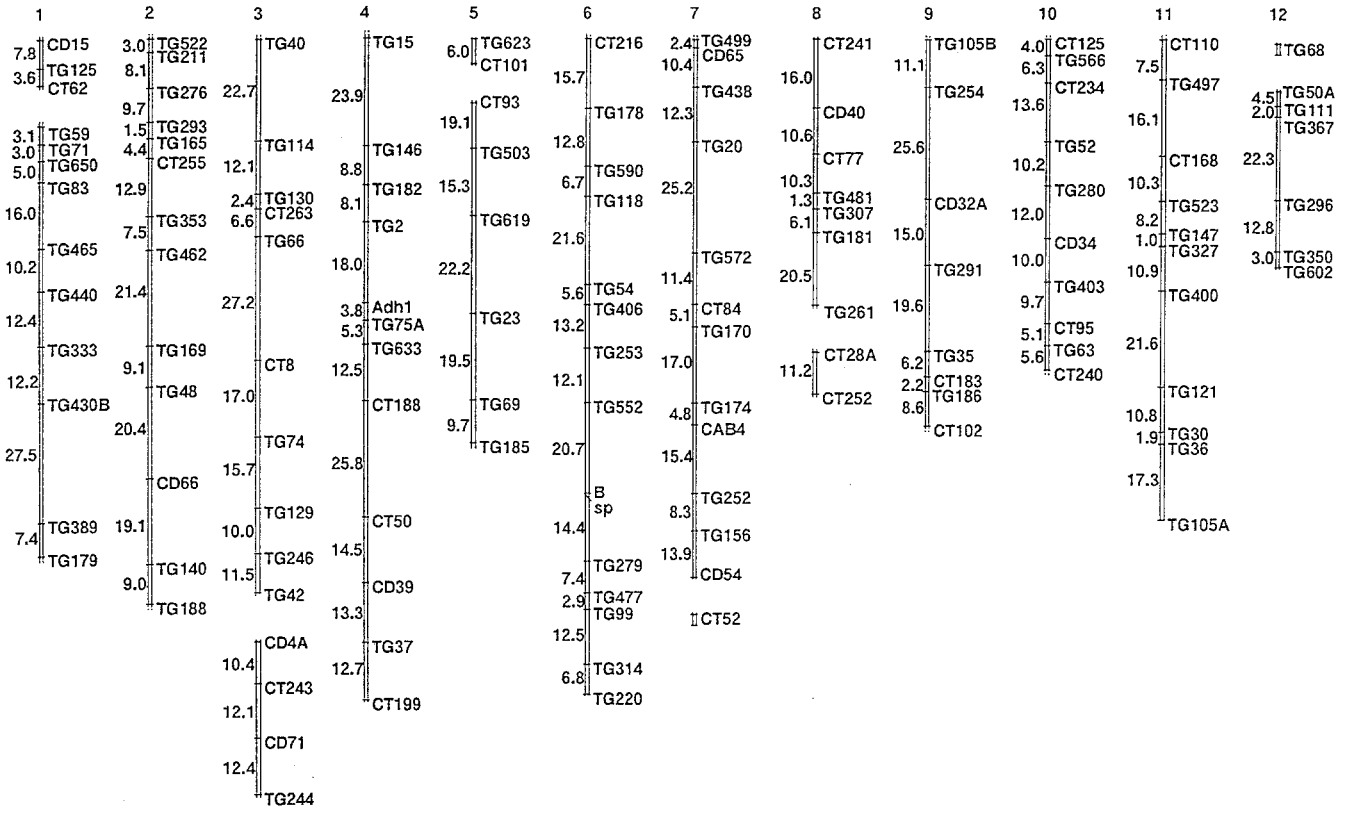

Fig. 2 Genetic map of recombinant inbred lines from the cross *L. esculentum* × *L. cheesmanii*. Chromosome numbers are recorded above the bars. *Numbers* to the left of the bar represent genetic distances in cM, calculated by the RI Plant Manager program. Marker names are to the right of the bar

Table 2 Comparison of percent recombinations for pairs of linked markers between the F_2 and F_7 generations and between expected and observed percent recombinants in F_7 generation of the cross *L.esculentum* × *L.cheesmanii*

Chrom.	Markers	%Rec		
		F_2	F_7[a] observed	F_7[b] expected
1	*CD15-TG125*	5.9	13.5*	16.7
	TG125-TG59	23.0	UL	33.3
	TG59-TG71	2.5	6.6*	5.6
	TG71-TG83	13.5	16.3	21.8
	TG83-TG465	15.1	24.1*	24.2
2	*TG48-CD66*	10.5	29.8**	19.8
3	*TG114-TG130*	8.2	19.4*	13.7
	TG130-TG74	31.5	UL	42.5
	TG74-TG42	25.1	UL	35.8
	TG42-CD4	17.3	UL	26.4
4	*TG15-TG182*	18.8	48.0**	28.5**
	TG182-TG2	9.2	14.7	15.2
	TG2-TG75	15.1	34.9**	24.2
	TG75-CD39	30.7	UL	41.8
	CD39-TG37	12.4	20.9*	20.6
6	*TG178-TG118*	6.7	32.9**	12.2**
	TG54-TG253	17.4	19.4	26.4
	TG253-B	14.7	49.3**	23.0**
7	*CD65-TG20*	12.2	17.9	20.6
	TG20-TG170	22.7	UL	32.4
	TG170-CAB4	20.8	34.1*	41.8
8	*CD40-TG181*	27.6	47.6**	38.2
9	*CD32-TG35*	24.1	UL	34.2
10	*TG52-CD34*	22.7	36.0*	33.3
11	*TG36-TG30*	3.5	4.4	6.5
	TG30-TG147	23.1	UL	35.8
12	*TG111-TG50*	10.9	9.0	18.0

*$0.01 < P < 0.05$
**$P < 0.01$
[a] Significance values refers to F_2–F_7 comparison. UL: unlinked
[b] Expected percent recombinants in F_7 (R) was derived from map distances in F_2 (r): $R = 2r/(1 + 2r)$. Significance values refer to the F_7 (observed)-F_7 (Expected) comparison

Discussion

We have constructed a molecular marker linkage map using RILs derived from the interspecific cross between *L. esculentum* and *L. cheesmanii*. The main objective in producing the RIL linkage map was to map genes that control qualitative and quantitative traits that segregate in the population. As an example of mapping genes for qualitative traits, the *Ve* gene that confers resistance to *Verticillium* race 1 was placed to chromosome 9 using the RIL map (Zamir et al. 1993a). The RILs were also utilized for high-resolution mapping of the genomic region in chromosome 11 which contains the *12* gene (Zamir et al. 1993b). This gene confers resistance to the fungal pathogen *Fusarium oxysporum* race 2. The use of RILs allowed for a fivefold increase of the mapping resolution in this region. Using the RILs, we detected five recombinants between markers that co-segregated in a similar-size F_2 population from a cross between *L. esculentum* and *L. cheesmanii* (Paran, Zamir and Fluhr, unpublished). The use of RILs for the analysis of quantitative traits, such as total soluble solids, fruit weight and seed weight, is described in a companion paper (Goldman et al. 1994).

The exceptations for maker segregation and recombination for RIL populations were examined in this cross and compared with the F_2 data. One of the objectives in comparing the F_2 and F_7 generations was to determine whether the segregation data in the RILs could be predicted from the F_2 generation. Deviation from a 1:1 ratio for each of the two homozygous classes was observed for most of the markers in the RILs as a result of overabundance of the homozygous E class. An indication of this directed deviation was already observed in the F_2 generation: out of 33 loci that were compared in the two generations, 17 that deviated towards E in the RILs had also deviated in the same direction in the F_2. The increase in segregation distortion from F_2 to F_7 probably resulted from a cumulative effect of selection against alleles of one of the parents during the propagation of the RILs.

The high degree of marker heterozygosity in the RILs could not have been predicted from the F_2 data because in the latter generation the heterozygous class was only slightly overabundant (a mean of 53% heterozygosity in the F_2 for the markers included in Table 1). Map expansion relative to the F_2 generation occurred in the RILs because markers spaced more than 20 cM apart in the F_2 were unlinked in the RILs. Similarly, linkage could not be determined between markers spaced more than 20 cM apart in maize RILs (Burr et al. 1988).

A few limitations of these RILs, however, may hamper their wide use as a mapping population in tomato: a low level of polymorphism, skewed segregation, and a high level of residual heterozygosity.

The RILs in this study were constructed from a cross between two closely related species. The choice of parents for the construction of interspecific mapping populations represents a compromise between the level of polymorphism detected between the species and the level of sterility in their progeny. The amount of polymorphism for DNA markers in the RILs was low compared to crosses in which the species used for mapping are more distantly related. However, the fertility of the RILs is much higher than that observed in the progeny of crosses with more distantly related species such as *L. pennellii*. Approximately one-quarter of the markers mapped in the *L. esculentum* × *L. pennellii* cross (Tanksley et al. 1992), using five restriction enzymes, were polymorphic in the RILs. Due to the relatively low level of polymorphism in the RILs, several gaps exist in the RIL map. Increasing the density of the map will require the screening of RFLP probes with additional restriction enzymes.

A second limitation of the RIL map is the skewed segregation favoring *L. esculentum* alleles observed for the majority of the markers. A similar observation was reported in an RFLP map using RILs obtained from a wide cross in rice (Wang et al. 1994). Skewed segregation in the RILs affected both the establishment of linkage groups and the estimation of recombinant frequency. Calculations of linkage usually assumes no segregation distortion and uses a recombination fraction of 0.5 for rejection of linkage. Skewed segregation, however, decreases the recombination fraction used to reject linkage and limits the RILs for detecting linkage among closely linked markers (Wang et al. 1994). Skewed segregation could also cause overestimation of recombinant frequency between linked markers. Unidirectional selection against C homozygotes increased the number of double heterozygotes in intermediate generations, because the observed level of heterozygosity in the RILs was much higher than expected, and therefore created more opportunity for recombinants to be formed in subsequent generations.

A high degree of heterozygosity was observed in the RILs, i.e., an average of 15% compared to the expected 1.5% heterozygosity in the F_7 generation. A heterozygosity of 15% is higher than that reported for maize RIL populations, namely, 1.6% and 2.7% (Burr and Burr 1991), or for *Arabidopsis* RILs (0.42%, Lister and Dean 1993). The level of heterozygosity in the tomato RILs could be the result of an unintentional selection against plants with low fertility during the propagation of the RILs, because only one-third of the original F_2 progenies were fertile enough to be propagated to the F_8. Low fertility could be expalined by the presence of homozygous chromosomes segments carrying C alleles. This phenomenon was observed for lines homozygous for particular segments that were introgressed from the wild species *L. pennellii* (Eshed and Zamir, unpublished). Introgression lines carrying these homozygous *L. pennellii* chromosome segments had lower yields than the same lines carrying the introgression in a heterozygous condition. For the RILs, the decrease in fertility observed during the propagation of the lines could be explained by the presence of homozygous C chromo-

some segments. Another explanantion for the high degree of heterozygosity could be cross-fertilization during propagation of the RILs. Continuous propagation of the RILs using conditions that minimize cross-fertilization will help to further decrease the degree of heterozygosity.

The availabilty of permanent mapping populations, such as the RILs and *L. pennellii* introgression lines (Zamir et al. 1993b), will greatly facilitate the mapping of new DNA clones in the tomato genome. Seeds of these lines will be distributed to different laboratories and mapping data will be added to the existing database. In addition to the mapping of new DNA clones, the RILs are currently being evaluated for various morphological quantitative traits in multiple environments in order to test the significance of genotype-by-environment interaction for these traits.

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